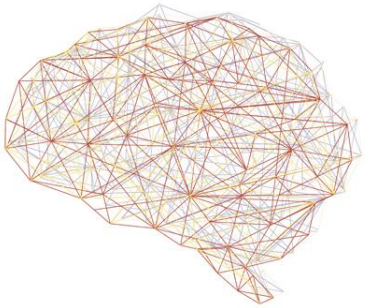

XV MEETING OF THE PORTUGUESE SOCIETY FOR NEUROSCIENCE

MAY 25-26, 2017 | Braga

ABSTRACT BOOK

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MELIÃ BRAGA HOTEL & SPA

VENUE:
Hotel Meliã Braga
Avenida General Carrilho da Silva Pinto 8,
4715-380 Tenões, Braga, Portugal
GPS Coordinates: 41.55663, -8.39663
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Sociedade Portuguesa de Neurociências

Life and Health Sciences Research Institute
Instituto de Investigação em Ciências da Vida e Saúde

Universidade do Minho
Escola de Medicina

PROGRAM – May 25, 2017

8h30	Registration
9h15	Welcome remarks
9h30	**Keynote Lecture – Repertoire and function of meningeal immunity in healthy and diseased brain.** **Jonathan Kipnis** | University of Virginia, USA.
10h30	Coffee break
11h00	**Session I – Neural circuits and behavior** Chairs: Nuno Sousa (ICVS, University of Minho) Rui Costa (Champalimaud Research)
11h00	**Mapping the mind with words.** **Sidarta Ribeiro** | Brain Institute, UFRN Selected oral communications
11h30	**Impairments in laterodorsal tegmentum to VTA projections underlie glucocorticoid triggered reward deficits.** Bárbara Coimbra - ICVS, University of Minho
11h45	**Activation of neuronal A2AR by ATP-derived adenosine facilitates hippocampal LTP: physiopathological role of CD73.** Francisco Queiroz Gonçalves - CNC, University of Coimbra
12h00	**γδ T cells are the major source of IL-17 in the meninges and control brain cognitive functions.** Julie Ribot - IMM, Lisboa
12h15	**Modulatory effects of an escape alternative in pro-social behavior.** Joana Carvalheiro - School of Psychology, University of Minho
12h30	Lunch
14h00	**Session II – Neurodegeneration** Chairs: João Cerqueira (ICVS, University of Minho) Odete Cruz e Silva (Ibimed, University of Aveiro)
14h00	**Cracking the code: protein posttranslational modifications and the molecular basis of neurodegeneration.** **Tiago Fleming Outeiro** | Univ. Medical Center Goettingen Selected oral communications
14h30	**Relevance of neuron-microglia vesicular trafficking, stress-related microRNAS and DAMPs in Alzheimer's disease.** Adelaide Fernandes - iMed.Ulisboa, Faculty of Pharmacy, University of Lisbon
14h45	**ATXN3 modulates mRNA splicing of Tau through ubiquitylation of splicing factors.** Andreia Neves-Carvalho - ICVS, University of Minho
15h00	**Sodium butyrate rescues dopaminergic cells from alphasynuclein-induced transcriptional deregulation and DNA damage.** Isabel Paiva - Dep. of NeuroDegeneration and Restorative Research, University Medical Center Goettingen
15h15	**Tau therapeutics in stress-driven brain pathology: exploring the path from depression to Alzheimer's disease.** Ioannis Sotiropoulos - ICVS, University of Minho
15h30	**Poster Session I** Coffee Break

17h30 **Session III – Neuropsychiatric disorders**
Chairs: Luísa Pinto (ICVS, University of Minho)
Rodrigo Cunha (CNC, University of Coimbra)

17h30 **My brain on a 24/7 schedule - impact on memory function.**
Luísa V. Lopes | IMM
Selected oral communications

18h00 **Exploring time-dependent anatomical and functional correlates of adult hippocampal cytogenesis in young-adult rats.**
António Mateus-Pinheiro - ICVS, University of Minho

18h15 **Chronic blockade of adenosine A2A receptors: gender-specific reprogramming of microglia morphology in the pre-frontal cortex.**
Carla Henriques - IBILI, University of Coimbra

18h30 **Autism-associated Caspr2 regulates synaptic AMPA receptors in the context of homeostatic synaptic plasticity.**
Dominique Fernandes - CNC, University of Coimbra

18h45 **Excitation/inhibition balance and glial function in mouse model of neurofibromatosis type 1: distinct susceptibility of hippocampus, prefrontal cortex and striatum.**
Joana Gonçalves - ICNAS, University of Coimbra

19h00 25th SPN Anniversary

19h15 SPN General Assembly

Dinner - Shuttles available from Meliã to dinner Venue - from 19:15 to 20:30

PROGRAM – May 26, 2017

9h00	**Session IV – Regeneration and therapies** Chairs: António Salgado (ICVS, University of Minho) Ana Paula Pêgo (INEB / i3S, University of Porto)
9h00	**Nanomedicine approaches to modulate neural stem cells for brain repair.** **Liliana Bernardino** | CICS, University of Beira Interior
	Selected oral communications
9h30	**A New Paradigm for Parkinson's Disease Regenerative Medicine based on the Secretome of Mesenchymal Stem Cells.** Bárbara Pinheiro - ICVS, University of Minho
9h45	**Gene silencing in a spinal cord injury model by local application of LNAgapmer antisense oligonucleotides.** Pedro Moreno - i3S, University of Porto
10h00	**Non-invasive silencing of mutant ataxin-3 alleviates motor and neuropathological deficits in a transgenic mouse model of Machado-Joseph disease.** Rui Jorge Nobre - CNC, University of Coimbra
10h15	**Local environment determines integration of transplanted neurons.** Sofia Grade - LMU/Helmholtz Zentrum, Munich
10h30	**Poster Session II** Coffee Break
11h30	MultiChannel Systems Live Demo – STARTS at ICVS (more info at MultiChannel Systems booth).
13h00	Lunch
14h30	**Session V – Neurodevelopment and Cell Biology** Chairs: Patrícia Maciel (ICVS, University of Minho) João Relvas (I3S, University of Porto)
14h30	**Transcriptional control of neurogenesis in time and space.** **Bassem Hassan** | ICM-Hôpital Pitié-Salpêtrière
	Selected oral communications
15h00	**Lipocalin-2 regulates adult neurogenesis and contextual discriminative behaviors.** Ana Catarina Ferreira - ICVS, University of Minho
15h15	**The transcription factor MyT1 counteracts the neural progenitor program to promote vertebrate neurogenesis.** Diogo S. Castro - Instituto Gulbenkian de Ciência
15h30	**Cortical neuronal migration entails A2A receptor-driven neuronal polarization and axon formation.** Sofia Alçada-Morais - CNC University of Coimbra
15h45	**WNT6 regulation in glioblastoma: mechanistic, functional and clinical implications.** Céline S. Gonçalves - ICVS, University of Minho
16h00	**Coffee break**
16h30	**Keynote Lecture - Molecular, cellular, and circuit mechanisms that link memories across time.** **Alcino Silva** | University of California, Los Angeles (UCLA)
17h30	Awards and closing remarks

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Macromolecular interactions and posttranslational modifications acting as Machado-Joseph Disease modifiers

ORAL PRESENTATIONS

SESSION: KEYNOTE LECTURE I

Oral presentation: O.01 | Jonathan Kipnis
Repertoire and function of meningeal immunity in healthy and diseased brain

Presenter: Jonathan Kipnis | University of Virginia
Jonathan Kipnis (1)
(1) Center for Brain Immunology and Glia (BIG), Department of Neuroscience, University of Virginia, Charlottesville, VA 22908, USA

Immune cells and their derived molecules have major impact on brain function. We have shown that a proper T cell compartment is critical for higher brain function. Mice deficient in adaptive immunity have impaired cognitive function compared to that of wild-type mice. Importantly, replenishment of the T cell compartment in immune deficient mice restored proper cognition. Our recent works also demonstrates the effect of the immune system on social behavior. Despite the robust influence on brain function, T cells are not found within the brain parenchyma, a fact that only adds more mystery into these enigmatic interactions between T cells and the brain. Our results suggest that meningeal space, surrounding the brain, is the site where CNS-associated immune activity takes place. We have recently discovered a presence of meningeal lymphatic vessels that drain CNS molecules and immune cells to the deep cervical lymph nodes. This communication between the CNS and the peripheral immunity is playing a key role in several neurological and psychiatric disorders and, therefore, may serve as a novel therapeutic target that is worth in-depth mechanistic exploration.

***References**: 1. Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, Derecki NC, Castle D, Mandell JW, Lee KS, Harris TH, Kipnis J. (2015) Structural and functional features of central nervous system lymphatics. Nature, Jul 16;523(7560):337-41. doi: 10.1038/nature14432; 2. Gadani SP, Walsh JT, Lukens JR and Kipnis J. (2015) Dealing with Danger in the CNS: The Response of the Immune System to Injury. Neuron. Jul 1;87(1):47-62. doi: 10.1016/j.neuron.2015.05.019.; 3. Louveau A, Harris TH and Kipnis J. (2015) Revisiting the mechanisms of CNS immune privilege. Trends Immunol, Oct;36(10):569-77. doi: 10.1016/j.it.2015.08.006.; 4. Filiano AJ, Xu Y, Tustison NJ, Marsh RL, Baker W, Smirnov I, Overall CC, Gadani SP, Turner SD, Weng Z, Peerzade SN, Chen H, Lee KS, Scott MM, Beenhakker MP, Litvak V, Kipnis J. (2016) Unexpected role of interferon-γ in regulating neuronal connectivity and social behaviour. Nature. Jul 21;535(7612):425-9., 5. Kipnis J. (2016) Multifaceted interactions between adaptive immunity and the central nervous system. Science. Aug 19;353(6301):766-71. doi: 10.1126/science.aag2638. Review.*

SESSION: KEYNOTE LECTURE II

Oral presentation: O.02 | Sidarta Ribeiro
Mapping the mind with words

Presenter: Sidarta Ribeiro | Brain Institute, UFRN
Sidarta Ribeiro (1)
(1) Brain Institute, Federal University of Rio Grande do Norte

Graph analysis provides a particularly useful tool for the computational phenotyping of verbal or written reports, constituting a fast and low-cost tool for the evaluation of discourse structure. In the past few years we have successfully applied graph analysis to the differential diagnosis of psychosis, to sort Alzheimer's disease from mild cognitive impairment, to track the cognitive gains of healthy children undergoing alphabetization, and to compare all these data to literary texts from the past 5,000 years. The applicability of the method extends far beyond psychiatry, reaching the various mental realms induced by sleep and dream states, mood and attention variations, meditation, psychoactive substances and psychiatric and neurological diseases. The method also has potential to reveal new perspectives on talking, reading and, most importantly, learning. The possibility to structurally compare verbal reports of subjects across the world, from a range of ages, socio-economic status, and cultures, with ancient and current texts, provides a broad perspective that is simply unprecedented.

SESSION: NEURAL CIRCUITS AND BEHAVIOR

Oral presentation: O.03 | Poster: P.005 | Bárbara Guimarães Salazar Coimbra
Impairments in laterodorsal tegmentum to VTA projections underlie glucocorticoid triggered reward deficits

Presenter: Bárbara Coimbra | ICVS, University of Minho
Bárbara Coimbra (1,2), Carina Soares-Cunha (1,2), Sónia Borges (1,2), Nivaldo AP Vasconcelos (1,2), Nuno Sousa (1,2) and Ana João Rodrigues (1,2)
(1) Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal; (2) ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal

Ventral tegmental area (VTA) activity is critical for motivated behaviours and reinforcement. Importantly, VTA activity is tightly modulated by afferents arising from the laterodorsal tegmentum (LDT). Disruption of this circuit can ultimately increase the risk for the development of neuropsychiatric disorders, including those associated with reward deficits, such as depression, anxiety, obsessive-compulsive disorder, obesity, addiction or antisocial behaviour. Additionally, the VTA region is particularly vulnerable to the effects of stress/glucocorticoids (GCs). Previous studies revealed that in utero exposure to glucocorticoids (iuGC) triggers prominent reward deficits later in life but nothing is known about the impact of this exposure in the LDT-VTA circuit. Here, we show that iuGC animals have long-lasting changes in the expression of cholinergic markers in the LDT, and in vivo singe-cell electrophysiology revealed that LDT basal activity was decreased. Interestingly, we observe a bidirectional effect in LDT-VTA inputs: upon LDT stimulation, iuGC animals present a decrease in the magnitude of excitation and an increase in the magnitude of inhibition in the VTA. While in control animals most of the inhibitory responses arise from putative GABAergic neurons, in iuGC group there is a shift in the type of cells presenting inhibitory responses, with a significant increase in the number of dopaminergic neurons. In agreement with LDT-VTA dysfunction, we show that iuGC animals present motivational deficits that are rescued by selective optogenetic activation of this pathway. Importantly, we also show that LDTVTA optogenetic stimulation is reinforcing, and that iuGC animals are more susceptible to the reinforcing properties of LDT-VTA stimulation.

***Funding**: BC, C-S C, and SB are recipients of Fundação para a Ciência e Tecnologia (FCT) fellowships (SFRH/BD/51992/2012; SFRH/BD/98675/2013; SFRH/BD/90374/2012; SFRH/BD/89936/2012). AJR is a FCT Investigator (IF/00883/2013). This work was co-financed by the Portuguese North Regional Operational Program (ON.2 – O Novo Norte) under the National Strategic Reference Framework (QREN), through the European Regional Development Fund (FEDER). This work was partially financed by BIAL grant 30/16.*
***Link between abstracts**: Blackberry*

Oral presentation: O.04 | Francisco Queiroz Gonçalves

Activation of neuronal a2ar by atp-derived adenosine facilitates hippocampal ltp: physiopathological role of cd73

Presenter: Francisco Queiroz Gonçalves | CNC-UC

Francisco Q. Gonçalves (1,3), João P. Lopes (1), Henrique B. Silva (1), Cristina Lemos (1), António C. Silva (1), Samira Ferreira (1), Nélio Gonçalves (1), Daniel Rial (1), Paula M. Canas (1), Detlev Boison (4), Ângelo Tomé (1,3), Catarina R. Oliveira (1,2), Paula Agostinho (1,2) and Rodrigo A. Cunha (1,2)

(1) CNC - Center for Neuroscience and Cell Biology, University of Coimbra, 3004-517 Coimbra, Portugal; (2) Faculty of Medicine, University of Coimbra, 3004-517 Coimbra, Portugal; (3) Faculty of Science and Technology, University of Coimbra, 3004-517 Coimbra, Portugal; (4) Legacy Emanuel Hospital, OR 97227, Portland, USA

ATP is stored in synaptic vesicles and released in an activity-dependent manner. Extracellular ATP is converted to adenosine in a process mediated by ecto-nucleotidases. Ecto-5'-nucleotidase (CD73) is the rate-limiting player controlling the formation of ATP-derived adenosine (Cunha et al, 2001), which is proposed to be required for the activation of adenosine A2A receptors (A2AR) in the striatum (Augusto et al., 2013). However, in the hippocampus, where the main role of A2AR is the control of synaptic plasticity, the link between CD73 activity and A2AR function remains unclear. Here we show that, in mouse hippocampus, ATP-derived adenosine resulting from CD73 activity selectively activates A2AR to control synaptic plasticity (long term potentiation – LTP) triggered by high frequency stimulation (100 Hz, 1 s) applied to Schaffer fiber-CA1 pyramid synapses in hippocampal slices from 10 weeks-old male C57/BL6 mice. The inhibition of CD73 with α ,β -methylene ADP (AOPCP, 100 μ M) decreased LTP by 29±1.2% (n=5; $P<0.05$), an effect abrogated in CD73 knockout (KO) mice. The effect of AOPCP was also occluded when slices were previously superfused with the selective antagonist of A2AR (SCH58261, 50 nM). AOPCP was also devoid of effects on LTP amplitude in global A2AR-KO mice and in forebrain neuronalselective A2AR-KO mice (CAM-KII-driven A2AR-KO), whereas AOPCP significantly decreasedLTP amplitude in astrocyte-selective A2AR KO mice (GFAP-driven A2AR-KO). Intracerebroventricular administration of the neurotoxic peptide Aβ1-42 (2 nmol) induced memoryrelated and synaptic plasticity deficits that were prevented in CD73-KO mice (n=5; $P<0.05$). Aβ1-42 icv injection also decreased pre- and post-synaptic protein markers in wild type but not in CD73KO mice. Overall, our results indicate that ATP-derived adenosine is strictly linked to the activation of neuronal A2AR to control synaptic plasticity in hippocampus, thus establishing CD73 as a novel target for the modulation of A2AR activity.

***References**: 1- Cunha, 2001, Neurochem. Res. 26:979; 2- Augusto et al., 2013, J. Neurosci. 33:11890*
***Funding**: Supported by FCT (PTDC/SAU-TOX/122005/2010, PTDC/NEU-NMC/4154/2014) and CNPq.*

Oral presentation: O.05 | Julie Cécile Caroline Ribot

γδ T cells are the major source of IL-17 in the meninges and control brain cognitive functions

Presenter: Julie Ribot | IMM, Lisboa

Miguel Ribeiro (1), Helena Brigas (1), Catia Santa (2), Claudia Valente (1), Mariana Temido-Ferreira (1), Diana G. Ferreira (1), Joana E. Coelho (1), Sara Omenetti (3), Brigitta Stockinger (3), Bruno Manadas (2), Luisa V. Lopes (1), Bruno Silva-Santos (1) and Julie C. Ribot (1).

(1) Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Portugal (2) Center for Neuroscience and Cell Biology – University of Coimbra, Portugal (3) The Francis Crick Institute, London, UK

γδ T cells are known to populate multiple tissues, such as the skin, gut or lung, where they make major contributions to local physiology. Here we investigated whether γδ T cells could play a role in normal brain function, given that αβ T cells were recently shown to be involved in learning behavior of mice: whereas IFN-γ producing subsets were detrimental (1), their IL-4-producing counterparts played a pro-cognitive role (2). We found that γδ T cells infiltrate the meningeal spaces from the brain of naive C57/BL6 mice already at birth and persisted throughout life. Strikingly, at 1 week of age, meningeal γδ T cells differentiated into IL-17 producers, which seemingly depended on IL-1β. In fact, γδ T cells were the major source of IL-17, whereas αβ T cells mostly provided IFN-γ in situ. To test whether IL-17-producing γδ T cells influenced the cognitive performance of mice, we scored the behavior of TCRδ-/-, IL-17-/- and respective WT littermate control mice in classical paradigms assessing learning capacities. We observed that, contrary to WT controls, mice deficient for γδ T cells or IL-17 displayed impaired short-term/working memory in the Y maze paradigm, but a normal long-term spatial memory in the Morris water maze. To identify the underlying molecular mediators, we performed a proteomic-based analysis of the hippocampus and the pre-frontal cortex from IL-17-/- and WT mice. Interestingly, our results highlighted a reduced plasticity of the glutamatergic synapses of IL-17-/- animals, as confirmed by Long Term Potentiation (LTP) measurements. Furthermore, we found that IL-17 enhanced Brain Derived Neurotropic Factor (BDNF) production by glial cells, thus providing a key mechanistic link between meningeal γδ T cells and neurons. Altogether, our data demonstrate that γδ T cells regulate brain cognitive functions through a novel IL-17-dependent mechanism.

***References**: (1) Monteiro S, Ferreira FM, Pinto V, Roque S, Morais M, de Sá-Calçada D, Mota C, Correia-Neves M, Cerqueira JJ. Absence of IFNγ promotes hippocampal plasticity and enhances cognitive performance. Transl Psychiatry. 2016 Jan 5;6:e707. doi: 10.1038/tp.2015.194.*

***Funding**: FCT*

Oral presentation: O.06 | Joana Rita da Silva Carvalheiro
Modulatory effects of an escape alternative in pro-social behaviour

Presenter: Joana Carvalheiro | School of Psychology, U. Minho
Joana Carvalheiro (1), Ana Mesquita (1), Ana Seara-Cardoso (1,2), Teresa Summavielle (3), Ana Magalhães (3,4)
(1) Neuropsychophysiology Lab, CIPsi, School of Psychology, University of Minho, Braga, Portugal; (2)Division of Psychology and Language Sciences, University College London, UK; (3) Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal; (4) Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal

Pro-social behaviour is known to occur in response to familiar rat's distress, but the motivations underlying helping behaviour remain elusive. In this study, we adapted the experimental setting of Bartal et al. (2011) to a paradigm that seeks to identify the motivation behind helping behaviour by giving individuals a chance to pursue a self-benefit goal (escape from distress). Inspired in the Light-Dark box test, we added a dark compartment to the Bartal pro-social arena to allow the free rat to escape through a open door, while a cage-mate rat was trapped in a restrainer that could only be opened from the outside. With the present design, we aimed to explore the motivation behind pro-social behaviour giving rats the possibility to relieve their own distress by escaping to a dark compartment. We investigated also how the trapped rat's behaviour influenced the pro-social behaviour. To test our hypothesis, 5 pairs of cage-mate male Wistar rats (PND 41-53) were tested in this arena. As a control, 4 pairs of cage-mate males were tested in the same arena but with the door to the dark area closed to eliminate the escaping possibility. Rats were video recorded in 12 sessions of 60 minutes. If the free rat failed to open the restrain door during the initial 40 minutes, the experimenter would open it in 45 degrees (not counted as door opening). Rats remained in the arena until the end of the session. The behaviour of both free and trapped rats was analysed by animal focal analysis. Our results showed that in the group of rats with the possibility to escape, the pro-social behaviour decreased significantly, when compared to rats that did not have the escape choice. The escape condition gives helper-rats an opportunity to escape, which seems to decrease their state of anxiety. This condition did not facilitate pro-social behavior, since animals showed greater latency to door opening. We observed that anxious affective state in the helper rat reduced pro-social behavior, whereas proactive/positive behavior seems to facilitate pro-social behavior in both conditions. Results also showed that the pro-social behavior of the free rat was not influenced by the distress of trapped rats. Furthermore, this distress also was not correlated with escaping choices of the free rat, suggesting that free rat's behavior was not modulated by the stress of the trapped rat. Interestingly, positive/proactive the behavior of the trapped rat, such as exploratory behavior, was negatively correlated with the latency to door opening. In summary, the motivation underlying pro-social behavior was not the trapped rat's distress, but its proactive behavior, such as restrainer exploration. This novel behavioral approach shed light on the importance of positive emotional states in pro-social behavior.

References: *Bartal, A., Decety, J., & Mason, P. (2011). Empathy and pro-social behavior in rats. Science, 334, 1427-1430.*

SESSION: NEURODEGERATION

Oral presentation: O.07 | Tiago Fleming Outeiro
Cracking the code: protein posttranslational modifications and the molecular basis of neurodegeneration

Presenter: Tiago Outeiro | Univ. Medical Center Goettingen
Tiago Fleming Outeiro (1)
(1) Department of Neurodegeneration, University Medical Center Goettingen

Aggregation of alpha-synuclein (ASYN) in Lewy bodies and Lewy neurites is the typical pathological hallmark of Parkinson's disease (PD) and other synucleinopathies. Furthermore, mutations in the gene encoding for ASYN are associated with familial and sporadic forms of PD, suggesting this protein plays a central role in the disease. However, the precise contribution of ASYN to neuronal dysfunction and death is still unclear. There is intense debate on the nature of the toxic species of ASYN, and little is still known about the molecular determinants of oligomerization and aggregation of ASYN in the cell. By harnessing the power of various model organisms, we are making progress towards the understanding of the basic molecular mechanisms underlying PD and other synucleinopathies. In order to clarify the effects of different posttranslational modifications on the toxicity and aggregation of ASYN, we exploit a variety of model systems. Glycation and acetylation are emerging as important modifications affecting ASYN aggregation. Altogether, our data shed light into the molecular underpinnings of synucleinopathies, opening novel perspectives for therapeutic intervention.

Oral presentation: O.08 | Adelaide Maria Afonso Fernandes Borralho

Relevance of neuron-microglia vesicular trafficking, stress-related microRNAS and DAMPs in Alzheimer's disease

Presenter: Adelaide Fernandes | iMed.Ulisboa, FFUP

Ana Rita Ribeiro (1), Mafalda Monteiro (1), Carolina Cunha (1), Ana Rita Vaz (1,2), Adelaide Fernandes (1,2), Dora Brites (1,2)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal; (2) Department of Biochemistry and Human Biology, Faculty of Pharmacy, Universidade de Lisboa, Portugal

Neuroinflammation is a known hallmark of Alzheimer's Disease (AD), where microglial cells play a major role. Vesicular trafficking mediated by cell-derived exosomes have recently gain increased interest in the context of AD, where they are crucial in the interplay between neurons and glial cells. Exosomes may carry endogenous inflammatory microRNAs (miRNAs) or Damage-associated molecular patterns (DAMPs) molecules, such as high mobility group box 1 (HMGB1) and S100B, which may change the phenotype of recipient cells. Here, we used an in vitro model of AD to investigate whether: (i) AD neuron-derived secretome affects microglia phenotype; (ii) if exosomal cargo, including inflammatory-related miRNAs and DAMPs recapitulate the donor cell ; and whether (ii) exosomes released by AD neurons are able to activate the recipient microglial cell. Human microglia cell line CHME3 was co-cultured with human neuroblastoma cells expressing amyloid-precursor protein (APP)695 Swedish mutation (SH-SY5Y APPSwe). After co-culture, the expression of miRNAs, their targets and inflammatory cytokines were evaluated by qRT-PCR. Exosomes from CHME3, SH-SY5Y and SH-SY5Y APPSwe were isolated by differential ultracentrifugation, characterized by NanoSight, and their content evaluated for miRNA profiling and HMGB1/S100B mRNA expression by qRT-PCR. In parallel, exosomes from AD neurons were fluorescently labelled with PKH-67 and incubated on CHME3 cells. Microglia were then evaluated for exosome internalization and lysosome processing. Exposure of CHME3 cells to SH-SY5Y APPSwe cells acutely increased the cellular expression of miR-155, followed by delayed upregulation of SOCS1 and miR-124, together with CEBP-α reduction. Increased levels of TNF-α, IL-1β, IL-6 and IL-10 occurred at 72 h of co-culturing. High expression of miR-124 was observed in the SH-SY5Y APPSwe cells, while miR-21 was increased in both SH-SY5Y APPSwe and CHME3 cells, and recapitulated in cell-derived exosomes. While HMGB1 mRNA expression was increased in SH-SY5Y APPSwe and not detected in their exosomes, S100B was decreased in SH-SY5Y APPSwe, when compared to SH-SY5Y, and reflected in their respective exosomes. Low mRNA levels of both DAMPs were found in CHME3 cells. Exosomes from SH-SY5Y and SH-SY5Y APPSwe showed similar concentration (~1.6 x106 cells/ml) and 2 populations with different sizes (~85 and ~135 nm). Microglia treated with SH-SY5Y APPSwe exosomes revealed an increased density of incorporated exosomes preferentially localized in lysosomes. Collectively, communication between SH-SY5Y APPSwe and CHME3 cells determine early microglia M1 phenotype with later heterogeneous M1/M2 cell subsets. Increase of stress-related miRNAs in both cells and their derived exosomes, suggest a key role of exosomes on the delivery of miRNAs and DAMPs from donor to recipient cells. Finally, neuronal exosomes are efficiently internalized by microglial cells and directed to lysosomal processing.

***Funding**: EU Joint Programme - Neurodegenerative Disease Research (JPND) project and Fundação para a Ciência e Tecnologia, Lisboa, Portugal (JPco-fuND/0003/2015 to DB, FCT-EXPL/NEU-NMC/1003/2013 to AF and UID/DTP/04138/2013 to iMed.ULisboa).*

Oral presentation: O.09 | Andreia Alexandra Neves Carvalho
ATXN3 modulates mRNA splicing of Tau through ubiquitylation of splicing factors

Presenter: Andreia Neves-Carvalho | ICVS, University of Minho
Andreia Neves-Carvalho(1,2), Bruno Almeida(1,2), Sara Duarte-Silva(1,2), Joana Silva(1,2), Sasja Heetveld(3), Ioannis Sotiropoulos(1,2), Peter Heutink(3), Ka Wan Li(4) and Patrícia Maciel(1,2)
(1) Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal; (2) ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal; (3) German Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany; (4) Vrije University Medisch Centrum (VUMC), Amsterdam, The Netherlands

Deubiquitylating (DUB) enzymes have been recognized as central players in the maintenance of the correct ubiquitylation/deubiquitylation balance in cells. Ataxin-3 (ATXN3) is a protein with DUB activity mutated in Machado-Joseph disease (MJD) (also known as Spinocerebellar ataxia type 3 – SCA3). To date, besides the involvement of ATXN3 in the Ubiquitin-proteasome pathway (UPP) its physiological function remain elusive and no substrates for its DUB activity have been identified. To identify potential candidates of the DUB activity of this protein, we characterized the ubiquitome of neuronal cells lacking ATXN3 (ATXN3shRNA cells) by mass-spectrometry. We found that a large proportion of the proteins with altered polyubiquitylation in ATXN3shRNA cells were proteins involved in RNA post-transcriptional modification. By transcriptomic analysis and using reporter minigenes we confirmed that splicing was globally altered in cells lacking ATXN3. Among the targets with altered splicing was SRSF7(9G8), a regulator of tau exon 10 splicing. Here we show that loss of function of ATXN3 leads to a deregulation of tau exon 10 splicing resulting in a decreased 4R/3R tau ratio. The fact that similar alterations were found in the brain of a mouse model of MJD, suggests that this mechanism might be contributing for the pathogenesis of MJD, and establishes a link between two key proteins involved in different neurodegenerative disorders.

***Funding**: FCT*

Oral presentation: O.10 | Poster: P.056 | Isabel Paiva de Castro

Sodium butyrate rescues dopaminergic cells from alpha-synuclein-induced transcriptional deregulation and dna damage

Presenter: Isabel Paiva | Uni. Medical Center Goettingen

Isabel Paiva(1), Raquel Pinho(1), Maria Angeliki S. Pavlou(1), Magali Hennion(2), Pauline Wales(1), Anna-Lena Schütz(2), Ashish Rajput(2), Éva Szego(1), Cemil Kerimoglu(3), Ellen Gerhardt(1), Ana Cristina Rego(4), André Fischer(3), Stefan Bonn(2), and Tiago F. Outeiro(1)

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Alpha-synuclein (aSyn) is considered a major culprit in Parkinson's Disease (PD) pathophysiology. However, the precise molecular function of the protein remains elusive. Recent evidence suggests that aSyn may play a role on transcription regulation, possibly by modulating the acetylation status of histones. Our study aimed at evaluating the impact of wild-type (WT) and mutant A30P aSyn on gene expression, in a dopaminergic neuronal cell model, and decipher potential mechanisms underlying aSyn-mediated transcriptional deregulation. We performed gene expression analysis using RNA-sequencing in Lund Human Mesencephalic (LUHMES) cells expressing endogenous (control) or increased levels of WT or A30P aSyn. Compared to control cells, cells expressing both aSyn variants exhibited robust changes in the expression of several genes, including downregulation of major genes involved in DNA repair. WT aSyn, unlike A30P aSyn, promoted DNA damage and increased levels of phosphorylated p53. In dopaminergic neuronal cells, increased aSyn expression led to reduced levels of acetylated histone 3. Importantly, treatment with sodium butyrate, a histone deacetylase inhibitor (HDACi), rescued WT aSyn- induced DNA damage, possibly via upregulation of genes involved in DNA repair. Overall, our findings provide novel and compelling insight into the mechanisms associated with aSyn neurotoxicity in dopaminergic cells, which could be ameliorated with a HDACi. Future studies will be crucial to further validate these findings and to define novel possible targets for intervention in PD.

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Oral presentation: O.11 | Ioannis Sotiropoulos

Tau therapeutics in stress-driven brain pathology: exploring the path from depression to Alzheimer's disease

Presenter: John-Ioannis Sotiropoulos | ICVS, University of Minho

Ioannis Sotiropoulos, Sofia Lopes, Joao Vaz-Silva, Chrysoula Dioli, Joana Silva, Monica Morais, Vitor Pinto, Osborne Almeida, Nuno Sousa

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Synaptic malfunction is a key pathomechanism in both depressive and Alzheimer´s disease (AD) pathologies with chronic stress and stress hormones, glucocorticoids (GC), being a risk factor for both disorders. Accumulating evidence has suggested the continuum between depression, impaired cognition and AD raising stress, a well-known sculptor of brain plasticity, as potential connecting factor. As Tau protein and its hyperphosphorylation have been implicated in neuronal/synaptic malfunction in AD brain, we hereby assessed whether Tau plays a critical role in stress-driven depressive pathology and associated cognitive decline. For that purpose, we exposed Tau knock-out (Tau-KO) mice and their wild-type (WT) littermates in chronic unpredictable stress. Our recent findings demonstrate, for the first time, that stress- and GC-driven neuronal deficits in wild-type mice are accompanied by synaptic missorting of Tau and enhanced Fyn/GluN2B-driven synaptic signaling assessed by both molecular (WB) and ultrastructural (TEM) analysis [1, 2]. In contrast, mice lacking Tau (Tau-KO) Tau-KO mice are resilient to chronic stress exhibiting no depressive-like behavior and cognitive impairments while, in contrary to WT, stressed Tau-KO also did not display neuronal/synaptic atrophy, reduction in synaptic plasticity and MRI-based neuronal activity [1]. Furthermore, our quantitative proteomic analysis of synaptosomal fractions, combined with TEM analysis, suggested a prominent role for mitochondria in the regulation of the effects of stress affecting their synaptic localization and function [3]. These findings identify Tau as an essential mediator in the orchestration of cellular cascades underlying dendritic and synaptic atrophy/loss in stress-evoked depressive pathology and associated cognitive deficits adding to our molecular understanding of how stress precipitates brain pathology.

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SESSION: NEUROPSYCHIATRIC DISORDERS

Oral presentation: O.12 | Luísa V. Lopes
My brain on a 24/7 schedule - impact on memory function

Presenter: Luísa V. Lopes | IMM
Luísa V. Lopes (1)
(1) IMM Lisboa, Portugal

The effects of a “24-7” schedule in our general physiology, as well as our cognitive capacities, are a growing social concern. A wealth of recent studies shows that the integrity of circadian rhythms is crucial for cognitive function possibly through direct disruption of its biological mechanism – neural plasticity. Circadian release of cortisol from the adrenal cortex is under tight regulation of this hypothalamic–pituitary–adrenal (HPA) axis. The hippocampus plays a crucial role in regulating HPA axis; and excessive glucocorticoid production disrupts the regulatory feedback from the hippocampus onto the hypothalamus. Impaired cortisol levels are observed in post-traumatic stress syndrome or major depression. Increased glucocorticoid activity has also been associated with greater hippocampal atrophy and memory impairment in the elderly. I will share evidence of age-related downregulation of glucocorticoid receptors (GR) in the hippocampus, and consequent desensitization of the regulatory feedback to the hypothalamus. Higher cortisol levels have been associated with more rapid Alzheimer’s disease (AD) progression and systemic administration of glucocorticoids or stress shown to potentiate memory impairments, hippocampal damage, β-amyloid formation and Tau accumulation in transgenic AD mice. We have recently described the interesting ability of novel drugs -adenosine A2A receptor (A2A) blockers- to rescue age-like memory deficits and anxiety in rodents, by re-establishing central GR levels, hippocampal feedback and consequent control of systemic CORT circadian levels. Plus, A2A deletion improved spatial memory and mitigates hippocampal Tau phosphorylation in AD-related THY-Tau22 mice. Altogether, such observations strongly suggest that A2A over-activation and GR dysfunction are key events in age-related hippocampal deficits and raise the possibility that both pathways might be interconnected. We recently provided the first demonstration of a direct impact of A2A modulation on GR function, a mechanism never hypothesized before. We found that A2A overexpression in forebrain neurons is sufficient to promote HPA-axis dysfunction, namely loss of plasmatic corticosterone circadian oscillation, and reduced GR hippocampal levels, both being age-related phenotypes; by modulating GR nuclear translocation and transcriptional activity. This supports the idea that the procognitive effects of A2AR antagonists, namely caffeine, on age-related cognitive impairments may be due, at least in part, to its ability to modulate GR actions and normalize HPA axis feedback. The concept of enhanced vulnerability to dementia by the existence of underlying circadian disorders is very appealing, supported by evidence and deserves further attention. Modern schedules have led humans to be the only animal species that routinely ignores its biological clock. This is a socially pervasive phenomenon, whose consequences to mental and general health are not fully understood. By identifying circadian modulators with impact on cognition we may contribute to novel strategies to ameliorate the effects of the ’24/7’ schedule on modern societies.

Oral presentation: O.13 | Poster: P.111 | António Maria Restolho Mateus Pinheiro

Exploring time-dependent anatomical and functional correlates of adult hippocampal cytogenesis in young-adult rats

Presenter: Antonio Mateus-Pinheiro | ICVS, University of Minho

Mateus-Pinheiro A, Patrício P*, Alves ND, Machado-Santos A, Loureiro-Campos E, Sardinha V, Oliveira J, Flint J, Sousa N, Pinto L.*

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Impaired ability to generate new cells in the adult brain has been linked to deficits in multiple emotional and cognitive behavioral domains. However, the mechanisms by which the abrogation of post-natal neural stem cells impacts on brain homeostasis and function remains controversial. Here, we used a transgenic rat line, the GFAP-Tk line, to selectively eliminate neural stem cells and assess the repercussion on different behavioral domains. We adopted two parallel experimental timeframes, to study both short-term and long-term effects of cytogenesis ablation (1 week post-ablation and 4 weeks post-ablation, respectively). Moreover, we conducted in vivo electrophsyiological analysis to assess the effects of cytogenesis ablation on the electrophysiological signatures of the hippocampal and prefrontal cortex regions. Our results show that the short-term repercussions of post-natal cytogenesis ablation are restricted to anxiety behavior. Contrastingly, cytogenesis abrogation promoted the late manifestation of anhedonic and anxiogenic deficits, along with multi-dimensional cognitive impairments. Furthermore, we found that cytogenesis ablation impaired electrophysiological function between the hippocampus and the prefrontal cortex, which are likely to contribute to the described cognitive alterations. Altogether, we describe a progressive time-dependent manifestation of emotional and cognitive impairments following cytogenesis ablation, supporting a differential role of immature vs mature cells in the modulation of different behavioral dimensions within the adult brain.

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Link between abstracts: GrayPhoenix

Oral presentation: O.14 | Poster: P.126 | Carla Filipa Simões Henriques

Chronic blockade of adenosine A2A receptors: gender-specific reprogramming of microglia morphology in the pre-frontal cortex

Presenter: Carla Henriques | IBILI, University of Coimbra

Carla Henriques (1, 2, 3), Joana Duarte (1, 2, 3), Helena Pinheiro (1, 2, 3), Rita Gaspar (1, 2, 3), Inês Almeida (1, 2, 4, 5), Patrícia Patrício (6, 7), António Mateus-Pinheiro (6, 7), Nuno Alves (6, 7), Beatriz Coimbra (6, 7), Sónia Henriques (1), Carina Cunha (7), Carlos A. Ribeiro (1, 2, 4, 5), Nuno Sousa (6, 7), Rodrigo A. Cunha (8, 2, 3), Ana João Rodrigues (6, 7), Luísa Pinto (6, 7), António Francisco Ambrósio (1, 2, 3, 5), Catarina A. Gomes (1, 8, 2, 3, 5)

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In adulthood, microglia (cells implicated in the genesis of anxiety) present morphologic differences between genders in physiological conditions (1). Under prenatal anxiogenic stimulus, these cells undergo gender-specific morphological remodelling. In these circumstances, the chronic blockade of adenosine A2A receptors (A2AR), a modulator of microglia, ameliorated microglia morphology and anxiety behaviour in males, but not in females (1). This dimorphic response is likely related with gender differences in the density of A2AR in the pre-frontal cortex (PFC), brain region strictly involved in anxiety disorders (1). It is thus imperative to study microglia morphology in the PFC from early neurodevelopment onwards, as well as the physiologic response to A2AR manipulation in males and females. The present study aims to clarify whether gender-specific microglia morphology is already present in newborns (post-natal day, PND 0) and infants (PND 7) in the PFC. Our second goal is to characterize the effect of the chronic blockade of A2AR in adulthood (PND 90) in microglia morphology in the PFC of male and female rats. Wistar rats were treated with SCH58261 (SCH) (0.1 mg/kg/day, intraperitoneal), a selective A2AR antagonist, for 21 consecutive days before PND 90. Microglia morphology was assessed by immunohistochemistry with a microglia marker (Iba-1). Confocal images obtained with a 63x objective, allowed the manual reconstruction of microglia in 3D using Neurolucida software. Statistic analysis was performed in GraphPad Prism: Student's t test was used to compare two independent means; differences were considered significant at $p<0.05$. Microglia volume at PND 0 was similar between genders (NT males: 2749 ± 391.2 µm3; NT females: 2376 ± 198.9 µm3, n=3, $p>0.05$); at PND 7 we still did not observe gender differences in ramifications number and length (n=3, $p>0.05$) in PFC microglia. At PND 90, the chronic blockade of A2AR reduced the number and length of microglial processes in females (NT females, n=6, vs SCH females, n=7, $p<0.05$), but did not affect males (n=4, $p>0.05$). In conclusion, the present data show that A2AR control microglia morphology in a gender-specific manner. On the other hand, we characterized for the first time microglia morphology in the PFC of newborn rats, showing that gender differences in microglia morphology are not present until PND 7, which coincides with an endogenous peak of A2AR expression.

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Oral presentation: O.15 | Dominique Moreira Fernandes

Autism-associated Caspr2 regulates synaptic AMPA receptors in the context of homeostatic synaptic plasticity

Presenter: Dominique Fernandes | CNC, University of Coimbra

Dominique Fernandes (1,2,3); Sandra Santos (1), Jessica L. Whitt (3), Ester Coutinho (4), M. Isabel Leite (4), Camilla Buckley (4), Hey-Kyoung Lee (3), Ana Luisa Carvalho (1,5)

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During development and learning/memory-related events, the mammalian brain undergoes constant changes that can compromise its function. To prevent this, homeostatic synaptic plasticity mechanisms come into play, allowing experience-based adaptations to occur while maintaining the activity of neuronal networks in-balance for proper brain function. One fundamental mechanism to achieve neuronal homeostasis is the dynamic regulation of AMPA receptors at glutamatergic synapses. Herein, we describe a novel role for the cell-adhesion molecule Caspr2, implicated in autism and other neuropsychiatric disorders, in the regulation of synaptic AMPARs in the context of homeostatic plasticity. We demonstrate that loss of Caspr2 not only decreases the basal synaptic content of GluA1-containing AMPARs in cortical neurons, but also hinders the triggering of homeostatic mechanisms that upscale synaptic AMPARs during prolonged periods of neuronal inactivity. Accordingly, Caspr2 is further required for experiencedependent plasticity in vivo, since its loss in the mouse visual cortex (V1) prevents the scaling of AMPAR-mediated mEPSC amplitudes following paradigms of chronic visual deprivation. Caspr2 is also a target antigen in autoimmune synaptic encephalitis. Remarkably, in vitro or in vivo incubation with patient-purified Caspr2 autoantibodies significantly decreases synaptic GluA1-AMPARs in cortical cultures and mEPSC amplitudes in V1. Overall, we uncover a novel function for autism-associated Caspr2 in the regulation of synaptic AMPARs and homeostatic plasticity. Importantly, this evidence hints at a potential disruption of neuronal homeostasis following Caspr2 dysfunction in the context of disease, which is consistent with accumulating data implicating glutamatergic synapse dysfunction and impaired neuronal homeostasis as common underlying pathologies of several cognitive disorders, including autism.

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Oral presentation: O.16 | Poster: P.127 | Joana Teresa Ferreira Gonçalves

Excitation/inhibition balance and glial function in mouse model of neurofibromatosis type 1: distinct susceptibility of hippocampus, prefrontal cortex and striatum

Presenter: Joana Gonçalves | ICNAS,UC

Joana Gonçalves (1,2,3), Inês Violante (4), José Sereno (1,2,3), Ricardo Alexandre Leitão (2,3,5), Ying Cai (6), Ana Paula Silva (2,3,5), Alcino José Silva (6), Miguel Castelo-Branco (1,2,3)

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Neurofibromatosis type 1 (NF1) is a monogenic developmental disorder, characterized by altered skin pigmentation, increased tumor predisposition and cognitive deficits. Increasing data have been proposed that alterations in the excitation/inhibition balance are the neural mechanism underlying NF1-mediated cognitive disabilities. Previous studies employing a mouse model of NF1 revealed that γ-aminobutyric acid (GABA) inhibitory neurotransmission were increased in several brain regions, including hippocampus and striatum. Nonetheless, we showed a reduction of GABA concentration in the visual and medial frontal cortex of human patients with NF1. Since the link between animal and human studies remains to be established, it is important to apply to the mice model the same techniques available to investigated the GABA levels in humans. Here, together with molecular and cellular methods, we used magnetic resonance spectroscopy in NF1 mouse model, as this is the only technique accessible to measure GABA in vivo in humans. We found that the excitation vs. inhibition and the pre- vs. post-synapatic phenotype is different in the NF1 mouse hippocampus, when compared to cortical and striatal regions. In fact, both hippocampal GABA and glutamate levels are reduced, without changes in the respective ratio. Moreover, hippocampal GABA(A) α1 subunit receptor levels were increased at the synaptosomal level. On the other hand, striatal and cortical GABA/glutamate ratios are significantly increased, while GABA(A) subunit levels were decreased mainly at synaptosomal level in prefrontal cortex and at the cytosolic level in the striatum. Further, imunolabelling confirmed these results and showed distinct patterns of receptor redistribution in all these structures with patchy zones of dense receptor clusters being more evident in the striatum of the mutant mice. Finally we found evidence that GABA dysfunction is accompanied by changes in astrocytes physiology. Changes in astrocytes physiology are consistent with increased glutamine vs. glutamate levels in the hippocampus and frontal cortex, suggesting abnormalities in the glutamine-glutamate cycle. Overall, our study reported distinct homeostatic mechanism in the hippocampus, prefrontal cortex and striatum induced by NF1 mutations at both neural and glial levels. These findings are crucial to design novel region specific therapeutics strategies that may need to improve cognitive disabilities in NF1 patients.

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SESSION: REGENERATION AND THERAPIES

Oral presentation: O.17 | Liliana Bernardino
Nanomedicine approaches to modulate neural stem cells for brain repair

Presenter: Liliana Bernardino | CICS, UBI
Liliana Bernardino (1)
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Parkinson's disease (PD), a neurodegenerative disorder characterized by the selective degeneration of the nigrostriatal dopaminergic pathway, is a major socio-economic burden in modern society. Presently there is no cure for PD, nevertheless, enhancing the number of neural stem cells (NSCs) and/or stimulating their differentiation into new neurons are promising therapeutic strategies. Many proneurogenic factors have been implicated in controlling NSCs activity, including several microRNAs. However, the current strategies described for the intracellular delivery of microRNAs involve mostly unspecific or inefficient platforms. Recently, we developed miR-124 loaded nanoparticles (NPs) able to efficiently deliver miR-124 into neural stem/progenitor cells and boost neuronal differentiation and maturation in vitro. In vivo, the intracerebroventricular injection of miR-124 NPs increased the number of new neurons in the olfactory bulb of healthy and 6-hydroxidopamine (6-OHDA) treated mice, a model for PD. Importantly, miR-124 NPs enhanced the migration of new neurons into the 6-OHDA lesioned striatum, culminating in motor function improvement. Given the recent advent of clinical trials for miR-based therapies and the theranostic applications of our NPs, we expect to support the clinical translation of our delivery platform in the context of PD and other neurodegenerative diseases which may benefit from enhancing microRNA levels.

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Oral presentation: O.18 | Bárbara Filipa Mendes Pinheiro

A New Paradigm for Parkinson's Disease Regenerative Medicine based on the Secretome of Mesenchymal Stem Cells

Presenter: Bárbara Pinheiro | ICVS, University of Minho

Barbara Mendes-Pinheiro (1,2), Fábio Teixeira (1,2), Sandra Anjo (3,4), Bruno Manadas (4,5), Leo Behie (6), Nuno Sousa (1,2), António Salgado (1,2)

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Parkinson's disease (PD) is a progressive movement disorder that results from the death of dopaminergic neurons, mainly in the nigrostriatal pathway, leading to the appearance of characteristic motor symptoms. Current PD treatments are focused on reducing the symptoms: none slow down or reverse the degenerative process, imposing the need for innovative therapeutical approaches. The use of adult stem cells cell-based strategy has emerged as a potential alternative therapy for PD, in which, among a number of promising stem cell sources, human mesenchymal stem cells (hMSCs) have stand out as a valid therapeutic option. Indeed, over the last years, a substantial effort has been performed in order to address the impact of hMSCs in central nervous system repair. Recently, and from an application point of view, several studies have claimed that the therapeutical effects of stem cells is mainly mediated by their trophic action namely, through their capacity of secreting a wide panel of neuroregulatory molecules (e.g. neurotrophic factors, cytokines, vesicles), which is defined as secretome. Thus, based in all these concepts, in this work we aimed to: 1) Characterize the secretome of hMSCs through proteomic-based approaches; 2) Determine the role of hMSCs secretome as a modulator of neuronal differentiation and 3) Investigate the effects of the hMSCs secretome in a rat model of PD, in comparison with cell transplantation. In vitro experiments revealed that MSCs secretome was able to differentiate human neural progenitor cells (hNPCs) towards a neuronal phenotype (MAP-2+ and DCX+ positive cells). Additionally, it was also possible to observe that the injection of the hMSCs secretome in a 6-hydroxydopamine (6-OHDA)-rat model of PD potentiated the recovery of dopaminergic neurons (estimated by neuronal densities in substantia nigra and striatum) when compared to the untreated group 6-OHDA, and those transplanted with cells. Similar outcomes were observed in the motor performance of these animals as assessed by the rotarod and staircase tests. Finally, proteomic characterization of hMSCs secretome revealed that these cells were able to secrete important molecules with neuroregulatory actions such as, Galectin-1, 14-3-3 proteins, PEDF, DJ-1, whereby may support the effects observed both in vitro and in vivo. Overall, we concluded that the use of secretome per se was able to partially revert the motor phenotype and the neuronal structure of PD animals, indicating that the secretome of stem cells could represent a novel therapeutic tool for the treatment of PD. In addition to these results, besides the dopaminergic survival, it has also been described that the modulation of neurogenesis may also play a role in the recovery of PD. Preliminary data of subependymal zone (SEZ) and striatum analysis indicates that hMSCs secretome seems to be more prone to induce dopaminergic neuronal differentiation in the lesion side.

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Oral presentation: O.19 | Pedro Miguel Duarte Moreno

Gene silencing in a spinal cord injury model by local application of LNA-gapmer antisense oligonucleotides

Presenter: Pedro Moreno | i3S

Pedro M.D. Moreno(1,2), Ana R. Ferreira(1,2), Daniela Salvador*(1,2), Ulf Tedebark(4,5), Mónica M. Sousa(1,3), Isabel F. Amaral(1,2), Jesper Wengel(6), Ana P. Pêgo(1,2,7,8)*

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In order to achieve nerve regeneration in the context of spinal cord injuries new molecular therapeutic strategies that block the growth inhibitory signals coming from the extracellular environment at the injury site and increase the intrinsic regenerative capacity of neurons are warranted (1). We have thus developed a strategy to down-regulate local gene expression of important molecules participating in the inhibition of nerve regeneration in a spinal cord injury setting. LNA-based antisense oligonucleotides (2) were designed against two targets of interest, RhoA (3) and GSK3 (4), and loaded in a fibrin gel matrix as an integrated system for their local release in vivo in a spinal cord injury setting. Fibrin gels prepared with AONs were found to contain the AONs co-localized with the fibrin fibers and a decreased fiber density suggesting some level of direct interaction between the fiber components and the AONs. An in vitro DRG explant culture system with the DRGs embedded in AON-containing fibrin gels was set-up to mimic a 3D culture condition with cells preserving the natural extracellular matrix components. Using a Cy5-AON containing gel we verified that the AONs were able to distribute throughout the whole DRG, penetrating to its core, and be uptaken by cells. An efficient reduction of targeted gene expression (>60% at RNA and protein level) was achieved when culturing DRG explants for 7 days in LNA-AON containing gels confirming that the AONs, besides being efficiently distributed throughout the DRG explant were also active inside the cells. In vivo experiments were conducted in a rat model of spinal cord injury (hemisection). The lesion was filled with the AON-containing fibrin gel and covered with an additional gel patch. Five days post lesion, an extensive distribution of the AON throughout the lesion site, but also traveling rostral and caudal to the lesion, was observed. When using functional LNA-AONs, we observed approximately 80 % gene down-regulation in vivo. AON-loaded fibrin gels could be a promising approach to modulate local cellular gene expression and activity lead toing enhanced regeneration in a spinal cord injury setting.

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Oral presentation: O.20 | Rui Jorge Gonçalves Pereira Nobre

Non-invasive silencing of mutant ataxin-3 alleviates motor and neuropathological deficits in a transgenic mouse model of Machado-Joseph disease

Presenter: Rui Jorge Nobre | CNC, University of Coimbra

Rui Jorge Nobre1,2, Joana Saraiva2, Clelia Fusco2, Susana Paixão2, Magda Santana2, Miguel Sena-Esteves3, Luis Pereira de Almeida1,4

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Machado-Joseph disease (MJD) is the most common dominantly-inherited ataxia. It is associated with the expansion of a (CAG)n tract in the coding region of the MJD1/ATXN3 gene. This abnormal over-repetition is translated into an expanded polyglutamine tract within ataxin-3, conferring toxic properties to this protein and resulting in severe clinical features. Although there is no medical treatment, several preclinical studies have demonstrated that silencing mutant ataxin-3 expression using RNA interference (RNAi) is a promising therapeutic approach for MJD. Our group showed that intracranial injection of viral vectors targeting mutant ataxin-3 significantly decreases the severity of the neuropathological abnormalities in rodent models of MJD (Alves et al., 2008, 2010; Nóbrega et al., 2013). However, this is an invasive procedure, which is associated with potential adverse effects and a limited vector distribution in the brain. The present study aimed to develop a non-invasive strategy to deliver RNA interference-based treatments to the brain by intravenous (iv) injection. For that, we used adeno-associated viral vector serotype 9 (AAV9), a vector that has a remarkable ability to bypass the blood-brain barrier (BBB) and transduce the central nervous system of mammals. AAV9 vectors encoding an artificial microRNA that targets the mutant form of ataxin-3 mRNA (AAV9-mirATAX3) were firstly generated. Their efficacy and specificity were tested in neuronal cell models and the therapeutic potential was then evaluated in a severely impaired transgenic mouse model of MJD. Mice were intravenously injected at postnatal (PN) day one (PN1); they were submitted to behavioral tests at 3 different ages and were sacrificed at PN95. We observed that AAV9-mirATAX3 vectors efficiently spread throughout the brain, transducing regions affected in MJD. Moreover, AAV9-mirATAX3's treatment reduced the number of protein aggregates and cerebellar neuropathology, leading to significant improvements in all behavioral tests. Overall, this study generated compelling evidences that a single systemic administration of the AAV9 system at postnatal day one is able to transpose the BBB, to transduce the brain of MJD mice, to silence mutant ataxin-3 in some cerebellar regions, and to alleviation of MJD motor phenotype and neuropathology.

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Oral presentation: O.21 | Poster: P.146 | Sofia Cristina Soares de Morais Grade
Local environment determines integration of transplanted neurons

Presenter: Sofia Grade | LMU/Helmholtz Zentrum, Munich
Sofia Grade (1,2), Leda Dimou (1,2,3), Karl-Klaus Conzelmann (4), Magdalena Götz (1,2,3)
(1) BMC, LMU Munich, (2) ISF, Helmholtz Zentrum Munich, (3) Synergy, LMU Munich, (4) Gene Center, LMU Munich

Cell transplantation aiming at replacing neurons lost upon brain injury or disease has been pursued, however, it remained elusive whether transplanted neurons can faithfully wire into mature circuits. We induced selective cell death in the primary visual cortex (V1) of adult mice, a confined and non-inflammatory injury, and transplanted neurons from the embryonic mouse neocortex. We demonstrate that neurons acquire mature morphologies, extend axons to correct targets through the host parenchyma, and develop synaptic specializations. Using rabies virus-based monosynaptic tracing we show that they receive area-specific, afferent projections matching those of endogenous V1 neurons, including topographically organized geniculate-cortical (graft) connections. Next, we compared neuronal integration in different environments: a traumatic invasive brain injury and the intact cerebral cortex. Transsynaptic tracing after transplantation into the stab wound injured cerebral cortex reveals a V1-specific connectome with no aberrant afferent areas, but an excess of local input. Conversely, neurons transplanted in the intact brain receive correct but considerably fewer connections per cell. Altogether our data indicate that neurons can integrate with great specificity into adult neocortical circuits, a central question for functional reconstruction of the brain, and pinpoint a key role for the local environment critically affecting the synaptic integration into host neuronal networks.

Funding: *SFB870 "Assembly and Function of Neuronal Circuits"*

SESSION: NEURODEVELOPMENT AND CELL BIOLOGY

Oral presentation: O.22 | Bassem Hassan
Transcriptional control of neurogenesis in time and space

Presenter: Bassem Hassan | ICM-Hôpital Pitié-Salpêtrière
Bassem Hassan
(1) Institut du Cerveau et de la Moelle Épinière (ICM) - Hôpital Pitié-Salpêtrière

The development of the nervous system requires the generation of neurons of the proper type, at the correct time and space and in the right ratios. These three axes of neurogenesis are essential for building circuits that process information in manner that results in appropriate behaviors. That neurons of different types are produced at different times and in different numbers suggests some level of coordination between the three fundamental axes of neuronal production. How these axes are coordinated in time and space is poorly understood. I will describe our efforts to contribute to these questions, focusing mainly on the gene regulatory mechanisms that underlie the spatio-temporally coordinated production of the correct numbers of neurons.

Oral presentation: O.23 | Ana Catarina Oliveira Ferreira
Lipocalin-2 regulates adult neurogenesis and contextual discriminative behaviors

Presenter: Ana Catarina Ferreira | ICVS, University of Minho
Ana Catarina Ferreira (1,2), Tiago Santos (3), Belém Sampaio-Marques (1,2), Ashley Novais (1,2), Sandro Dá Mesquita (1,2), Paula Ludovico (1,2), Liliana Bernardino (3), Margarida Correia-Neves (1,2), Nuno Sousa (1,2), Joana A. Palha (1,2), João Sousa (1,2), Fernanda Marques (1,2)
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In the adult mammalian brain, newborn granule cells are continuously generated from resident adult neural stem cells (NSCs), and integrated into hippocampal circuits, thus contributing for hippocampal function. In fact, hippocampal neurogenesis has been proposed to participate in a myriad of behavioral responses, both in basal states and in the context of neuropsychiatric disorders. Thus, the identification of factors that control NSCs maintenance, differentiation and integration is essential. In recent years, the iron trafficking protein lipocalin-2 (LCN2) has emerged as a novel regulator of brain processes and behaviour, involved in neural structure and remodelling, anxiety-like behaviours and cognitive function. Here, we show that the deletion of LCN2 induces deficits in NSCs proliferation and commitment, with impact on the hippocampal-dependent contextual fear discriminative task. Mice deficient in LCN2 present a significant increase in the NSCs population, as a consequence of a G0/G1 cell cycle arrest induced by increased endogenous oxidative stress. Of notice, treatment with the iron-chelating agent deferoxamine rescues NSCs oxidative stress, promotes cell cycle progression and neurogenesis, and improves contextual fear conditioning. This indicates that LCN2 is a novel key modulator of neurogenesis that, through iron cell content modulation, controls NSCs cell cycle progression and death, self-renewal, proliferation and differentiation and, ultimately, hippocampal function. Altogether, our findings identify novel mechanisms of hippocampal neurogenesis regulation in the adult brain, and opens perspectives in understanding the role of iron and iron-related regulators in the (patho)physiology of neuropsychiatric disorders affecting hippocampal neuroplasticity and cognitive function.

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Oral presentation: O.24 | Poster: P.170| Diogo Pinto da Cruz Sampaio e Castro

The transcription factor MyT1 counteracts the neural progenitor program to promote vertebrate neurogenesis

Presenter: Diogo S. Castro | Instituto Gulbenkian de Ciência

Francisca F. Vasconcelos(1), Alessandro Sessa(2), Cátia Iaranjeira(1), Alexandre A.S.F. Raposo (1), Daniel W. Hagey(3), Jonas Muhr(3), Vania Broccoli(2) and Diogo S. Castro(1)

(1) Instituto Gulbenkian de Ciência, Oeiras, Portugal, (2) San Raffaele Scientific Institute, Milan, Italy, (3) Ludwig Institute for Cancer Research, Karolinska Institute, Stockholm, Sweden

In the developing vertebrate embryo, generation of neurons at the correct time and location requires a fine balance between gene expression programs that regulate differentiation and maintenance of neural stem cells. This is to large extent regulated by the opposing forces of the Proneural and Notch pathways. While recent studies have focused on characterizing the differentiation genes activated by proneural factors such as Ascl1, less is known on the mechanisms that suppress progenitor cell identity. Here, we show that Ascl1 induces the transcription factor MyT1 at the onset of neuronal differentiation. We investigate the function of MyT1 at this critical stage by combining acute functional experiments in the mouse telencephalon, with the characterization of its transcriptional program. We found that MyT1 binding occurs mostly at active regulatory regions in undifferentiated neural stem/progenitor cells and is associated with transcriptional repression genome-wide. We further show that MyT1 acts at multiple levels to antagonize the inhibitory activity of Notch signaling, targeting both Notch pathway components and downstream targets. Notably, MyT1 promotes the downregulation of Hes1, a determinant step for the onset of neurogenesis, by competing with Rbpj for binding to the Hes1 promoter. Our results reveal a function of Ascl1 in inhibiting Notch signaling cell-autonomously, showing how activation of neuronal differentiation is tightly coordinated with repression of the progenitor program.

***Funding**: Fundação para a Ciência e Tecnologia (PTDC/NEU-NMC/031572012) and EU (Marie Curie CIG 303644)*

Oral presentation: O.25 | Poster: P.171 | Patrícia Sofia Alçada Tomás de Morais
Cortical neuronal migration entails a2a receptor-driven neuronal polarization and axon formation

Presenter: Sofia Alçada-Morais | CNC, University of Coimbra

Sofia Alçada-Morais (1,2), Veronica Moreno-Juan (3), Nélio Gonçalves (1), Belén Andres (3), Sofia Ferreira (1,2), Joana M. Marques (1), Xinli Xu (1,2), Rodrigo A. Cunha (1,4), Guillermina López-Bendito (3), Ricardo J. Rodrigues (1)

(1) Center for Neuroscience and Cell Biology, University of Coimbra, Portugal (2) Instituto de Investigação Interdisciplinar, University of Coimbra, Portugal; (3) Instituto de Neurociencias de Alicante, CSIC-UMH, Spain. (4) Faculty of Medicine, University of Coimbra, Portugal

Neuronal migration is a fundamental process in brain development. Indeed, impairment in neuronal migration is one of the major causes of cortical malformation, which has been associated to several neurological and psychiatric disorders [1]. Hence, it is of upmost importance to unravel the mechanisms driving neuronal migration. In this regard, it was recently shown that adenosine A2AR controls interneurons migration [2]. We now aimed to evaluate if A2AR is also involved in the migration of cortical principal neurons. For that purpose, we first evaluated the impact of the genetic deletion (A2AR KO) or the pharmacological blockade of A2AR on mice cortical neurons migration during embryonic development. In comparison to their wild-type littermates, embryos lacking the A2AR showed a delayed migration of cortical principal neurons at embryonic day 17 (E17). Similarly, embryos exposed to the A2AR antagonist SCH58261 (daily 0.1mg/kg i.p. injection in pregnant females from E13 to E16) have shown delayed migration, when compared with embryos exposed to vehicle. This should be due to A2ARs expressed by migratory neurons since in utero electroporation of a plasmid encoding shRNA specific for A2AR (E14-E17) also delays migration. The neuronal migration delay occurs mostly in the intermediate zone, where it was observed an accumulation of neurons. It is well-known that it is required a transition from a multipolar to a bipolar shape at the intermediate zone and the establishment of an axon-like leading process in order to the neurons to proceed their migration into the cortical plate [3]. Accordingly, we found in mice cortical neurons that the pharmacological blockade of A2AR with the selective antagonist SCH58261 (50 nM) leads to a reduction in the number of axons (SMI-31 positive neurites) and in their length (DIV 0-3), and we could observe that the knockdown of A2ARs leads to an impairment both in neuronal polarization and axon formation in the migratory neurons. Finally, the observation of a similar delayed cortical principal neurons migration in the CD73-KO mice, which lacks the ecto-5'-nucleotidase that converts AMP into adenosine, indicates that the adenosine that is activating the A2ARs derives from the extracellular catabolism of ATP. This is further heralded by the observation of immunoreactivity for the vesicular nucleotide transporter in the developing mice cortex at E13-E17. Altogether, these results show that A2ARs activated by ATP-derived adenosine are required for cortical principal neuronal migration, in particular for the transition from the intermediate zone into the cortical plate by controlling the establishment of neuronal polarity and axon formation.

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Oral presentation: O.26 | Poster: P.166 | Céline Saraiva Gonçalves
WNT6 regulation in glioblastoma: mechanistic, functional and clinical implications

Presenter: Céline S. Gonçalves | ICVS, University of Minho
Céline S. Gonçalves (1,2), Marta Pojo (1,2), Ana Xavier-Magalhães (1,2), Joana Vieira de Castro (1,2), Vera Miranda-Gonçalves (1,2), Afonso A. Pinto (3), Ricardo Taipa (4), Manuel Melo Pires (4), Fernando Pardal (5), Fátima Baltazar (1,2), Rui M. Reis (1,2,6), Nuno Sousa (1,2), Bruno M. Costa (1,2)
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Background: Glioblastoma (GBM) is the most common and most malignant type of glioma, a heterogeneous group of primary brain tumors. While the clinical outcome of GBM patients is unpredictable, patients are equally treated with a standardized approach. Thus, the identification of new biomarkers is crucial. HOXA9 overexpression in GBM is associated with poor prognosis and a more aggressive tumor phenotype. We recently found that HOXA9 transcriptionally activates the WNT pathway; here, we explore how WNT6, a WNT ligand/activator, may contribute to the malignant behavior of GBM. Material and Methods: Gene set enrichment analysis (GSEA) was used to query the HOXA9 transcriptome. Quantitative PCR, Western blot, chromatin immunoprecipitation (ChIP), methylation-specific PCR (MSP), and immunohistochemistry were performed in GBM cell lines, in vivo xenografts, or in patient samples to study WNT6 at various molecular levels. The functional effects of WNT6 in cell viability (MTT/Trypan blue), proliferation (BrdU), invasion (transwell matrigel), migration (ibidi inserts), angiogenesis (Chick Chorioallantoic Membrane), cell death after treatment with temozolomide (TMZ; Annexin/PI staining) and stemness capacity (limiting dilution assay) were assessed after silencing WNT6 with shRNA. U373+/-WNT6 cells were intra-cranially implanted in NSG mice to evaluate implications in survival. TCGA dataset was assessed for WNT6 status and clinicopathological correlations. Results: We found that the Wnt pathway is over-activated in HOXA9-positive GBM cells. Specifically, WNT6 is a direct transcriptional target of HOXA9 and is overexpressed in a subset of GBM patients. Additionally, we observed that WNT6 expression correlates with higher glioma grades and with the GBM proneural subtype, whose patients do not benefit from more intensive therapies. Interestingly, we demonstrated that WNT6 expression is also regulated by DNA methylation in GBM patients. In vitro, WNT6-positive cells showed increased viability, migration, invasion and resistance to TMZ, and decreased cell death, when comparing to their negative counterparts. When cultured in stem-cell conditions, WNT6-positive cells show increased viability and capacity to form neurospheres than WNT6-negative cells. In addition, mice bearing WNT6-positive tumors presented faster glioma-related symptomatology and a significantly shorter overall survival (p=0.0042). Importantly, we provide the first evidence of the clinical prognostic value of WNT6 in GBM patients from TCGA and at the protein level in a cohort of Brazilians patients, implicating high levels of WNT6 as a novel independent negative prognostic marker. Conclusion: Together, our findings provide mechanistic, functional and prognostic insights into the role of WNT6 in GBM, creating opportunities to novel therapeutic approaches to treat this highly-aggressive cancer.

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***Link between abstracts**: qCEJA*

SESSION: KEYNOTE LECTURE II

Oral presentation: O.27 | Alcino Silva
Molecular, cellular, and circuit mechanisms that link memories across time

Presenter: Alcino Silva | UCLA
Alcino Silva (1)
(1) Departments of Neurobiology, Psychiatry, and Psychology, Integrative Center for Learning and Memory, Brain Research Institute, University of California, Los Angeles, USA

Studies of the molecular, cellular and circuit mechanisms of learning and memory have focused almost exclusively on how single memories are acquired, stored and edited. By comparison, very little is known about the mechanisms that integrate and link memories across time. Recent studies from our laboratory showed that learning triggers CREB activation and a subsequent temporary increase in neuronal excitability in these circuits that for a time biases the allocation of a subsequent memory to the neuronal ensemble encoding the first memory. Recently, we have used state of the art in vivo imaging methods and other approaches to show that in the hippocampus, this mechanism can link memories across time, such that the recall of one memory increases the likelihood of recalling the other memory. Interestingly, we also showed that this mechanism is disrupted in older mice, and that artificially manipulating neuronal excitability with a chemo-genetically strategy can rescue these deficits, a result that implicates this mechanism in memory linking and in age-related cognitive decline.

***Link between abstracts**: Edeota*

POSTER PRESENTATIONS

SESSION: NEURAL CIRCUITS AND BEHAVIOR

Poster: P.001 | Sónia Isabel Nunes Guerra Gomes
The role of astrocytic calcium signaling in the cognitive function

Presenter: Sónia Guerra-Gomes | ICVS, University of Minho
Sónia Guerra-Gomes (1,2), Vanessa Morais Sardinha (1,2), Gabriela Tavares (1,2), Joana Sofia Correia (1,2), Inês Caetano (1,2), Eduardo Loureiro-Campos (1,2), Nuno Sousa (1,2), Luísa Pinto (1,2), João Filipe Oliveira (1,2)
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Astrocytes play an active role in the regulation of synaptic transmission and plasticity. They have the ability to regulate and dynamically respond to changes in the microenvironment, namely by increasing intracellular calcium levels through the activation of inositol 1,4,5-trisphosphate receptors type 2 (IP3R2) [1,2]. We hypothesized that modulation of astrocytic calcium signaling might have implications in neurotransmission, metabolism and brain homeostasis, whose disruption might be associated with alterations in learning and memory processing. We used the IP3R2KO mouse model, to disclose its implications in brain networks by performing a behavioral, morphological and molecular characterization. We assessed cognitive function in IP3R2KO mice and wild-type littermates by performing Morris Water Maze and Fear Conditioning. Our results suggest that astrocyte calcium signaling is related with a modulation of hippocampal-dependent behavior. Since this model is a constitutive knock-out, we raised the possibility that, during development, these mice might adapt to the loss of IP3R2 by the activation of compensatory mechanisms. So, we performed a neurodevelopmental evaluation which indicates that "silencing" astrocytes induces no alterations during neurodevelopment. Moreover, we observed that mice with impairment in astrocytic calcium signaling do not present alterations in the morphology of hippocampal neurons. In order to identify molecular signaling pathways and transcription factors altered in our mouse model of astrocytic dysfunction, we performed microarray analysis (Affimetrix GeneChip) of control and IP3R2KO mice brain. This characterization will provide us with novel roles for astrocytic calcium signaling in the modulation of neural activity, namely in cognitive processes.

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***Link between abstracts**: astrogang*

Poster: P.002 | João Filipe Pedreira de Oliveira
Astrocytes support hippocampal-prefrontal theta synchronization and cognitive function

Presenter: João Filipe Oliveira | ICVS, University of Minho

Vanessa Morais Sardinha (1,2), Sónia Guerra-Gomes (1,2), Gabriela Tavares (1,2), Manuella Martins (1,2), Joana Santos Reis (1,2), Joana Sofia Correia (1,2), Andreia Teixeira-Castro (1,2), Luísa Pinto (1,2), Nuno Sousa (1,2), João Filipe Oliveira (1,2,3)

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Astrocytes interact with neurons at the cellular level through modulation of synaptic formation, maturation and function, but the impact of such interaction into behavior remains unclear. Here, we studied the dominant negative SNARE (dnSNARE) mouse model to dissect the role of astrocyte-derived signaling in cortico-hippocampal circuits, with implications for cognitive processing. We found that the blockade of gliotransmitter release in astrocytes triggers a critical desynchronization of neural theta oscillations between dorsal hippocampus and prefrontal cortex. Moreover, we found a strong cognitive impairment in tasks depending on this network. Importantly, the supplementation with D-serine completely restores hippocampal-prefrontal theta synchronization and rescues the spatial memory and long-term memory of dnSNARE mice, providing a novel mechanism of long distance network modulation by astrocytes, with direct implications to cognitive function.

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***Link between abstracts**: astrogang*

Poster: P.003 | Inês Caetano Costa Campos
D-serine modulates neural oscillations in the hippocampus-prefrontal cortex network

Presenter: Inês Caetano | ICVS, University of Minho
Inês Caetano (1,2), Vanessa Morais Sardinha (1,2), Sónia Guerra-Gomes (1,2), Joana Santos Reis (1,2), Joana Margarida Silva (1,2), Ioannis Sotiropoulos (1,2) Nuno Dias (1,2,3), Nuno Sousa (1,2), João Filipe Oliveira (1,2,3)
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LFPs reproduce summated individual conductance and synaptic inputs of networks composed by ensembles of firing neurons and surrounding glia, and therefore are an excellent readout of network dynamics. LFPs reflect the temporal pattern of activity that acts on local networks that are directly connected. Importantly, the dorsal hippocampus (dHIP) and the medial PFC are tightly linked. The neuronal oscillations in the theta band within this network are fairly synchronized to support cognitive behavior and this synchrony was shown to be affected in the dnSNARE model, that lacks astrocyte signaling. In this work, we are addressing how the supplementation of D-serine in either the hippocampus or prefrontal cortex can revert the loss of synchrony found in dnSNARE mice without astrocyte signaling. We measured spectrograms of neuronal activity and quantified the levels of coherence established between the dorsal hippocampus and the prefrontal cortex. This readout is a valuable tool to assess the role of astrocytes in the modulation of neuronal oscillations.

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***Link between abstracts**: Astrogang*

Poster: P.004 | Gabriela Pires Tavares
Validation of an open source tool to study 3D astrocytes morphology

Presenter: Gabriela Tavares | ICVS, University of Minho
Tavares G. 1,2, Sardinha V.M. 1,2, Guerra-Gomes S. 1,2, Martins M. 1,2, Correia J. S. 1,2, Neves S.P., Marques F. 1,2, L. Pinto 1,2 N. Sousa 1,2, J.F. Oliveira1,2,3
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Astrocytes have gained recognition in the past decades mainly because of their function in neuron-astrocyte interactions responsible for synaptic modulation and plasticity. Astrocytes present a complex and ramified morphology that is closely related with their function, state and population. However morphometric analysis of astrocytes has been poorly explored in the vast majority of the studies, due to difficulties related to the reconstruction softwares available. For instance, most of the available softwares either display a license cost, or are computationally and operationally demanding. Therefore, it was essential to find an effective computational method that balanced time expended on tracing, data accuracy and reproducibility and accessibility. Simple Neurite Tracer (SNT) is an open source Plugin of Fiji-Image J that presents an intuitive workflow that allows an easy 3D reconstruction in z-stack confocal images in a semi-automatic manner. Here we present a validation of this software including a comparative study by different users and different softwares to confirm data reproducibility and reliability. To assess software sensibility and discrimination to different morphometric structures, astrocytes from a model of astrogliosis were analyzed. SNT was confirmed as a practical, reliable, and economic tool that provides less user-dependent errors to assess 3D astrocytes morphology. It allowed discriminative and accurate morphometric analysis of astrocytes by quantifying their total process length, number of processes, process thickness and complexity by Sholl analysis.

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***Link between abstracts**: Astrogang*

Poster: P.005 | Oral presentation: O.03 | Bárbara Guimarães Salazar Coimbra
Impairments in laterodorsal tegmentum to VTA projections underlie glucocorticoid triggered reward deficits

Presenter: Bárbara Coimbra | ICVS, University of Minho
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Ventral tegmental area (VTA) activity is critical for motivated behaviours and reinforcement. Importantly, VTA activity is tightly modulated by afferents arising from the laterodorsal tegmentum (LDT). Disruption of this circuit can ultimately increase the risk for the development of neuropsychiatric disorders, including those associated with reward deficits, such as depression, anxiety, obsessive-compulsive disorder, obesity, addiction or antisocial behaviour. Additionally, the VTA region is particularly vulnerable to the effects of stress/glucocorticoids (GCs). Previous studies revealed that in utero exposure to glucocorticoids (iuGC) triggers prominent reward deficits later in life but nothing is known about the impact of this exposure in the LDT-VTA circuit. Here, we show that iuGC animals have long-lasting changes in the expression of cholinergic markers in the LDT, and in vivo singe-cell electrophysiology revealed that LDT basal activity was decreased. Interestingly, we observe a bidirectional effect in LDT-VTA inputs: upon LDT stimulation, iuGC animals present a decrease in the magnitude of excitation and an increase in the magnitude of inhibition in the VTA. While in control animals most of the inhibitory responses arise from putative GABAergic neurons, in iuGC group there is a shift in the type of cells presenting inhibitory responses, with a significant increase in the number of dopaminergic neurons. In agreement with LDT-VTA dysfunction, we show that iuGC animals present motivational deficits that are rescued by selective optogenetic activation of this pathway. Importantly, we also show that LDTVTA optogenetic stimulation is reinforcing, and that iuGC animals are more susceptible to the reinforcing properties of LDT-VTA stimulation.

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***Link between abstracts**: Blackberry*

Poster: P.006 | Carina Isabel Soares da Cunha
Dissecting the role of nucleus accumbens D1- and D2-MSNs in reinforcement

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The nucleus accumbens (NAc) is a key region for reward/reinforcement. In the NAc, two distinct GABAergic neuronal populations, constituted by medium spiny neurons (MSNs) that express either dopamine receptor D1 (D1R, D1-MSNs), or dopamine receptor D2 (D2R, D2-MSNs), process dopaminergic signals arising from the ventral tegmental area (VTA). While activation of D1-MSNs has been traditionally associated with reinforcement and positive stimuli, D2-MSNs activation has been associated with aversion and negative stimuli. Nonetheless, recent data has shown that both types of MSNs may have a convergent role in reinforcement. In the present work, we used optogenetic tools to specifically stimulate/inhibit NAc D1- and D2-MSNs and assess its effects in behavioral conditioning. Activation of either of these neuronal populations is sufficient to induce preference, while their inhibition causes aversion. Interestingly, we were able to modulate preference by changing the type of optogenetic stimulation performed. This change in preference was related to different accumbal-evoked responses in downstream target regions, namely the ventral pallidum and the VTA. In conclusion, our data suggests that a concerted action of both types of MSNs may be required for (positive and negative) reinforcement, challenging the traditional view of D1- and D2-MSNs opposing roles in behavior.

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***Link between abstracts**: Blackberry*

Poster: P.007 | Sónia Maria de Sousa Borges
The role of dopamine receptor 2-expressing neurons in social behavior

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Social behavior is highly rewarding therefore, it is modulated by neural systems implicated in other rewards such as food, sex and drugs of abuse. Though the mesolimbic system has been linked to social interaction, less is known about the function of nucleus accumbens (NAc) different neuronal populations in these behaviors. At least one study has shown that optogenetic activation of dopamine receptor D1-expressing neurons in the NAc leads to increase in social interaction time (Gunaydin et al. 2014). Less explored is the role of D2 neurons in these type of behaviors. We used optogenetics to study the role of Nac D2 neurons in a resident-intruder home cage test. Activation of NAc D2 neurons increases social interaction in control animals. Furthermore, it rescues the social impairments previously observed in an in utero glucocorticoid (iuGC) animal model. These findings suggest that additional studies have to be made in order to evaluate the contribution of different types of NAc neurons in social behaviors.

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***Link between abstracts**: Blackberry*

Poster: P.008 | Ana Margarida Ferreira da Cunha

Side specific impact of peripheral neuropathic lesions in goal-directed and impulsive decision making: potential role of nucleus accumbens' dopamine

Presenter: Margarida Cunha | ICVS, University of Minho

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Mechanisms associated with decision-making impairments in chronic pain (CP) remain poorly understood. We have therefore studied the impact of CP in the habit- to goal-directed decision-making transition and the involvement of Nucleus Accumbens (NAc) dopaminergic pathways in the process. For that, food restricted Wistar-Han rats with neuropathic lesions in left (Spared Nerve Injury (SNI)-L) or right (SNI-R) posterior paw were trained in an operant chamber (OC) to press a lever to receive a reward (sugar pellets or sucrose 20%). The ratio between action and outcome increased along the days until it reached a random ratio of 20 lever presses/reward (RR20). An early devaluation test was then performed: rats had ad libitium access to one of the rewards (devaluated reward) for 1h and were placed in the OC in extinction for 5 min to lever press counting. In the following days rats were submitted to 6 RR20 sessions to induce habit formation after which a late devaluation test was performed. In other experiment, we analyzed the impact of the same neuropathic lesion in the NAc's dopamine (DA) and DA receptors (D1R and D2R) expression by HPLC and qPCR, respectively. Finally, we performed the same habit- to goal-directed decision task in left and right DA depleted animals (by 6-Hydroxydopamine (6-OHDA) injection in the NAc). All animals (both in SNI and 6-OHDA lesions experiments) increased lever pressing along sessions, progressing the same way, and decreased the number of lever presses in the devaluated condition in the early test. SNI-L rats (but not SNI-R) presented a defective devaluation ability in the late test, indicating an impairment in habit- to goal-directed decisions. When compared with Sham, both SNI-L and SNI-R show an increase in D1R and D2R expression towards the contralateral side of the lesion but no differences in the DA levels. Animals with a 6-OHDA lesion in the left or right NAc showed the same decision-making impairments observed in SNI-R and SNI-L animals, respectively, indicating a lateralized role of NAc in decision-making. Considering these results and previous results from the group that show that SNI-R (but nor SNI-L) rats have higher impulsivity in the variable delay-to-signal (VDS) test when compared to sham, we hypothesize that peripheral neuropathic lesions impact specifically the contralateral NAc, leading to selective impairments in the devaluation (in SNI-L) or impulsivity (in SNI-R).

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***Link between abstracts**: M&M&M*

Poster: P.009 | Madalena Curva Esteves
Accumbal activity predicts impulsive action

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The nucleus accumbens (NAcc) is widely known to be associated with critical aspects of decision-making, e.g. motivation and reward. Its role in behavioral inhibition is however less known. NAcc activity was registered in 5 Wistar-Han rats implanted with nichrome single wire electrodes during the execution of an impulsivity task - Variable Delay-to-Signal (VDS). Animals learnt to nosepoke a small aperture after a 3 s delay (signaled with a light) to earn a sugared pellet. Premature nosepokes (during the delay) were punished with a timeout and no reward was provided. Local Field Potentials (LFPs) were acquired, amplified and band-pass filtered (3-250Hz). Processing was performed in Matlab using the Chronux toolbox and included downsampling, referencing, Notch application (75 and 150Hz), artifact removal, event alignment, power estimation using a multitaper method, normalization to baseline and averaging. Power in theta (3-7Hz) and beta (7-40Hz) ranges peaked around the nosepoke, preceding it when the response was premature but following when timed. Higher frequency ranges, namely low gamma (40-75Hz), high gamma (75-120Hz) and high frequency oscillations (120-200 Hz), presented higher power up to 1500 ms before a premature nosepoke and abruptly decreased after the response. Correct nosepokes on the other hand, were associated with a decrease in power in these frequencies in the 750 ms before and an abrupt increase after the end of the response. Additionally, logistic regression showed that responses could be predicted based on inter-trial interval power. Increased beta and decreased higher gamma and high frequency oscillations were significantly associated with higher probability of correct response, even after controlling for the result of the last trial. In conclusion, our results suggest a physiological role of the NAcc in impulsive behavior. The electrophysiological profile differs between premature and timed responses, and, importantly, it predicts the direction of the decision-making outcome.

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***Link between abstracts**: M&M&M*

Poster: P.010 | Paulo de Castro Aguiar
Encoding time in recurrent neuronal populations – a study with the COBA model

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Introduction: Introduction: Coordinated motor tasks, such as playing a musical instrument, require precisely clocked patterns of neuronal activity which are supposed to rely on recurrent networks with strong internal connections [1]. The neuronal mechanisms supporting measures of time in the scales of tenths of milliseconds to several seconds are still not well understood, and whether these mechanisms are dedicated or intrinsic remains an open question [2]. In this study we explored temporal features of the COBA model, a recurrent random network of integrate-and-fire neurons, and assessed the possibility for such neuronal architectures to be used as time keepers. In these computer simulations we tested the hypothesis of specific time delays being represented as specific spatial patterns of activity, a coding mechanism known as population clocks [1]. Methods: The open source simulator BRIAN [3] was used for the simulation of the COBA (conductance-based) model. The COBA model, implemented as in [4], consists of a random recurrent network of 4000 neurons, described by the integrate-and-fire (I&F) model, capable of self-sustained activity. The I&F neuronal model is a simplified version of the Hodgkin-Huxley model of neuronal activity, which is adequate for simulations of large networks. The recurrent network of 4000 neurons was composed of 3200 excitatory and 800 inhibitory neurons, with exponential synaptic currents. In the simulations, the neurons' state is updated synchronously with spike times bounded to a discrete time grid. To avoid transient effects, the network activity is simulated for 20 minutes of network time, and the spikes occurring on the last 200ms of simulation were analysed. A normalized symmetric co-activation function (NCAF) was developed for the quantification of the synchronization between the spike times of two neurons, allowing a delay of 10ms. A Boolean matrix, M, 4000x4000 was built with 1 if NCAF>0.96 and 0 otherwise. By construction, the 4000 neurons can be regarded as the vertices of a graph G having adjacency matrix M, with no self-edges. The problem of finding subpopulations of coactive neurons is solved if we can find all the maximal subgraphs of G. For that we have used an implementation of the Bron-Kerbosch algorithm for finding maximal cliques on G [5, 6]. Results: Several coactive subpopulations of neurons were found, with distinct number of neurons and spikes. With more than 2 neurons and more than 2 spikes, we obtained a total of 124 subpopulations. From those, the largest one has 8 neurons firing 3 spikes each and the more active, two sets were found, have 3 neurons firing 6 spikes each. Conclusion: While the self-sustained activity in the COBA recurrent network appears completely random we have shown that the activity of several groups of neurons can become synchronized. Since different subpopulations take different delays to become synchronized, readout neurons could in principle be used to signal (clock) different times.

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***Link between abstracts**: milhafreazul*

Poster: P.011 | Ana Rosa Maço Abreu

The physiology of ion-channels in pain sensing neurons – relevance to chronic orofacial pain

Presenter: Ana Rosa Abreu | Faculdade de Ciências UL
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Introduction: Chronic orofacial hyperalgesia is associated with disorders such as migraine and trigeminal neuralgia and without adequate therapy can become profoundly debilitating. Due to the poor knowledge of the underlying pathophysiological mechanisms, the available treatments provide only temporary relief and have several undesirable side effects[1]. The orofacial pain stimuli are transmitted by the trigeminal nerve branches that converge on the trigeminal ganglion (TG). These fibers are in a higher excitability state in situations of chronic pain, a reflection of changes on the activity of the ion channels involved in the maintenance of neuroexcitability[2]. Such alterations on firing patterns may be caused by modifications on the biophysics of voltage-gated potassium (Kv) channels [3]. Since the trigeminal system has distinct symptomatic responses to injury and inflammation when compared to its spinal counterparts, the dorsal root ganglia (DRG)[2],[4], the present work aims to characterize the different Kv current components from TG neurons in chronic pain conditions and to compare them to those obtained from DRG neurons. Methods: Wistar rats were used to establish an animal model for chronic inflammatory orofacial hyperalgesia, complete Freund's adjuvant (CFA) was subcutaneously administered to the whisker pads of the animals[5]. The presence and extend of sensory disturbance was evaluated through alterations of natural and evoked behavioral responses for 28 days[6]. After this period, rats were euthanized by decapitation after anesthesia with pentobarbital and TG and DRG were dissected and enzymatically dissociated to obtain acutely isolated cells, which were used within 8 hours. Whole-cell voltage-clamp technique was used to record Kv currents. Animal care and experimental studies were in accordance with Directive 2013/63/EU. Results and Perspectives: The results show that obtained Kv currents in TG neurons are characterized by two current components as the current decays are better fitted by a sum of 2 exponential functions. One, here termed Ifast, inactivates within ten of milliseconds and the second, termed Islow, decays within hundreds of milliseconds. The obtained current traces were similar to those obtained from small diameter DRG neurons. Moreover, an identical voltage-dependence of activation and inactivation was found in both TG and DRG which suggests a similar biophysical nature of the underlying Kv channels. However, further characterization of the biophysics of the Kv currents/channels is needed in order to identify the channels involved. Finally, the alterations in the Kv current components in the TG CFA pain model are presented.

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***Link between abstracts**: passarobrilhante*

Poster: P.012 | Beatriz Szwarc dos Santos
K+ Channels in Neurons from rat Dorsal Root Ganglia reveal new Therapeutic Leads for Inflammatory and Neuropathic Chronic Pain

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Introduction: Chronic pain affects 21% of the human population and, to date, treatments are only partially effective[1]. In this situation, nociceptive fibers that are responsible for the transmission of pain stimuli are in a higher state of excitability. Such neural excitation degree is controlled by sets of ion channels [2]. Of all sensory fibers, small diameter dorsal root ganglia (DRG) neurons are found to have an augmented activity in chronic pain patients [3], which may be caused by abnormal firing patterns modulated by voltage-gated potassium (Kv) channels. Our goal is to elucidate the specific mechanisms of neuropathic chronic pain in rat models by studying the biophysics of Kv currents/channels in small diameter DRG neurons. We aim to identify which channels are relevant and how should we affect them in new therapeutic strategies. Methods: Animal care and experimental studies with Wistar rats were in accordance with Directive 2013/63/EU. To study neuropathic pain, a chronic constriction injury (CCI) model was performed by making 4 loose ligations in the right sciatic nerve of the animals (120g-140g rats)(n=9) and the model was maintained throughout 4 weeks[4]. Seven naïve animals were used as controls for each model. Mechanical sensibility was scored based on von Frey monofilament stimulations as experimental paradigm [6]. After 4 weeks of injury, rats were euthanized by decapitation after anesthesia with pentobarbital. L4-L6 DRGs were removed and enzymatically dissociated to isolated single cells, which were used within 8 hours. Whole-cell voltage-clamp technique was used to record Kv currents. Results & Conclusions: Maximal mechanical nociceptive scores validated the CCI chronic pain model. Whole-cell voltage-clamp results demonstrate that neuropathic pain alters the biophysical properties of the Kv currents. Specifically, data revealed that the fast-inactivating K+ current component is smaller in CCI-derived DRG neurons when compared to Naïve without voltage sensitivity alteration which is in line with the idea of pain-induced hyperneuroexcitability. Surprisingly, the slow-inactivating K+ current component is augmented in CCI, also not showing alterations in voltage-sensitivity. Moreover, regarding the voltage dependence of inactivation, the CCI pain model showed a depolarized inactivation curve shift, which, together with the previous result, contradicts the idea of hyperexcitbility. In addition, we found that Kv1.3 is overexpressed in CCI-derived DRG neurons suggesting kv1.3 as the channel underluying the augmented slow current in CCI. Importantly, all results point out for the identification of K+ current components that are differentially expressed in the chronic pain models, thus revealing new therapy leads.

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***Link between abstracts**: passarobrilhante*

Poster: P.013 | Inês Alexandra Teixeira de Almeida
Should eye trust you: How the OFC and amygdala represent predictive cues of monetary outcome in a Trust economic game

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The ability to form associations between ecological cues and significant outcomes predicted by them is a central aspect guiding behaviour, being crucial for survival. Learning of this association is particularly relevant during decision-making in which one wants to maximize reward and diminish punishment. In social economic interactions, facial cues play an important role in outcome prediction. Trustworthiness can be inferred by patterns of eye gaze (e.g. direction), while establishing contingencies with behaviour. Studies show that direct eye contact is more trustworthy than avoidance of eye contact, but how these facial cues are represented in the brain to guide decision towards rewarding outcomes is still largely unknown. In the animal model, recordings of orbitofrontal cortex (OFC) and amygdala neurons show these areas become selective for cues predicting both more and less appetitive outcomes. When learning of the association cue-outcome is reversed, the OFC is important to establish new contingencies. In humans, less invasive methods such as functional neuroimaging (fMRI) have been used. Here we apply fMRI to study how eye gaze type (Directed, DIR; Averted, AV) (predictive cue, PC) guides economic decisions during a Trust Game (N=19) when the former is linked to the money received (outcome, O). In a previous task, we linked DIR gaze (reward PC) with higher return of money, and AV gaze (punishment PC) with smaller returns. In the current task, we reversed the association of eye gaze type (PC) and money received (O), with AV now signalling higher returns and DIR smaller ones. We investigated how the OFC and amygdala represent both PCs during the Video (PC presentation) period, by performing ROI analyses in the amygdala (left, L, right, R), medial OFC, and lateral OFC (L, R). We predict that differences between the first run of relearning (run 5), in which the association PC-O is reversed, and the following runs (6, 7 and 8), in which learning is consolidated, will be reflected in the direction of the contrast DIR vs. AV gaze. Crucially, we separated the participants into learners (G1, able to learn the new PC-O association) and non-learners (G2, not able to notice the new PC-O association). In run5, the lateral OFC (L, R) differentiated eye gaze type (DIR>AV) in G1 but not in G2. In run 7, the lateral OFC (L, R) differentiated eye gaze (AV>DIR) in G1, whereas in G2 this was observed in medial OFC (DIR>AV). In run 8, the amygdala (L, R) differentiated eye gaze (AV>DIR) in G2, whereas only the R one did so in G1 (AV>DIR). Differences in OFC between DIR and AV gaze in the run of relearning disappeared or were reversed in later runs when learning was consolidated, whereas the amygdala seemed to represent true PC-O. Our results show that OFC and amygdala play similar but separate roles when processing cue-outcome associations, guiding decision in operant learning games. Future work should address how these representations are used to form decisions.

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Funding: *Projeto Estratégico (PEst) FCT UID/NEU/04539/2013; COMPETE POCI-01-0145-FEDER-007440*
Link between abstracts: *SETYFMRI*

Presenters are required to stand by their poster at least during 1h
Odd poster number - First hour
Even poster number - Second hour

Poster: P.014 | Sara Dias dos Santos
Should eye trust you again? An fMRI study on decision-making

Presenter: Sara Santos | IBILI, FMUC
Sara Santos,(1) Inês Almeida (1), Miguel Castelo-Branco (1, 2)
(1) IBILI, Institute for Biomedical Imaging and Life Sciences, Faculty of Medicine, University of Coimbra, Coimbra, Portugal,
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The face is a source of information from which we are able to perform judgements regarding others, in particular of trustworthiness. Furthermore, the direction of eye gaze is a facial cue frequently used to predict others' behavior and intentions. Studies show that directing gaze to others makes people to be perceived as more trustworthy than those who avoid eye contact. These perceptions of others are of particular relevance, since they are known to influence our decisions. However, it is not clear how facial trustworthiness cues, when linked to certain behaviors, influence and guide the process of decision-making. In this study, using fMRI, participants (N=19) performed a trust game with two trustees with pre-established reputations: one trustworthy, who would overall return more, and one untrustworthy, returning overall less money. Two tasks were performed: during the first, directed gaze (DIR) was linked to higher outcomes and averted gaze (AV) to lower outcomes (congruent prior). This association was later reversed (learning of incongruency): DIR would lead to lower returns and AV to higher ones. Thus, eye gaze type worked as a cue for the trustee's behaviour, being linked to the amount of money received. The reversal of the association between gaze and outcome allowed to test if brain regions were responding only to the gaze cue itself or if they would also signal its affective value during the relearning process. The results here described concern only to the learning task. Two groups were considered for the analysis of the 4 task periods (video, expectation, investment and outcome): those who were able to learn the new association between gaze type and outcome and those who did not. An ANOVA RFX revealed a main factor of Group (learning, L, vs non-learning, NL) in the precuneus, parahippocampal and precentral gyrus, putamen, cerebellum, cingulate, superior temporal gyrus (STG) and thalamus. As we were interested if learning differences between groups were related with the differentiation of eye gaze types, we performed ROI analyses in these regions testing for group differences for contrast 'DIR vs AV' for the 4 task periods. Differences were found in the cerebellum during the video period (NL>L), and in the thalamus and STG for the expectation period (L>NL). These regions are known to be involved in various cognitive processes (non-motor functions of the cerebellum) and to participate in associative learning and event-driven attention (associative part of the thalamus), while others are related to the integration of previous actions and successful outcomes into one's decision-making strategy (STG). These results suggest that the STG, thalamus and cerebellum were involved in the learning and interpretation of the different eye gaze cues, influencing the decision whether to trust or not in another person, integrating previous interactions. Future studies would allow to elucidate the precise role of these regions in decision-making processes.

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***Link between abstracts**: SETYFMRI*

Poster: P.015 | Ana Isabel Magalhães Gamito Carrilho

Comparison of two short periods of maternal separation on adolescent rat social behavior and drug reward

Presenter: Ana Magalhães | I3S-IBMC

Marlene Nogueira(1), Renata Alves*(2,3), Joana Bravo(3), Cecília Juliana Alves(3), Ana Raquel Pereira(4), Ana Mesquita(1), Liliana de Sousa(5), Fernando Barbosa(2), Teresa Summavielle(3), Ana Magalhães(3,5).*

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The social environment is critical in the development of drug-related problems, with social features playing an important role in the initial use, maintenance and recovery from addictions. In this study, a maternal separation (MS) paradigm was used as an animal model of physical mother absence, a relevant issue in modern societies. Mother-pup relationship is a dynamic and reciprocal interaction with important roles in modulating behaviour and shaping neuronal networks. Therefore, it is expected that MS affects both members of this dyad. In this study we examined if short periods of early MS may modify maternal behaviour and disrupt the way adolescent rats interact with others, as well as their susceptibility to drug abuse. Two periods of MS, postnatal day (PND)2-6 and PND10-14, for 2 hours/daily were investigated, on different social behaviour paradigms, on adolescent Wistar rats. Collected data were correlated with the expression profile of oxytocin receptor (OXTR) gene. Sensitivity to the conditioned reward of cocaine was also evaluated. Results showed that MS during PND2-6 highly reduced social affiliation/motivation and social novelty preference, indicating inability to establish strong bonds. A tendency to express cocaine place preference was also observed in these rats. After MS PND10-14, dams increased maternal behaviour, which seems to be reflected in the offspring OXTR expression in prefrontal cortex and in an increase in the affiliative behavior. This study reveals that early short periods of MS are able to shape the adolescent rat social behavior and affect the reward circuit. Results also suggest that PND10-14 may be a key period for parental care investment and a critical window for the mother-infant bonding quality.

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***Link between abstracts**: wonderland*

Poster: P.016 | Haissa de Castro Abrantes
Evaluation of the receptor-mediated function of lactate in neuronal activity

Presenter: Haissa de Castro | University of Lausanne - DNF
Haíssa de Castro Abrantes (1), Marc Briquet (1), Stefan Offermanns (2), Jean-Yves Chatton (1)
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Since the introduction of the astrocyte-neuron lactate shuttle hypothesis lactate started being recognized as an energy substrate for neurons. In this model, lactate is provided to neurons by transport from astrocytes. Besides the metabolic functions of lactate, the recent discovery of a G protein-coupled receptor (GPCR) for lactate in neurons of the central nervous system, called hydroxycarboxylic acid receptor 1 (HCA1R), has pointed to additional non-metabolic effects of lactate on neuronal network activity.The aim of this work was to characterize the intracellular pathway mediated by the activation of HCA1R in neurons, and to investigate the cooperation between HCA1R and other GPCRs for the modulation of neuronal network activity. The non-metabolized agonists of HCA1R, 3,5-DHBA and 3-Cl HBA, reversibly decreased the spontaneous spiking activity of primary cortical neurons of wild-type mice by 40%. Neither compounds affected the activity of neurons prepared from HCA1R knock-out animals. We observed that HCA1R in neurons mediates its effect through the inhibition of adenylyl cyclase, decreasing cAMP levels and PKA activity. These results together with previously published data on the Gi protein deactivator PTX ability to reverse L-lactate effect in neurons, strongly supports the notion that HCA1R in the central nervous system is mediating its effect through a Gi protein, as was previously demonstrated in adipocytes. A characteristic feature of GPCRs is their ability to cross-talk with other GPCRs. We found that HCAR1 cooperates with the adenosine A1 receptor, GABAB receptor, and α2-adrenergic receptor for the modulation of the neuronal network activity. Our results underlines the requirement of HCA1R activation and the non-metabolic nature of the lactate effects on neuronal activity. This study supports the idea that lactate can be considered a gliotransmitter able to modulate the neuronal activity through GPCRs.

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Poster: P.017 | Ana Carla David Pereira

Astrocytic metabotropic receptors type 5 in the infralimbic cortex tonically promote descending facilitation in monoarthritic, but not in healthy rats

Presenter: Ana David-Pereira | ICVS, University of Minho

Ana David-Pereira (1,2), Sara Gonçalves (1,2), Armando Almeida (1,2), Filipa Pinto-Ribeiro (1,2)

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The importance of the limbic-cognitive component of pain has recently been highlighted with increasing evidence showing the medial prefrontal cortex (mPFC) is involved in pain processing. Metabotropic glutamate receptor 5 (mGluR5) partly mediates mPFC output and regulates its activity in pain. mGluR5 are present not only in neurons but also in astrocytes, which are important in development, maintenance and perception of chronic pain. This work assessed the role of infralimbic cortex (IL) mGluR5 in the descending modulation of pain in healthy and monoarthritic (ARTH) rats and the contribution of astrocytic mGluR5 towards this effect. For intracerebral injection of drugs, guide cannulas were implanted in the IL of healthy and ARTH (kaolin/carrageenan model) rats. Primary and secondary hyperalgesia were assessed using the pressure application measurement (PAM) in the affected knee joint and the Hargreaves test in the ipsilateral paw, respectively, before and after microinjection of mGluR5 agonist/antagonist. To evaluate the role of astrocytes in IL-mGluR5 nociceptive modulation, the gliotoxin L-α aminoadipate was microinjected in the IL to ablate astrocytic function. Twenty-four hours later, nociceptive behavior was assessed after IL mGluR5 activation/inhibition and the results compared to those of rats without astrocytic ablation. In healthy animals, mGluR5 agonist in the IL facilitated both mechanical and thermal nociceptive behavior, but mGluR5 antagonist had no effect. Four weeks post-induction, ARTH animals presented primary mechanical hyperalgesia, but not secondary thermal hyperalgesia. mGluR5 agonist produced thermal hyperalgesia but did not worsen the primary mechanical hyperalgesia. mGluR5 antagonist induced thermal antinociception in ARTH rats and reversed primary mechanical hyperalgesia. Ablating astrocytes in the IL did not alter baseline behavior or mGluR5 agonist action in both experimental groups and stimulation modalities. However, the antinociceptive effect of mGluR5 antagonist in ARTH animals' primary and secondary nociceptive behavior was lost after astrocyte ablation. The results indicate the IL contributes to the descending modulation of nociception, an effect partly mediated by mGluR5 in healthy and ARTH animals. Moreover, tonic alterations in mGluR5 activity elicited by chronic inflammatory pain seem to be partly dependent on astrocytic mGluR5.

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Poster: P.018 | Ana Filipa Domingues Gerós
Automatic quantification of laboratory animal behavior using 3D video recording

Presenter: Ana Gerós | INEB/i3S
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(1) INEB/i3S, University of Porto, (2) FEUP, University of Porto

Introduction: Animal behavioral experiments are a core pillar in neuroscience research, and behavioral patterns characterizations are used to assess the effects of pharmacological manipulations, rehabilitation protocols, neurological diseases, etc.. Unfortunately, the methods to achieve precise quantifications in ethology are still in their early stages since behavior experiments are extremely complex to analyze and often end up relying on human judgement for manual quantification and classification [1]. To overcome these limitations, several studies have addressed the behavioral quantification task by applying computer vision methods to automatically track and characterize the behavior of different animals [1, 2, 3]. However, these systems have important limitations such as manual intervention, poor performance or being limited to 2D analysis (impairing 3D postures characterization). Here, it is presented research aimed to develop a 3D video-tracking software, taking advantage of a RGB-D camera to provide a robust and feasible tool to automatically characterize animal behavior. Materials and Methods: The Microsoft Kinect v2 camera was used to acquire both colour and depth frames from laboratory rats inside an open field cage. A background subtraction technique was implemented to perform tracking and the volume centroid was calculated to produce 3D real-time estimates of animal's position from the camera video streaming. The algorithm was embedded in a user-friendly graphical interface, to support data acquisition and processing in a laboratory environment. Results and Discussion: The validation results from video tracking of Wistar rats revealed that 3D information regarding animals' centroid can be extracted from the developed method and further used to completely describe animal's trajectories. When compared to published methodologies, the proposed tool presents advantages regarding low cost, easy installation/setup, and ability to work in low contrast/dark conditions, providing a 3D real-time tracking method to support the behaviour recognition task.

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Poster: P.019 | Ana Patrícia Figueiredo Rocha Simões
ATP-derived adenosine activates A2A receptors controlling long-term potentiation in the amygdala

Presenter: Ana Patrícia Simões | CNC, University of Coimbra
Ana Patrícia Simões (1), Henrique B. Silva (1), Sara Reis (1), Rodrigo A. Cunha (1,2)
(1) CNC-Center for Neuroscience and Cell Biology, (2) Faculty of Medicine, University of Coimbra, Portugal

Introduction: The amygdala is a central brain region for processing affective memories (1). In rodents, the association between specific cues and an aversive experience involves synaptic plasticity, namely long-term potentiation (LTP), of excitatory synapses at the lateral amygdala (1). Recently, we showed that the blockade or downregulation of adenosine A2A receptors (A2AR) in the basolateral amygdala decreased LTP at cortico-lateral amygdala synapses and affected the learning and memory of conditioned fear (2). However, the source of the extracellular adenosine responsible for A2AR activation in the amygdala remains unknown. Aim: Since it has been shown in other synapses that ATP-derived adenosine is responsible for A2AR activation (3,4), we now tested the impact of inhibiting ecto-5´-nucleotidase (CD73, converting extracellular AMP into adenosine) on A2AR-mediated control of amygdala LTP. Materials and methods: Extracellular recordings at cortico-lateral amygdala synapses were performed in horizontal brain slices (400 µm) from C57BL/6 mice (8-12 weeks-old) wild type (WT), CD73 knock-out (CD73 KO) and forebrain A2A knock-out (fbA2AR KO). Test stimuli were delivered at 0.05 Hz and the amplitude of the resultant population spike (PS) was measured. LTP was induced by high frequency stimulation (HFS, 3 pulses of 100 Hz with an interval of 5 seconds) and LTP magnitude quantified by comparing PS amplitudes 10 min before and 50-60 min after. Results are mean±SEM of n animals and unpaired Student's t test or one-way ANOVA plus Bonferroni's post hoc test were used in the statistical analysis. Results: In WT mice, the A2AR selective antagonist (SCH58261, 50 nM) decreased LTP (179.4±12.9% in control vs 133.8±9.8% with SCH58261, n=6, $p<0.05$). Accordingly, LTP amplitude in fbA2AR KO mice was lower (134.2±4.8%, n=6, $p<0.01$) than in WT littermates (175.7±10.7%, n=5). In WT mice, 100 µM AOPCP (inhibitor of CD73), in the presence of a general antagonist of ATP P2 receptors, PPADS (20 µM), also decreased LTP amplitude (129.7±5.8% vs 160.5±9.9% in control, n=5-6, $p<0.05$) and occluded the effect of SCH58261 (127.0±4.2%, n=5). In WT littermates from the fbA2AR KO colony the same results were observed: AOPCP+PPADS decreased LTP (128.5±10.3% vs 175.7±10.7% in control, n=4-5, $p<0.05$) and occluded the effect of SCH58261 (130.8±3.1%, n=2). Also, in fbA2AR KO mice, AOPCP+PPADS had no effect on LTP (154.2±10.0% vs 134.2±4.8% in control, n=5-6). Accordingly, in CD73 KO mice, SCH58261 was devoid of effect on LTP (179.4±27.99% in the presence of SCH58261 vs. 156.5±26.42% in control, n=4). Conclusions: These results indicate that ATP-derived adenosine is responsible for activation of A2AR to control amygdala LTP, prompting CD73 as a novel target to control the activation of A2AR in the amygdala.

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Poster: P.020 | Ana Patrícia Tomé Francisco
Commensal bacteria and essential amino acids control food choice behavior and reproduction

Presenter: Patrícia Francisco | Champalimaud Research
Ricardo Leitão-Gonçalves (1), Zita Carvalho-Santos(1)*, Ana Patricia Francisco (1)*, Gabriela Tondolo Fioreze(1), Margarida Anjos (1), Célia Baltazar (1), Ana Paula Elias (1), Pavel M. Itskov (1), Matthew D. W. Piper (2), Carlos Ribeiro (1)*
(1) Behavior and Metabolism Laboratory, Champalimaud Neuroscience Programme, (2) School of Biological Sciences, Monash University

Choosing the right nutrients to consume is essential to health and wellbeing across species. However, the factors that influence these decisions are poorly understood. This is particularly true for dietary proteins, which are important determinants of lifespan and reproduction. We show that in Drosophila melanogaster, essential amino acids (eAAs) and the concerted action of the commensal bacteria Acetobacter pomorum and Lactobacilli are critical modulators of food choice. Using a chemically defined diet, we show that absence of any single eAA from the diet is sufficient to elicit specific appetites for AA-rich food. Furthermore, commensal bacteria buffer the animal from the lack of dietary eAAs: both increased yeast appetite and decreased reproduction induced by eAA deprivation are rescued by the presence of commensals. Surprisingly, these effects do not seem to be due to changes in AA titers, suggesting that gut bacteria act through a different mechanism to change behavior and reproduction. Thus, eAAs and commensal bacteria are potent modulators of feeding decisions and reproductive output. This demonstrates how the interaction of specific nutrients with the microbiome can shape behavioral decisions and life history traits.

Poster: P.021 | Ana Paula Ventura Silva
Microbiota mediates social and cognitive deficits in a mouse model of Caesaeran section

Presenter: Paula Ventura Silva | APC Microbiome Institute
Livia H. Morais(1,2), Ana Paula Ventura-Silva(1)*, Timothy Dinan(1,3), John F. Cryan(1,2)*
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There is a growing realization of the importance of the gut microbiome in all aspects of health including brain health. Alterations in the composition of the microbiome in early life has been shown to affect neurodevelopment and behavior in mouse models (1). The first major contact with bacteria happens during the birth process whereby infants are passed on the microbiome from their mothers. Thus in infants born by Caesarean section (C-section) this seeding does not occur and the microbiome is different to that of vaginally born infants (2). The longterm functional aspects of being born by C-section are unclear in terms of behavior thus we established a mouse model of C-section and observed that delivery by C-Section induces deficits in social behavior, cognition and anxiety. In order to confirm that the microbiota is driving some of these changes we took advantage of the coprophagic aspects of murine behavior and co-housed one C-section born mice with three vaginal born mice. For this, at weaning, animals were divided into four different groups: vaginal born (VB), C-section or co-housed (VB or C-section) mice. The behavior of these animals was evaluated in adulthood (8-14 weeks of age). Our data show that, when compared with VB mice, C-section born offspring exhibited deficits in social memory in the three-chamber sociability task and deficits in cognition measured in the novel object recognition test. Additionally, C-section mice also have an increase in anxiety-like behavior assessed through the elevated-plus maze. Interestingly, C-section born mice that were co-housed with VB mice showed improvements both in social behavior and in working memory but not in anxiety-like behavior. In conclusion, using co-housing as a strategy to manipulate the microbiota of C-section born mice, we were able to selectively reverse the effect of delivery mode in social and working memory.

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***Funding**: Science Foundation Ireland (12/RC/2273), Science Without Borders*

Poster: P.022 | Ana Rita Andrade Pereira da Costa
Effects of opioids on descending pain facilitation during neuropathic pain

Presenter: Ana Rita Costa | FMUP/ I3S
A.R. Costa 1,2,3 , P. Carvalho 1,2,3 , G. Flik 4 , S.P. Wilson 5 , I. Martins 1,2,3 , I. Tavares 1,2,3
1 Departamento de Biomedicina - Unidade de Biologia Experimental, Faculdade de Medicina da Universidade do Porto, Portugal 2 i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal 3 IBMC - Instituto de Biologia Celular e Molecular, Universidade do Porto, Portugal Portugal 4 Brains On-Line BV, Groningen, The Netherlands 5 Department of Pharmacology, Physiology and Neuroscience, University of South Carolina School of Medicine, Columbia, SC, USA

Opioids play a crucial role in pain modulation but their role in the control of supraspinal areas involved in descending pain facilitation is not fully understood mainly in chronic pain states. This is important inasmuch as descending pain facilitation is exacerbated during chronic pain. The dorsal reticular nucleus (DRt) is an area located in the medulla oblongata that belongs to the endogenous pain control system. The DRt plays a unique and exclusive pain facilitatory role and is under opioidergic modulation since local neurons express μ-opioid receptors (MOR). Here we studied how opioids modulate the DRt in naïve and neuropathic animals (spared nerve injury-SNI- model). First, we evaluated the expression of MOR and the release of the endogenous opioid peptides Met- and Leu-enkephalin by in vivo microdialysis, at the DRt of naïve and SNI animals. Then, we used pharmacological and genetic approaches targeted to the DRt to study the local effects of opioids on the nociceptive behavior. Naïve male Wistar were used for the injection of a lentiviral vector knocking down MOR or the implantation of a guide cannula at the DRt. Neuropathic-animals, two weeks after SNI-induction, were also implanted with a guide cannula into the DRt. One week after stereotaxic procedures, naïve and SNI animals were injected DAMGO or CTAP, MOR agonist and antagonist, respectively, through the guide cannula or submitted to in vivo microdialysis. Microdialysates collected at the DRt were analysed by mass spectrometry. Pain behaviour was assessed by the von Frey test which evaluates mechanical sensitivity. MOR expression was evaluated by immunohistochemistry. Neuropathic-animals showed higher levels of Met- and Leu-enkephalin peptides while MOR expression was significantly lower compared to naïve animals. In naïve animals, MOR knock down induced a decrease of mechanical thresholds, DAMGO produced the opposite and CTAP produced no effects. DAMGO produced a dose-dependent increase of mechanical thresholds in naïve animals and no effects in SNI animals. Together these results indicate that the DRt is under inhibitory opioidergic modulation which is impaired during neuropathic pain. It remains to ascertain if this accounts to enhance descending pain facilitation.

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Poster: P.023 | Anna Vladimirovna Pliássova
Dissecting the transducing systems involved in the control of long-term potentiation (LTP) in the amygdala by adenosine A2A receptors

Presenter: Anna Pliássova | CNC, University of Coimbra
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Caffeine intake correlates inversely with the incidence of depression [1]. Our lab reported recently that adenosine A2A receptors (A2AR) antagonists mimic the ability of caffeine to prevent mood and memory dysfunction induced by chronic stress [2]. Importantly, A2AR control long-term synaptic potentiation (LTP) in the cortico-amygdala pathway and selective amygdala A2AR downregulation normalizes LTP and reduces fear memory [3]. This suggests that A2AR in the amygdala are important players in the pathophysiology of depression. The main focus of this work is to characterize the transducing systems operated by A2AR in the amygdala. For that purpose, we are recording excitatory postsynaptic currents (EPSC) in pyramidal cells of the basolateral amygdala (BLA) upon stimulation of the lateral amygdala (LA) in horizontal slices from Wistar rats. High-frequency stimulation (100Hz) and depo-pairing (2Hz coupled with postsynaptic depolarization) protocols are being used, in the absence and presence of the selective A2AR antagonist, SCH58261 (50 nM), or of the A2AR agonist CGS21680 (30 nM) to unravel how A2AR modulate LTP in the LA – BLA circuitry. We are mingling the use of pharmacological inhibitors and of patch pipette-applied peptide inhibitors of different transducing systems that are proposed to be involved in the A2AR-mediated signalling in different cells, to attempt unravelling key transducing systems formatting synaptic plasticity in the amygdala.

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Poster: P.024 | Catarina Raposo de Oliveira Lima
The role of chronic stress on addictive vulnerability – focus on the nucleus accumbens

Presenter: Catarina Lima | ICVS, University of Minho
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Chronic stress is an established risk factor for the development of addiction disorders, but its role in mediating susceptibility to drug effects remains poorly understood. Although acute stress exposure has been shown to enhance self-administration of drugs of abuse, the impact of isolated chronic stress on addictive vulnerability has not been described. Here, the conditioned place-preference paradigm was used to evaluate this effect. Furthermore, to try to clarify the role of the NAcc main subdivisions in mediating addictive behavior, the core and shell, we assessed neuronal activity through c-FOS expression. Our results showed chronic stress exposure differentially affects NAcc subregions, resulting in increased neuronal activation in the shell as compared to the core and this effect is associated with higher conditioning to morphine, as revealed in the CPP apparatus.

Poster: P.025 | David Vilhena Catarino Brito
Mimicking age-associated Gadd45γ depletion results in memory impairments in young mice

Presenter: David Brito | U. Heidelberg, Germany
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With an increasingly aged population age-associated cognitive decline is a major health and socio-economical burden. Understanding the mechanisms underlying progressive cognitive loss is required to develop future therapies. The underlying causes of age-associated cognitive decline are largely unknown. Interestingly, overexpression of the DNA damage (Gadd45) family orthologue D-GADD45, in the central nervous system, increases drosophila lifespan1. Additionally, two of the mammalian Gadd45 proteins, Gadd45β and Gadd45γ are induced upon learning2,3 and plasticity4 events in the hippocampus. We hypothesized that altered Gadd45 family activity could be involved in age-associated cognitive decline. To test this hypothesis 18-month-old mice were trained in an object recognition task and levels of Gadd45 family members were compared to 8-week-old mice. We found a selective 39% and 37% reduction in basal and induced Gadd45γ hippocampal mRNA levels, respectively. Next, we selectively decreased Gadd45γ hippocampal levels in 8-week-old mice and performed an object displacement task and contextual fear conditioning. We detected a robust trend for memory impairments when compared to age-matched controls. Vast evidence highlights that Gadd45 proteins interact with JNK and p38 signaling pathways during tumorigenesis5. We next knocked-down Gadd45γ levels in dissociated hippocampal neurons and analyzed phosphorylated versions of these proteins upon neuronal activity. We found that pJNK and pp38, but not pERK, were decreased, which suggests Gadd45γ is required for MAPK signaling during plasticity-associated events. We next hypothesized that these effects could mediate abnormal CREB signaling. Indeed, reducing Gadd45γ levels seem to decrease stimulus-dependent CREB phosphorylation. We next analyzed the mRNA and protein levels of the well characterized plasticity-associated CREB target genes c-fos and arc. Impaired Fos and Arc levels were detected while Npas4, a learning-associated gene without CRE sites4 was unaltered. This data suggests that age-associated decrease in Gadd45γ expression may be involved in cognitive deficits possibly by abnormal MAPK and CREB signaling activation.

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Poster: P.026 | Diana Alexandra da Silva Amorim
Minocycline reduces mechanical allodynia and depressive-like behaviour in type-1 diabetes mellitus in the rat

Presenter: Diana Amorim | ICVS, University of Minho
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Painful diabetic neuropathy (PDN) is a common and devastating complication of diabetes mellitus that can be accompanied by comorbid emotional disorders such as depression. A few studies have suggested that minocycline, an FDA approved drug, inhibits microglia and may attenuate pain hypersensitivity in PDN. Moreover, a recent study reported that minocycline has an acute antidepressive-like effect in diabetic animals. Here we studied whether: (i) prolonged minocycline treatment suppresses pain behaviour in PDN; (ii) the minocycline effect varies with submodality of pain; and (iii) the suppression of pain behaviour by prolonged minocycline treatment is associated with antidepressive-like effect, in a streptozotocin-induced rat model of type-1 diabetes. Pain behaviour was evoked by innocuous (monofilaments) and noxious (paw pressure) mechanical stimulation, innocuous cold (acetone drops) and noxious heat (radiant heat). Depression-like behaviour was assessed using forced swim test. Minocycline treatment (daily 80 mg/kg per os) of three-week duration started four weeks after induction of diabetes. Diabetes induced mechanical allodynia and hyperalgesia, cold allodynia, heat hypoalgesia, and depression-like behaviour. Minocycline treatment significantly attenuated mechanical allodynia and depression-like behaviour, while it failed to produce significant changes in mechanical hyperalgesia, cold allodynia or heat hypoalgesia. The results indicate that prolonged per oral treatment with minocycline has a sustained mechanical antiallodynic and antidepressive-like effect in PDN. These results support the proposal that minocycline might provide a treatment option for attenuating sensory and comorbid emotional symptoms in chronic PDN.

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Poster: P.027 | Ernesto Saias Soares
Towards EEG-clamp: reduced EEG spectral entropy through visual negative feedback

Presenter: Ernesto Soares | Brain Institute, UFRN, Brasil
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Electroencephalography (EEG)-based neural control is an active field of research with major scientific and therapeutic potential applications, both acute (e.g. delivering temporally precise stimulation to disrupt epileptic seizures or parkinsonian tremor), and chronic (e.g. effecting brain connectivity changes through synaptic plasticity triggered by the reliable and repetitive precise timing of endo- and exogenous events). Distinct control strategies studied so far differ in such parameters as the type of EEG data used (e.g. instantaneous EEG phase or power), and in the feedback stimulation method used: invasive, such as electrical Deep-Brain Stimulation (DBS) or non-invasive, such as Transcranial Magnetic Stimulation (TMS). Here we describe research towards a non-invasive naturalistic visual-based EEG neural control technique, dubbed “EEG-clamp”. We used intermittent periodic visual stimulation, which causes the increase of EEG power at the stimulation frequency and its harmonics over occipital visual regions (the Steady-State Visual Evoked Potential, SSVEP), as feedback signals in a novel type of non-invasive Brain-Machine Interface, called Sensory Brain-Machine Oscillator (SBMO). Based on occipital EEG power calculated near real-time (delay <150ms), the SBMO continuously alternates between two visual stimuli delivered to the subject through a computer screen, either an inverting (20Hz) monochromatic radial checkerboard (ON state) or a homogeneous gray screen (OFF state). ON and OFF states where triggered when occipital EEG power (20 and 40 Hz, during the latest 500ms of data), or its derivative, crossed below or above, respectively, a predetermined threshold. We tested 3 distinct types of negative feedback (controllers) in 8 subjects. For each controller, a 3 min closed-loop trial was performed, when all stimuli were calculated in real-time, followed by a 3 min open-loop trial, when the stimuli were an exact repetition of the closed-loop trial. We found slow (~1.1Hz) and reliable closed-loop SBMO-induced occipital EEG power oscillations that are spectrally less entropic, and therefore less random, than corresponding open-loop-induced oscillations. These results indicate that the operation of closed-loop SBMOs drives EEG activity into regimes that are inaccessible through conventional open-loop experimental designs.

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Poster: P.028 | Filipa Lima Ramos Santos Júlio
A novel ecological assessment of executive function in Huntington's disease

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Background - Impairments in executive functions are common in Huntington's disease (HD), even in prodromal and early disease stages, and are thought to significantly influence the patient's functional status [1,2]. Reliable ecological tools to assess and predict the impact of executive dysfunction in daily performance are needed [3,4,5]. Aims - This study aims to get a comprehensive picture of the everyday executive deficits reported in HD by using two new assessment tools – the "EcoKitchen" task (objective measure) and "IAFAI - Inventário de Avaliação Funcional de Adultos e Idosos" (subjective measure). Methods - Participants were assigned to one of three groups (controls, premanifest HD and early manifest HD) and performed a virtual reality task with an increasing executive load that simulates daily-life like routines usually done in a kitchen setting ("EcoKitchen"). Timing and error variables were extracted from the participants' performance. Additionally, participants had to answer questions of the "IAFAI" based on their self-perceived competence to perform simple and complex activities of daily living. Disability scores were assigned to each participant in regard to cognitive, emotional and physical dimensions of everyday performance. Results - The results indicate that both premanifest HD and manifest HD participants showed statistically significant differences in the timing and error variables considered in the objective measure "EcoKitchen", when compared to controls. The clinical groups showed an overall slower cognitive and motor performance, and a higher number of attention errors. Furthermore, in the computerized tasks with higher executive demands, the performance of both clinical groups deteriorated and slowed down. The two HD groups also showed a significantly higher disability score in the subjective measure "Inventário de Avaliação Funcional de Adultos e Idosos", when compared to controls. Conclusion - The more ecological assessment protocol created to evaluate the executive functioning of HD patients seems to be sensitive to early deficits in this domain. Importantly, both "EcoKitchen" task and "IAFAI" can potentially identify subtle changes in the daily functioning of premanifest individuals and differentiate them from controls even with small sample sizes. This comprehensive assessment of everyday executive function in HD will contribute to a better understanding of the phenotype of this disease, and also to a better identification and management of the patients' real-life deficits.

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Poster: P.029 | Helena Filipa da Cunha Fernandes
Silicon-based 3D optrode for optogenetic applications

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The brain is a complex and heterogeneous organ, formed by neurons with diverse morphologies and molecular composition, connected by complex patterns. This complexity makes it difficult to understand neural circuits functions and interactions. The ability to activate or inhibit specific cell type or connect them on a certain time space may indicate which function is fulfilled by each cell or connection. There are many developed technologies that aim to help neuroscientists to understand the behaviour of the neural tissue. One promising neuromodulation technique is optogenetics that combines bioengineering, light and genetics to control neural activity. This method is based on the genetic modification of targeted brain cells to express light sensitive proteins (opsins), giving the neuron the ability to respond to optic stimulus. Therefore, when exposed to a light stimulus at a specific wavelength, these opsins will activate or inhibit neuronal activity, allowing neuroscientists to know which neurons are inherent to the normal or pathological functions of the brain. The BRAIN-LIGHTING project, led by INOVA+ in partnership with University of Minho and Champalimaud Foundation, proposes the development of a new technological solution in the neurology field that aims to contribute to the evolution of neuroscience and to deepen the existing knowledge about the functioning of the brain and its constituents. For this purpose, it is intended to develop an optogenetic device (referred as optrode) using optic neuronal probes provided with optical stimulation and electrical recording of neural activity and fully integrated microelectronics, including a wireless communication interface. By making the genetic modification of neurons to express the specific opsins, and using the optical stimulation and electrical recording mechanisms, it is possible to develop a tool to modulate neuronal activity. The biocompatible 3D matrix with elongated neuronal probes allows to reach targeted neuronal cells at different depths. Micro-LEDs will be directly delivering light to cells, optimizing irradiance, which is a major issue for optical fiber and waveguide-based optrodes. Since opsins are triggered by specific wavelengths, several micro-LEDs with different wavelengths will be coupled to stimulate/inhibit different types of opsins, and consequently different types of cell functions, and thereby increase the device application range. The wireless communication module allows the total mobility of the subjects. The project will also focus on the development of an intuitive software module that aims to ease the acquisition, storage, visualization, analysis and processing of acquired brain signals. The ambition is to create a modular framework able to easily add new models and action modes for different types of conditions or pathologies. Finally, the optrode will be tested ex-vivo and in-vivo, which is essential to validate the final solution and improve the system requirements.

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Poster: P.030 | Inês Costa Laranjeira

Selective optogenetic inhibition of prelimbic excitatory neurons during delay-period reverses pain-related working memory deficits

Presenter: Inês Laranjeira | FMUP

Inês Laranjeira (1-4), Pedro Paiva (1-3), Clara Monteiro (1-3), Helder Cardoso-Cruz (1-3), Vasco Galhardo (1-3)*

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Working-memory (WM) is a specific type of memory requiring the active maintenance of information (delay-period) that is pertinent to ongoing behavior, and stability of local prefrontal cortex (mPFC) networks and output activity is thought to be critical for sustaining these cognitive processes. Dysfunction of the mPFC has been identified as a leading cause to memory deficits in several in chronic pain conditions; however the underlying mechanisms remain poorly determined. Prefrontal pyramidal cells are considered the neuronal substrate for information maintenance and memory retention during the delay-period. Here, we evaluated the effects of selective optogenetic modulation of local mPFC pyramidal cells activity during a food-reinforced delayed non-match to sample WM task performance. Behavioral performance was evaluated using different retention delay-intervals. Extracellular single-unit recordings were made from prelimbic area (PL) of the mPFC, and dorsal hippocampal CA1 field (dCA1) or nucleus accumbens core (NAcc). Optogenetic inhibition of local PL pyramidal cells activity was promoted using selective adeno-associated viral vectors encoding halorhodopsin (eNpHR, version 3.0) under the control of the CaMKIIa or HSyn promoters. Light was delivery unilaterally in PL or NAcc brain areas using a continuous led-generated orange solid pulse (620 nm) with a fix intensity of 5 mW at the implanted fiber tip. Within-subject behavioral performance and patterns of neuronal activity were assessed before and after the onset of persistent neuropathic pain using the Spared Nerve Injury (SNI) model of neuropathic pain. Our results showed that peripheral nerve lesion caused an impairment of WM performance that is temporally associated with changes in local PL neural activity and PL output to dCA1 and NAcc brain areas. These disruptions were reversed by partial delay-period selective direct (CaMKIIa-expressing rats) and indirect (HSyn-expressing rats) suppress of local PL pyramidal cells. In addition, we found that direct inhibition of PL pyramidal cells in CaMKIIa-expressing SNI rats do not produce antinociceptive effects, whereas the PL pyramidal cells indirect inhibition in HSyn-expressing rats via corticostriatal circuit produces a strong analgesic effect. Together, our findings suggest that disruption of local balanced mPFC network activity may be crucial for the clinical neurological and cognitive deficits observed in patients with painful syndromes.

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Poster: P.031 | Irene Chaves-Coira

Different Basal Forebrain neuronal populations display different cortical projection pathways to process information of different modalities

Presenter: Irene Chaves-Coira | Medical School. UAM
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The mammalian cerebral cortex receives consistent projections from cholinergic and non-cholinergic neurons bearing the basal forebrain (BF). Different structures and nuclei have been included in BF by different the medial septum, the horizontal and vertical limbs of the diagonal band of Broca (HDB and VDB, respectively), the substantia innominata (SI), and the nucleus basalis magnocellularis (B nucleus; Maynert basal magnocellular nucleus in humans). Anatomical studies indicate that the basal forebrain cholinergic neurons project practically over every layer of all cortical areas. BF structures provide the majority of the cholinergic innervation to sensory, motor and prefrontal cortices, and hippocampus. However, specific reciprocal projections between different BF structures and cortical targets are necessary to control sensory information processed in the different cortices. The aim of present work is to elucidate if different neuronal populations of the BF modulate specific sensory cortical areas throughout separate neuronal networks. Sprage-Dawley rats have been utilized in the experiments. All animal procedures were approved by the Ethical Committee of the Autonomous University of Madrid, in accordance with Council Directive 2010/63/UE of the European Community. Efforts were made to minimize animal suffering as well as to reduce the number of animals used. Neuronal tracing techniques by injecting retrograde and anterograde tracers in cortical areas and in different BF nuclei were combined with sensory and optogenetic stimulation to determine the functionality of the different reciprocal pathways between the BF and cortical areas, in the rat. Anatomical and optogenetic results indicate that while the ventral and horizontal diagonal band of Broca (VDB/HDB) nuclei display more specific cortical targets, the B nucleus shows more widespread targets in the cortex. In addition, the VDB/HDB entertains reciprocal projections to the medial prefrontal and somatosensory cortices. Although B nucleus is involved in somatosensory projecting pathways, it does not project to the medial prefrontal cortex. Both VDB/HDB and B nuclei are also connected with motor and cingulate cortices. Optogenetic stimulation of these BF areas confirms our anatomical data showing the existence of an important modulation of whisker responses in primary somatosensory cortex and medial prefrontal cortex when blue-light stimulation was addressed to HDB. Therefore, results strongly suggest that there are two different pathways processing sensory-motor stimuli of different modalities.

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Poster: P.032 | Isabel Catarina Castro Duarte
Rhythmic Motor Performance in Neurofibromatosis Type 1 as assessed by EEG and fMRI

Presenter: Catarina Duarte | ICNAS Coimbra
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Introduction: Neurofibromatosis type 1 (NF1) is the most common autosomal dominant condition with an estimated prevalence of 1 in 3000 individuals (Williams et al. 2009). NF1 has a wide range of clinical manifestations and behavioral difficulties (Kayl & Moore 2000). This condition is also associated with motor deficits, but their neural correlates are still poorly understood (Hyman et al. 2005; Levine et al. 2005). To our knowledge, there is no study addressing the neurobiological correlates of impaired motor performance in NF1. We used a motor coordination task at different levels of pacing to investigate the disease as function of performance, electroencephalography (EEG) and functional magnetic resonance (fMRI). Methods: Twenty-one adults with NF1 and 20 controls were recruited. The pool of participants with NF1 had a definite diagnosis of NF1 in accordance with the NIH criteria. Exclusion criteria for all participants included psychiatric disorder, neurologic illness other than NF1, IQ<75, epilepsy and traumatic brain injury. The clinical group (age range 23.8 – 51.8, mean age ± standard deviation [SD] 36.7 ± 6.7, 12 females) and the control group (age range 22.5 – 55.0, mean age ± SD 36.8 ± 7.1, 12 females) were matched for age (U=205.5, p=0.907, ns) and gender ($\chi 2$ (1) =0.034, p=0.853, ns). The motor task required the participant to tap both index fingers at the pace of a cueing sound, beeping at three incremental frequencies: 1, 3 and 5 Hz. The task was performed both during fMRI scans and during separate EEG recordings. Results: To have an overview of the task performance, we compute the power of the tapping at the frequency of each condition. The control group performed better at 1Hz and 3Hz both synchronous (Mann-Whitney, p=0.006 and p=0.043, respectively) and alternated (Mann-Whitney, p=0.025 for 1Hz and unpaired t-test, p<0.001, t=4.045, df=35, for 3Hz); while no differences were found for the 5 Hz condition. Both groups show a strong periodical variation of the beta frequencies, which matches the behavioral data. For the frequency band 20-26 Hz, we computed the power for each tapping frequency. Nevertheless, behavioral data showed more consistent group differences than time-frequency spectra. Concerning the fMRI results, differences between groups were found for the fastest condition (tapping at 5 Hz). The NF1 group presented decreased activity in regions involved in motor control and rhythmic pacing in the basal ganglia, cerebellum, midbrain and medial prefrontal cortex when compared with the control group. Discussion: Our results corroborate other studies reporting motor deficits in NF1 and further show impaired precision in rhythmic pacing behavior. Our neuroimaging data suggest that the differences in rhythmic motor performance is linked to changes in activity regions involved in these processes such as the basal ganglia, cerebellum and midbrain. These regions are not accessible in scalp recordings as assessed by time-frequency spectra.

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Poster: P.033 | Joana Amorim Freire
Adenosine A1 receptor antagonism prevents DSI in hippocampal CA1 pyramidal cells

Presenter: Joana Amorim Freire | Instituto de Medicina Molecular
Joana Freire (1,2), Diogo Rombo (1,2), Ana Sebastião (1,2)
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The widely consumed psychoactive drug cannabis, containing cannabinoid compounds, and/or caffeine, with adenosinergic antagonizing proprieties, exert their central actions by affecting cognitive operations such as learning and memory. Indeed, endogenous adenosine and endocannabinoids (eCB) are known to interfere with physiological synaptic plasticity phenomena that represent the neuronal substrate of memory formation (Chevaleyre et al., 2006; Sebastião et al., 2014). Here we focused on the interplay between these two neuromodulatory systems and how adenosine actions interfere with a short-term form of neuronal plasticity, the depolarization-induced suppression of inhibition (DSI), a phenomenon that is completely dependent on eCB function. Whole-cell voltage-clamp recordings (Vh=-70mV) were performed on hippocampal CA1 pyramidal cells of 3 to 5 weeks-old C57BL/6 mice. Slices (350 µm thick) were perfused with artificial cerebrospinal fluid (aCSF) supplemented with glutamate receptor antagonists (CNQX, 25 µM and DL-APV, 50 µM) to block glutamatergic transmission and isolate GABA-mediated responses. Inhibitory postsynaptic currents (IPSCs) were evoked every 3 seconds through a stimulation electrode placed in stratum radiatum. The recording electrode was filled with a CsCl-based intracellular solution (Rombo et al., 2014) and DSI was evoked through a 5 seconds voltage step of +80 mV (Chevaleyre et al., 2004). The magnitude of DSI was measured 9 seconds after the depolarizing step and DSI recovery was evaluated between 30-60 seconds after depolarization. When recording eCB-mediated DSI we observed a decrease in electrical-evoked IPSC amplitudes to 81.0 ± 5.4 % of baseline ($p < 0.01$, n= 14) that fully recovered to 90.2 ± 5.4 % after 30-60 seconds. The adenosine A1 receptor antagonist, DPCPX (100nM), prevented DSI, recordings showing a non-significant change in IPSCs amplitude to 95.1 ± 12.0 % of baseline ($p= 0.3473$, n= 10) that was maintained throughout the recovery period (87.1 ± 12.0 %). These results suggest that tonic adenosine A1 receptor activation is necessary for the occurrence of DSI. The mechanisms involved in this process remain unclear and need further investigation.

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Poster: P.034 | Joana Fernandes Coutinho
Neural Basis of Self-other Distinction during an fMRI empathic task

Presenter: Joana Coutinho | School of Psychology, U. Minho
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The ability to empathize with others implies the capacity to separate our own emotions from others´ in order to regulate the emotional arousal generated by the exposure to other´s feelings. This ability to distinguish between the representations of our own actions, perceptions, sensations and emotions, and those of others is known as Self-other distinction. Accurate self–other distinction is an important component of our empathic ability as it prevents the occurrence of our own personal distress when dealing with other´s emotions and in this way it prevents that our own affective state biases how we empathize with others. This is especially important when we are the target of other´s emotional expression which happens, for example, in intimate relationships. In this study we designed an experimental task that differentiates the individual´s processing of his/her partner´s emotional expression from his/her own emotional experience. Twenty-one heterosexual couples (N=42 participants), who reported being in monogamous romantic relationship with the duration of 1 year completed this fMRI Task. In this task each element of the couple watched a series of video-vignettes of his/her partner expressing negative and positive contents and was asked to elaborate on his/her spouse´s experience (other condition) and on his/her own experience when listening to that content (self condition). These vignettes were extracted from a real interaction task, previously recorded in the lab, thus its content was real and highly idiosyncratic. Two blocks (Self and Other) were conducted with 3 conditions each. Trials of positive, negative and neutral videos were presented in a pseudo randomized order in each block. Neutral videos were extracted from the (EMDB) Emotional Movie Database. After preprocessing of the fMRI acquisitions, statistical analysis were performed using general linear model (GLM). First level analysis was conducted to establish for each block the participant's voxel-wise activation for each condition. Contrasts were then inserted in paired sample t-test to compare brain activation between blocks. Anatomical labeling was defined by a combination of visual inspection and Anatomical Automatic Labeling atlas (AAL). In Self-Other discrimination of emotional stimuli our preliminary results revealed a higher brain activation during the self condition when compared with the other condition in three main clusters: left and right cuneus; left insula and left superior temporal gyrus; left and right SMA and middle frontal gyrus. These differences in brain activation during processing of the participants 'own emotions when compared with the processing of other´s emotions were more pronounced during the visualization of negative vignettes. The clinical implications of these preliminary results for empathy in intimate relationships, especially in situations of relational conflict where the occurrence of personal distress is more likely to occur, will be discussed.

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Poster: P.035 | João Miguel Guerreiro Covita

Peptidergic modulation of pain and anxiety: Forebrain relaxin-3/RXFP3 networks and descending control of nociception in mice

Presenter: João Covita | UBordeaux & UMelbourne

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Pain is a complex biological process that plays a pivotal role in the survival of animals. However, persistent pain can hinder normal function with a serious negative impact on quality-of-life. In persistent pain conditions, patients develop comorbid conditions such as anxiety and sleep-related disorders that worsen pain sensation, creating a positive feedback mechanism between pain and its comorbidities. These comorbidities have been linked to impaired function of brain areas where the neuropeptide relaxin-3 has been detected. Since its discovery 15 years ago, relaxin-3 has been linked to the control of a wide range of behaviors such as arousal, and anxiety- and reward-seeking behaviors [1-3], through activation of its cognate receptor, relaxin/insulin like family peptide receptor 3 (RXFP3). The role of relaxin-3 in these brain functions led us to hypothesize that there might be a link between activation of RXFP3 and control of pain sensitivity. Initially, our research aimed to assess the effect of RXFP3 activation/inhibition on the control of mechanical and thermal pain sensitivity in normal and persistent pain conditions in mice. We observed that central intracerebroventricular (icv) injection of the RXFP3 agonist peptide, RXFP3-A2, produces relief of mechanical, but not thermal, pain sensation ($n=5$ mice/group, $p<0.01$). These effects were associated with decreased activity of nociceptive neurons in spinal cord ($n=6$ mice, $p<0.05$). Moreover, icv injection of the RXFP3 antagonist peptide, R3(B1-22)R, augments mechanical and thermal pain sensitivity ($n=7$ mice/group, $p>0.05$). These data suggest relaxin-3/RXFP3 signaling has a tonic effect in maintaining mechanical and thermal pain thresholds in mice, and that treatments that activate RXFP3 may produce pain relief in the clinic. Additionally, we aimed to identify the neural circuits that are linked to the observed effects. We used the retrograde tracer, fluorogold, to determine which brain areas that receive relaxin-3 inputs are connected to the rostroventral medulla (RVM), a structure that acts as a gateway for descending pain control. Fluorogold immunoreactivity was detected in the vicinity of relaxin-3 inputs in the central amygdala, hypothalamus and bed nucleus of the stria terminalis. These areas have been previously reported as being related to pain sensation and impairment of their normal functions has been linked to pain comorbidities. Altogether, our data point towards RXFP3 as a potential therapeutic target for pain management, although further studies to reveal more about the specific mechanisms that underlie such control are warranted.

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Poster: P.036 | José Carlos Barreiro Mateus
A DIY system for in vitro multielectrode recording

Presenter: José Mateus | i3S, Universidade do Porto
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Electrophysiology “on a chip” in the form of multielectrode array (MEA) systems has become a powerful tool for studying electrical activity in neuronal networks. MEA studies have described fundamental properties of network activity patterns, plasticity and learning; but have also shown promise from a clinical perspective in pharmacological testing or disease modelling (1, 2). In vitro MEA systems typically consist of MEA chips, an headstage for recordings, an amplifier and a data acquisition computer (3). MEAs generally incorporate planar microelectrodes embedded in a substrate that is used as a cell culture dish. The extracellular recordings allow for non-invasive, simultaneous and controllable long-term recordings of extracellular field potentials. However, subthreshold synaptic signals are undetected and individual spikes can only be inferred though spike sporting algorithms (1). Commercially available MEA systems have helped greatly in the field expansion (4), but are expensive and limited in the range of circuit schematics. These limitations still impair the number of possible users and applications. With these limitations in mind we developed a low-cost and versatile DIY system for simultaneous multielectrode neural recordings up to 16 independent channels. The system is composed of a 16 analog channels DAQ system (National Instruments, model USB-6356), a differential AC amplifier (AM-Systems, model 1700), an isolated pulse stimulator (AM-Systems, model 2100), and an in-house 3D printed headstage. In addition to the core system, other constructs were designed and 3D printed, to address specific cell culture and recording constraints. Care was taken to make the whole system compatible with commercially available MEA chips. Nevertheless, the system can be adapted to different MEA schematics as needed. Taking advantage of the versatility of this system, we have been exploring the design, fabrication and use of non-planar mushroom-shaped microelectrodes. These biocompatible arrays of protruding electrodes potentially enable the detection of activity normally unattainable with traditional planar MEAs. This work provides some degree of freedom for small laboratories to initiate MEA-based studies.

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Poster: P.037 | José Tiago da Costa Pereira
Descending noradrenergic modulation recruitment in paclitaxel-induced neuropathic pain

Presenter: José Tiago da Costa-Pereira | FMUP/I3S
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Chemotherapeutic agents are among the first approaches used for cancer treatment. One of the reported drawbacks of chemotherapy is the development of peripheral neuropathy known as chemotherapy-induced neuropathy (CIN). Paclitaxel is one of the most frequently used chemotherapeutic drug which induces CIN. Upon paclitaxel treatment, patients report mechanical and cold allodynia and spontaneous pain, which often persist for several months or years after cessation of the treatment. Antidepressants, namely noradrenaline reuptake inhibitors, which act by increasing and potentiating the antinociceptive effects of noradrenaline at the spinal cord, are amongst the drugs used to treat CIN-pain. Nonetheless, it is presently unknown whether descending noradrenergic modulation is altered during CIN. Here we used a rat model of CIN, induced by paclitaxel, to study the influence of paclitaxel on descending noradrenergic pain modulation. One month after CIN induction, we evaluated the spinal noradrenergic innervation and also the effects of the α2-adrenoreceptor (AR) agonist clonidine, delivered at the spinal cord, on pain responses. CIN was induced on male Wistar rats by intraperitoneal injection of paclitaxel (Taxol, 2.0 mg/Kg) on four alternate days (day 1, 3, 5 and 7). Control animals were treated with the vehicle solution 4% Dimethyl Sulfoxide (DMSO) or saline. In order to validate the development of neuropathic pain following paclitaxel treatment, the von Frey test, which evaluated mechanical sensitivity, was performed before and once a week after CIN induction, for a month. The open-field test, which allows studying motor integrity, was performed at one month. Clonidine (1 and 10 µg) was delivered into the 4th lumbar segment through a catheter implanted intrathecally one week earlier. The effects of clonidine were assessed by the von Frey and the cold plate tests. The noradrenergic innervation of the spinal lumbar segments 4 and 5 was studied by immunohistochemical evaluation of the expression of dopamine beta hydroxylase (DBH), a noradrenaline biosynthetic enzyme expressed in noradrenergic nerve terminals. Paclitaxel-treatment induced mechanical allodynia from the first week until the last time point evaluated (one month) without compromising motor integrity. At one month, paclitaxel-treated animals also developed cold hyperalgesia. Clonidine, at both doses, induced antinociception but with more pronounced effects in paclitaxel-treated animals. DBH expression was increased in paclitaxel-treated animals. The effects of clonidine suggest an enhancement of α2-AR antinociceptive potency. It remains to ascertain if DHB upregulation results in increased spinal noradrenaline levels. We are currently evaluating if there are changes in the recruitment of descending inhibitory noradrenergic modulation which probably represents a compensatory mechanism in response to the development of neuropathy.

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Poster: P.038 | Liliana Patrícia Carvalho Amorim
Exploring the path between subjective sleep quality and cognitive performance

Presenter: Liliana Amorim | ICVS, University of Minho
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Cognitive performance is highly influenced by sleep. Some studies have indicated that a poor sleep quality is associated with cognitive deficits and decline, as well as vulnerability and/or aggravation of the symptoms of certain diseases (e.g. Alzheimer´s Disease). However, the psychosocial and clinical factors affecting the relation between sleep quality and cognitive performance are poorly understood. In the present study, we use path analysis to explore the association between PSQI, sociodemographic factors and clinical parameters with cognitive performance and some specific brain correlates, in a community-based sample of older individuals. For this, a cohort of 120 individuals residing in the community was recruited. Information regarding subjective sleep quality (PSQI), Geriatric Depression Scale (GDS), sociodemographic factors, clinical parameters and performance in different cognitive domains (processing speed, memory, mental flexibility and general cognition) were obtained. A MRI acquisition was also acquired. Results indicate that there is a moderation effect of height in the relation between PSQI and Cognitive Performance and a mediation effect between PSQI and GDS in the association with Cognitive Performance. In conclusion, our results show that the inclusion of psychosocial and clinical variables is critical to obtain a more appropriate model on the sleep and cognition association.

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Poster: P.039 | Liliana Sofia da Silva Carvalho
Spinal cord plasticity in brain injury-induced motor deficits and long-term recovery

Presenter: Liliana S. Carvalho | DBM - FMUP
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Stroke and traumatic brain injury (TBI) are leading causes of death and disability. Both clinical conditions lead to motor impairments, such as hemiparesis and hemiplegia, which are relevant causes of disability, poor quality of life and social isolation. Brain pathology associated with stroke and trauma involves plastic changes at the level of the spinal cord, which can be manifested as postural asymmetry (PA) due to alterations in muscle tone and motor reflexes. PA induced by brain injury is regarded as a model of pathological spinal cord memory or pathological plasticity, since it persists after the spinal cord transection. We addressed the issue of whether or not the spinal cord plasticity contributes to injury-induced impairments of motor functions. Adult rats were subjected to surgical ablation of the left somatosensory motor cortex to induce lesions similar to those resulting from stroke or TBI. The physiological and behavioral measurements were performed in this group and in the sham-treated group before the surgery, 1 day after the surgery and weekly during the first month of the recovery period. The following measures were taken: the conduction velocity in the tibialis muscle in both limbs, the difference, in mm, between the extension-induced flexion of the left and right hind limbs (PA) and the number of errors (slips) made by rats on the beam-walking test (BWT) when stepping with the left and right hind limbs. Subsequently, the animals were perfused and their brains were processed for histological evaluation of the brain lesion. Before the surgery there were no differences between the groups and between the left and right limbs in any measure. Starting from the second week after the surgery there was a decrease in the conduction velocity in the right leg of injured rats vs. the left leg and vs. the sham group. This decrease persisted until the end of the recovery period. One day after the surgery, injured animals showed marked right PA, i.e. increased flexion of the right leg in comparison with the left leg. This right PA persisted during the four weeks. In the BWT test, rats in both groups committed no or only a few errors with left hind limb either before or after the surgery. One day after the surgery, there was a strong increase in the number of right leg slips. At weeks 1, 2, 3 and 4 after injury, the mean number of right leg slips gradually decreased, suggesting significant motor recovery. This reduction of slips was accompanied by a postural adjustment aimed to equilibrate on the surface of the wooden beam. When corrected for this behavioral adaptation, the ablation group showed significant motor deficits when compared to sham group and basal values. Significant correlations between the PA and behavioral indices were found only for the first measurements following the surgery. These data suggest that the spinal cord plasticity contributes to the injury-induced motor impairments, but not to the long-term functional recovery.

Poster: P.040 | Lorena Itati Petrella
Connectivity of brain structures related to emotional processing: a study in Nf1+/- mice models

Presenter: Lorena Petrella | ICNAS, University of Coimbra
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Neurofibromatosis type-1 (NF1) is a common neurogenetic disorder that results from mutations in the NF1 gene that encodes the protein neurofibromin. This protein is a negative regulator of cellular proliferation and differentiation [1]. Besides intellectual disabilities and social deficits [2,3], emotional problems have been reported in children with NF1 [3]. Mice models of NF1 have been studied in which concerns brain and behavioral aspects of the disease [4]. Diffusion tensor imaging (DTI) allows in vivo assessment of connectivity between brain structures. In this study we analyze connectivity of brain structures involved in emotional processing. We used a heterozygous mice model of NF1 (Nf1+/-). DTI images were acquired with a 9.4 T equipment, including 9 wild type (WT) and 13 Nf1+/- mice. Average mean diffusivity (MD) and fractional anisotropy (FA) were assessed on diverse brain regions including: neocortex (NEO), cingulate cortex (CIN), hippocampus (HIP), hypothalamus (HYP), periaqueductal gray (PAG) and Septum (SEP). Differences between WT and Nf1+/- groups were evaluated using Student's test (alpha=0.05) followed by false discovery rate (FDR) correction (Q=0.05). Pearson's correlation coefficients (R) between brain structures were analyzed (alpha=0.05), followed by FDR correction (Q=0.05). Additionally, we evaluated differences between R(WT) and R(Nf1) through Fisher transformation (alpha=0.05). MD: (i) Higher MD in Nf1+/- mice was observed for all the analyzed brain structures; (ii) Significant correlations were found for WT and Nf1+/- mice between the following structures: NEO, CIN, HIP and HYP, and for CIN vs. PAG. Some correlations were significant only for Nf1+/- mice: SEP versus all the remaining structures, and PAG versus NEO, HIP and HYP; (iii) Marginally significant differences between R(WT) and R(Nf1) were found for CIN vs. SEP (p=0.0694, z=1.48, R(WT)=0.7417, R(Nf1)=0.9361), and CIN vs. HIP (p=0.0885, z=1.35, R(WT)=0.8897, R(Nf1)=0.9709). FA: (i) Higher FA was observed at HIP for Nf1+/- mice; (ii) Significant correlations were found on WT and Nf1+/- mice for CIN vs. SEP. Additional significant correlations were exclusive for Nf1+/- mice, as that for HYP versus CIN and SEP; (iii) No significant differences between R(WT) and R(Nf1) were found for FA. Our results evidence a generalized MD increase in Nf1+/-, and it is in agreement with our previous voxel-by-voxel analysis [5]. Also increases in FA are observed. These two characteristics suggest an increase in cell and axonal proliferation [6] as expected by defects in neurofibromin. Moreover, the presence of significant correlations of MD and FA values only on Nf1+/- mice, as well as differences between R(WT) and R(Nf1) for some structures, suggest higher connectivity in Nf1+/- involving all the studied brain structures, but more pronounced for SEP, PAG and HYP.

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Poster: P.041 | Marcelo Duarte Dias Mendonça de Sousa
Assessment of movement sequence kinematics in dopamine depleted animals

Presenter: Marcelo D Mendonça | Champalimaud Research
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Background: Models of basal ganglia function frequently focus on initiation deficits. This contrasts with what is observed in Parkinson's Disease (PD) where chronic dopamine depletion is associated not only with "slowness of initiation" but also with "progressive reduction in speed and amplitude of repetitive actions" (Bradykinesia). The role of basal ganglia on movement speed and amplitude (movement vigour) remains unclarified, but it seems reasonable to hypothesize that dopamine has a pivotal role. Methods: Repetitive finger tapping is commonly used to assess movement speed and amplitude in PD. Using this as an inspiration we developed a novel self-paced operant task, in which mice learn to perform a particular sequence of actions, using only one forelimb. The task was designed to collect data regarding the spatial position, speed and acceleration of the mouse forelimb and lever. A miniature epifluorescence microscope (~1.9g) was used to image GCaMP6f fluorescence (a calcium indicator) in dopaminergic Substantia Nigra pars compacta (SNpc) cells while TH-cre mice performed the task. After animals learned the task we induced partial dopamine depletion by unilateral intrastriatal 6-Hydroxydopamine injection. Results: With our task we were able to assess with high spatial and temporal resolution the movement kinematics of unilateral forelimb lever-press. The maximal angular velocity of lever presses correlates with task-relevant features (Number of rewards obtained, number of lever presses/sequence) but not with the total number of lever presses/session. Preliminary results showed that after dopamine depletion there is a redistribution of movement speed with an increase in slower movements and with longer within-sequence inter-press intervals. We also found changes in the sequence microstructure, including the number of lever presses/sequence. Using in vivo calcium imaging we identified phasic activity of SNpc dopaminergic accompanying the start of a learned lever-press sequence both in healthy and partially dopamine depleted animals. Conclusion: We developed a clinically-relevant task for movement sequence kinematics and vigour assessment in mice, and identified SNpc correlates of movement. Ongoing analysis using the combination of these 2 tools will allow us to clarify the role of SNpc dopaminergic neurons in different type of movements (slow vs. fast movements), in healthy and chronic dopamine depleted mice. This will have impact in our comprehension on the role of basal ganglia dysfunction in PD symptoms.

Poster: P.042 | Maria José Braga Marques Ribeiro
Impaired noradrenergic modulation in healthy ageing: evidence from pupillography and electroencephalography

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Noradrenaline is a neuromodulator produced in the locus coeruleus, a small brainstem nucleus. The noradrenergic system controls behavioural arousal and alertness, plays a role in attention, memory, and inhibitory control, and protects the brain against neurodegeneration. These facts highlight the importance of studying this system to elucidate the mechanisms underlying age-related changes in cognitive function. Indeed, lower locus coeruleus neuronal density is associated with higher rates of age-related cognitive decline. In this study, we probed noradrenergic function in humans using pupil dilation measurements shown to correlate with locus coeruleus activity. The electroencephalogram (EEG) was acquired simultaneously in order to probe cortical function. We were interested in understanding the role of noradrenergic activation in response preparation and how this might be affected in older adults. In order to address this question, we applied a cued auditory reaction time task consisting of two different task conditions, go/no-go and simple reaction time, in a group of younger (mean age = 22 years) and a group of older (mean age = 61 years) adults. Reaction time data showed a significant taskXgroup interaction, with older adults slowing down their responses more in the more difficult condition (go/no-go) than the younger group. The effect of group was not significant. This observation suggested that older individuals were able to respond as fast as young adults in simple tasks, but took longer in more complex tasks. In order to determine if differences in noradrenergic function might explain these differences in task performance, we measured baseline pupil diameter, and stimulus evoked pupil dilation and EEG responses. Baseline pupil diameter was found to be reduced in older adults suggesting that tonic noradrenergic activity is reduced in this group. As expected, the cue elicited a pupil dilation response associated with the preparation to respond to the incoming stimulus, and an electrophysiological correlate of motor preparation, the contingent negative variation (CNV), a slow negative event-related potential. Both the amplitude of the pupil dilation response and the amplitude of the CNV revealed a significant group interaction. In the younger group, the amplitude of these responses were higher in the go/no-go task than in the simple reaction time task, thereby presenting a modulation according to task difficulty. In contrast, in the older group, the responses were similar in both tasks, suggesting a failure to modulate the preparatory effort and noradrenergic activity according to task requirements. In addition, the CNV amplitude was significantly increased in the older group, indicating that these participants required higher effort to perform even the simpler of the tasks. These findings suggest that impaired noradrenergic tonic activity and phasic modulation might play a role in the slowdown of motor responses observed in healthy ageing.

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Poster: P.043 | Maria Luisa Vasconcelos
Carbon dioxide sensing in the innate olfaction center of Drosophila melanogaster

Presenter: Maria Luisa Vasconcelos | Champalimaud Research
Nélia Varela, Miguel Gaspar, Sophie Dias, Maria Luísa Vasconcelos
(1) Champalimaud Research, Champalimaud Centre for the unknown, Lisbon, Portugal

It is thought that the innate olfactory responses in the fruit fly are processed at the Lateral Horn (LH) where the different odors may be segregated into the categories of attractive, repellent and pheromone. However this role has never been directly demonstrated because LH neurons are very poorly described. We set out to characterize LH neurons in the context of behavior, more specifically the behavioral response to carbon dioxide that generates a strong avoidance. We did a neuronal silencing screen for LH neurons that are required for carbon dioxide avoidance. We identified two positive lines. Interestingly, the neurons in each line are quite distinct in terms of the areas that they innervate in the LH and where they connect in the brain. We addressed whether these neurons are involved in general odor responses and whether they are involved in general avoidance responses. Strikingly both lines revealed specificity to carbon dioxide response. We are now imaging the activity of the neurons to assess their response profile to different odorants. Our findings directly demonstrate a role of the LH in an innate olfactory response. Furthermore, they identify neurons that are involved in the response to an odor rather than a category of odors suggesting that we are in the early stages of understanding LH organization.

Poster: P.044 | Marta Cristina de Pinho Teixeira
Neural responses to Illuminant Change - An EEG/ERP Oddball Study

Presenter: Marta Teixeira | IBILI-FMUC
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Introduction. Color constancy is the ability of a visual system to maintain object color appearance across variations in the intensity and spectral composition of the illumination. Being color a property of the objects, its invariance is a property of the brain. Distinct physical sources can produce similar results in the perceived visual signal. Do neural responses correlate with the distinct physics of light or do they correlate with perception irrespective of such changes? In this study we try to enlighten these questions, experimentally simulating natural daylight spectral and spatial variations on a colored surface, at different perceptual levels. Methods: An Oddball EEG/ERP task was conducted at IBILI-UC. 20 healthy volunteers with normal color vision participated in the experiment (mean ages=30 YO; SD=9; 9 males and 11 females). Previous to EEG, psychophysical discrimination thresholds for illuminant variation were tested for each participant. These thresholds were used to set subject-adapted subliminal, at threshold, and supraliminal Target stimuli for the Oddball. Stimuli consisted of simulations of isoluminant reddish-grey/center-surround surfaces, illuminated by simulated radiant power distributions. Targets stimuli were randomly displayed as deviant relative to a frequent Standard, in 3 conditions of spectral and spatial illuminant distribution x 3 levels of perceptual detection. Conditions: Global center-surround Illuminant distribution; Local congruent center-surround Illuminant distribution (congruent direction along daylight locus, different center-surround spectral profiles); Local incongruent center-surround Illuminant distribution (opposite daylight directions center-surround spectral profiles). Results and Conclusion. Behavioral results show target detection rates for subliminal variations at chance level, monotonically increasing with perceptual difference for the 3 conditions. ERP results show a significant posterior effect at P1 component latency for subliminal Global variation. This effect is not observed for threshold and supraliminal levels, nor for local congruent and incongruent conditions at any awareness level. Modulating differences in signal at perceptual threshold and supra-threshold levels, systematically started at the posterior N2-P3 complex latencies for all Illumination conditions, spreading over parietal(P3b) and frontal regions(N2a/P3a wave-like modulations). Comparisons within each perceptual level confirmed a significant sensory effect for globally distributed illuminant at subliminal variation, with faster processing latencies than local conditions. Supraliminal level showed significant posterior N1-N2 pronounced negativities for the incongruent condition. We conclude that conscious access to illuminant detection is sharply demarcated and operates at perceptual-dependent saliency levels of processing, and that unconscious low-level wavelength-dependent computations can be detected, which might contribute to color constancy perception.

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Poster: P.045 | Marta Moita

Freezing to inescapable repeated threats is modulated by walking velocity and mediated by a hitherto unidentified pair of descending neurons in fruit flies

Presenter: Marta Moita | Champalimaud Research
Ricardo Zacarias, Maria Luísa Vasconcelos and Marta Moita (*Authors contributed equally)*
(1) Champalimaud Research at Champalimaud Center for the Unknown

Practically all animal-species are preyed upon, having evolved various detection and response mechanisms to avoid or survive a potentially deadly encounter with a predator. Much is known regarding threat detection mechanisms, but when and how different defensive behaviors are executed remains illusive. The defensive behavior, freezing, a widely used behavioural readout in Neuroscience, is pervasive across animals. Despite its adaptive value, its instantiation is underexplored. Hence, it is essential to understand whether there are general principals that govern freezing and how different animals, with different bodies and brains, implement this seemingly simple behavior. To tackle the mechanisms of freezing and its modulation by the environment we use D. melanogaster. Escape responses of fruit flies triggered by threatening looming stimuli have been described in detail. Testing the response of fruit flies to inescapable repeated looming, we found that most flies freeze. Freezing was sustained for long periods of time, up to the five minutes of stimulation. Although prevalent in our experimental conditions, not all flies froze. A set of flies ran away from the looming stimulus instead. Interestingly we found that the walking velocity of flies just prior to the looming stimulus, determined in great part whether flies froze or ran in response to looming. Leveraging the genetic tractability of fruit flies we explored the neural underpinnings of freezing by screening for descending neurons, which send motor commands from the brain to the spinal cord, that might mediate this behavior. We found a hitherto unidentified pair of descending neurons, P9, that when silenced prevented flies from freezing in response to looming. Importantly, other defensive responses such as running or jumping were spared or even increased. Finally, optogenetic activation of P9 neurons led to an initial burst of running followed by freezing. Our findings show sustained freezing behavior that is modulated by the walking speed of flies and unravel a crucial role of new pair of descending neurons in the implementation of this defensive response.

Poster: P.046 | Matheus Augusto Farias da Silva
Sound of silence: Neural mechanisms of freezing behavior as an alarm cue

Presenter: Matheus Farias | Champalimaud Research
Ana Pereira, Matheus Farias, Alexandra Medeiros and Marta Moita
(1) Champalimaud Research at Champalimaud Center for the Unknown

Animals use a myriad of social cues to detect impending danger. Despite its importance, the neural mechanisms by which animals use these cues to infer the presence of a threat remain largely unknown. In a previous study we found that rats use freezing as an alarm cue, which is sensed through a sudden cessation of movement-evoked sound. To determine the neural circuit underlying silence triggered freezing, we used a combination of optogenetics, immunohistochemistry, anatomical tracing and pharmacology. Optogenetic inactivation of the lateral amygdala (LA), a sub-nucleus of the amygdala essential for acquisition and expression of freezing triggered by conditioned sounds, impairs freezing upon the onset of silence. In addition, we found that within the auditory thalamus, which conveys auditory information to the amygdala, the dorsal sub-division (MGd), shows increased expression of c-fos, a neural activity marker, when rats are exposed to sound of movement interrupt by moments of silence. Optogenetic inactivation of MGd also impaired freezing triggered by silence. As the MGd projects directly to the LA and indirectly via two regions of secondary auditory cortex, we also tested the role of these cortical regions through pharmacologically inhibition. Preliminary evidence suggests that the antero-ventral cortex, which directly projects to LA, but not posterior-dorsal cortex, associated with sound localization, is required for silence triggered freezing. These studies unravel a cue used by rats to infer danger from the behavior of conspecifics and its underlying neural circuitry while identifying a behavioral function of the dorsal subdivision of the auditory thalamus.

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Poster: P.047 | Mirjam Heinemans

If you say freeze, I'll freeze with you… how learning from self-experience affects the response to the display of defense behaviors of others

Presenter: Mirjam Heinemans | Champalimaud Research
Andreia Cruz, Mirjam Heinemans and Marta Moita
(1) Champalimaud Research at Champalimaud Center for the Unknown

It has been previously shown that rats with prior experience with shock respond to the display of freezing by con-specifics, i.e. show they observational freezing, whereas naïve rats do not. We hypothesized that freezing becomes an alarm cue during exposure to shock, as rats associate their own freezing responses with shock. To address this issue we exposed rats to different stimuli that allowed dissociating the aversive stimulus, freezing and fear learning and then tested observational freezing during a social interaction. Exposure to shock that does not lead to freezing nor fear learning did not result in subsequent observational freezing. Exposure to shock such that rats do not express freezing but still learn to fear the context in which they were shocked, still do not show observational freezing. Only rats that experienced shock, froze during shock exposure and showed contextual fear learning displayed subsequently observational freezing. In addition, we exposed rats to stimuli that trigger innate freezing, such as 2MT, an odorant derived from fox urine, and looming stimuli, an expanding shadow that simulates a large object on collision course. 2MT effectively induced innate freezing. However, rats showed no sign of fear learning (contextual freezing was absent) or observational freezing. Looming stimuli also triggered robust innate freezing but only very low freezing to the context or during the social interaction. Together our results support the hypothesis that freezing becomes an alarm cue through its association with aversive shock. These findings suggest that learning the meaning of ones own behavioral responses allows the use of the behaviors of others as cues about the environment.

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Poster: P.048 | Natália da Costa Madeira
Synaptic cooperation and competition in lateral amygdala

Presenter: Natália Madeira | IGC / CEDOC NOVA Medical School
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The synaptic-capture hypothesis of memory is a conceptual framework that explains how weak events, only capable of inducing transient, short-term memories, can be stabilized into long-term memories if occurring in the context of other behavioural relevant experiences1. This cooperation of memory consolidation is achieved by sharing a common pool of plasticity-related proteins (PRPs) that are distributed among activated synapses to maintain synaptic plasticity. Interestingly, memories can also compete, interfering with each other. How do memories cooperate and compete? What are the rules that determine whether a particular memory is consolidated or lost? To tackle these questions, we have studied the interaction between the cortical and thalamic afferents to projection neurons of the lateral amygdala (LA), a circuitry necessary for the formation of fear memories. The leading cellular model underlying auditory fear conditioning is a form of Hebbian LTP, induced by the association between the auditory thalamic and auditory cortex projections (CS) and the nociceptive input (US). To test whether cortical and thalamic synapses cooperate, we have recorded synaptic potentials in pyramidal neurons in the LA, elicited by thalamic and cortical inputs stimulation. We found that cortical synapses can cooperate with thalamic synapses, even within an extended time window of 15 minutes2. Thalamic-cortical cooperation is dependent on the sharing of PRPs between the two groups of activated synapses and results in the re-enforcement of both inputs in an associative manner. Interestingly, we found that synaptic cooperation between cortical and thalamic synapses is bi-directional but temporally asymmetrical. The time window of thalamic cooperation is limited by activation of the endocannabinoid receptor CB1 (CB1R). Thalamic and cortical synapses also compete for the availability of PRPs. The stimulation of an additional thalamic projection leads to an unbalance between the number of activated synapses and PRPs availability, leading to competition. Synaptic competition is also modulated by time: extending the time window of the second thalamic stimulation to 30 minutes, decreases synaptic competition. As for cooperation, CB1R activation also modulates synaptic competition. Since endocannabinoid signalling has been implicated in fear generalisation, it is conceivable that by limiting the time window in which thalamic synapses can cooperate with cortical synapses, this cellular mechanism is limiting incorrect associations and therefore limiting generalization3. We are currently testing this hypothesis using discriminative fear-learning. Our results show that cortical and thalamic inputs to the LA can interact with each other within large time windows. This observation has a profound impact on the conceptual framework of associative fear learning, as it provides a cellular mechanism for continuous integration of information at amygdala synapses.

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***Funding**: FCT Investigator Starting Grant, IF/01359/2014*

Poster: P.049 | Sandra Cristina Henriques Vaz
Astrocytes, gliotransmitters and BDNF: working together to shape synaptic plasticity?

Presenter: Sandra Vaz | iMM and FMUL, U. Lisboa
Sandra H. Vaz (1,2), João Jesus (1,2), Joana Gomes (1,2) and Ana M. Sebastião (1,2)
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Astrocytes are glial cells that play an important role in neuronal information processing. Hippocampal θ-burst long-term potentiation (LTP) is a form of synaptic plasticity that can be modulated by astrocytes since these cells release gliotransmitters that are crucial for the LTP maintenance [1]. LTP is also modulated by the neurotrophic factor BDNF [2] that enhances LTP magnitude in an adenosine A2A receptor-depend manner [3]. The aim of the present work was to investigate the role of astrocytes upon the action of BDNF on LTP, and to identify the gliotransmitters involved in the crosstalk between astrocytes, BDNF and LTP. To study the role of astrocytes upon synaptic plasticity two approaches were used: pharmacological astrocytic metabolism reduction with fluorocitrate (FC) and the dn-SNARE mice model in which the SNARE-dependent release of gliotransmitters was selectively impaired in astrocytes (through the administration of doxycycline (Dox) in the drinking water (25 µl/ml)). LTP of synaptic potentials (fEPSP) was recorded from the CA1 area of hippocampal slices prepared from male Wistar rats, WT and transgenic dn-SNARE (6-12 weeks) mice. LTP was induced by theta-burst stimulstion of the Schaffer collaterals/CA1, by 3 trains separated by 200 ms, 3 pulses 100Hz each (mild-LTP). The magnitude of LTP was 22±10% in WT (+Dox) mice, and this was increased to 55±6.8% ($p<0.05$, n=5) by BDNF (20 ng/ml) ($p<0.05$, n=5), corresponding to a significant LTP potentiation of 150%. In dn-SNARE (-Dox) mice the facilitatory effect of BDNF upon LTP was lost (LTP magnitude 24±4.2% in the absence of BDNF and 29±2.8% in its presence, n=3 per condition). In Wistar rats LTP magnitude was 16 ± 5.9% in control conditions and 39 ± 4.7% in the 2nd pathway of the same slices but in the presence of BDNF (20 ng/ml) ($p<0.05$, n=5). In slices pre-treated with FC, LTP was abolished and BDNF could not facilitate LTP. These results suggest that BDNF effect upon LTP is under astrocytic control. Since activation of adenosine A2A receptor is crucial for BDNF mediated effects on LTP [3], we hypothesised that astrocytes were the source of adenosine involved in this processes. To test this hypothesis we assessed the action of BDNF in hippocampal slices treated with FC or from dn-SNARE (-Dox) mice but superfused with the selective A2AR agonist, CGS21680 (30nM), before the treatment with BDNF (20ng/ml). CGS21680 (30nM) rescued the facilitatory effect of BDNF upon LTP, both in FC-treated slices (from Wistar rats) and dn-SNARE (-Dox) mice. These results show for the first time that astrocytes play an active role in the facilitatory action of BDNF upon LTP, and suggest that they do so by being a source of the gliotransmiter adenosine and/or its precursor ATP.

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Poster: P.050 | Sara Cristina Lourenço dos Reis
Different impact of the adenosinergic system in the dorsal versus ventral hippocampus

Presenter: Sara Reis | CNC
Sara Reis (1), Henrique B. Silva (1), Ana Patrícia Simões (1), Rodrigo A. Cunha (1,2)
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Introduction: The hippocampus is a brain region critically involved in learning and memory. It is currently accepted that learning and memory mostly involves information processing in the dorsal hippocampus (DH), which has different electrophysiological properties, patterns of gene expression and connectivity with cortical and subcortical areas of the ventral hippocampus (VH), which has a predominant role in emotional processing (1). Adenosine (Ado), acting mainly through A1 and A2A receptors, critically modulates neurotransmission and synaptic plasticity in the hippocampus (2); however, the impact of adenosine modulation along the hippocampus dorsal-ventral axis it is still poorly studied. Aim: To understand if there is a distinct adenosinergic tonus and modulation of synaptic plasticity in the dorsal versus ventral hippocampus. Materials and methods: Extracellular recordings were performed at the Schaffer collaterals - CA1 synapses in transversal hippocampal slices (400 µm) from C57BL/6 mice or from Wistar rats (8-12 weeks-old). Test stimuli were delivered at 0.05 Hz and the slope of the resultant field excitatory postsynaptic potential (fEPSP) was measured. Long-term potentiation (LTP) was induced with a high frequency stimulation train (100 Hz, 1 s) and paired pulse facilitation (PPF) was induced by paired stimuli with different interpulse intervals (20, 40, 100, 200 and 400 ms). Results are means ± SEM of n animals and paired Student's t test was used to assess statistical significance. Results: Our results show that the adenosinergic tonus is higher at the DH comparing to the VH: 2-chloroadenosine (0.1-3 µM) caused a greater inhibition of synaptic transmission at the DH and clearance of extracellular Ado, using adenosine deaminase (2 U/mL), increased fEPSPs more at the DH (circa 20% above baseline comparing to circa 10% in the VH). In addition, the magnitude of the LTP at the DH tended to be higher (156.9±14.9% vs 138.2±9.2% at the VH, n=5) as well as the PPF at all interpulse intervals. Finally, the selective antagonist of A2AR, SCH58261 (50 nM), tended to decrease LTP amplitude only at the DH (156.9±14.9% in control vs 138.1±11.6% with SCH58261, n=5, p=0.08) and was devoid of effect at the VH (138.2±9.2% in control vs 137.1±18.1% with SCH58261, n=5, p=0.86). Conclusion: These findings suggest that the adenosinergic system is more effective in the DH than in the VH. In particular, the impact of A2AR on synaptic plasticity is only evident at the DH, which may predict a different relevance of these receptors at DH- vs VH-associated behavior.

***References**: 1-Strange et al., 2014, Nat. Neurosci. Rev. 15:665-69; 2- Cunha et al., 2016, J. Neurochem. 139: 1019-1055.*
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Poster: P.051 | Luísa da Rocha Santa Marinha
The role of phospholipase D in hippocampal synaptic plasticity

Presenter: Luísa Santa Marinha | ICVS, University of Minho
Luísa Santa Marinha (1), Vítor Pinto (1), Tiago Gil Oliveira (1)
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Since lipids are one of the major constituents of the brain, the modulation of its levels can potentially have an impact in its functioning (1). In fact, defects or alterations in the enzymatic regulation of lipids have been related to the pathogenesis of nervous system diseases (1, 2, 3), such as Alzheimer's disease (AD) (4, 5, 6), Parkinson's disease (7), mood disorders (8, 9) or cancer (10, 11). One of the enzymes that can modulate the levels of signaling lipids is phospholipase D (PLD). In mammals there are three two main isozymes of PLD: PLD1 (12) and PLD2 (13, 14) and they are both responsible for the generation of phosphatidic acid (PA) from phosphatidylcholine (5, 15, 16). PA is a central signaling lipid with membranar fusogenic properties (15, 17). Consequently, the modulation of its levels can potentially alter synaptic properties with an impact in neuronal functioning. For example, concerning pathological conditions, PLD and PA were shown to have a fundamental role in AD pathogenesis (5). Since the precise role of PLD1 and PLD2 in brain functioning is still unclear, we propose to study the impact of PLD ablation in adult hippocampal synaptic functioning, by evaluating the Long Term Depression and Long Term Potention of PLD1 KO mice and PLD2 KO mice.

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Funding: *BIAL 253/14*

SESSION: NEURODEGENERATION

Poster: P.052 | Ana Catarina Rodrigues Neves
The retina as a mirror of the alterations observed in Alzheimer's disease brain? Evaluation of structural, cellular and molecular changes

Presenter: Catarina Neves | IBILI
Catarina Neves (1,2), Samuel Chiquita (1,2), Rafael Carecho (1,2), Filipa Baptista (1,2), Paula Moreira (2,3), A. Francisco Ambrósio (1,2)
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Alzheimer's disease (AD) is the most common dementia that affects more than 35 million of people worldwide. AD has no cure and its diagnosis is difficult, relying on expensive and/or invasive methods. There is an urgent need to identify better and early biomarkers for AD diagnosis. Since AD patients have visual problems even before AD diagnosis and the retina is part of the central nervous system, we aimed checking whether the retina could be used as a reliable window to look into the brain. In this work, we investigated whether the retina of an AD animal model is affected and mirrors the molecular and cellular alterations observed in AD brain, namely, synaptic loss, neuronal cell death and inflammatory features, at 4 and 8 months old (early stages) of the disease. Male triple transgenic (3xTg-AD; AD model) and age-matched wild-type (WT; C57BL6/129S) mice were used to evaluate retinal structural changes by optical coherence tomography. Retinas, hippocampus and total cortex homogenates were used to evaluate proteins related with synaptic loss and glial reactivity. Distribution and/or the reactivity of glial cells were assessed by immunohistochemistry in retina cryosections. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was used to assess cell death. At 8 months, but not at 4 months, there was a significant reduction of retinal thickness of 3xTg-AD mice compared with WT, assessed with line scans (179.96±1.07 µm vs 188.60±2.34 µm) and circular scans (176.49±1.28 µm vs 186.75±2.36 µm). In the cortex of 3xTg-AD mice, there was an increase (198.0±22.6% of control vs 100.0±10.6%) of syntaxin protein levels at 4 months, but not at 8 months. Retina and hippocampus did not present alterations in the levels of syntaxin and synaptophysin at both time points. The brain and retina of 3xTg-AD and WT mice did not show any TUNEL+ cells at both time points. In the retina of 3xTg-AD, there were no alterations in vimentin (Müller cells marker) levels. In the cortex of 3xTg-AD mice, no changes were detected in glial fibrillary acidic protein (GFAP; astrocyte marker) protein levels at 4 and 8 months. However, in the retina of 3xTg-AD, it was observed a significant decrease (43.7±7.0% of control vs 100.0±20.8%) of GFAP protein levels at 8 months old. In the hippocampus there was a significant increase (212.2±27.0% of control vs 100.0±17.2) of GFAP protein levels at 4 months that did not persist to 8 months. These results show that in the early stages of the disease there is a decrease in retinal thickness. Despite this, no synaptic loss and cell death was detected in the retina, neither in brain. The levels of GFAP were affected in the retina and hippocampus, at different time points, suggesting a possible glial plasticity that needs to be further explored. In summary, the retinal thickness is early affected in this animal model, but no cellular or molecular correlates were found in the retina and brain, at least in early time points.

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***Link between abstracts**: Window*

Poster: P.053 | Rafael José Monteiro Carecho
Are the main features of Alzheimer's brain mirrored by the retina?

Presenter: Rafael J. M. Carecho | IBILI, FMUC
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Alzheimer's disease (AD) is the most frequent neurodegenerative disorder. Pathologically, AD is characterized by the accumulation of amyloid-beta plaques followed by neurofibrillary tangles of hyperphosphorylated tau protein leading to a massive neuronal loss and brain homeostasis disruption. Moreover, loss of cholinergic neurons by decrease of acetylcholine neurotransmitter may account some of the alterations observed in AD such as decline of memory and learning. However, these cognitive symptoms arise only after the occurrence of significant neuronal deterioration and its diagnosis is very difficult, relying on expensive and invasive methods. The retina, as part of central nervous system, is an extension of the brain and, in fact, previous studies have shown that retina is affected in several neurodegenerative diseases. Taking this into account, a relatively new concept claims that the retina might be used as a window to look into the brain. Since there are few studies analyzing changes in both regions in the context of AD, we aim to clarify when they start appearing in the retina and brain, which regions are mostly affected, how changes progress and try to correlate all data. In this study, we evaluated the content of several proteins associated with the main features of AD pathology by western blotting in cortex, hippocampus and retina using an animal model of AD (3xTg-AD) along two early time points (4 and 8 months of age). At 4 and 8 months of age, we found a significant increase in the levels of amyloid beta in 3xTg-AD mice in both hippocampus (288.0±57.2% and 615.0±164.0% of nonTg, respectively) and cortex (220.2±37.7% and 697.8±91.4% of nonTg, respectively) comparing with non-transgenic mice, whereas amyloid-beta was not detected in the retina, neither in the controls nor in the transgenic mice. At 4 months of age, the protein levels of p-Tau increased not only in the cortex (185.2±27.0% of nonTg) and hippocampus (203.2±37.3% of nonTg) but also in the retina (151.2±12.8% of nonTg) in 3xTg-AD, increase that persisted until 8 months in the hippocampus (224.8±28.0% of nonTg). Moreover, the levels of amyloid precursor protein are increased at 8 months in hippocampus (147.2±6.2% of nonTg), whereas no changes were observed in the cortex and retina in the timepoints studied. The content of beta-secretase 1 and choline acetyltransferase remained unaltered at 4 and 8 months of age in all regions analyzed. These preliminary results suggest that the triple transgenic model is developing the AD pathology, although it seems that the main alterations in AD firstly appear in the brain, mainly in hippocampus, in opposition to the retina. We expect giving new insight into the AD pathology and find new biomarkers for a better and early AD diagnosis filling the gaps regarding changes in the retina and brain by assessing later time points of disease progression.

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***Link between abstracts**: window*

Poster: P.054 | Ana Francisca Rodrigues Vaz Bravo
The impact of phospholipase D functional ablation in physiology and in disease models in Caenorhabditis elegans

Presenter: Francisca Bravo | ICVS, University of Minho
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Lipids are a major constituent of the brain, and specifically signaling lipids have been shown to regulate brain functioning[1]. Moreover, lipid signaling modulation has been demonstrated to have the potential to be an effective therapeutical option for neurological disorders, such as Alzheimer´s Disease (AD) [1]. Growing evidence suggests that a group of enzymes called phospholipases, that modulate signaling lipids, have an impact in neuronal physiology. In this study, we focused on a specific enzyme, phospholipase D (PLD), which converts phosphatidylcholine to phosphatidic acid (PA). PA plays an important role in multiple aspects of cell physiology [1]. In order to study the role of this enzyme, a multitude of behavioral, imaging and biochemical tests were performed using a nematode model with PLD ablation [2]. A decrease in PLD activity was confirmed in this model by mass spectrometry analysis, as well as mechanistically through evaluation of ethanol susceptibility, since PLD has an increased affinity to primary alcohols. Our data indicates that although PLD is a key signaling modulator, it is not essential for the survival or for the normal performance in a myriad of behavioral tests in a C. elegans PLD mutant model. Interestingly, the biometric analysis shows that, PLD mutant worms are wider and have an increased volume in both fed and starved conditions when compared to WT worms. Since the mechanism by which this phenotype arise is still elusive, we are testing the hypothesis of possible lipid dysregulation due to a lipid droplets and cholesterol accumulation. In addition, we are performing an assay to recover the body size phenotype using metformin. Besides this, in order to better understand the potential impact of PLD functional ablation in neurodegenerative diseases we are performing established behavioral tests [3, 4]. Our results indicate that PLD functional ablation has a protective effect in motor and thrashing tasks, modulates the susceptibility to a proconvulsivant drug (pentylenetetrazol), protects against deleterious effects of serotonin, leads to an increase in associative learning and in body size in C. elegans neurodegenerative disease models[5]. Overall, our data shows that PLD functional ablation has an important role in neurodegeneration and the specificity of some of our phenotypes shed some light in potential mechanisms.

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Poster: P.055 | Ana Isabel Marques Duarte

Changes in brain glucose metabolism render middle-aged females less vulnerable to chronic type 2 diabetes-related alzheimer disease

Presenter: Ana I. Duarte | CNC, University of Coimbra

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Aging, type 2 diabetes (T2D) and female sex are known risk factors for Alzheimer disease (AD). However, their precise subcellular interactions remain elusive. Since several common molecular mechanisms link T2D and AD, there is an increasing need for a highly specialized personalized, sex-specific medicine. This, together with our previous studies, led to the hypothesis that sex differently affects brain glucose metabolism in T2D, predisposing to neurodegenerative events. Therefore, we aimed to analyze the role of sex on brain glucose metabolic pathways in middle-aged T2D rats. As such, we used brain cortical homogenates from middle-aged (8-month-old) male and female Wistar and T2D Goto-Kakizaki (GK) rats to determine key markers for glucose transport and energy metabolism by immunoblotting and colorimetric approaches. We observed that besides the higher glycemia in both male and female GK rats, this was even higher in both female cohorts. This was mirrored by a slightly higher density of glucose transporter 3 (GLUT3) in both control and T2D females' brains. However, a massive decrease in brain glucose levels was found in both Wistar females and GK males, suggesting either an impairment in their brain glucose transport and/or its immediate metabolism. Additionally, both control and T2D females had tendentiously lower brain pyruvate levels and lactate dehydrogenase activities than males, suggesting a middle-aged female-related attenuation in brain glycolysis. Similarly, our preliminary results point towards an inhibition of brain pentose phosphate pathway in both Wistar and GK rat females, as given by their lower glucose-6-phosphate dehydrogenase activity. Although our preliminary measurements showed an inhibition in one of the initial enzymes from Krebs cycle (citrate synthase) in both female cohorts, the activities of the intermediate enzymes α-ketoglutarate dehydrogenase and succinate dehydrogenase were tendentiously higher, especially in Wistar female rat brains. Though this may arise from the use of alternative amino acids as substrates for the latter two enzymes to compensate for the attenuation in Krebs cycle in females' brains, it may not be enough to rescue this metabolic pathway, as malate dehydrogenase (the last enzyme of the cycle) activity was decreased in middle-aged control females brain. In sum, the attenuation in brain glucose metabolism in middle-aged females may protect them against the previously-observed oxidative stress and AD-like neuropathology.

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Poster: P.056 | Oral presentation: O.10 | Isabel Paiva de Castro
Sodium butyrate rescues dopaminergic cells from alpha-synuclein-induced transcriptional deregulation and DNA damage

Presenter: Isabel Paiva | Uni. Medical Center Goettingen

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Alpha-synuclein (aSyn) is considered a major culprit in Parkinson's Disease (PD) pathophysiology. However, the precise molecular function of the protein remains elusive. Recent evidence suggests that aSyn may play a role on transcription regulation, possibly by modulating the acetylation status of histones. Our study aimed at evaluating the impact of wild-type (WT) and mutant A30P aSyn on gene expression, in a dopaminergic neuronal cell model, and decipher potential mechanisms underlying aSyn-mediated transcriptional deregulation. We performed gene expression analysis using RNA-sequencing in Lund Human Mesencephalic (LUHMES) cells expressing endogenous (control) or increased levels of WT or A30P aSyn. Compared to control cells, cells expressing both aSyn variants exhibited robust changes in the expression of several genes, including downregulation of major genes involved in DNA repair. WT aSyn, unlike A30P aSyn, promoted DNA damage and increased levels of phosphorylated p53. In dopaminergic neuronal cells, increased aSyn expression led to reduced levels of acetylated histone 3. Importantly, treatment with sodium butyrate, a histone deacetylase inhibitor (HDACi), rescued WT aSyn- induced DNA damage, possibly via upregulation of genes involved in DNA repair. Overall, our findings provide novel and compelling insight into the mechanisms associated with aSyn neurotoxicity in dopaminergic cells, which could be ameliorated with a HDACi. Future studies will be crucial to further validate these findings and to define novel possible targets for intervention in PD.

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Poster: P.057 | Marco Rafael Machado Guimarães
Social behavior is impaired in chronic pain conditions

Presenter: Marco Rafael Guimarães | ICVS, University of Minho
Marco Rafael Guimarães (1,2), Ana Margarida Cunha (1,2), Nuno Dinis Alves (1,2), Madalena Esteves (1,2), Nuno Sousa (1,2), Armando Almeida (1,2), Hugo Leite-Almeida (1,2)
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Chronic pain (CP) affects 7-10% of worldwide population and it often accompanied by dramatic changes in the quality of life. The complex relationship between sensory and contextual (i.e., cognitive, emotional, and motivational) impairments in CP conditions has been widely studied; nevertheless, the psychological and social domains of pain have yet to be fully elucidated. Therefore, in this study we aimed to elucidate the impact of a CP animal model in social interaction. Twenty-two males and sixteen females Wistar-han (8 weeks) rats were used. We used the spared nerve injury (SNI) neuropathic pain model in the left- (SNI-L) or in the right-hindpaw (SNI-R) to compare the familiar and unfamiliar social behavior in a neutral arena after 6 hours of isolation at 1 and 2 months of neuropathy. Finally, the neuronal activation patterns in medial prefrontal cortex (mPFC), orbital frontal cortices (OFC), nucleus accumbens (NAc), paraventricular nucleus (PVN) and amygdala (AMY) from male and female brain slices were analyzed by c-Fos immunohistochemistry. SNI-L and SNI-R present i. similar values in mechanical allodynia (a hallmark of neuropathic pain); ii. SNI males and females (no sex differences) present similar phenotype during the interaction period (15 min) when compared with Sham-operated animals and that is inversely correlated with the weigh; iii. The social interaction time is similar in familiar or unfamiliar pairs; iv. The social interaction does not affect the nociceptive responses in SNI animals; v. Both SNI-R males and females interact lesser that SNI-L animals, demonstrated a lateralized effect of SNI in social behavior and vi. such interaction results in c-Fos expression alterations in different brain regions such as the PVN, AMY, Prelimbic and Infralimbic cortex from mPFC. Collectively, our results demonstrated that SNI neuropathic pain model promotes lateralized deficits in social behavior as well as different neuronal activation. This work highlights the importance of social dimension in neuropathic pain conditions and allows the study of underlying mechanisms of social impairments in rodent and human pain conditions.

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Poster: P.058 | Sandro Gabriel Ferreira Dá Mesquita
Meningeal lymphatic function in models of Alzheimer's disease

Presenter: Sandro Dá Mesquita | University of Virginia
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The brain parenchyma is devoid of lymphatic vasculature1, 2. Hence, the excretion of brain metabolic byproducts and waste, such as amyloid beta (Aβ), is in part achieved through the bulk flow of interstitial fluid (ISF) from the parenchyma into the cerebrospinal fluid (CSF)2. However, the presence of functional lymphatic vessels embedded in the brain meninges challenged the conventional perception of fluid dynamics within the central nervous system (CNS)1, 3. These lymphatics actively drain both molecules and cells from the CSF into the cervical lymph nodes (cLNs), serving as a previously unappreciated route of CNS waste clearance1, 3. Herein, we show that impaired drainage of CSF by ligation of lymphatic vessels afferent to the deep cLNs or ablation of the meningeal lymphatics results in decreased clearance of Aβ42 from the brain. Additionally, impaired meningeal lymphatic drainage results in an accumulation of Aβ42 in the CSF, in the formation of amyloid aggregates in the brain meninges as well as aggravation of memory deficits in transgenic mouse models of Alzheimer's disease. Altogether, our findings suggest that the meningeal lymphatic system plays an important role in Aβ drainage from the brain, which could be relevant in the context of neurodegenerative disorders like Alzheimer's disease.

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Poster: P.059 | Ana Inês Marques Morgado

Impact of neuronal adenosine A2A receptor overexpression on glial phenotype of the hippocampus

Presenter: Inês Marques-Morgado | Instituto de Medicina Molecular
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Chronic stress, brain insult and Alzheimer's disease share a common feature of dysregulation of adenosinergic system, characterized by a long-term robust upregulation of A2A receptors (A2AR) in cortical and hippocampal regions that leads to excitotoxicity phenomena, dendritic retraction and impairment in memory and synaptic plasticity (Batalha et al, 2013, Mol Psychiatry). Although little is known on the mechanism involved in this A2AR upsurge, different studies have found similarities between this type of dysregulation and those found in aged animals (Cunha et al, 1995, Neuroreport.; Lopes et al, 1999, J Neurochem.), suggesting that this A2AR upregulation is an early-aging phenotype and may be instrumental in driving neurodegeneration. To address this issue we used transgenic rat model with a postnatal neuronal-specific overexpression of human A2AR under the control of the CaMKII promoter [Tg(CaMKII-hA2AR)]. These animals display age-like alterations in hippocampal function, with cognitive, synaptic and molecular impairments that are reversed upon A2AR blockade (Coelho et al, 2015, Front Psychiatry; Batalha et al, 2016, Sci Rep). In order to clarify the involvement of different cellular subsets in the A2AR hippocampal dysregulation, we characterized microglial and astroglial phenotypes in Tg(CaMKII-hA2AR) model, assessing cell density, reactivity and morphology, comparing to wildtype littermates (WT). Microglial number, reactivity and morphology were assessed by a combined approach of Flow Cytometry, Western Blotting and Immunohistochemistry. Astroglial reactivity and morphology were evaluated using Western Blotting and Scholl Analysis of Immunohistochemistry images. Along with an increase of microglial cell number ($n=4$; $P<0.05$) and protein levels of the microglial marker Iba1 ($n=7$; $P<0.05$) we found morphological differences in microglial cells of CA1 area of the hippocampus of transgenic animals, namely a decrease in the area of cellular influence due to process retraction ($n=2$-3; $P<0.0001$), despite no significant changes in the basal levels of cytokines as measured by qPCR. The levels of astrocytic GFAP were reduced in the hippocampus of transgenic animals ($n=7$; $P<0.05$), but we did not observe any morphological changes neither in the length nor in the branching of astrocytic processes in CA1 area. These data show that A2AR overexpression in forebrain neurons is sufficient to drive significant changes in glial cell function, inducing a mild priming of microglia that resembles early states of activation process, associated to an asthenic phenotype of astrocytes. This suggests that the pathological process of A2AR dysregulation may derive from a synergy of synaptic and glial dysfunction which deserves further attention.

***References**: Batalha et al, Molecular Psychiatry. 2013, 18: 320–331; Cunha et al, Neuroreport. 1995, 6: 1583–1588; Lopes et al, Journal of Neurochemistry. 1999, 73: 1733–1738; Coelho et al, Frontiers in Psychiatry. 2014, 5:67 Batalha et al, Scientific Reports. 2016, 6: 31493*
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Poster: P.060 | Ana Rita Alves Malheiro
The (ether) link between phospholipids and myelination in the central nervous system

Presenter: Ana Malheiro | i3S
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Nervous tissue is highly enriched in plasmalogens, a class of ether-phospholipids, whose biosynthesis initiates in peroxisomes and is required to attain the high levels of these phospholipids in myelin and neurons. The importance of plasmalogens to human health is emphasized by the severe clinical presentation of Rhizomelic Chondrodysplasia Punctata (RCDP). The neurological involvement in RCDP, with impaired myelination, seizures and intellectual disability combined with the observation of plasmalogen deficiencies in several neurodegenerative disorders underscores the role and function of these phospholipids in neurons and myelinating glia. In Gnpat knockout (KO) mice, we show that lack of plasmalogens causes myelination defects in the central nervous system. In spinal cord, a defect in plasmalogens leads to an adult onset demyelination without axonal loss or loss of oligodendrocytes. Astrocytosis and microgliosis were also a feature in the white matter of spinal cords from KO mice. In optic nerve, the lack of plasmalogens initially impairs myelination (dysmyelination) and is followed by a rapid and progressive demyelination. Surprisingly, dysmyelination or demyelination of optic nerves in KO mice was not accompanied by axonal pathology. Unlike spinal cord, optic nerves from plasmalogen-deficient mice did not exhibit microgliosis or axonal damage, only revealing astrocytosis during the late stage demyelination. In summary, contrarily to the characteristic demyelination and axonal loss that occurs in the peripheral nervous system of KO mice, the CNS axons are preserved despite a severe demyelination. Besides revealing the importance of plasmalogens for myelination and myelin maintenance, our results highlight that in the presence of oligodendrocytes, loss of myelin has minimal consequences to axon integrity despite differential susceptibility of neurons to demyelination.

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Poster: P.061 | Andreia Cristiana Teixeira de Castro
Serotonergic signaling suppresses proteotoxicity

Presenter: Andreia Castro | ICVS, University of Minho
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Our previous results support a therapeutic role of serotonergic signaling in Spinocerebellar ataxia type 3 (SCA3). In a C. elegans screen of FDA-approved small molecules for suppressors of mutant ataxin-3 (ATXN3) induced neurotoxicity, a selective serotonin re-uptake inhibitor (SSRI) was found to rescue mutant ATXN3-mediated toxicity in vivo. SSRI chronic and early symptomatic treatment in SCA3 mice led to a striking amelioration of motor symptoms, to a reduction of mutant ATXN3 aggregation and neuronal loss. But how late in disease progression can treatment be initiated? How is this antidepressant rescuing SCA3 pathogenesis? Is this relevant for other diseases of protein misfolding? In C. elegans, chronic citalopram treatment stalled disease progression and restored survival. Moreover, the extent of drug exposure period was found to be critical and maximum protection required early treatment. In mice, treatment initiated after symptom onset still ameliorated motor coordination and balance, in parallel with restoration of cerebellar calbindin positive neurons, but with no major impact on protein aggregation. These results suggest that small molecule modulation of serotonergic signaling represents a promising therapeutic target for SCA3 even after symptom onset, while showing that early initiation of treatment may lead to increased efficacy. We are also using genetic, pharmacological and transcriptomic approaches to determine which components of 5-HT signaling are key for neurodegeneration offset. Suppression of ATXN3 aggregation suggests that the increase in 5-HT availability, early in disease progression, affects folding and conformational stability of ATXN3. The toxic effects caused by other aggregation-prone proteins are also ameliorated by the 5-HT pathway. This parallels with activation of protein homeostasis subnetworks and increased folding capacity in live neuronal cells. Our results suggest that small molecule modulation of serotonergic signaling represents a promising therapeutic approach for conformational disorders, and support the emerging role of this signaling pathway in the modulation of proteostasis, and strengthening the conceptual basis for future human clinical trials.

Poster: P.062 | Belina Simões Ferreira Rodrigues
From nutrient intake to brain regions volumes

Presenter: Belina Ferreira-Rodrigues | ICVS, University of Minho
Belina Ferreira-Rodrigues(1,2,3), Carlos Portugal-Nunes(1,2,3), Teresa Costa Castanho (1,2,3), Paulo Marques (1,2,3), José Miguel Soares (1,2,3), Nuno Sousa (1,2,3), Nadine Santos (1,2,3)
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Studies have found a significant relationship between cognitive tests and regional brain volumes in cognitively healthy older adults. Moreover, cognitive function and brain volume were associated with different circulating plasma nutrient biomarker patterns. In a more comprehensive approach, a cohort of community-dwelling old adults (n=120) was assessed through a battery of cognitive tests and a 24-hours dietary recall. Individuals underwent a MRI and blood samples were drawn. This data will be used to study whether a relationship can be established between dietary intake and brain regions, using the nutrient biomarkers to ensure the status of the relevant dietary constituents. This analysis is part of a study on cognitive ageing (Switchbox, TEMPO and PANINI cohorts).

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Poster: P.063 | Bruno Miguel Barroso Rodrigues Almeida
Macromolecular interactions and posttranslational modifications acting as Machado-Joseph Disease modifiers

Presenter: Bruno Almeida | ICVS, University of Minho
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Machado-Joseph disease (MJD) is caused by a polyQ-tract expansion within the DUB enzyme Ataxin-3 (ATXN3). Similar to other polyQ diseases, in MJD, the mutant ATX3 self-assembles into amyloid fibrils and aggregates into neuronal inclusions. Macromolecular interactions and post-translational modifications, modifiable on a time and tissue-dependent manner, are nowadays being regarded as an explanation for the late-onset of the disease. Indeed, ATXN3 surfaces involved in aggregation overlap with some interaction binding sites, indicating a role for molecular interactors in ATXN3 self-assembly protection. We found that the ATPase p97 forms a high affinity complex with the brain-predominant ATXN3 isoform that is increased by ATXN3 SUMOylation. This association reduces the amount of ATXN3 toxic fibrils and promotes the formation of putatively non-toxic ATXN3 aggregates. Importantly, we observed reduced SUMOylation levels of the mutant ATXN3, which impacts on the aggregation behavior. Additionally, our data also suggests that ATXN3 might interact with and regulate the turnover of several S/R-rich splicing factors in neurons and that this may be compromised in MJD leading to splicing deregulation and neurotoxicity. By using biochemical (baculovirus-driven protein production), biophysical (surface plasmon resonance) and cell biology approaches (cell culture and animal models) we are characterizing these interactions and evaluating the relevance of RNA-splicing deregulation for MJD.

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Poster: P.064 | Carla Maria Nunes Lopes

Huntington's disease patient-derived induced pluripotent and neural stem cells offer additional insights into mitochondrial-based pathological mechanisms

Presenter: Carla Lopes | CNC

Lopes, Carla (1,2), Pereira de Almeida, Luís (1,3), Daley, George Q. (4), Rego, Ana Cristina (1,5)

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Huntington's disease (HD) is the most common polyglutamine expansion disorder, caused by increased number of CAG repeats in the HTT gene, coding for huntingtin [1]. The mutation is translated into an abnormally long glutamine tract at the N-terminal, of mutant protein, conferring a deleterious gain of function. Selective loss of striatal medium spiny neurons is a major hallmark of HD, with symptoms ranging from psychiatric disturbances, involuntary movements and cognitive deficits, leading to dementia [2]. Multiple mechanisms have been implicated in HD pathogenesis, including defects in mitochondrial and energy metabolism associated with increased oxidative stress [3]. Induced pluripotent stem cell (iPSC) technology used for disease modeling has given new insights into the analysis of potentially modified "early" disease mechanisms. In this study, we sought to understand mitochondrial and metabolic (dys)function in HD iPSC and derived neural stem cells (NSC) versus respective control cells. Mitochondria from heterozygous human iPSC (HD-iPSC) (72 CAGs) and differentiated neural stem cells (HD-NSC) appear more fragmented with immature round shape morphology linked to decreased levels of OPA1, required for mitochondrial fusion. Furthermore, HD-iPSC and HD-NSC were shown to dependent more on glycolysis for energy production than oxidative phosphorylation, as displayed by lower basal respiration and decreased activity of complex III. Additionally, decreased mRNA levels of nuclear and mitochondrial encoded complex III subunits were observed. PGC-1□ and downstream transcription factor TFAM were also downregulated, suggesting decreased mitochondrial biogenesis. Interestingly, mitochondria from these cells were hyperpolarized due to ATP synthase reversal and exhibited increased mitochondrial calcium accumulation, when compared to control iPSC and NSC. Both HD-iPSC and HD-NSC showed decreased ATP/ADP levels, which were reduced after glycolysis inhibition. Enhanced levels of mitochondrial superoxide anion and hydrogen peroxide were also observed in HD-iPSC and HD-NSC versus control cells. Enhanced intracellular levels of GSH were further observed probably due to an upregulation of GSH biosynthesis, as verified by increased mRNA levels of glutamate-cysteine ligase catalytic subunit. In addition, HD-iPSC and HD-NSC displayed increased phosphorylation of pyruvate dehydrogenase (PDH) E1α subunit at Ser232, 293 and 300, which correlated with increased mRNA levels of PDH kinase 1 and reduced mRNA levels of PDP1 (PDH E1α phosphatase) in HD-iPSC, indicating reduced PDH catalytic activity. In conclusion, HD-iPSC and HD-NSC exhibited diminished activity of PDH and complex III linked to reduced oxidative phosphorylation, hence largely relying on glycolysis for ATP generation and producing high levels of ROS. This study evidences mitochondrial dysfunction and metabolic deregulation as early events in HD pathogenic cascade.

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Poster: P.065 | Carla Sofia Pais Fonseca
Neuroprotective and anti-inflammatory effects of Klotho in ventral midbrain cell cultures

Presenter: Carla Fonseca | CICS-UBI
Daniela J. Alexandre, Graça Baltazar, Carla P. Fonseca
(1) CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal

Parkinson's disease (PD) is an age-related disease characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta. The pathogenesis of PD is not fully understood, but it is known that neuroinflammation and increased oxidative stress may contribute to dopaminergic cell loss. Klotho is an anti-aging protein capable of extending lifespan in mice, decreasing oxidative stress and enhancing synaptic plasticity and cognition. Previous studies have shown that circulating levels of Klotho decrease with age, which has been associated with an increased risk for developing age-related diseases. Few studies have focused on the relevance of Klotho in PD. Therefore, in this work we aimed at investigating the role of this anti-aging protein in cellular models of PD. Our results show that Klotho, applied exogenously and prior to 1-methyl-4-phenylpyridinium (MPP+) exposure, prevents the loss of ventral midbrain dopaminergic neurons. A maximal neuroprotective effect was observed for the concentration of 0.2 µg/mL of Klotho, applied to the cells 1 h to 4 h before the toxic insult. Furthermore, our data suggest that the presence of astrocytes is required for Klotho's neuroprotective effect. Moreover, we also show that Klotho is able to modulate microglial reactivity by preventing LPS-induced release of nitric oxide by these cells. Taken together, the present study show, for the first time, that exogenous administration of Klotho protects dopaminergic neurons from an oxidative insult and promotes anti-inflammatory effects. The effects of Klotho should, in the future, be investigated in animal models of PD in order to disclose if Klotho may constitute a therapeutic target in the treatment of this disease.

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Poster: P.066 | Catarina Alexandra Barbosa Ezequiel
SOD1G93A transduced microglia show a depressed inflammatory response to LPS

Presenter: Catarina Ezequiel | iMed.ULisboa/FFULisboa
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Immune unbalance plays a crucial role in Amyotrophic Lateral Sclerosis (ALS) and microglia dysfunction was shown to be associated with neuronal injury and to influence the onset and and progression of the disease [1]. Indeed, there are evidences that microglia can either be highly reactive in early stages or irresponsive to stress stimuli [2]. However, it is not known the signaling pathways that are affected in the mutated ALS microglia that may be responsible for the dual microglia signatures, mainly when stimulated by a proinflammatory stimulus as lipopolysaccharide (LPS). Our previous data showed that LPS stimulation of microglial N9 cells switch to a prevalent M1 polarization [3]. With this is mind we proposed to evaluate the resultant effects of overexpressing human SOD1G93A mutation, one of the most common in ALS, in the reactivity of microglia towards LPS. For that, we used mouse N9 microglial cell line, expressing WT human SOD1 (WT-MG) or containing the G93A mutation (mSOD1-MG), either incubated or not with 300 ng/ml of lipopolysaccharide (LPS) for 48 h. Upon incubation, phagocytic capacity was evaluated by quantifying the number of ingested beads and mRNA was isolated to evaluate the expression of different M1/M2-associated cell polarization markers. We observed that mSOD1-MG significantly lost the arginase-1 associated M2 phenotype as well as the expression of the IL-10 anti-inflammatory cytokine, an effect that was exacerbated in the presence of LPS. In addition, the mSOD1-MG showed an increased expression of the stress-related HMGB1 and lower capacity to upregulate S100B levels or MHCII expression upon interaction with LPS. Intriguingly, mSOD1-MG still sustained a moderate ability to increase some markers of M1 microglial phenotype, either in the absence or in the presence of LPS, such as TNFα, microRNA(miR)-155 and miR-146a. In terms of the phagocytic ability, the mSOD1-MG was able to ingest an increased number of beads than the WT-MG, but not in the presence of LPS. Overall, our study provides a model to characterize microglial heterogeneity in ALS, and data indicate that ALS microglia, although sustaining a moderate inflammatory response to LPS, show increased levels of the alarmin HMGB1 and low expression of MHCII expression that may lead to suboptimal Th cell response during neuroinflammation along the progression of the disease (4).

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Poster: P.067 | Claudia Almeida
The Alzheimer's risk factors Bin1 and CD2AP polarize the endocytic generation of β-amyloid

Presenter: Claudia G. Almeida | CEDOC
*Florent Ubelmann (1), Tatiana Burrinha (1), Laura Salavessa (1) and Claudia G. Almeida (1)**
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The mechanisms driving pathological beta-amyloid (Aβ) generation in late-onset Alzheimer's disease (AD) are unclear. Two late-onset AD risk factors, Bin1 and CD2AP, are regulators of endocytic trafficking, but it is unclear how their endocytic function regulates Aβ generation in neurons. We identify a novel neuron-specific polarisation of Aβ generation controlled by Bin1 and CD2AP. We discover that Bin1 and CD2AP control Aβ generation in axonal and dendritic early endosomes, respectively. Both Bin1 and CD2AP loss of function raise Aβ generation by increasing APP and BACE1 convergence in early endosomes however via distinct sorting events. When Bin1 levels are reduced, BACE1 is trapped in tubules of early endosomes and fails to recycle in axons. When CD2AP levels are reduced, APP is trapped at the limiting membrane of early endosomes and fails to be sorted for degradation in dendrites. Hence, Bin1 and CD2AP keep APP and BACE1 apart in early endosomes by distinct mechanisms in axon and dendrites (Ubelmann et. al, 2017). We are currently investigating the impact of AD mutations in CD2AP and Bin1 function in endosomal amyloid production. Individuals carrying variants of either factor would slowly accumulate Aβ in neurons increasing the risk for late-onset AD.

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Poster: P.068 | Daniela Isabel Ferreira Madeira
Astrocytic ATP release under Alzheimer´s disease conditions

Presenter: Daniela Madeira | CNC, FMUC
Daniela Madeira (1,2), Patrícia Santos (1), Paula M Canas (1), Rodrigo A. Cunha (1,2), Paula Agostinho (1,2)
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Astrocytes, the most abundant cells in the brain, modulate the synaptic transmission through the release of gliotransmitters; such as ATP and glutamate. Increasing evidences suggest that astrocytes are involved to pathogenesis of Alzheimer´s disease (AD), in which the first signs of memory deficits are associated to synaptic dysfunction and loss that is thought to be triggered by the accumulation of amyloid-beta (Aβ) oligomers [1]. Previous studies from our group showed that astrocytes have adenosine A2A receptors (A2AR), which have also been implicated in AD; and that the blockade of these receptors prevents the astrocytic reactivity and dysfunction triggered by Aβ peptides [2]. Alterations on gliotransmittion by astrocytes can contribute to exacerbate the pathology, however it remains to be established how astrocytic ATP and glutamate release are affected by AD conditions. In this study, we aim to investigate: i) how AD conditions affect the release of ATP by astrocytes and ii) the role of astrocytic A2AR in controlling ATP release. To achieve these goals we used cultured astrocytes from cortical brain of Wistar rats exposed acutely or chronically to Aβ1-42 peptides (1µM), and the ATP was quantified by a bioluminescence assay [2,3]. The data obtained showed that Aβ exposure caused a marked astrogliosis, which was assessed by increased in GFAP and connexin 43 reactivity. The acute exposure to Aβ1-42 increased the astrocytic ATP release by 163.0±54.0% (n=5, $p<0.05$) as compared with untreated (control) cells, similar results were obtained with chronic Aβ1-42 exposure. Currently, we are studying the effect of selective antagonist of A2AR, SCH58261 (50nM) on ATP release by astrocytes. These findings may contribute to elucidate the impact of astrocytes on AD pathogenesis, and pave the way to develop novel therapeutic strategies against this neurodegenerative disorder.

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Poster: P.069 | Daniela Monteiro Fernandes
Identifying the molecular underpinning of altered corticosteroid milieu in brain - impact on Tau phosphorylation profile

Presenter: Daniela Monteiro | ICVS, University of Minho
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Current lifestyle places individuals under increasingly greater loads of psychological and physical stress. Although the mechanisms that are triggered by stress and stressful stimuli are primarily adaptive and vital for survival facilitating the restoration of physiological and behavioral homeostasis, chronic stress and altered corticosteroid milieu may become maladaptive. In the brain, corticosteroid alterations, often accompanied by biochemical and structural changes, are responsible for both the adaptive and maladaptive (when secreted in excess) effects of stress. Neuronal atrophy and dysfunction are among the best known effects of stress and excess GC secretion, and stress/GC are now considered important disruptors of neuroplasticity. As our recent studies identify Tau, a cytoskeletal protein, as a key regulator of neuronal plasticity induced by chronic stress and GC, this study monitors the effect of chronic alterations of corticosteroids of Tau phosphorylation profile related to neurostructural changes in different brain areas such as hippocampus, prefrontal cortex and hypothalamus. Our findings highlight the importance of corticosteroid imbalance on Tau phosphorylation dynamics and neuroplasticity.

Poster: P.070 | Elisabete Baptista Ferreiro

Chronic hyperglycemia impairs the maturation of newly-generated hippocampal neurons and exacerbates memory loss in a mouse model of Alzheimer's disease

Presenter: Elisabete Ferreiro | CNC, University of Coimbra

Elisabete Ferreiro (1,2), Mariagrazia Lanzillo (1), Paulo Pinheiro (1), Diogo Canhoto (3), António M. Carvalho da Silva (1,2,4), Giorgia Mastrella (1), Ana Raquel Fontes (1), Jorge Valero (1,2, 5), Ana Cristina Rego (1,3)

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Alzheimer's disease (AD) is the most prevalent neurodegenerative disease. Metabolic alterations, like those in glucose metabolism, are believed to occur early in the onset of AD, contributing to the development of the disease. Prolonged insults caused by chronic hyperglycemia may affect normal brain function and capacity to cope with insults. This may lead to reduced brain cognitive reserve and capacity to compensate age-related neuronal loss, which can be related to a sustained decrease in adult hippocampal neurogenesis, early before the appearance of AD symptoms. In order to evaluate whether hyperglycemia aggravates alterations in hippocampal adult neurogenesis in AD, and further impair memory, 3xTg-AD male mice were treated with an elevated dose of sucrose for 6 months. Upon induction of hyperglycemia, the spatial memory of 3xTg-AD mice was significantly compromised when compared to untreated 3xTg-AD mice. This exacerbation in memory loss might be related to the observed decrease in the total number of newly-generated neurons, reduction of dendrite complexity and number of PSD95-positive puncta in the dendritic branches of immature neurons in the dentate gyrus (DG) outer molecular layer (OML). In untreated 3xTg-AD mice, newly-generated neurons presented increased dendrite arborization and increased number of PSD95-positive puncta in the DG OML, which could compensate the decreased number of immature neurons and play an important role in the memory performance of these mice. Electrophysiological recordings showed no significant synaptic potentiation at the lateral perforant path to DG synapses after high frequency stimulation in any of the experimental groups, as expected for mice of this age, in the presence of intact inhibitory neurotransmission. However, for individual animals, there was a significant negative correlation between the mean degree of change in synaptic efficacy after high frequency stimulation and the animal's levels of hyperglycemia in 3xTg-AD group, with more hyperglycemic mice displaying reduced synaptic facilitation. These data suggest that hyperglycemia enhances AD pathology, contributing for the impairment in neurogenesis, defective learning and memory loss.

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Poster: P.071 | Graça Maria Fernandes Baltazar
Inhibition of glial step as a protective strategy in experimental models of parkinson's disease

Presenter: Graça Baltazar | CICS-UBI
Rita Videira(1), João Carlos Ferreira Barbosa(1), Carla Fonseca(1), Ana Saavedra(2-4), Graça Baltazar(1)
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Protein phosphorylation regulates multiple cellular processes and depends on the balance between phosphorylation by kinases and the opposite action of phosphatases. STEP (from STriatal Enriched protein tyrosine Phosphatase) is a neural specific tyrosine phosphatase highly expressed in the striatum, and its activity is regulated by dopamine and NMDA receptors. Dysregulation of STEP levels has been reported in several neurodegenerative and psychiatric disorders. For instance, in Alzheimer's disease increased STEP levels result in disruption of synaptic transmission and cognitive deficits. Recent studies from our group have shown that STEP levels are also increased in brains of Parkinson's disease (PD) patients, as well as in animal models of the disease (Kurup et al., 2015). In the present study we investigated whether a reduction of STEP levels/activity could protect dopaminergic neurons in in vitro and in vivo models of PD. Our results show that genetic deletion or pharmacological inhibition of STEP protects DA neurons against MPTP-induced lesion in vivo. Nevertheless, our in vitro approaches show that the STEP inhibitor TC-2153 was neuroprotective against MPP+-induced toxicity in neuron-astrocyte midbrain co-cultures, but not in mesencephalic neuronal cultures or in the neuroblastoma cell line SH-SY5Y, does suggesting the involvement of astrocytes. In fact, our results indicate that astrocyte conditioned medium (ACM) from TC-2153-treated midbrain cultures protects DA from MPP+ toxicity, and this effect could be reproduced by ACM from cortical cultures. Finally, by neutralization and pharmacological studies, we show that inhibition of astrocytic STEP has neuroprotective effects on DA neurons that are dependent on BDNF, but not on GDNF. Taken together, our results suggest that inhibition of glial STEP can be used as a strategy to protect the nigrostriatal pathway.

***References**: Kurup et al Proc Natl Acad Sci U S A. 2015 Jan 27;112(4):1202-7*
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Poster: P.072 | Helena Isabel Alvites Cavaleiro

Is TRPV1 involved in pain and bladder dysfunction associated with multiple sclerosis? An experimental study in the rat

Presenter: Helena Cavaleiro | FMUP

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Multiple sclerosis (MS) is an autoimmune inflammatory and demyelinating disease that affects the central nervous system. It is a severe neurodegenerative disease, presently without cure, that affects more than 2 million people globally. With a strong economic and social impact, MS is the commonest progressive neurological disorder in young people, with a mean age at onset between the 20 and 40 years old, causing irreversible physical and mental disability. Among the most incapacitating symptoms, neuropathic pain and bladder dysfunction are reported by the majority of patients. The transient receptor potential vanilloid 1 (TRPV1) is a receptor described to have an important role in neuropathic pain, bladder dysfunction and inflammation. A recent study showed that TRPV1 knockout mice were protected from MS, presenting delayed disease onset, reduced clinical scores and reduced demyelination. Protection conferred by TRPV1 absence was related to reduce BBB permeability, preventing inflammatory cell extravasation into the CNS and consequent myelin degradation.The present project aims to investigate the contribution of TRPV1 to MS-induced pain and bladder dysfunction. We will use the established MS-model of Experimental Auto-immune Encefalitis (EAE), induced by a single injection in the flank of a solution of myelin basic protein (MBP; a major constituent of the myelin sheath) in an emulsion of Complete Freund's adjuvant (CFA). Preliminary results indicate that EAE female rats developed neuropathic pain, as shown by the presence of mechanical allodynia and hypersensitivity to thermal stimuli. As in the majority of patients, pain severity fluctuated during the experimental period (4 weeks). Cystometries performed at this time point showed signs of neurogenic detrusor overactivity. We hope to detect signs of demyelination and glial changes in spinal cord samples from these animals, as well as changes in TRPV1 expression. In subsequent experiments, we will desensitize TRPV1 and hopefully alleviate pain and bladder symptoms.

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Poster: P.073 | Inês Dinis Aires
Intravitreal injection of adenosine A2A receptor antagonist reduces neuroinflammation and vascular leakage in the retinas of diabetic mice

Presenter: Inês Dinis Aires | FMUC
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Diabetic retinopathy is a complication of type 1 and type 2 diabetes and it is among the main causes of blindness worldwide. Diabetic retinopathy is characterized by blood-retinal barrier breakdown and a low grade chronic inflammatory response that involves the activation of microglial cells. Mounting evidence, gathered by us and others, demonstrate that the blockade of adenosine A2A receptors (A2AR) affords protection through the control of microglia-mediated neuroinflammation. Herein, we hypothesized that the blockade of A2AR is able to reduce retinal microglia reactivity in diabetes, thus preventing retinal cell death through the control of neuroinflammation. Type 1 diabetes was induced in 4-5 months old C57BL/6 mice by a single intraperitoneal injection of 150 mg/kg of streprozotocin. After one month of diabetes, SCH 58261 (2 µl of 100 nM solution), a selective A2AR antagonist, was administered intravitreally once a week, for a period of four weeks. Vehicle (2 µl of 0.9% NaCl) was administered to the contralateral eye. The levels of pro-inflammatory mediators in the retina were evaluated by western blot. Microglia reactivity and cell death were assessed by immunohistochemistry and TUNEL assay in retinal cryosections, respectively. Furthermore, vascular leakage was assessed in vivo by fluorescein angiography. Intravitreal administration of SCH 58261 decreased the levels of the pro-inflammatory markers TNF and iNOS and the reactive microglia marker mitochondrial translocator protein (18 kDa) (TSPO). Furthermore, microglia reactivity was reduced in the retinas of diabetic mice treated with the A2AR antagonist, as determined by the decrease in the number of MHC II-immunoreactive cells. The intravitreal administration of SCH 58261 also decreased retinal cell death induced by diabetes and the permeability of the blood-retinal barrier. The results demonstrate that, in an animal model of type 1 diabetes, treatment with A2AR antagonist decreases retinal microglia reactivity and neuronal degeneration, also preventing blood-retinal barrier breakdown. Hence, our results suggest that intravitreal injection of SCH 58261 can be envisaged as a new therapeutic option for the treatment of retinal degeneration in diabetic retinopathy.

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Poster: P.074 | Inês Margarida Dias Cabaço Amaral
Intracerebroventricular administration of a gliotoxin impairs hippocampal synaptic function

Presenter: Inês Amaral | CNC, University of Coimbra
Inês Amaral(1), Marlene Pereira(1), João P. Lopes(1), Paula M. Canas(1), Rodrigo A. Cunha(1,2), Paula Agostinho(1,2)
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Astrocytes are key players involved in a bi-directional communication in synapses, shaping synaptic plasticity that constitutes the neurophysiological basis of memory. Interfering with the integrity of the neuron-glial network in the hippocampus is known to impair integrated responses, like learning and memory [1]. Thus, astrocytic pathology has been proposed to underlie the pathogenesis of mood or cognitive disorders, as heralded by the impact of the gliotoxin, L-alpha-aminoadipic acid (L-AA) in brain regions such as prefrontal cortex or amygdala [2,3]. In the present study, we aimed to characterize the effects of L-AA, and therefore of astrocytes elimination, in hippocampal synaptic plasticity and memory. To tackle this question, male C57BL/6 adult mice were injected bilaterally in the lateral ventricle with either 160 µg of L-AA or saline solution (control). After 3 days, mice were behaviourally evaluated in a hippocampal-dependent memory paradigm, the novel object recognition test, and hippocampal synaptic function was evaluated by electrophysiological recordings of synaptic plasticity, namely long-term potentiation (LTP) triggered by high frequency stimulation (HFS, 100Hz/1s). Immunohistochemistry and Western blot analysis were also performed to assess alterations of astrocytic markers. Mice injected with L-AA displayed decreased LTP amplitude by 25% (82.0 ±11.4% in control and 56.9 ±8.4% in slices from L-AA-injected mice, n=4; $p>0.05$), without alteration in basal synaptic transmission, when compared to control mice. Mice injected with L-AA also displayed a reduction in glial fibrillary acidic protein (GFAP) and glutamine synthetase (GS) immunoreactivities in the CA1 region, a finding confirmed by Western blot analysis of these glial markers in hippocampal extract from L-AA-injected mice. Taken together, these data suggest that L-AA is able to trigger astrocytes dysfunction in hippocampus and this likely contributes to impair synaptic plasticity. This paves the way to explore the contribution of astrocytes in the deficits of synaptic plasticity and memory that are characteristic of many neurodegenerative disorders, such as Alzheimer's disease.

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Poster: P.075 | Inês Margarida Lourenço Figueira
Dietary (poly)phenol metabolites neuroprotection in a Parkinson's disease 3D cell model

Presenter: Inês Figueira | iBET/ITQB-NOVA

Inês Figueira (1,2), Joana Godinho-Pereira (1,2), Ana Paula Terrasso (1,2), Marcel Leist (3), Catarina Brito (1,2), Cláudia Nunes dos Santos (1,2)

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The aged population, with an increasing prevalence of age-related disorders keeps growing. Parkinson's disease (PD) is one of most common age-related neurodegenerative disorders, and it is still without cure. Epidemiological and clinical studies have showed that dietary (poly)phenols are able to reduce the incidence and prevalence of such disorders, exhibiting remarkable multipotent ability to modulate several pathways. The prevention and treatment of neurodegeneration, characterized by a mechanistic complexity, requires novel therapeutic strategies targeting multiple targets, such as (poly)phenols can do. Our goal was to investigate potential protective effects of selected metabolites of dietary (poly)phenols identified in an human intervention study in a physiologically-relevant human cell model of PD. To generate the latter, Lund human mesencephalic neural progenitor cells (LUHMES) were differentiated in 3D cultures. Differentiated neurospheres were enriched in dopaminergic neurons and presented evoked synaptic activity. A PD-like phenotype, with reduction of tyrosine hydroxylase levels, was induced with 1-methyl-4-phenylpyridinium (MPP+), a specific mitochondrial respiratory chain inhibitor. The neuroprotective effect of (poly)phenol metabolites was observed in the PD cell model implemented. The 3D PD in vitro model developed allowed us to disclose some of the mechanistic pathways involved in (poly)phenol metabolite neuroprotection and is a novel valuable tool for screening and studying potential therapies for PD.

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Poster: P.076 | Jessica Faria da Eira
Actin cytoskeleton disruption as a novel player in the pathogenesis of familial amyloid polyneuropathy

Presenter: Jessica Eira | I3S, University of Porto
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Familial Amyloid Polyneuropathy (FAP) is a neurodegenerative disease characterized by deposition of amyloid fibrils of mutated transthyretin (TTR) in the peripheral nervous system, leading to a dying-back axon degeneration. Abnormalities in cytoskeletal organization are a common feature of many neurodegenerative disorders. In this work we investigated the hypothesis that cytoskeleton damage occurs downstream of TTR deposition. In primary cultures of mouse dorsal root ganglia (DRG) neurons, a relevant cell type for FAP studies as mutant TTR accumulates close to the DRG, axons treated with TTR oligomers presented a marked reduction of the growth cone area, with disruption of the typical morphology of the growth cone which lacked the lamellipodial actin structures. Additionally, using a FAP Drosophila model in which the amyloidogenic mutant TTR Val30Met is expressed in the photoreceptor cells resulting in roughening of the eye, we observed decreased axonal projection of photoreceptor neurons, that presented more compact growth cones lacking the spread distribution of filopodia and lamellipodia actin structures. A genetic screen was subsequently performed by crossing the TTR Val30Met flies with readily available fly lines for the knockdown or overexpression of candidate genes whose function is associated with cytoskeleton dynamics. In this screen we determined that the Rho GTPase family-the major regulator of actin dynamics modulates TTR-induced rough eye phenotype. Using cell based assays of DRG neurons treated with TTR oligomers, we are currently validating the results obtained in the Drosophila genetic screen by comparing the activity of key Rho GTPases and performing a cytoskeleton phospho-antibody array which includes 141 specific phosphorylation antibodies mainly involved in actin signaling pathways. With this work we will dissect the cascade of events that underlie alterations in axonal cytoskeleton dynamics induced by TTR.

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***Funding**: JE is a FCT fellow (SFRH/BD/116343/2016); Norte-01-0145-FEDER-000008 - Porto Neurosciences and Neurologic Disease Research Initiative at I3S; FEDER - Fundo Europeu de Desenvolvimento Regional funds through the COMPETE 2020 - Operacional Programme for Competitiveness and Internationalisation (POCI), Portugal 2020, and by Portuguese funds through FCT - Fundação para a Ciência e a Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior in the framework of the project "Institute for Research and Innovation in Health Sciences" (POCI-01-0145-FEDER-007274).*

Poster: P.077 | Joana Carolina Marinho Cunha

Differently aged microglia from the spinal cord of SOD1G93A mice show diverse polarized phenotypes, which are exacerbated by co-incubation with their counterpart astrocytes

Presenter: Carolina Cunha | iMed.Ulisboa, FFUP
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Neuroinflammation plays a critical role in Amyotrophic Lateral Sclerosis (ALS) [1,2]. Microglia actively contribute to motor neuron degeneration [3], but their phenotypic transition over disease course is poorly understood. Recently, we have shown that astrocytes isolated from SOD1G93A mice have a reactive/neurotoxic phenotype (unpublished data). However, few studies explored the dynamics between astrocytes and microglia interaction in ALS. Here we aimed to (i) assess the phenotypic changes in the polarization of SOD1G93A microglia when aged in culture; (ii) evaluate the effect of reactive/neurotoxic SOD1G93A astrocytes on microglia subtype distribution. For that, primary mixed glial cultures were obtained from the spinal cord of 7-day old SOD1G93A mice and after 21 days in vitro (DIV), microglia was isolated and kept in culture for 2 and 16 DIV, to mimic reactive and aged-like phenotypes, as we recently published [4]. In parallel experiments, astrocytes were isolated from the same animals and co-cultured with the reactive 2 DIV microglia for 48 h. We assessed microglia phenotype markers, inflamma-miRs, NF-κB and NLRP3-inflammasome pathways. Non transgenic littermates (wild type, wt) were used as controls. Upregulation of MHC-II, iNOS and microRNA (miR)-155 suggests M1-polarization in the 2 DIV cultured SOD1G93A microglia. Accordingly, we found decreased M2-associated SOCS1 and arginase 1 markers, as well as miR-124 and miR-21 expression levels. Despite the increase in pNF-kB nuclear translocation, IL-1beta expression and nitric oxide (NO) release, NLRP3-inflammasome and IL-18 levels did not change in 2 DIV SOD1G93A microglia. Contrasting with these cells, aged SOD1G93A microglia showed mixed anti-inflammatory, inflammatory and senescent-like subtypes based on the upregulation of miR-155, miR-124 and miR-146a, together with the downregulation of NOS2, MHC-II, IL-1β, SOCS1 and TGF-β1, when compared with aged-matched wt cells. Interestingly, SOD1G93A astrocytes induced wt microglia M1-polarization, with upregulation of miR-155 and IL-1beta levels, as well as NO release. Most relevant, these astrocytes changed the M1-polarized SOD1G93A microglia into mixed microglia populations, showing both M1 (MHC-II and iNOS) and M2 (IL-10, miR-124 upregulation and NO decrease) markers. Interaction of SOD1G93A astrocytes with microglia showed to additionally trigger the NLRP3-inflammasome activation. Overall, our data indicate that the predominant M1 spinal SOD1G93A microglia switch to heterogeneous mixed populations when aged in culture, which may relate with phenotypic transitions as disease progresses and with the complex and unique microglia signature in ALS disease. In addition, SOD1G93A astrocytes not only revealed to produce harmful effects in healthy microglia, but also to induce the representation of mixed cell populations in the SOD1G93A microglia, further compromising their neuroprotective function in ALS.

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Microglia change from a reactive to an age-like phenotype with the time in culture. Front Cell Neurosci 8:152 doi:10.3389/fncel.2014.00152
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Poster: P.078 | Joana Margarida Cardoso Serra Martins
Activation of adenosine A3 receptor prevents pressure-induced retinal neuronal cell death

Presenter: Joana Margarida Martins | IBILI
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Glaucoma is the second leading cause of blindness in the world and is characterized by progressive loss of retinal ganglion cells (RGCs) and optic nerve atrophy. It is a multifactorial disease, being intraocular pressure (IOP) the only modifiable risk factor and the main target for therapeutic interventions, but vision loss progresses despite successful IOP control. Therefore, it is of utmost importance to develop novel effective treatments for glaucoma, and neuroprotection of RGCs is considered an alternative therapy. The activation of adenosine A3 receptor (A3R) is neuroprotective against several insults in CNS. Thus, the main objective of this work was to investigate whether activation of A3R prevents retinal neuronal death induced by elevated hydrostatic pressure (EHP), which mimics increased IOP. Retinal primary neural cell cultures and retinal organotypic cultures were pre-treated with 1 µM of 2-Cl-IB-MECA, a selective A3R agonist, and were exposed during 24 h to EHP (70 mmHg above normal atmospheric pressure). Control cells were incubated in a standard cell incubator (normal atmospheric pressure). Cell death was assessed by TUNEL assay, neuronal survival by immunolabeling of neurons with neuronal nuclear protein (NeuN), and the survival of RGCs was assessed by immunostaining with Brn3a (a marker of RGCs). The protein levels of caspase 3 were determined by western blot. In retinal primary neural cell cultures, the exposure to EHP for 24 h significantly increased the number of TUNEL-positive cells and decreased the number of neurons (NeuN-immunoreactive cells) in the culture. The activation of A3R prevented cell death and neuronal loss triggered by EHP. Moreover, the exposure to EHP for 24 h increased the protein levels of cleaved caspase-3 protein, but the activation of A3R did not prevent cleaved caspase-3 increase, suggesting that the protection conferred by A3R activation occurs by other mechanism. In retinal organotypic cultures, the exposure to EHP for 24h elicited a significant decrease in the number of RGCs, and the activation of A3R prevented the EHP-induced RGC loss. Our results demonstrate that the activation of A3R is neuroprotective for retinal cells against damage induced by elevated pressure, thus opening the possibility of a new pharmacologic strategy for the treatment of glaucoma.

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Poster: P.079 | Joana Margarida Gonçalves Mota Silva
Chronic stress triggers Tau pathology through autophagy inhibition and induction of stress granules

Presenter: Joana M Silva | ICVS, University of Minho

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Imbalance of neuronal proteostasis associated with misfolding and aggregation of Tau protein is a common neurodegenerative feature in Alzheimer's disease (AD) and other Tauopathies. Consistent with suggestions that lifetime stress maybe an important precipitating factor of AD , we previously reported that environmental stress and high glucocorticoid (GC) levels evoke accumulation of aggregated Tau [1][2]; however, the underlying molecular mechanisms remain unclear. We now demonstrate that chronic stress and GC trigger an mTOR-dependent inhibition of autophagic process, the cardinal clearance pathway for aggregated proteins, leading to accumulation of Tau aggregates and cell death in mice and cells stably expressing P301L-Tau. Considering the interplay of autophagy with Stress granules (SGs) dynamics, we also show that environmental stress/GC stimulate the induction of SGs, recently shown to promote Tau misfolding, aggregation and neurotoxicity. Notably, pharmacological intervention that stimulates autophagic process (Temsirolimus) attenuates the GC-driven elevation of Tau, SGs and cell death. This work provides novel insights into the mechanisms through which neuronal cells convey the detrimental impact of prolong environmental (HPA-related) stress to intracellular "stress" signaling, causing Tau-driven brain pathology.

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Poster: P.080 | Joana Margarida Marques Branco dos Santos
Protein phosphatase 1 modulates mutant huntingtin aggregation and toxicity

Presenter: Joana Branco dos Santos | ITQB
Joana Branco-Santos (1,3), Federico Herrera (1), Gonçalo M. Poças (2), Yolanda Pires-Afonso (2), Flaviano Giorgini 3, Pedro M. Domingos (2), Tiago Fleming Outeiro (4,5,6)
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Huntington's disease (HD) is an incurable neurodegenerative disorder caused by a polyglutamine (polyQ) expansion in the N-terminal region of the huntingtin protein (HTT). The first 17 amino acids of the protein (N17), immediately preceding the polyQ tract, are a critical functional domain for HTT function and HD pathogenesis. Recent studies suggest that double N17 phosphorylation at serines 13 and 16 reduces mutant HTT aggregation and toxicity. However, double phosphorylation events are less likely to occur than single phosphorylation events and often require overexpression of specific kinases. Here, we analyzed the effect of single NT17 phosphorylation events (at Thr3, Ser13 or Ser16) in HTT oligomerization, aggregation and toxicity in cell and Drosophila models of HD. The generation of large aggregates, but not oligomeric species, was completely abolished by single phosphomimic mutations in living cells, while phosphoresistant mutants did not produce overt phenotypes. Thr3 phosphorylation decreased HTT aggregation in both larva and adult flies and exacerbated climbing impairments of HD flies. Strikingly, and in line with these observations, we found that pharmacological or genetic inhibition of protein phosphatase 1 prevented HTT aggregation and potentiated neurotoxicity in Drosophila. Our findings suggest that phosphorylation of the HTT N-terminal region plays a critical role in HD pathogenesis, and support the targeting of specific N17 phosphorylatable sites and protein phosphatases for HD therapy. Key words: Huntington's disease, huntingtin, phosphorylation, bimolecular fluorescence complementation, Drosophila melanogaster, neurodegeneration

***Funding**: European Huntington Disease Network (EHDN); Fundação para a Ciência e a Tecnologia (FCT); Medical Research Council (MRC)*

Poster: P.081 | Joao Carlos Bettencourt de Medeiros Relvas
Caveolin-1-mediated internalization of the vitamin C transporter SVCT2 in microglia triggers an inflammatory phenotype

Presenter: João Bettencourt Relvas | I3S, University of Porto

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Vitamin C is essential for the development and function of the central nervous system (CNS). The plasma membrane sodium-vitamin C co-transporter 2 (SVCT2) is the primary mediator of vitamin C uptake in neurons. SVCT2 specifically transports ascorbate, the reduced form of vitamin C, which works as a central reducing agent in the CNS. Here we demonstrated that ascorbate uptake through SVCT2 is critical for the homeostasis of microglia, the resident myeloid cells of the CNS that are essential for proper functioning of the nervous tissue. We found that depletion of SVCT2 from the plasma membrane triggered a proinflammatory phenotype in microglia and was required for microglia activation. Src tyrosine kinase-mediated phosphorylation of caveolin-1 at tyrosine 14 in microglia induced the internalization of SVCT2. Ascorbate treatment, SVCT2 overexpression, or blocking SVCT2 internalization prevented the activation of microglia. Overall, our work demonstrates the importance of the ascorbate transport system for microglial homeostasis and hints that its dysregulation might play a role in neurological disorders.

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Poster: P.082 | João Filipe Fonseca Gomes
Impact of BDNF receptor cleavage on AD pathophysiology

Presenter: João Fonseca-Gomes | Ana Sebastião Lab - iMM, Lisboa
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Alzheimer's Disease (AD) is the most common form of dementia worldwide and the accumulation of amyloid-beta (Aβ) peptide in the brain is considered a main hallmark of this disease [1]. In AD, Brain-derived neurotrophic factor (BDNF) signalling is seriously impaired, which compromises its physiological functions: neuronal survival, differentiation and plasticity [2]. Actually, decreased levels of BDNF and its TrkB Full-Length receptor (TrkB-FL) were described in several pathologies including AD. It is also widely known that there is an upregulation of TrkB truncated isoforms, which act as negative modulators of BDNF signaling [3,4]. Recently, we described that accumulation of Aβ peptide leads to calpain overactivation, through the increase of intracellular calcium levels mediated by eNMDAr, and subsequent TrkB-FL cleavage. As consequence, TrkB-FL levels decrease and two fragments are generated: a membrane-bound truncated receptor (TrkB-T') and an intracellular fragment (TrkB-ICD) [5]. Accordingly, this work aimed to characterize the TrkB-ICD fragment and its consequences on cell signalling. Experiments, performed in human neuroglioma (H4) cell line and in primary rat cortical neurons, revealed that: i) TrkB-ICD is a stable fragment (half-life time ~8h); ii) it accumulates within the cell nucleus overtime and iii) it has tyrosine kinase activity, leading to the phosphorylation of nuclear, somal and axonal proteins. Importantly, data using using post-mortem human brain samples (frontal cortex) from patient with AD and an age-matched control also demonstrate that TrkB-FL receptors are decreased and TrkB-ICD levels are increased in the AD brain. Overall, these evidences support the hypothesis that TrkB-ICD fragment might have a biological role on the AD pathophysiology. Moreover, attending to the extremely relevant role of BDNF signalling for the endogenous neuroprotection, it was clear for us the relevance of studding whether the prevention of calpains mediated cleavage of TrkB-FL could be a good strategy to prevent the loss of BDNF functions. Accordingly, we performed structural predictions, using PEP-FOLD software [6], in order to obtain a specific peptide to act as a substrate for calpains (TAT-TrkB). In this way, using this approach, in future, we will disclose whether the prevention of TrkB-FL cleavage will be a feasible approach to avoid the loss of BDNF actions on pathologies associated to exacerbated activation of calpain activity. Taken together these data suggest that TrkB-ICD could have a role on the pathophysiology of disorders where calpains are overactivated, such as in AD. Moreover, our structural prediction of TAT-TrkB will allow us to further prove the importance of TrkB-FL cleavage prevention on pathologic contexts.

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Poster: P.083 | João Manuel do Canto Gomes
Impact of immune system aging on Multiple Sclerosis' forms of progression: study of the thymic function

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Multiple Sclerosis (MS) is a neuronal autoimmune disorder characterized by progressive demyelination of neurons in a process mediated by T-lymphocytes produced by the thymus. As a condition highly disabling that lacks effective treatment, its progression leads to cognitive and emotional deficits. Studies showed that children with MS and individuals with relapsing-remitting form of the disease have premature T-lymphocytes aging in comparison to healthy controls. The main goal of this study is to investigate how thymic function relates to T-lymphocytes aging and how this overall impacts on progression of MS and on manifestation of different forms of the disease. The hypothesis in study is that the reduced thymic activity impacts on the aging process of T-lymphocytes and that this aging process may be essential for the pathophysiological phenomena observed in multiple sclerosis. For this, in blood samples from MS patients with distinct forms of the disease and from healthy individuals, thymic output is currently being determined (by quantification of recent thymic emigrants by flow cytometry and of signal joint T-lymphocytes receptor excision circles by nested-PCR) and related to immunological aging and telomere length (determined by quantification of the CD45RA vs. CD45RO ratio by flow cytometry and flow-FISH, respectively). The outcome provided by the current study may lead to the better understanding of the role of the immune system's aging and thymic function on the different forms of progression of multiple sclerosis and therapies based on thymic modulation may be adapted and adopted to improve disease outcome.

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Poster: P.084 | Joao Miguel Seabra Castelhano
Magnetic Resonance and PET imaging of a transgenic mouse model of Alzheimer's disease: an in vivo longitudinal study

Presenter: Joao Castelhano | IBILI/ICNAS, Uni. Coimbra
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It is generally believed that neurodegeneration is accompanied by blood–brain barrier (BBB) and neurometabolic impairments. Contradictory findings have been reported regarding BBB disruption leading to an ongoing controversy in Alzheimer's disease (AD). Regarding magnetic resonance spectroscopy quantification, previous studies also show disparate findings (e.g. no observed difference in Glu+Gln and reduction of Glu+Gln in AD). Other studies suggested that NAA and myo-inositol are highly discriminative in diagnosing AD. Volumetric analysis of brain structures (e.g. hippocampus and striatum) and white/gray matter quantification have also been proposed as new biomarker tools. In vivo imaging studies with an emphasis on longitudinal analysis are needed to shed light on this issue. It is important to quantify and temporally localize abnormalities during ageing and determine their role in the pathophysiology of cognitive decline and dementia in particular in AD. We performed a longitudinal study including localized proton spectroscopy (H-MRS; 9.4T Bruker MRI), structural MRI and contrast-enhanced Perfusion MRI (with measures that allow estimating the BBB permeability index) and Voxel Based Morphometry (VBM) in the mouse brain to assess functional/synaptic dysfunction, at 4, 8 and 12 months of age in 3xTg-AD model (n=7; and C57BL6/129S, as controls, WT, n=6). Furthermore, we used 11C Pittsburgh compound B (PiB) and PK11195 PET imaging to analyze amyloid distribution and to evaluate brain neuroinflammation and microglia activation, respectively. Here we report a preliminary analysis of spectroscopy, structural and perfusion MRI and PET data in 4 distinct regions of interest (Striatum, Hippocampus, Parieto-frontal cortex and Visual cortex). We did not find differences in the perfusion levels between groups up to 12M, thus suggesting that the BBB is intact. Regarding the MRS data, we found between-group differences in the hippocampus and striatum mainly for Taurine (Str 8M and 12M, WT>TG, $p=0.022$; Hip all time points WT>TG, $p<0.005$), Inositol (Str 8M WT<TG, $p=0.008$) and GABA (Str 4M WT>TG, $p=0.003$). It is known that Taurine prevents the neurotoxicity of ß-amyloid. We found Taurine is reduced in TG animals comparing to WT, in an age-dependent manner. In the VBM analysis, WT and TG groups show an initial significant difference of Hippocampus gray matter volume, that is significantly reduced in TG group ($p<0.05$ FWE), suggesting that structural changes might be present early on (4M). The quantification of PK PET data revealed TG animals have increased uptake of PK at 8M in comparison to the WT group (Mann-Whitney U test: frontal cortex $p=0.032$; Hip $p=0.047$; Str $p=0.002$). This suggests that neuroinflammatory processes are already taking place at this stage. The evidence presented here may help clarify the contradictory findings of BBB state and MRS results and confirm the previous findings about grey matter and inflammation.

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Poster: P.085 | João Paulo de Sá e Silva
Spinal Muscular Atrophy: LARP4 is a novel regulator of SMN expression

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Spinal muscular atrophy (SMA) is the most common autosomal recessive cause of infant mortality. It is a neuromuscular disease characterized by the degeneration of motor neurons in the anterior horn of the spinal cord, caused by low levels of the survival motor neuron (SMN) protein due to mutation of the Survival of Motor Neuron 1 gene (SMN1). This gene is locate on chromosome 5 that in humans presents a duplication of the SMN1 region containing a centromeric copy of this gene, called SMN2. The two genes differ in five nucleotides, only one of which is located on a coding exon (exon 7). This variation is a silent transition (C->T) that results in inefficient splicing of exon 7 leading to the predominant (90%) production of an unstable truncated protein. Nevertheless, SMN2 can still produce a small amount of full-length functional Smn protein (10%), thus making it an important target for therapy. Most efforts have focused on increasing Smn protein levels through the activation of SMN2 transcription and splicing. However, few studies have focused on the modulation of SMN2 mRNA stability and translation. The identification of proteins binding to SMN1/2 mRNA would provide a greater insight into the mechanisms regulating its expression and ultimately uncover novel targets that favor mRNA stabilization and/or increased translation. In this project we aim to identify and characterize the post-transcriptional mechanisms that regulate the SMN2 mRNA expression using a luciferase reporter system to monitor the effects of the 5´UTR and 3´UTR regions. We have shown that the 3´UTR region has a global positive effect on SMN2 expression, in contrast with the 5´UTR region, which has a negative regulatory effect due to the putative presence of a uORF or a secondary structure element. We have further tried to identify RNA binding proteins that bind to the 3´UTR of SMN2 and which may be involved in mRNA stability control. Using RNA-protein pull downs coupled to mass spectrometry, we have identified LARP4 as a SMN1/2 mRNA binding protein and show that it has a positive effect on the expression of a SMN 3'UTR luciferase reporter by promoting its translation. Furthermore, our data suggest that endogenous SMN levels are also positively regulated by LARP4 at the level of translation. Interestingly, genetic screening studies in Drosophila have identified the LARP4 homologue as a regulator of dendrite spine overgrowth and a modifier of SMN mutations. These results suggest that LARP4 is a positive regulator of SMN mRNA translation that is also active in neuronal cell types, providing novel insights into alternative approaches to modulate SMN levels with potential therapeutic application.

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Poster: P.086 | Maria Clara Ferreira de Oliveira Quintas
Astroglial proliferation induced by adenosine is mediated by A2A/2B receptors, coupled to the PKA-ERK pathway and is under control of microglia

Presenter: Clara Quintas | FFUP, LAQV-REQUIMTE
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In the central nervous system, astrocytes and microglia are the main cells coordinating the inflammatory response. During inflammation, dying or temporarily damaged cells release ATP, as a danger-associated molecular pattern, inducing astrogliosis and promoting clearance of the debris by immune cells such as microglia (1). Adenosine, that results from ATP metabolism, also induces astrogliosis (2). However, the effects of adenosine on astrogliosis may be more complex since it also modulates microglia phenotype (3) and microglia have been shown to prevent excessive astroglial proliferation mediate by nucleotides (4). In this context, ATP and adenosine are assumed as relevant signaling molecules in the control of astrogliosis and its modulation by microglia. However, it is still unknown whether and how microglia modulate adenosine-mediated astrogliosis. The present study aims to clarify the impact of microglia in the control of adenosine-induced astrogliosis. Two types of primary glial cultures were prepared from cortical hemispheres of newborn rats (P0-P2): co-cultures of astrocytes containing approximately 10% of microglia, and “pure” cultures of astrocytes, where microglia was almost absent (< 1%). These cultures were used to evaluate the effect of P1 agonists on methyl-[3H]-thymidine incorporation and to evaluate A2 receptors expression either by western blot or immunocytochemistry analysis. In “pure” cultures of astrocytes, adenosine (0.001-0.3 mM) increased astroglial proliferation up to 172 ± 5% (n=7; $P<0.05$), an effect attenuated to 131 ± 5% (n=5; $P<0.05$) by 30 nM SCH 58261, a selective A2A antagonist; and to 125 ± 6% (n=5; $P<0.05$) by 10 nM MRS 1706, a selective A2B antagonist. When selective agonists of P1 receptors were tested, only the A2A agonist CGS 21680 (1-100 nM; up to 155 ± 3%; n=4; $P<0.05$), and the A2B agonist Bay 60-6583 (1-100 nM; up to 167 ± 7%; n=4; $P<0.05$) induced astroglial proliferation; the A1 agonist CPA (1-100 nM) and the A3 agonist 2-Cl-IB-MECA (1-100 nM) had no effect. Furthermore, the proliferative effect of adenosine (100 microM; 179 ± 4%; n=5, $P<0.05$) was attenuated to 107 ± 7% (n=3; $P<0.05$) by inhibition of protein kinase A (PKA) with 1 microM H-89 and to 120 ± 6% (n=4, $P<0.05$) by inhibition of mitogen-activated protein kinase kinase 1/2 (MEK1/2) with 10 microM U0126. In co-cultures, the proliferative effects induced by adenosine, CGS 21680 and Bay 60-6583 (concentrations as above) were lower than those obtained in “pure” cultures: up to 142 ± 8% (n=4; $P<0.05$), to 126 ± 5% (n=4; $P<0.05$) and to 121 ± 2% (n=4, $P<0.05$), respectively. Western blot and immunocytochemistry analysis indicated that A2A receptors are expressed either in pure cultures of astrocytes and in co-cultures being present in both types of cells. The results show that astroglial proliferation induced by adenosine is mediated by A2A and A2B receptors coupled to the intracellular PKA-ERK pathway, an effect attenuated by microglia.

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Poster: P.087 | Maria da Glória Correia da Silva Queiroz
Microglia P2Y13 receptors modulate astrocyte proliferation mediated by P2Y1/12 receptors

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Cerebral inflammation is a common feature of several neurodegenerative diseases contributing to the loss of cognitive functions. It is mediated by astrocytes and microglia that acquire reactive phenotypes to participate in neuronal repair mechanisms (1, 2). During brain inflammation, adenine nucleotides, such as ATP and ADP, are released into the extracellular medium and acting, on P2 receptors, modulate astrogliosis, through mechanisms that involve communication between astrocytes and microglia (1). In previous studies, two models of primary cultures, “pure” astrocyte cultures (with less than 1% of microglia) and co-cultures of astrocytes and microglia (with about 10% microglia), were used to investigate the influence of microglia on astroglial proliferation mediated by the stable nucleotide ADP analogue, ADPβS (P2Y1/12 and P2Y13 receptor agonist). The results indicated that, in astrocyte cultures, ADPβS (0.001-0.3 mM) increased proliferation up to 204±9 % (n=4; P<0.01). This effect seems to be mediated by activation of P2Y1 and, in less extent, by activation of P2Y12 receptors. Interestingly, it was abolished in co-cultures. The loss of the ADPβS-mediated effect could not be explained by a microglia-induced degradation of ADPβS or alterations on cellular localization of P2Y1/12 receptors. Since the proliferative effect of ADPβS in astrocyte cultures was also attenuated in the presence of the supernatant of ADPβS-treated microglial cultures (3), the mechanism involved in the microglia-prevention of the proliferative effect of ADPβS in co-cultures was further investigated, namely the role of P2Y13 receptors. The effect of ADPβS in astroglial proliferation was evaluated by measuring methyl-[3H]-thymidine incorporation and expression of P2Y13 receptors either by western blot or by immunocytochemistry analysis. The results obtained by western blot indicated that P2Y13 receptors are expressed in co-cultures; immunocytochemistry studies showed that they were co-localized mainly with microglia. Furthermore, in co-cultures, simultaneous blockade of P2Y12 and P2Y13 receptors, with the selective antagonists AR-C66096 (10 µM) and MRS 2211 (10 µM), respectively, rescued most of the ADPβS (0.3 mM) proliferative effect, from 119±2 % (n=4; P<0.05) to 186±2% (n=2); an effect closer to that observed in pure astrocyte cultures 204±9 % (n=4; P<0.05). The involvement of IL-1β and/or IL-4 in in microgliaattenuation of astroglial proliferation is a possibility, since IL-1β (3 ng/mL) and IL-4 (1 ng/mL) attenuated the proliferative effect ADPβS (0.3 mM) in astrocyte ultures from 219±11 % (n=4; P<0.01) to 137±5 % (n=4; P<0.05) and 167±5 % (n=4; P<0.05), respectively. The results indicate that microglia P2Y13 receptors control astroglial proliferation mediated by P2Y1/12 receptors and it may involve the release of interleukins (namely IL- 1β and/or IL-4).

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Funding: *MedInUP, Faculty of Pharmacy, University of Porto*

Poster: P.088 | Maria Soares Cachide de Almeida
Genetic risk factors in a dementia subgroup of a primary care-based portuguese cohort

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ApoE is a significant genetic determinant for Alzheimer's disease (AD), the most common form of dementia among the elderly. In particular, the ε4 allele is known as the major genetic risk factor while carriers of ApoE ε2 allele exibited increased longevity and were less prone to be demented. This study was carried out in a primary care-based cohort (pcb-cohort), that includes 590 volunteers, from which 568 met the inclusion criteria. ApoE genotyping of the study population of the pcb-cohort, revealed that the most frequent genotype was ε3ε3 and that about 20% of the population was allele ε4 carriers and 8% was allele ε2 carriers. Further, ApoE allele specific associations with other comorbidities, as the case of cardiovascular disease and diabetes were identified. Other AD genetic variants, namely BIN, CLU and CR1, were likewise addressed in a pcb-cohort dementia subgroup and correlations were explored between ApoE and other chronic conditions. This study addressed the correlation of ApoE, with dementia but also identified additional risk factors in the pcb-cohort. This is particularly relevant since it contributes to characterization of the population at the regional level, allowing for a more preventive medicine.

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Poster: P.089 | Mariana Temido Mendes Ferreira
Reversion of age-related changes in long-term depression by adenosine A2A receptor blockade

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Aging and Alzheimer's disease (AD) are associated with hippocampal alterations and cognitive impairments. Such deficits are connected with a hippocampal upsurge in adenosine A2A receptors (A2AR). Epidemiological data showed that regular caffeine consumption, a non-selective A2AR antagonist, attenuates memory disruption during aging and decreases the risk of developing memory impairments in AD (van Boxtel et al, 2003, Pharmacol. Biochem. Behav.; Hameleers et al, 2000, Hum. Psychopharmacol. Clin. Exp.; Ritchie et al, 2007, Neurology). Blocking A2AR in aging and AD models prevents, or even reverts, hippocampus-related impairments, namely long-term potentiation (LTP) (Batalha et al, 2013, Mol. Psychiatry; Laurent et al, 2015, Mol. Psychiatry; Vieira da Silva S, 2016, Nat Comm). Consequently, hippocampal A2AR function dysregulation may drive some detrimental processes leading to aging and AD. However, the effect on long-term depression (LTD) has not been addressed so far. We performed field extracellular recordings in CA1 hippocampal neurons from transgenic rats with a neuronal-specific A2AR overexpression in forebrain areas [tg(CaMKII-hA2AR)] versus wildtype animals. A low frequency stimulation train (LFS) failed to induce long-term depression (LTD) in tg(CAMKII-hA2AR) animals (n=6; $P<0.05$). This LTD shift was due to a NMDAR differential activation, since a complete rescue to WT levels was achieved with AP5 perfusion, 50 µM (n=3-7; $P<0.05$). A2AR acute blockade (SCH 58261, 50 nM, and caffeine, 30 µM) abolished this shift in LTD (n=3-6; $P<0.05$). Furthermore, chronic blockade (KW6002, 5 mg/kg/day) not only rescued LTD shift but also reverted the NMDAR differential activation observed in tg(CaMKII-hA2AR) animals (n=3-6; $P<0.05$). Since A2AR are overexpressed upon aging and present increased ability to facilitate synaptic transmission (Lopes et al, 1999; Rebola et al, 2003, J. Neurophysiol.), we performed behavioral and electrophysiological experiments and compared aged (18 months-old) versus young (3-4 months old) rats. In fact, aged animals presented cognitive and memory deficits, since in the Y-maze test the preference for the novel arm was lost (n=10, $P<0.05$), and in the Morris Water Maze test a decrease in the acquisition was observed (n=8-10, $P<0.05$). In terms of synaptic plasticity, when LFS is applied, lack of LTD induction is observed in aged animals, while in young animals a significant LTD was obtained (n=5, $P<0.05$). Importantly, acute blockade with SCH 58261, 50 nM, in aged animals partially rescued the lack of LTD back to young animals values (n=5, $P<0.05$), further emphasizing A2AR as a central mediator of synaptic dysfunction observed in aging. Altogether, these data show that A2AR overexpression causes NMDAR overactivation leading to LTD disruption, as observed in AD and in models of aging, strongly suggesting that A2AR overexpression is a key event in AD- and age-associated glutamatergic synaptic dysfunction.

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Poster: P.090 | Marlene Cristina Faria Pereira
Impact of astrocytes on hippocampal synaptic plasticity

Presenter: Marlene C. Pereira | CNC, University of Coimbra
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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 element of synapses. Thus, astrocytes release and uptake gliotransmitters within the synaptic cleft to fine-tune synaptic transmission1,2. The present study aims to evaluate the impact of astrocytes on synaptic plasticity in hippocampal circuits to later understand the role of astrocytes to control memory deficits in pathological conditions, such as in Alzheimer's disease. To silence astrocytic contribution in synaptic plasticity we used three gliotoxins previously described as pharmacological tools to blunt astrocytic function3,4: i) L-α-aminoadipate (L-AA) that inhibits glutamine synthetase; ii) trifluoroacetate (TFA), which is able to interfere with the TCA cycle; iii) dihydrokainate (DHK), glial glutamate transporter-1 inhibitor. Electrophysiological recordings of synaptic plasticity in Schaffer fiber-CA1 pyramid synapses were performed in hippocampal slices from adult C57/Bl6 mice, by applying a high frequency stimulation (HFS, 100 Hz/1s) to induce long-term potentiation (LTP)5. Exposure to L-AA (100 µM, 2h) had no effect on basal synaptic transmission, but decreased LTP amplitude by 35.3±6.8% ($p<0.05$; n=9), when compared to control hippocampal slices (not exposed to L-AA). Another gliotoxin, TFA (100 µM, 2h) also decreased LTP amplitude (31.8±6.1% lower than control, n=3; $p<0.05$) and occluded the effect of L-AA on LTP (in the presence of L-AA, TFA was not able to alter LTP amplitude when compared with L-AA per se, n=3). Likewise, DHK (15 µM, 40min) also reduced LTP amplitude (25.1±6.5% lower than control, n=4; $p<0.05$) and tended to occlude the effect of L-AA on LTP. Altogether, these data suggest that L-AA, by silencing astrocytes, decreases hippocampal synaptic plasticity, which might have impact in memory function and play a role in memory deficits in pathological conditions such as Alzheimer's disease.

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Poster: P.091 | Marta Daniela Araujo Costa

Genetic anti-aging manipulations differentially modify disease manifestations and protein aggregation in a Caernohabditis elegans model of Machado-Joseph disease

Presenter: Marta D. Costa | ICVS, University of Minho
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Machado-Joseph disease (MJD) is an autosomal dominant inherited neurodegenerative disorder caused by a CAG expansion in the causative gene ataxin-3. This genetic alteration gives rise to a protein harboring an expanded polyglutamine (polyQ) segment, which tends to aggregate in specific neuronal populations. In patients, despite the ubiquitous expression of expanded ATXN3 since birth it only leads to neuronal toxicity late in life, an evidence that puts the aging phenomenon as a relevant risk factor for the development of this disease. To understand in more detail the role of aging in onset and progression of MJD, we are currently manipulating aging through genetic alterations in conserved pathways and comparing the impact of this action in important features of MJD modelled in C. elegans, including motor performance and neuronal mutant ATXN3 aggregation. Such longevity-determining pathways include the insulin/IGF-1 receptor and mTOR signaling pathways, and modification of mitochondrial function. Here we demonstrate that the majority of lifespan-increasing mutations reduced mutant ATXN3(mATXN3) aggregates in neurons, although at different timepoints of animals' adulthood, the exception being the dietary restriction-causing mutation in eat-2, which increased mATXN3 neuronal aggregation along aging. Moreover, not all genetic modifications that delay aging were able to ameliorate the motor phenotype of adult animals. These results suggest that different mechanisms can be recruited when life span is extended, with differential impact in MJD pathogenesis. Transcriptomic analysis of the single and double mutants revealed the importance of the activation of Skn-1/Nrf-2 stress response pathway in the nervous system and the consequential modulation of oxidative stress, as part of the disease-modifying process. In a disease that remains without an effective cure, this kind of approach can contribute not only with novel knowledge about the pathogenic mechanism of this disease but most importantly, it can reveal potential therapeutic targets.

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Poster: P.092 | Marta Isabel da Silva Rodrigues Barbosa

Recovery of miR-146a levels through overexpression reverts astrocyte reactivity in cells from ALS mice and modulate astrocytic exosome cargo

Presenter: Marta Barbosa | iMed-UL

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Amyotrophic Lateral Sclerosis (ALS) is a disease characterized by the loss of motor neurons (MNs) from the motor cortex and spinal cord, although it is still not clear where the disease begins and disseminates (1). In addition, glial cells are determinant for disease progression, and recent data from our group point out that astrocytes from cortical brain of mice pups (7 days) expressing the G93A mutation in human superoxide dismutase 1 (mSOD1) have an aberrant/reactive phenotype profile characterized by SOD1 high/GFAP low/S100B high/Glutamate transporters low/Ki-67 high/vimentin high/miRNA-146a low (unpublished data). These astrocytes revealed neurotoxic properties when co-cultured with NSC34-like MNs. Such neurotoxic potential may be exerted by astrocyte-derived exosomes. Therefore, this work aimed to evaluate whether the recovery of the miR-146a expression (i) will impact on the astrocyte reactivity and (ii) on their exosome miRNA profiling. For that, we used astrocytes isolated from the cortical brain of mSOD1 mice pups and cultured for 13 days. Cell were then modulated for miR-146a expression by using pre-miR-146a (mSOD1 astrocytes pre-146a). Non-transgenic littermates (wt) were used as controls. Exosomes were isolated by differential ultracentrifugation. Isolated mSOD1 astrocytes showed increased levels of S100B and connexin-43 (Cx43) gene expression, as well as reduced ones GFAP mRNA and protein levels, thus corroborating their aberrancy. We identified decreased levels of miRNA-21 and unchanged alterations of miR-155 in these astrocytes, as compared with wt astrocytes. After successfully increase of miR-146a expression in mSOD1 astrocytespre-146a, we were able to observe a reduction on both miR-155 and miR-21 expression. In addition, in mSOD1 astrocytes pre-146a we obtained a reduction of S100B and Cx43 mRNA levels, as well as increased levels of GFAP protein, thus reverting, at least in part, the aberrancy of mSOD1 astrocytes. Remarkably, astrocyte-derived exosomes showed to recapitulate the miRNA expression pattern of the modulated mSOD1 astrocytes pre-146a, e.g. miR-155 downregulation and miR-146a upregulation relatively to their untreated counterparts. Overall, our results highlight the influence of miR-146a upregulation in reversing astrocyte aberrant phenotype. Therefore, miR-146a may be a potential target to driven therapies. Furthermore, modulatory intervention in astrocytes was able to also trigger an increase of exosomal content in miR-146a, which may have a broader impact in preventing, halting or even reverting the harmful effects of astrocytes on MNs in ALS disease.

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Poster: P.093 | Nádia Raquel Henriques Rei

Expression of vegf and vegf receptors in amyotrophic lateral sclerosis (als) mice model at different disease stages

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the progressive degeneration of motor neurons in corticospinal tract, leading to muscle weakness, atrophy, paralysis and death. Recent studies support that vascular endothelial growth factor (VEGF) has a neuroprotective function, protecting motor neurons from degeneration in ALS models(1). VEGFA binds both VEGF-R1 and VEGF-R2, but with more affinity to VEGF-R1, which may negatively regulate VEGF function, preventing its binding to VEGF-R2; VEGFB binds only to VEGF-R1(2). We now evaluated if there is any changes in the levels of VEGF molecules (VEGFA and VEGFB), and VEGF receptors (VEGF-R1 and VEGF-R2) at the cerebral cortex (CTX) and spinal cord (SC) of a model of ALS, SOD1(G93A) mice. A qReal-time PCR was used to evaluate mRNA levels (n=3-7) and an ELISA assay (n=4-6) was performed to evaluate the expression of VEGF and VEGF receptors protein levels. Pre-symptomatic (4-6 weeks old) and symptomatic (12-14 weeks old) mice were analysed and data compared with age-matched wild type mice. Significant differences were considered at $p<0.05$. Concerning mRNA expression of VEGF and its receptors, at the CTX of pre-symptomatic mice, there was an increase in the expression of mRNA levels for VEGFA and VEGFB with no significant change in the expression of mRNA for VEGF receptors. Interestingly, in CTX of symptomatic mice there was a decrease of the mRNA expression for VEGFA and VEGF-R2 and an increase of VEGFB. At the SC of pre-symptomatic mice there was an increase in mRNA levels of VEGF-R1 and VEGFB and a decrease of mRNA levels of VEGFA and VEGF-R2 in the SC of symptomatic mice. Regarding the protein levels, there are no significant changes for VEGFA expression at both, CTX and SC and there is a significant increase of VEGFB at SC of pre-symptomatic mice. Concerning the VEGF receptors, there is an increase at SC of pre-symptomatic mice of both receptors (VEGF-R1 and VEGF-R2) and a decrease at CTX of symptomatic mice. Overall, the data suggest an early dysfunction of VEGF-mediated mechanisms, with an enhanced expression at pre-symptomatic stage, which reverses to a decrease at the symptomatic stage.

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Poster: P.094 | Neide Marina Vieira Pereira
Exploring Sorting Nexin 27 role in Pain

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Sorting nexins (SNXs) are a large family of phosphoinositide-binding proteins that play fundamental roles in orchestrating cargo sorting through the endosomal network. SNX27 is one of the most studied members and has a well-established role in glutamate receptors trafficking regulation [1,2]. Glutamatergic transmission is critical for nociception and underlies the onset and maintenance of hypersensitivity in chronic pain conditions [3] but surprisingly, no previous study assessed SNX27 role in pain. Based on this rationale we studied the manifestation of pain in a mice model with reduced expression of SNX27. Male SNX27 -/+ mice and littermate controls were used to assess pain-related behaviors in models of acute noxious thermal stimulus (Hargreave's test), tonic inflammatory stimulus (formalin) and chronic neuropathic pain (spared nerve injury, SNI). No differences were found between genotypes regarding the threshold to noxious heat, in the acute phase of the formalin test, or regarding chronic neuropathic pain, in the SNI model. However, Snx27-/+mouse model displayed significantly less pain-related behaviors in the tonic phase of the formalin response suggesting decreased sensitization at the spinal dorsal horn level. In conclusion, while having no evident role in the conduction of the nociceptive information, Snx27 appears to be a fundamental element of the biochemical cascaded mediating the emergence of hyperalgesia. The knowledge generated in this work is expected to contribute to the understanding of SNX27 role in pain, particularly, its impact on synaptic plasticity in nociception, and unravel new pathways of intervention for pain management.

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Poster: P.095 | Pedro Filipe Marques Pascoal
S100B-RAGE axis is increased in Experimental Autoimmune Encephalomyelitis and reduced upon dimethyl fumarate treatment

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Multiple sclerosis (MS) is an autoimmune disease with strong inflammatory and neurodegenerative components, characterized by severe effects on motor and cognitive functions. Accordingly, most of the disease-modifying drugs used in MS therapeutics, as dimethyl fumarate (DMF), have been used to reduce inflammation and neurotoxicity. S100B is mostly expressed by astrocytes and act as a signaling molecule through engagement of the receptor for advanced glycation end-products (RAGE). S100B exerts beneficial or detrimental effects in a concentration-dependent manner. We recently detected high S100B levels in the CSF and serum of MS patients, accompanied by increased expression of both S100B and RAGE in MS lesions of post-mortem brain samples. Using a demyelination ex vivo model, we also detected a massive S100B expression upon demyelination, which was involved in the observed increased inflammation and glial reactivity, as well as impaired neuronal function and oligodendrocyte maturation. Here, we wanted to evaluate (1) the expression of S100B and RAGE in an in vivo MS model, the Experimental Autoimmune Encephalomyelitis (EAE) and (2) to understand if DMF is able to alter the expression of this axis. Brain slices (20 µm) of the EAE-induced model treated in the presence or absence of DMF were stained with hematoxylin in order to assess overall histology, and with Luxol to evaluate the degree of axon demyelination. Additionally, it was evaluated the expression of astrocytes (GFAP), S100B and RAGE by immunohistochemistry in three most affected regions: (I) fimbria (II) internal capsule and (III) perivascular zone. Induced-EAE presented a great loss of myelin that was reduced after DMF treatment. We observed an overall increase of S100B and GFAP ($p< 0.05$) in the EAE animals with no differences between the different zones studied, that decreased after DMF treatment by more than 25%. Interestingly, when we evaluated the density of astrocytes expressing S100B we saw a high number in EAE animals, namely in region II and III, that was reverted by DMF treatment. RAGE staining was also increased in EAE animals ($p<0.05$) with a most intense expression in regions II and III, while DMF treated group showed RAGE levels comparable to control ones ($p<0.01$). Overall, our results showed that EAE animals highly express S100B and RAGE, and DMF counteracts the expression of both proteins in parallel to the reduced loss of myelin fibers. This suggests that S100B-RAGE may be a new and more specific target for MS therapeutic intervention.

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Poster: P.096 | Ricardo José da Silva Magalhães
The dynamics of stress: a longitudinal MRI study of brain structure and connectome

Presenter: Ricardo Magalhães | ICVS, University of Minho

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Stress is a well-established trigger factor for several neuropsychiatric disorders, as it alters the structure and the function of the central nervous system. Herein, we conduct a transversal and a longitudinal neuroimaging assessment of the impact of the exposure to stress in the structure and in the functional connectome of the rat brain. Using a cluster segmentation based on endocrine and behavioral outcomes, animals were classified in high- and low-responders to stress. Our results show that stress increases the functional connectivity of a complex neuronal network, whose principal nodes include brainstem areas, sub-cortical regions and anterior cortical areas . When analyzing the contrast between high- and low-stress responders, we could discriminate the role of thalamic and subicular/entorhinal nodes as critical for determining resilience to stress exposure. Moreover, the comparison of acute- versus chronic stress exposure displayed a contrasting pattern of changes in the functional connectivity. Using a voxel based morphometry approach we found similar patterns of structural alterations. Finally, our results reveal important networks and nodes for predicting the response to stress that are critical for determining individual's resilience/vulnerability to stressful conditions.

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Poster: P.097 | Rita Alexandra Figueira Belo
Exploring Kyotorphin and Amidated-Kyotorphin actions upon synaptic transmission and calpain and caspases activation

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Kyotorphin (KTP) is an endogenous dipeptide - L-tyrosyl-L-arginine, initially described as a powerful analgesic (1). Recently, it was shown that Alzheimer´s disease (AD) patients have decreased levels of KTP in cerebrospinal fluid (2). This opened a new line of research focused on KTP as a possible drug to treat AD patients. Given the KTP inability of crossing the blood-brain barrier, an amidated form, the amidated-kyotorphin (KTP-NH2) was produced (3). Recent evidences showed that administration of KTP-NH2 prevents memory deficits in an AD model by an unknown mechanism of action (4). To better understand the mechanism of actions of KTP and KTP-NH2, we studied its actions upon hippocampal synaptic transmission and upon calpains and caspases activation, enzymes usually overactivated in neurodegenerative disorders. Field-excitatory post-synaptic potentials (fEPSP) were recorded from the CA1 area of hippocampal slices taken from 10-17 week old C57BL/6J mice. The effect of increasing concentrations (5nM, 50nM, 500nM, 5µM, 50µM, and 5mM) of KTP and KTP-NH2 were evaluated by percentage of change (mean±SEM %) of fEPSP amplitude when compared to the baseline. Calpains and caspases activation were evaluated through the analysis of the αII-spectrin protein breakdown product by western-blot in primary neuronal cultures (DIV 14) incubated with KTP and KTP-NH2 (0.5nM, 1nM, 5nM, 50nM, and 5µM) for 24 hours. The data was analysed by the one-way ANOVA test corrected with the Dunnett test. KTP induced a concentration/effect bell-shape curve, and a statistical effect was achieved at 50nM (11.9 ± 3.0 % increase on the fEPSP amplitude compared to the baseline, n=6). On the contrary, KTP-NH2 induced a constant decay on fEPSP amplitude, and a statistical effect was achieved at 50µM (27.9 ± 8.1 % decrease on the fEPSP amplitude, n=5) and at 5mM (53.0 ± 6.9 % decrease on the fEPSP amplitude, n=5). Both peptides severely compromised the synaptic transmission at 5mM, being this effect partially recovered by the KTP or KTP-NH2 washout. Moreover, the product levels generated by the αII-spectrin protein breakdown, which are related with caspases and calpaines activation, do not showed any statistically differences between conditions (n=3-5). In sum, our data show that KTP and KTP-NH2 have opposite effects on hippocampal synaptic transmission which might reflect different mechanisms of action. Moreover, neither KTP nor KTP-NH2 significantly affect the activation of both caspases and calpains.

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Poster: P.098 | Sandra Isabel Nogueira Tenreiro
Synucleins levels and distribution are altered in mouse retinal neurons with ageing and diabetic retinopathy

Presenter: Sandra Tenreiro | CEDOC -NMS |UNL
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Alpha-synuclein (aSyn) misfolding and aggregation is a hallmark of Parkinson's disease (PD), the most common neurodegenerative movement disorder. Recently other synuclein (Syn) members, beta-(bSyn) and gamma-synuclein (gSyn) were associated with pathological mechanisms. Interestingly, deletion of all three Syn genes in mice results in age-dependent retinal dysfunction and blindness. All Syn members are expressed in the synapse-rich layers of the retina. Moreover, inclusions positive for a, b, and gSyn were found in retina from patients with different neurodegenerative diseases. However, the physiological and the pathological roles of the Syn proteins in the retina are completely unexplored. Retinal neurodegeneration was recently found to occur in PD and in early stages of Diabetic retinopathy (DR), the leading cause of irreversible vision loss in working-age adults. Evidences point to a common pathophysiology in the visual impairment of PD patients and in DR. Disruption of the dopaminergic system was observed in both diseases and may result from loss of dopaminergic amacrine cells in the retina. Dopamine-restoring treatments improve visual symptoms in both PD and diabetic models. However, the underlying molecular mechanism for this effect is not clear. Here, we hypothesize that retinal neurodegeneration in PD and DR could involve both i) loss of Syn function and ii) gain of toxic function due to the formation of Syn oligomers. Using mouse models we are performing a detailed characterization of a, b, and gSyn levels and distribution in the different neuronal populations of the retina, and how they are affected by ageing and diabetic retinopathy progression. Our preliminary work indicates that aSyn is mainly localized in the inner plexiform layer. With aging and diabetes progression, aSyn is also observed in neuronal cell somas at the inner nuclear layer and at the ganglion cell layer. aSyn protein levels tend to decrease in the retina with aging, but are increased in the retina of a mouse model of diabetes. bSyn presents a broader distribution in the inner nuclear and plexiform layers as well as in the ganglion cell layer. Finally, gSyn is mainly localised in the ganglion cell layer but is also present in the inner nuclear layer. Interestingly, with ageing and diabetes, the colocalisation of aSyn with bSyn or gSyn increased. In conclusion, our preliminary data opens novel research directions in the search for novel targets for therapeutic intervention for these complex disorders.

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Poster: P.099 | Sara Carina Duarte da Silva
Neuroinflammation assessment in a transgenic mouse model of Machado-Joseph disease

Presenter: Sara Duarte-Silva | ICVS, University of Minho

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Machado-Joseph disease (MJD), also known as spinocerebellar ataxia type 3, is a genetically determined neurodegenerative disease of adult onset, caused by expansion of a polyglutamine (PolyQ) tract within the protein ataxin-3. Astrogliosis is observed in MJD patients' brains post-mortem, but it has classically been interpreted as a reaction to neuronal demise. Our goal in this study was to determine the contribution of glial cells for disease initiation and progression in MJD, using a well characterized animal model of the disease, the CMVMJD135 transgenic mouse. We found a region-specific astrocytic pathology in symptomatic mice (34 weeks of age): while in the substantia nigra and spinal cord (two affected regions) a classical astrocytic reactivity was observed, in the pontine nuclei (another affected region) we found a general hypotrophy of the astrocytes. Nevertheless, we found no differences in the expression levels of the glutamate transporter EEAT2 in the brainstem and spinal cord suggesting functional astrocytes. Expression analysis in young symptomatic mice (22 weeks) of inflammation-related molecules revealed an up-regulation of anti-inflammatory molecules such as arginase-1 and Il4. Interestingly, as the disease progresses, we observed a shift towards a mixed profile: in 34 weeks-old animals, an up-regulation of Tnfa, Il1b, Ccl2, CD86 and Il10 (pro and anti-inflammatory cytokines) was detected in the brainstem and spinal cord of these mice. Intriguingly, the expression levels of arginase 1 and peroxiredoxin-2, anti-inflammatory-related enzymes, were significantly decreased at this age. No differences were found in other inflammation-related molecules such as Il-6, iNOS, Cxcl10, Cxcl12, Cxcl14, Cxcl17, Il4, Tgfb1, Cx3cr1, Mac2, MHCII and Nfkb1. Altogether, these results point to the importance of glial cells in the pathogenesis of MJD, and suggests a bi-modal pattern of neuroinflammation in this disorder.

***Funding**: FCT, NAF, Ataxia UK*

Poster: P.100 | Sara Francisca Ramalhosa Guerreiro
A Novel Role for Tau Protein in Mood and Cognitive Deficits Induced by a Peripheral Neuropathy

Presenter: Sara Guerreiro | ICVS, University of Minho

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Peripheral neuropathic pain is known to induce maladaptive brain plasticity associated with memory decline and other behavioural deficits. Tau is a cytoskeletal protein involved in neuroplastic and signalling events related to neuronal function and malfunction. Tau hyperphosphorylation is causally related to different brain pathologies such as Alzheimer's disease and chronic stress suggesting that it can also be a key player on chronic pain associated deficits. To test this hypothesis, Tau-KO and wild-type animals with a right peripheral neuropathy (spared nerve injury model, SNI) were tested in a battery of behavioural paradigms for mood and cognition, 6 and 12 weeks after surgery; sham-operated animals were used as controls. Hippocampal tissue was dissected and Tau phosphorylation profile was analysed by western blot. We found that anxious behaviour was absent in both groups at any time point. However, memory deficits and depressive-like behaviour were found in WT mice, but not TauKO, 12 weeks after SNI surgery. At the molecular level, SNI-operated WT animals showed Tau accumulation in the cytoplasmic fraction followed by Tau hyperphosphorylation in different epitopes suggesting Tau involvement in brain damage provoked by peripheral neuropathy. As Tau accumulation and hyperphosphorylation is causally related to neuronal malfunction and cognitive deficits, our findings suggest the potential role of Tau and its malfunction in the SNI-driven memory and mood deficits. These findings open a novel window of understanding the potential mechanisms through which peripheral neuropathy driven by SNI causes neuronal damage and cognitive deficits.

Poster: P.101 | Sara Luísa Ramalho Tanqueiro
Inhibition of extrasynaptic NMDA receptors prevents the loss of BDNF function induced by amyloid β

Presenter: Sara Tanqueiro | Instituto de Medicina Molecular
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The brain-derived neurotrophic factor (BDNF) plays important functions in cell survival, neuronal outgrowth, differentiation and plasticity. In Alzheimer's disease (AD) BDNF signaling is known to be impaired, which is in part due to the amyloid β (Aβ)-induced truncation of its main receptor, TrkB-full length (TrkB-FL), by calpains. This results in the formation of an intracellular domain (ICD) fragment and in the BDNF loss of function (1,2). Since calpains are Ca2+-dependent proteases, we hypothesized that N-methyl-d-aspartate receptors (NMDARs), in particular extrasynaptic receptors (eNMDARs) are a source of the increased intracellular levels Ca2+ that induce their activation. Indeed, eNMDARs are involved in cell death signaling pathways and are known to be over activated in AD (3). To test our hypothesis, we investigated if the prevention of eNMDAR activation in primary rat neurons or hippocampal slices exposed to Aβ could inhibit the truncation of TrkB-FL by calpains, restoring the functions of BDNF upon synapses. Our results showed that the inhibitor of eNMDAR, memantine, reduces significantly the activation of calpains, leading to an increase in TrkB-FL levels and a decrease in ICD levels. Importantly, the inhibition of eNMDAR prevented the loss of BDNF actions on the number of dendritic spines in the presence of Aβ. Finally, the inhibition of eNMDAR reestablished the capacity of BDNF to enhance long-term potentiation in hippocampal slices, the physiological basis for learning and memory. These findings highlight the functional consequence of the Aβ-induced cleavage of TrkB-FL receptors and point eNMDAR activation as a source of increased Ca2+ concentration that is involved in the truncation of TrkB-FL receptors.

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***Funding**: Work supported by FCT. BDNF was a gift from Regeneron.*

Poster: P.102 | Sofia Pereira das Neves
Thymic alterations in MOG-induced experimental autoimmune encephalomyelitis

Presenter: Sofia Neves | ICVS, University of Minho
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The experimental autoimmune encephalomyelitis (EAE) model is the most widely used to study multiple sclerosis (MS) [1-3]. In MS, auto-reactive T cells react against the myelin sheath, ultimately leading to demyelination [4-6]. The thymus, via negative selection and maturation of regulatory T cells, plays an important role in preventing auto-immunity [7,8]. For that reason, in this work, besides studying a widely-affected area of the central nervous system, the cerebellum, we also explored the alterations occurring in the thymus throughout disease development. We induced EAE in female mice, and sacrificed them on days 16 (onset phase) and 23 (chronic phase) post-disease induction. At the onset phase of disease, EAE animals presented an increased percentage of area occupied by demyelinating lesions and inflammatory infiltrates, and increased expression levels of T helper (Th) 1 and Th17 cytokines, compared to non-induced animals and EAE animals at the chronic phase of disease. Regarding thymic alterations, it was possible to observe thymic atrophy in both disease timepoints. In addition, at the onset phase of disease, we observed a significant decrease in the percentage of CD4+CD8+ thymocytes and an increase in the CD4 and CD8 single positive populations; an increase in the medullary/cortical ratio; and an increase in the expression levels of genes important for T cell maturation, namely Interleukin 7. On the other hand, during the chronic phase of disease, the thymi of EAE animals were similar to the thymi of non-induced animals. This work contributed to improve the knowledge about some pathological alterations occurring in the cerebellum and the thymus during EAE development, and showed that several of these alterations can be reverted to almost control levels, at later stages of the disease, even in a chronic model of MS.

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Poster: P.103 | Sónia Andreia Silva Puga
Mercury contamination alters the season-dependent variation in brain morphology of wild fish (Liza aurata)

Presenter: Sónia Puga | ICVS, University of Minho

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Mercury (Hg) is a potent neurotoxicant that induces important adverse effects on fish brain morphology in controlled laboratory studies. Noteworthy, fish cope with social and environmental challenges by adapting their physiology and behaviour, which is supported and influenced by neural plasticity. Yet, a deeper understanding is lacking regarding how the environmental exposure to Hg contamination affects the neural plasticity of wild fish in specific brain regions. In this work, we evaluated the relative volume and cell density of the lateral pallium, hypothalamus, optic tectum and molecular layer of the cerebellum on wild Liza aurata captured in Hg-contaminated (LAR) or non-contaminated (SJ) sites of a coastal system (Ria de Aveiro, Portugal). Given the seasonal variations in the environment that wild fish are naturally exposed, this assessment was performed in the winter and summer. In fish from the SJ site, the lateral pallium relative volume and the cell density of the hypothalamus and optic tectum were higher in the winter than in summer. Thus, season-related stimuli strongly influenced the size and/or cell density of specific brain regions in the non-contaminated environment, pointing out the ability of fish to adapt to environmental and physiological demands. Conversely, Hg contamination induced a different profile of seasonal variations in brain morphology; LAR fish presented a larger optic tectum in the summer, as well as a larger molecular layer of the cerebellum with higher cell density. Moreover, Hg exposure impaired the winter-summer variation of the lateral pallium relative size, and triggered a deficit in cell density of hypothalamus during the winter, showing therefore a region-specific susceptibility to Hg. Altogether, seasonal variations in fish neural morphology and physiology should be considered when performing ecotoxicological studies in order to better discriminate the Hg influence.

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Poster: P.104 | Tânia Cristina Soares Martins
Phosphoprotein profiles triggered by OA involved in neuronal death and cytoskeletal organization

Presenter: Tânia Martins | iBiMED - University of Aveiro
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Abnormal protein phosphorylation is an event linked to many diseases, including Alzheimer's disease (AD). AD is histopathologically characterized by the presence of senile plaques and neurofibrillary tangles as a result of Tau hyperphosphorylation; both hallmarks contribute to neuronal degeneration and death. Okadaic acid is a neurotoxin capable of inhibiting protein phosphatases like PP1, PP2A, involved in Tau dephosphorylation. Thus OA represents a great model to address phosphorylation dependent processes. The cellular and molecular mechanisms involved in OA induced neuronal toxicity are not completely understood. In this study OA was added to primary cortical neurons, phosphoproteins extracted and MS/MS analysis carried out followed by a bioinformatic approach. Particular emphasis was given to the cell death and cytoskeletal related processes, as they are central processes modulated by phosphorylation. Apoptosis is a key event in many neurodegenerative diseases, including AD, as well as cytoskeletal alterations, which by themselves can lead to neuronal death. Results showed that 245 phosphoproteins were significantly increased and 75 significantly decreased in response to OA. Unraveling the cellular and molecular mechanisms involved in OA-induced neurotoxicity will aid in the development of novel therapeutic strategies for diseases where PP inhibition is a key hallmark.

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Poster: P.105 | Tatiana Marisa Andrade Burrinha
Why is Aging the major risk factor for Aβ accumulation and AD development?

Presenter: Tatiana Burrinha | CEDOC
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Age-related cognitive decline increases the risk of neurodegenerative diseases, such as Alzheimer's disease (AD). Neurons of the aging brain show synaptic decline by unknown mechanisms. β-Amyloid (Aβ) is a primary trigger in AD. Exacerbated accumulation/oligomerization Aβ impacts synaptic function, with eventual loss of synapses early on before the onset of the disease. Aβ can accumulate in the "normal" aging human brain and in aged mice. We hypothesize that aging-driven alterations of cellular homeostasis could potentiate Aβ production and thus cause synaptic decline. Aβ is produced upon the endocytosis of amyloid precursor protein (APP) and subsequent cleavage by β- and γ-secretases. We used primary neuronal cultures that undergo a stereotyped process of differentiation, maturation and aging in 28 days in vitro (DIV) to study neuronal aging alterations. At 28DIV, neurons show early signs of aging like lipofuscin accumulation and we found that intracellular Aβ rises due to higher APP processing. We have started to uncover the yet unknown mechanisms that underlie Aβ rise with aging. Defects in endocytosis had been previously described for transferrin receptor at 28 DIV. We started investigating APP trafficking by measuring APP endocytosis. Unexpectedly, we found an enhancement of APP endocytosis in aged neurons. Accordingly, we found early endosomes to be enlarged and lysosomes to be more distal, suggestive of endosomal abnormalities, that we are currently investigating. Although the cellular levels of APP are unchanged in older neurons, APP localization changes with aging becoming more present at the neurites. Importantly, aged neurons with increased Aβ show synaptic decline with prominent loss of spines. We thus asked if this synaptic decline could be reversed by inhibition of Aβ production. We found that both γ- and β-secretase inhibition can partially rescue aging-synaptic decline. Overall our data indicates that an increase in APP endocytosis is responsible for the aging accumulation of Aβ which contributes to aging-synaptic decline.

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Poster: P.106 | Teresa de Jesus da Costa Castanho
Validating use of rapid testing through technology and informant reports for cognitive assessment in aging: an holistic overview

Presenter: Teresa Castanho | ICVS, University of Minho

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To provide feasible and rapid assessment tools to assist in cognitive diagnosis in research and clinical practice, as well as to establish thresholds for cognitive impairment, adapted to the Portuguese context, the applicability of rapid cognitive screening and informant instruments (TICSM-PT and IQCODE respectively) was explored. Results indicate that the TICSM-PT presents associations not only with global cognitive measures, but also with a number of cognitive and psychological instruments performed in-person. Moreover, it demonstrated to be a practical tool for rapid cognitive assessment, and a valid method of screening cognition by telephone. Using a different technological approach, but the same instrument, it was further demonstrated that is possible to carry out accurate and reliable cognitive assessments using videoconference. Findings indicate that the videoconference administration method yields comparable results to the traditional face-to-face administration and supports the hypothesis of good acceptability among the older participants. Finally, results also indicate that the IQCODE, when applied in community-dwelling older individuals, may not be an informative tool due to the lack of variability in the responses of informants. Although family members or close companions remain important sentinels in helping in the detection of cognitive impairment, herein the results raise important concerns, regarding their strengths and weaknesses.

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Poster: P.107 | Vitor Manuel da Silva Ferreira
Immune profile characterization of an Alzheimer's disease mice model

Presenter: Vitor Ferreira | ICVS, University of Minho
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Alzheimer's disease (AD) is known as the most common neurodegenerative disorder, corresponding of 60 to 80% of dementia cases around the world. Memory impairments are the principal complain of AD patients with a huge impact on the society, in a variety of personal and professional dimensions.(1) The cognitive impairments developed are believed to be due to two hallmarks of the disease: the production and accumulation of amyloid-β (Aβ), in neuritic or amyloid plaques, as well as, the accumulation of an abnormal hyperphosphorylated form of microtubule associated tau protein in neurofibrillary plaques or tangles, outside and inside the neurons, respectively. Although the irrevocably importance of these proteins in AD appearance and progression, it is already known that AD is the result of several pathological factors, namely neuroinflammatory responses.(2) Despite the importance of the inflammatory process on AD development, as well as, the prevalence of some leukocytes populations and peripheral organs on amyloid-beta (Aβ) clearance; the impact of peripheral immune cells in AD is not so well assessed. Therefore, in this work we aim to characterize the immune peripheral alterations, in blood and peripheral organs (thymus and spleen), on the J20 AD-mice model by flow cytometry at the age of 1, 3 and 10 months old (MO). Our results show differences in adaptive immune leukocyte subpopulations in the thymus and blood between the J20 AD mice model and their littermate controls. Herein we showed, a decrease in the CD4+CD8+ cells (at 3MO) with an increase in the CD4+CD3+ and CD8+CD3+ cells, at both 1 and 3MO in the thymus of J20 mice when compared with the age-matched controls. Additionally, at the blood we showed, at 3MO, an increase of CD8+CD3+ cells in the J20 animals. Although no differences were found at the different immune cell populations at the spleen, when spleenocytes from J20 and control animals were stimulated with concanavalin-A the J20 animals seems to have less production of IFN-γ. In the future more studies are needed to clarify if these alterations in the peripheral immune cell populations are playing a role in the Aβ clearance from the brain or if they are a consequence of AD pathology.

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Poster: P.108 | Pedro Elói Antunes Dionísio
Microglial Parkin at the crossroads between neuroinflammation and necroptosis

Presenter: Pedro Dionísio | iMed.Ulisboa, FFUP
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Necroptosis is a regulated form of cell death that depends on the kinase activity of receptor interacting protein 3 (RIP3) and RIP1, being negatively regulated by caspase-8 activity. Parkin is an E3 ubiquitin ligase whose activity is downregulated in Parkinson's disease (PD). Recently, Parkin was described to be a player in linear ubiquitination events, which are involved in the modulation of several signaling pathways that regulate inflammation and necroptosis. Moreover, Parkin was also implicated in inflammation, a mechanism involved in necroptosis. Here, we investigate the role of Parkin in lipopolysaccharide (LPS) vs. zVAD-mediated necroptosis/inflammatory response in murine BV2 microglial cells. Incubation of BV2 cells with the pan-caspase inhibitor zVAD for 24 h induced high levels of cell death, as measured by MTS/LDH assays. Importantly, co-incubation of zVAD with necrostatin-1 (Nec-1), an inhibitor of RIP1 kinase activity, abrogated cell death, thus implicating RIP1-dependent necroptosis as the cell death mechanism. Moreover, zVAD exposure for 5h induced RIP1/RIP3 necrosome assembly, as determined by sequestration of RIP1/RIP3 in insoluble fractions. MLKL phosphorylation, a key marker of necroptosis, was also significantly increased after zVAD exposure, which was abolished by Nec-1 co-treatment. Also, zVAD exposure led to TNFα secretion, which may in turn contribute to autocrine induction of necroptosis. Parkin siRNA-mediated knockdown protected BV2 cells from necroptosis and induction of pro-inflammatory mediators in this cellular context. Interestingly, this effect appears to be independent of pro-inflammatory mechanisms related to Toll-like receptor 4, since its activation by LPS in cells after Parkin knockdown resulted in higher expression of inflammatory genes and TNFα secretion. Further, this effects appears to be elicited exclusively by NFkB and JNK over-activation, an effect not observed in BV2 cells exposed to zVAD. In conclusion, we suggest that Parkin contributes to zVAD-induced necroptosis in BV2 cells. Moreover, Parkin presents alternative roles during LPS- vs. zVAD-driven inflammation, possibly by altering linear or non-linear ubiquitination patterns. Further work in this field may this unravel unsuspected roles for Parkin in PD pathogenesis, inflammation in general, and necroptosis.

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***Link between abstracts**: cellfun*

Poster: P.109 | André Filipe Ferreira Nadais
Protein aggregation and phosphorylation in a dementia subgroup of a primary care-based cohort

Presenter: André Nadais | iBiMED - University of Aveiro
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In an emerging elderly society, aging is a subject of paramount importance, related with several neurodegenerative disorders of which Alzheimer's disease (AD) is the most common. Neuropathologically, AD is mostly characterized by the presence of extracellular senile plaques (SP) and intraneuronal neurofibrillary tangles (NFTs). These neuropathological phenomena are associated with distinct but overlapping molecular events including TAU hyperphosphorylation in the case of NFTs and the deposition of extracellular β-amyloid peptides (Aβ) in the case of SPs. In TAU, there are 85 phosphorylatable residues, of which 45 have been found in the AD brain, namely serine (S), threonine (T), and tyrosine (Y) residues. From these phosphorylation sites, the most studied phospho-epitopes in Cerebrospinal fluid (CSF) are p-TAU181 and p-TAU231 which are localized at the half-N-terminus and C terminus outside the microtubule binding domain. pTAU231 appears early in AD while pTAU181 has a later onset. Particularly, phosphorylation at T231 by GSK-3β plays a role in diminishing the ability of TAU to bind to microtubules resulting in protein aggregation and ultimately in the formation of NFTs. Hyperphosphorylation and abnormal phosphorylation may be useful as diagnostic markers. On the other hand SPs result from the build-up of extracellular A□, either with 40 amino acids (Aβ40) or 42 amino acids (Aβ42), which are produced through the metabolism of the Amyloid Precursor Protein (APP) after being sequential cleaved by specific secretases (β- and γ-secretases). Although A🚲40 is the most abundant variant, A🚲42 is more likely to form aggregates. The function of A🚲 is a matter of some debate and subject to intense research. From a diagnostic standpoint it is important to quantify protein aggregates in human samples. This was carried out using samples from a primary care-based cohort (pcb-cohort) of 23 putative AD patients and 23 controls. This analysis was performed by flow cytometry and spectrophotometry. Using the same samples it was also evaluated if pTAU231 can be used as a blood biomarker in the diagnosis of AD.

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***Link between abstracts**: Tesselate*

SESSION: NEUROPSYCHIATRIC DISORDERS

Poster: P.110 | Joana Sofia da Silva Correia
Exploring the astrocytic neuroprotective functions in a chronic mild stress model of depression

Presenter: Joana Correia | ICVS, University of Minho
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Depression is a multidimensional psychiatric disorder affecting millions of people worldwide. In order to unveil the pathophysiology of depression increasing significance is given to the study of abnormal neurotransmission in brain areas affected. Particularly, glutamatergic excitotoxicity has been suggested as one of the possible processes underlying the installation of this disorder. Moreover, previous studies have suggested that glutamate release is increased in depressed subjects [1,2]. Astrocytes play an important role in removing synaptic glutamate mainly through glutamate transporter 1 (GLT-1), promoting reduction of excitotoxicity. Interestingly, the β-lactamic antibiotic ceftriaxone (CEF) was described to increase GLT-1 expression thus increasing glutamate uptake [3]. These findings may suggest GLT-1 as a target for disruption in depression and provide clues for development of novel therapeutic strategies. To assess the potential effect of CEF administration at behavioral and molecular level, an unpredictable chronic mild stress (uCMS) protocol was used to mimic depressive-like behavior in rats. Furthermore, two different antidepressants (fluoxetine and imipramine) already known to positively impact on behavior and neuronal morphology of uCMS exposed animals were used as comparative controls [4]. Animals were exposed continuously to different types of stressors during six weeks and daily administered either with CEF (200mg/kg; i.p.), fluoxetine (10mg/kg; i.p.) or imipramine (10mg/kg; i.p.) during the last two weeks of the uCMS protocol. At the end of the protocol three behavioral dimensions commonly affected in depressive patients were assessed: mood, anxiety and cognition. For comparison between groups One-way ANOVA statistical analysis was applied and statistical significance was accepted for $P < 0.05$. As previously reported uCMS animals presented deficits in all three behavioral domains that were, at least partially, reversed by fluoxetine or imipramine administration. Similarly to antidepressants, CEF administration significantly reversed anhedonic profile of uCMS animals in the sucrose preference test. Regarding anxiety-like behavior, CEF administration could partially reverse the latency to feed in the novelty suppressed feeding test. GLT-1 gene and protein expression levels were assessed to determine the effect of CEF and further correlate with behavioral results. Interestingly, fluoxetine decreased significantly GLT-1 protein levels in the dorsal hippocampus. Contrarily, CEF increased GLT-1 protein levels in the same brain region, although no statistical significance was found. In summary, using the uCMS animal model of depression we can suggest that CEF has a putative role in the reversion of anhedonic behavior after chronic stress exposure. Further analyses are now being conducted to elucidate the role of GLT-1 increased expression in the reversion of excitotoxicity processes in the hippocampus and prefrontal cortex synapses. This study will possibly reveal the potential use of drugs targeting GLT-1 expression in the treatment of depression, paving the way for the development of new therapeutic strategies.

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***Link between abstracts**: Gray Phoenix*

Poster: P.111 | António Maria Restolho Mateus Pinheiro

Exploring time-dependent anatomical and functional correlates of adult hippocampal cytogenesis in young-adult rats

Presenter: Antonio Mateus-Pinheiro | ICVS, University of Minho

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Impaired ability to generate new cells in the adult brain has been linked to deficits in multiple emotional and cognitive behavioral domains. However, the mechanisms by which the abrogation of post-natal neural stem cells impacts on brain homeostasis and function remains controversial. Here, we used a transgenic rat line, the GFAP-Tk line, to selectively eliminate neural stem cells and assess the repercussion on different behavioral domains. We adopted two parallel experimental timeframes, to study both short-term and long-term effects of cytogenesis ablation (1 week post-ablation and 4 weeks post-ablation, respectively). Moreover, we conducted in vivo electrophsyiological analysis to assess the effects of cytogenesis ablation on the electrophysiological signatures of the hippocampal and prefrontal cortex regions. Our results show that the short-term repercussions of post-natal cytogenesis ablation are restricted to anxiety behavior. Contrastingly, cytogenesis abrogation promoted the late manifestation of anhedonic and anxiogenic deficits, along with multi-dimensional cognitive impairments. Furthermore, we found that cytogenesis ablation impaired electrophysiological function between the hippocampus and the prefrontal cortex, which are likely to contribute to the described cognitive alterations. Altogether, we describe a progressive time-dependent manifestation of emotional and cognitive impairments following cytogenesis ablation, supporting a differential role of immature vs mature cells in the modulation of different behavioral dimensions within the adult brain.

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***Link between abstracts**: GrayPhoenix*

Poster: P.112 | Patrícia Carvalho Patrício
Relevance of adult cytogenesis for behavior in female rats and the role of female hormones

Presenter: Patrícia Patrício | ICVS, University of Minho
Patrícia Patrício (1,2), António Mateus-Pinheiro (1,2), Joana Sofia Correia (1,2), Emanuel Novais (1,2), Nuno Dinis Alves (1,2), Ana Rita Machado dos Santos (1,2), Nuno Sousa (1,2), Luísa Pinto (1,2)
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Adult cytogenesis in the mammalian brain is a complex phenomenon encompassing several steps. This process allows the generation of both neurons and astrocytes that are capable of integrating in the pre-existing neural networks. The subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) is one of the brain regions where new neurons and glial cells are generated during adulthood. Newborn neurons generated in the hippocampal DG have been proposed to participate in pattern separation, contextual and spatial memory, memory consolidation, stress response and antidepressants actions. Strikingly, most studies on the relevance of adult hippocampal cytogenesis have been performed in male rodents, disregarding putative sex disparities and the role of female hormones. Indeed, previous studies have suggested a hormonal modulation of neurogenesis. To further address this topic, we have used a transgenic GFAP-thymidine kinase (TK) rat model treated with ganciclovir (30mg/kg) for 18 days, to ablate adult cytogenesis in adult female rats, and performed a comprehensive behavioral characterization. Additionally, we monitored the estrous cycle after each behavioral test, and measured the levels of serum glucocorticoids and glucocorticoids receptors in the hippocampal DG. Behavioral analysis revealed that cytogenesis suppression was sufficient to induce anxiety-like behavior, while producing no significant effects in hedonic, depressive-like or social-interaction behaviors. Interestingly, the precipitation of anxiety-like behavior was only evident when discriminating females in non-proestrus phases from those in proestrus, as the latter phase was associated with heightened anxiety in both genotypes that masked the anxiogenic effect of cytogenesis ablation. Accordingly, animals with suppressed cytogenesis presented increased serum corticosterone levels and decreased expression of glucocorticoid receptors in the hippocampal DG. Cytogenesis ablation also promoted impairments in behavioral flexibility, but not in the spatial reference task in the Water Maze test. Interestingly, increased levels of serum glucocorticoids were accompanied by decreased expression of the glucocorticoid receptor (GR) and serotonin receptors in the hippocampus of female rats after cytogenesis ablation. Overall, we report that cytogenesis suppression induces hypercorticosteronemia in adult female rats, which is accompanied by heightened anxiety and behavioral flexibility deficits.

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***Link between abstracts**: GrayPhoenix*

Poster: P.113 | Ana Rita Machado dos Santos
Glial plasticity as a key mechanism underlying the pathophysiology of depression

Presenter: Ana Rita Santos | ICVS, University of Minho
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Major depression (MDD) is a prevalent disorder that poses a significant social burden in society nowadays, affecting approximately 350 million people, worldwide. The pathophysiology of this disease is still poorly known but growing evidence suggests that impaired neuroplasticity may be a key underlying mechanism. Recent studies showed an important role for astrocytes in the pathophysiology of this disorder evidenced by astrocytes loss in MDD. However, the role of astrogliogenesis in the precipitation of and recovery from MDD is unknown, constituting a promising area of research for developing therapeutic strategies. We aim here to dissect the role of adult astrogliogenesis in the precipitation of and recovery from depressive-like behaviour in rodents both untreated and treated with antidepressants (ADs), in a longitudinal manner. Here, we provide some consistent evidences for the causative implication of newborn and pre-existing astrocytes in the pathophysiology of depression, having significant impacts in the long-term development and maintenance of cognitive deficits, as well as in the long-term recovery of those impairments by different ADs. We also show with in vitro experiments, using hippocampal primary cultures, that desipramine (and not norfluoxetine) regulates the differentiation of newborn cells into astrocytes rather than neurons.

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***Link between abstracts**: GrayPhoenix*

Poster: P.114 | Cláudia Filipa Cunha Antunes
Role of Tet3 in brain function

Presenter: Cláudia Antunes | ICVS, University of Minho
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The role of epigenetic modifications in the regulation of the nervous system is one of the most remarkable issues of contemporary neuroscience. Its relevance in brain plasticity processes such as learning, memory and in neurological and psychiatric disorders has been subject of intense investigation. Particularly pertinent in this field are the dynamic changes in DNA modifications, namely the hydroxylation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) catalyzed by TET dioxygenases (Santiago et al., 2014). It has been shown that high 5hmC content is positively correlated with gene transcription and is a feature of both neuronal progenitors and post-mitotic neurons. Tet genes have also been shown to be highly transcribed in the brain, with Tet3 being the most abundant. Recently, it was shown that Tet3 knockdown in hippocampal neurons cultures elevated glutamatergic synaptic transmission, whereas overexpressing Tet3 decreased it. At molecular level, RNA-seq analyses revealed a pivotal role of Tet3 in regulating gene expression in response to global synaptic activity changes (Yu et al, 2015). Our work aims to understand the role of TET3 in brain function using an in vivo model approach. Therefore, to specifically study the biological function of Tet3 in forebrain post-mitotic neurons, Tet3 C57BL/6N floxed mice (Peat et al., 2014) carrying an inducible Camk2a-Cre ERT2 gene were generated, in which the Tet3 gene can be deleted selectively in neurons at adult stage by the administration of tamoxifen. Six-week-old mice received 2 mg tamoxifen/20 g body weight for 5 days and then, after a 1-week interval, the administration period was repeated. Behavioural analysis were performed at mood and cognition level. The results obtained suggest that conditional deletion of Tet3 in post-mitotic neurons increase the anxiety in females and leads to a cognitive impairment in both sexes. Namely, in Morris Water Maze test, the analysis of the strategies adopted to reach the escape platform showed that the Tet3 cKO mice have a tendency to maintain test performances comparable to control group by delaying the switch from non-hippocampal dependent strategies to hippocampal ones. Also, the Tet3 cKO deletion reduced the recognition index in Tet3 ckO animals, indicating deficits of short-term memory. The results obtained so far suggest a role for TET3 in regulating some behavioural dimensions particularly related to anxiety and cognition but further analyses are being performed in order to understand the potential impact at neuronal morphology and molecular level of this conditional ablation.

***References**: Santiago et al., Genomics 2014; 104: 334–340; Yu et al, Nature Neuroscience 2015; 18:836-843; Peat et al., Cell Reports 2014; 9: 1990-2000*
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***Link between abstracts**: GrayPhoenix*

Poster: P.115 | Eduardo Manuel Loureiro de Campos
AP2gamma transcription factor role in the modulation of adult glutamatergic neurogenesis in depression

Presenter: Eduardo Loureiro-Campos | ICVS, University of Minho
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Major depression is a multidimensional psychiatric disorder that poses a significant burden in society nowadays. Despite of its importance in modern societies the processes underlying its pathophysiology remain poorly understood. It is accepted that this complex disorder involves genetic-environment interactions, but the genetic and environmental substrates remain largely unknown. Several hypotheses have been proposed to clarify the neurobiological mechanisms underlying this disease, being the link between adult hippocampal neurogenesis and depression a central topic in the past decades. Previous studies have identified AP2 transcription factor as a key regulator of embryonic and adult hippocampal neurogenesis in mice, acting as a regulator of basal progenitors, promoting proliferation and neuronal differentiation[1,2]. Thus, we wanted to further explore the impact of AP2 in brain neurophysiology and behavior during development and at adult stages, dissecting also its mechanisms both in health and depression.
We were able to understand the impact of AP2 in post-natal development and during juvenile age, through the AP2 constitutive knockout (KO) model, in which the deletion of AP2 occurs since embryonic development. Through developmental milestones protocol we did not find any major impairment in the behavioral performance of KO mice, since all parameters analyzed were within the typical range for appearance of the developmental milestones. However, in the juvenile mice, where we performed an emotional characterization trough the open-field and sucrose splash tests, and in the hippocampal glutamatergic neurogenesis analysis, impairments were found, since KO mice showed anxious-like behavior and decreased proliferation of immature neurons. To study the impact of modulating the transcription factor AP2 in depression we exposed both constitutive and conditional KO animal models to an unpredictable chronic mild stress protocol, which efficiently induced core depressive-like symptoms, since different weight gain patterns and a disruption of the hypothalamic-pituitary axis were observed. Through a multidimensional behavioral analysis after chronic stress exposure, we observed that reduced expression of AP2 (constitutive KO mice) produced significant deficits in cortico-dependent cognitive tasks, which is likely a consequence of cortical developmental deficits. However, this deletion since embryonic development proved to be beneficial for hippocampal dependent cognitive functions, namely working and spatial memory tasks. Possibly the cognitive improvement observed in these mice was due to a compensatory increase of several genes important to promote hippocampal glutamatergic neurogenesis, as measured through western-blot, namely Sox2, Pax6 and Tbr2, ameliorating in this way the deleterious effects induced by chronic stress. This compensatory mechanism in the constitutive AP2 KO mice is further supported by the fact that these genes were normally expressed and cognitive deficits were found in the conditional KO mice after chronic stress exposure, in which the AP2 deletion only occurs in an adult phase, being promoted by tamoxifen usage. The reported results not only support the involvement of AP2 in the transcriptional network that modulates the juvenile and adult neurogenic process, but also highlight the potential of this molecule as a future therapeutical tool in neuropsychiatric disorders, in which neurogenesis is impaired.

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***Link between abstracts**: GrayPhoenix*

Poster: P.116 | Nuno Dinis Lopes Oliveira Alves
Adult hippocampal neuroplasticity triggers susceptibility to recurrent depression

Presenter: Nuno Dinis Alves | ICVS, University of Minho
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Depression is a highly prevalent and recurrent neuropsychiatric disorder associated with alterations in emotional and cognitive domains. Neuroplastic phenomena are increasingly considered central to the etiopathogenesis of and recovery from depression. Nevertheless, a high number of remitted patients experience recurrent episodes of depression, remaining unclear how previous episodes impact on behavior and neuroplasticity and/or whether modulation of neuroplasticity is important to prevent recurrent depression. Through re-exposure to an unpredictable chronic mild stress protocol in rats, we observed the re-appearance of emotional and cognitive deficits. Furthermore, treatment with the antidepressants fluoxetine and imipramine were effective to promote sustained reversion of a depressive-like phenotype, however their differential impact on adult hippocampal neuroplasticity triggered a distinct response to stress re-exposure: while imipramine re-established hippocampal neurogenesis and neuronal dendritic arborization contributing to resilience to recurrent depressive-like behavior, stress re-exposure in fluoxetine-treated animals resulted in an overproduction of adult-born neurons along with neuronal atrophy, accounting for an increased susceptibility to recurrent behavioral changes typical of depression. Strikingly, cell proliferation arrest compromised the behavior resilience induced by imipramine and buffered the susceptibility to recurrent behavioral changes promoted by fluoxetine. This study shows that previous exposure to a depressive-like episode impacts on the behavioral and neuroanatomical changes triggered by subsequent re-exposure to similar experimental conditions and reveals that the proper control of adult hippocampal neuroplasticity triggered by antidepressants is essential to counteract recurrent depressive-like episodes.

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***Link between abstracts**: GrayPhoenix*

Poster: P.117 | Cláudia Sofia Serre Miranda
The absence of αβ T cells leads to altered depressive-like behavior but normal cognitive performance

Presenter: Cláudia Serre Miranda | ICVS, University of Minho
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The interplay between the central nervous system and the immune system has been proved to be essential for the establishment and maintenance of a proper function of the brain. However, how these interactions influence the brain has been mostly studied in pathological conditions and to a lesser extent on the maintenance of health. Recent data on such interplay led to the current vision that the brain, rather than being an immune privileged organ, enjoys the privilege of being modulated by the immune system in several functions, namely in cognition (1-3). Mice with no T cells have been suggested to presented cognitive deficits that were reverted when the T cell pool was reconstituted, which was not observed with other cellular populations (4,5). Among T cells, it was observed that CD4+ T cells-depletion led to impaired cognition and reduced hippocampal neurogenesis (6). This observation led to the hypothesis that the CD4+ T cells are essential for the maintenance of the cognitive function. There are two main T cell populations. A central difference is on the composition of the T cell receptor (TCR), which is composed by an α and β for the αβ T cells and γ and δ chain for γδ T cells. The most prevalent T cell population in circulation is composed by αβ T cells, while γδ T cells reside mostly within the mucosa. To test if αβ T cells are essential for cognitive performance we analyze the behavior of TCRα knockout (KO) mouse model, that lack these cells. We observed that male TCRα KO mice present no differences when compared to their wild-type (WT) littermate controls in terms of locomotor activity and anxious-like behavior, but are less depressive-like than WT. To evaluate cognitive behavior, we performed the Morris-water-maze and the novel object recognition task, and the TCRα KO mice presented no differences in terms of cognitive behavior when compared to WT mice. The role of γδ T cells on the maintenance of the cognitive function in mice lacking αβ T should be explored in the future.

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***Link between abstracts**: NIRD*

Poster: P.118 | Susana Roque
The lack of the anti-inflammatory interleukin 10 (IL 10) leads to cognitive impairment

Presenter: Susana Roque | ICVS, University of Minho
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The crosstalk between the immune and the nervous systems is known to be essential for the maintenance of the body homeostasis in general and, in particular, for the correct functioning of the central nervous system. In fact, disruption in the brain/immune communication is associated with mood and cognitive alterations (Kipnis 2016; Miller and Raison 2016). Previous studies from our lab revealed that mice lacking the expression of the anti-inflammatory interleukin 10 (IL10KO mice) present depressive-like behavior, a phenotype that is rescued through IL10 administration (Mesquita et al. 2008). Recently we observed that the absence of IL10 is associated with cognitive impairment in female mice. Moreover, female IL10KO mice also present alterations on the blood immune cells profile, such as a decrease in total CD8+T, CD8+ central memory T, B and NK cells and an increase in granulocytes and CD4+ effector memory T cells. Accordingly, in humans CD4+ effector memory T cells have been showed to be a significant predictor of cognitive performance (Serre-Miranda et al. 2015). Currently, we are unraveling the mechanisms that could be associated with the alterations observed in the cognitive performance of IL10 KO female mice.

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Link between abstracts: *NIRD*

Poster: P.119 | Andrea Catarina Amaro de Campos Lobo
Methamphetamine-induced changes in neuronal morphology are regulated by rhoGTPases signaling

Presenter: Andrea Lobo | I3S-IBMC
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Methamphetamine (METH) is a highly neurotoxic psychostimulant that leads to long-term dysfunction in monoaminergic and glutamatergic neurons. Besides these effects, METH also alters neuronal morphology in several brain regions. Here, we hypothesized that modulation of RhoGTPases, highly expressed in the nervous system and key regulators of neuronal morphology may impact METH-induced changes in hippocampal neuronal morphology, providing new targets for therapies development. To test that, a set of experimental approaches was applied to primary hippocampal neuronal cultures obtained from mice embryos. We observed that METH promotes neurite outgrowth, by analyzing total neurite length and arborization in neurons transfected with enhanced green fluorescent protein (EGFP). METH incubation did not induce neuronal death or caused mitochondrial damage. Furthermore, we observed that METH increased dendritic spine density of hippocampal neurons, particularly regarding filopodia and mushroom spines. We evaluated the activation state of classic rhoGTPases- rhoA, rac1 and cdc42- using specific Förster resonance energy transfer (FRET) reporter probes to assess differential topographical activation. Our results evidence that, in the neurites of hippocampal neurons, rhoA activity suffers a downregulation, while rac1 activity shows a sustained increase. We also observed that cdc42 activity was increased in dendritic spines of hippocampal neurons. When rac1 levels were downregulated, or when a constitutively active form of rhoA was expressed, METH-induced effects in neurite outgrowth were abolished, showing that both effects are required for METH-induced increase in morphological complexity to occur. Furthermore, we analyzed the effects of the expression of cdc42 dominant negative and constitutively active forms in neurite outgrowth, as well as in dendritic spine density. We also observed that the expression of several genes related to cdc42 signaling pathways was increased, particularly genes that encode for proteins involved in cdc42 cycling between activaton and inactivation state, and for effector proteins downstream cdc42 activation. We further explored the signaling pathways involved in neuronal METH exposure, which might underlie the activation/inactivation of rhoGTPases, and its subsequent effects on neuronal morphology. Noteworthy, in the nucleus accumbens of mice exposed to cocaine, increased neuronal complexity was associated with rac1 inhibition, showing that these mechanisms are region specific, and that pharmacological therapies targeting rhoGTPases signaling must be carefully evaluated.

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***Link between abstracts**: wonderland*

Poster: P.120 | Joana Alexandra Moreira Ribeiro
New possible therapeutic targets in methamphetamine exposure

Presenter: Joana M. Ribeiro | I3S-IBMC

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Methamphetamine (METH) is a highly addictive psychostimulant drug whose consumption leads to long-term dysfunction of dopaminergic and glutamatergic neurons, glial impairment and high oxidative stress. Interestingly, exposure to psychostimulants also affects neuronal morphology, increasing neurite length and dendritic spine density in several brain regions. Addicted individuals usually present severe pathophysiological conditions, but treatment is scant and the mechanisms by which addiction develops are still elusive. To explore this issue, we conducted an RNAseq analysis from both soma and neurite fraction of cultured hippocampal neurons, which revealed differential expression patterns in numerous genes in METH exposed neurons. In a first step, we selected genes differentially expressed in the soma, related to metabolism, cell signaling, cell cycle or transcription/ transduction. From those we elected 5 genes that were likely to be associated with neuroinflammation and neuron/glia interplay and: Optn, Olfm4, Rasgrp1, RelB and Ebf3. We conducted RT-qPCR to validate the expression of the selected candidates, and confirmed that upon exposure to METH, Olfm4 mRNA expression levels were significantly increased in primary cultures of hippocampal neurons. Olfma4 protein expression levels were however decreased either when evaluated through western blot or immunocytochemistry. Because Olfm4 is known to interact with integrins and metalloproteinases and has been linked to inflammatory processes, we assessed METH effect on extracellular matrix related proteins (Cldn5, ZO-1, MMP2, Src and RhoA) by western blot, immunocytochemistry, zymography and FRET assays. We show that in hippocampal cultures METH does not affect Cldn5 or ZO-1 expression. METH exposure also does did not significantly affect MMP2 activity in these neuronal cells. In opposition, Src activation was increased by METH, which is concomitant with down regulation of RhoA activity. Of note, Src activation seems to decrease when Olfm4 is knocked down. In summary, METH exposure may target a pathway that involves Src activation and consequent RhoA inactivation, and that may contribute to the METH-induced neuronal morphological alterations.

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***Link between abstracts**: wonderland*

Poster: P.121 | Joana Catarina da Silva Bravo
Methamphetamine and neuroinflammation: The crosstalk between microglia and neuronal cells

Presenter: Joana Bravo | I3S-IBMC
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Exposure to psychostimulants has been classically associated with the disruption of the dopaminergic system, concomitant with terminal degeneration and eventual neuronal death. However, increased attention has been given the interaction between neuronal and glial cells as a relevant factor to the building and maintenance of addiction. We hypothesize that the long-term adverse consequences occurring within the brain's reward circuitry under psychostimulant exposure may be due, at least in part, to the underlying neuroinflammatory process, and that limiting inflammation may be relevant to control the addictive behaviour. Contrary to the common held view, our preliminary data revealed that methamphetamine (METH), a potent psychostimulant frequently associated to neuroinflammation, cannot stimulate microglia cells in a cell-autonomous manner. Therefore, we are interested in clarifying the role of different types of neuronal cells on this inflammatory process. We have already observed that the conditioned medium obtained from primary astrocytes cultures exposed to METH is effective in inducing an inflammatory profile in primary microglia. Here, we explored the crosstalk between microglial cells and different types of neurons typical from brain regions well known to be affected by METH exposure in vivo. Therefore, hippocampal, striatal and mesencephalic neuronal cells were exposed to well-characterized doses of METH (10 and 100µM). Co-cultures of striatal and mesencephalic neuron were also used. After different time points neuronal conditioned medium was collected and added to primary microglial cells. Microglia activation was evaluated by morphological criteria through iba1 immunocytochemistry, and the presence of a pro-inflammatory profile was evaluated using an antibody for iNOS. Phagocytosis was assessed using fluorescent latex beads. Our results show that the different neuronal conditioned mediums used were not able to increase iNOS expression or the phagocytic efficiency in microglia, showing that microglia did not change into a reactive state. In addition, microglia exposed to escalating dopamine doses also did not change into an activated profile. Of note, these results are not yet sufficient to exclude a role for neuronal cells in microglia activation and further studies using different paradigms need to be performed.

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***Link between abstracts**: wonderland*

Poster: P.122 | João Duarte Magalhães
Neurolastin as a possible regulator of methamphetamine-induced changes in neuronal morphology

Presenter: João Magalhães | I3S-IBMC

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Methamphetamine (METH) is one of the most addictive psychostimulant drugs. Drug addiction is considered a chronic and relapsing brain disease associated to severe systemic disorders, which raises relevant socio-economic and public health concerns. Although addicted individuals usually present severe pathophysiological conditions, addiction treatment is currently scant. Therefore, it is essential to further explore the addictive process to find new possible therapeutic targets. METH in particular, leads to long-term dysfunction of dopaminergic and glutamatergic neurons, glial impairment and high oxidative stress. Interestingly, exposure to psychostimulants also affects neuronal morphology, increasing neurite length and dendritic spine density in several brain regions. To explore this issue, we conducted a RNAseq analysis from both soma and neurite fraction of cultured hippocampal neurons, which revealed differential expression patterns in numerous genes in METH exposed neurons. In a first step, we have selected genes differentially expressed in the soma or neurite fractions, related with cytoskeletal dynamics and regulation of RNA translation. The genes selected for validation by RT-qPCR were Rab7, Rnf112 and Tristetraprolin. PCR data corroborated that Rnf112 (or neurolastin), but not Rab7 and TTP, mRNA levels expression were significantly altered after METH exposure in neurites fraction, making it a suitable candidate for posterior research regarding protein expression. Structural studies suggest neurolastin is an unusual dynamin-like protein with two functional domains - RING and GTPase - that presents high homology with atlastin. Neurolastin is a brain-specific protein that localizes in endosomal vesicles, regulating their size and trafficking patterns through its RING domain. Moreover, the intrinsic GTPase activity is involved in synaptogenic processes modulating spine formation with direct repercussion in synaptic transmission. Although no differences were observed in neurolastin protein expression (all cell) after a METH challenge, Rnf112 knockdown in cultured hippocampal neurons downregulates PSD-95 and VGluT1 protein levels. Furthermore, when Rnf112 knockdown, METH exposure fails to induce increased arborization in hippocampal primary cultures, evidencing a possible role for Rnf112 in this process.

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***Link between abstracts**: wonderland*

Poster: P.123 | Teresa Correia Soares Canedo
Methamphetamine-induced neuroimmune response: focus on astrocyte-microglia crosstalk

Presenter: Teresa Canedo | I3S-IBMC
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Methamphetamine (METH) is a highly addictive psychostimulant that was associated neuroinflammation in chronic human users. Microglia, the myeloid resident cells of the CNS, modulate neuroinflammation and the addictive behaviour in inflammatory processes. Recent in vivo and in vitro studies pointed microglia as a possible major mediator of METH-induced neurotoxicity. Here, we investigated the role of METH on microglia activation. We show that METH induced neither a pro- nor an anti-inflammatory phenotype in primary microglial cells. We however found that that METH could activate microglia via astrocytes. Conditioned media from astrocytes (ACM) exposed to METH induced a pro-inflammatory phenotype in microglia, significantly increasing ROS production, iNOS expression and phagocytic activity, when compared with microglia incubated with conditioned media from naïve astrocytes. In order to isolate the astrocytic factor(s) responsible for activating microglia upon METH exposure, we analyzed the production of pro-inflammatory cytokines (IL-1B; IL-6 and TNF) by qRT-PCR and glutamate release by FRET-based nanosensors in primary astrocytes. We observed no change in pro-inflammatory cytokine production and a huge increase in glutamate release in astrocytes exposed to METH. To gain mechanistic insight into astrocyte-induced microglia activation, we explored the pathways involved in astrocytic glutamate release. Previous studies show that TNF controls glutamate release from astrocytes and the METH-induced glutamate release was largely attenuated in TNF knockout astrocytes. We also revealed that METH and TNF regulated astrocytic glutamate release via mobilization of Ca2+ from the endoplasmic reticulum to the cytosol in an IP3R-sensitive manner. Cytosolic Ca2+ rise, triggered by METH or TNF-induced IP3R activation, promotes the release of glutamate from astrocytes via SNARE-dependent exocytosis. In accordance, TNF knockout mice exposed to METH do not display METH-induced behavioural alterations when tested in the elevated plus maze. Overall, here we described that METH activates microglia via glutamate release from astrocytes, which is mediated by METH-promoted TNF production leading to astrocytic glutamate release through IP3/Ca2+-dependent exocytosis.

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***Link between abstracts**: wonderland*

Poster: P.124 | Teresa Summavielle
The antigenic effect of ethanol in adult mice requires TNF production from microglia

Presenter: Teresa Summavielle | I3S - IBMC
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Alcohol is a licit psychoactive substance largely abused worldwide. Excessive alcohol intake leads to immune cell dysfunction and behavioural impairment. Microglia, the immune resident cells of the central nervous system (CNS), are major players in inflammatory responses in the brain. After inflammatory stimuli microglia become activated releasing cytotoxic mediators like Tumor Necrosis Factor (TNF) and glutamate, which can potentially induce behavioural impairment. Here, we dissected the signaling events associated with ethanol-induced TNF production and glutamate release from brain microglia, and associated this pathway with the alterations caused by ethanol on anxiety-like behaviour in adult mice. We show that TNF production and glutamate release in microglia required the sustained activation of the tyrosine kinase Src. While forced Src activation mimicked the ethanol effect in promoting TNF production and in triggering glutamate release, the knockdown or the pharmacological inhibition of Src abrogated the ethanol effects. The ethanol/Src-mediated glutamate release was prevented in microglia obtained from TNF deficient mice, indicating that ethanol triggered glutamate release through a Src-induced TNF production. Ethanol administration to adult mice altered microglia homeostasis and induced TNF production in the brain without triggering microgliosis. Adult wild type mice exposed to ethanol, displayed an increase in anxiety-like behaviour, an effect that in observable in TNF deficient animals. Overall, our work suggests that blocking the TNF signaling pathway in adult brain microglia could constitute a potential strategy for preventing part of the ethanol-mediated alterations in the brain.

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***Link between abstracts**: wonderland*

Poster: P.125 | Christina Miskolczi

From childhood social adversities to abnormal aggressive behaviour in adulthood – does the central serotonergic system have an active role this transition?

Presenter: Christina Miskolczi | IEM HAS

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Studies have shown that childhood social adversities (e.g. social neglect) are consistently linked to violent behaviour in adulthood, though changes underlying this phenomenon are unclear. To investigate the effects of social neglect, our laboratory has developed a model in which rats are subjected to post-weaning social isolation. Isolated rats display abnormal aggression and increased stress-reactivity in adulthood. Currently we aimed to investigate the effects of social isolation and aggressive interaction (i.e. fighting) on gene expression in our model. Since the serotonergic system is crucially involved in the regulation of aggression, our targets included the human aggression-related serotonin metabolizing enzyme monoamine-oxidase A (MAOA) and serotonin receptors 5HT1A and 5HT1B. We also targeted epigenetic regulator enzymes DNA-methyltransferase 1 and 3a (DNMT1, DNMT3a), since epigenetic mechanisms might also play a role in the emergence of abnormal aggression. Samples were taken from brain areas relevant to the control of aggression: the prefrontal cortex (PFC), the medial (MeA) and the basolateral amygdala (BLA). In accordance with our previous findings, isolated animals displayed abnormal aggression in adulthood. Social isolation affected gene expression in all observed brain areas: it increased MAOA expression and lowered 5HT1B expression in the PFC, increased 5HT1A and lowered 5HT1B expression in the BLA and increased MAOA expression in the MeA. Fighting caused an increase in DNMT3a expression in the PFC and BLA of socially housed and isolated animals, whereas fighting selectively decreased MeA MAOA in isolated animals. Our findings show that both long- and short-term effects influence gene expression. Particularly, social isolation causes marked changes in the serotonergic components of all observed aggression-related brain areas and gene expression is regulated differently in isolated animals. Our results outline the importance of the serotonergic system in mediating the effects of early-life social neglect, and may provide a further step in designing effective treatments for abnormal aggression stemming from childhood social adversities.

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Poster: P.126 | Oral presentation: O.14 | Carla Filipa Simões Henriques

Chronic blockade of adenosine A2A receptors: gender-specific reprogramming of microglia morphology in the pre-frontal cortex

Presenter: Carla Henriques | IBILI, University of Coimbra

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In adulthood, microglia (cells implicated in the genesis of anxiety) present morphologic differences between genders in physiological conditions (1). Under prenatal anxiogenic stimulus, these cells undergo gender-specific morphological remodelling. In these circumstances, the chronic blockade of adenosine A2A receptors (A2AR), a modulator of microglia, ameliorated microglia morphology and anxiety behaviour in males, but not in females (1). This dimorphic response is likely related with gender differences in the density of A2AR in the pre-frontal cortex (PFC), brain region strictly involved in anxiety disorders (1). It is thus imperative to study microglia morphology in the PFC from early neurodevelopment onwards, as well as the physiologic response to A2AR manipulation in males and females. The present study aims to clarify whether gender-specific microglia morphology is already present in newborns (post-natal day, PND 0) and infants (PND 7) in the PFC. Our second goal is to characterize the effect of the chronic blockade of A2AR in adulthood (PND 90) in microglia morphology in the PFC of male and female rats. Wistar rats were treated with SCH58261 (SCH) (0.1 mg/kg/day, intraperitoneal), a selective A2AR antagonist, for 21 consecutive days before PND 90. Microglia morphology was assessed by immunohistochemistry with a microglia marker (Iba-1). Confocal images obtained with a 63x objective, allowed the manual reconstruction of microglia in 3D using Neurolucida software. Statistic analysis was performed in GraphPad Prism: Student's t test was used to compare two independent means; differences were considered significant at $p<0.05$. Microglia volume at PND 0 was similar between genders (NT males: 2749 ± 391.2 µm3; NT females: 2376 ± 198.9 µm3, n=3, $p>0.05$); at PND 7 we still did not observe gender differences in ramifications number and length (n=3, $p>0.05$) in PFC microglia. At PND 90, the chronic blockade of A2AR reduced the number and length of microglial processes in females (NT females, n=6, vs SCH females, n=7, $p<0.05$), but did not affect males (n=4, $p>0.05$). In conclusion, the present data show that A2AR control microglia morphology in a gender-specific manner. On the other hand, we characterized for the first time microglia morphology in the PFC of newborn rats, showing that gender differences in microglia morphology are not present until PND 7, which coincides with an endogenous peak of A2AR expression.

***References**: Caetano L and Pinheiro H et al. Mol Psychiatry 2016; 00: 1–9.*

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Poster: P.127 | Oral presentation: O.16 | Joana Teresa Ferreira Gonçalves
Excitation/inhibition balance and glial function in mouse model of neurofibromatosis type 1: distinct susceptibility of hippocampus, prefrontal cortex and striatum

Presenter: Joana Gonçalves | ICNAS,UC

Joana Gonçalves (1,2,3), Inês Violante (4), José Sereno (1,2,3), Ricardo Alexandre Leitão (2,3,5), Ying Cai (6), Ana Paula Silva (2,3,5), Alcino José Silva (6), Miguel Castelo-Branco (1,2,3)

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Neurofibromatosis type 1 (NF1) is a monogenic developmental disorder, characterized by altered skin pigmentation, increased tumor predisposition and cognitive deficits. Increasing data have been proposed that alterations in the excitation/inhibition balance are the neural mechanism underlying NF1-mediated cognitive disabilities. Previous studies employing a mouse model of NF1 revealed that γ-aminobutyric acid (GABA) inhibitory neurotransmission were increased in several brain regions, including hippocampus and striatum. Nonetheless, we showed a reduction of GABA concentration in the visual and medial frontal cortex of human patients with NF1. Since the link between animal and human studies remains to be established, it is important to apply to the mice model the same techniques available to investigated the GABA levels in humans. Here, together with molecular and cellular methods, we used magnetic resonance spectroscopy in NF1 mouse model, as this is the only technique accessible to measure GABA in vivo in humans. We found that the excitation vs. inhibition and the pre- vs. post-synapatic phenotype is different in the NF1 mouse hippocampus, when compared to cortical and striatal regions. In fact, both hippocampal GABA and glutamate levels are reduced, without changes in the respective ratio. Moreover, hippocampal GABA(A) α1 subunit receptor levels were increased at the synaptosomal level. On the other hand, striatal and cortical GABA/glutamate ratios are significantly increased, while GABA(A) subunit levels were decreased mainly at synaptosomal level in prefrontal cortex and at the cytosolic level in the striatum. Further, imunolabelling confirmed these results and showed distinct patterns of receptor redistribution in all these structures with patchy zones of dense receptor clusters being more evident in the striatum of the mutant mice. Finally we found evidence that GABA dysfunction is accompanied by changes in astrocytes physiology. Changes in astrocytes physiology are consistent with increased glutamine vs. glutamate levels in the hippocampus and frontal cortex, suggesting abnormalities in the glutamine-glutamate cycle. Overall, our study reported distinct homeostatic mechanism in the hippocampus, prefrontal cortex and striatum induced by NF1 mutations at both neural and glial levels. These findings are crucial to design novel region specific therapeutics strategies that may need to improve cognitive disabilities in NF1 patients.

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Poster: P.128 | Ana Rita Gonçalves Gaspar
Microglia morphology in the dorsal hippocampus of female rodents: a comparative study with the prefrontal cortex

Presenter: Rita Gaspar | IBILI -FMUC

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During brain development, microglial cells are implicated in the formation, maturation and elimination of newborn synaptic contacts, functions supported by the immune capability of these cells. Microglia are highly sensitive to changes in the immune environment, which may perturb neurodevelopment and result in behavioural alterations. Our previous work, using a rodent model of gestational exposure to an immunomodulator (in utero exposure to dexamethasone, iuDEX), characterised by chronic anxiety and by morphologic remodelling of microglia in the prefrontal cortex (PFC), strengthens the role of these cells in the developmental genesis of anxiety. Females exposed to iuDEX, besides anxious-like behaviour, present deficits in recognition memory, an observation that prompted us to perform a comparative ontogenic analysis of microglia morphology between the PFC and the dorsal hippocampus (dHIP). The goal of the present study was to quantify specific parameters of microglia morphology (number and length of cellular processes) in the HIP in the post-natal period and at adulthood (postnatal day, PND7 and PND90), tracking eventual differences between brain regions in female Wistar rats in physiological conditions and upon iuDEX (1 mg/kg, subcutaneous injection to pregnant rats). Morphometric analysis was performed by immunohistochemistry (myeloid marker, Iba-1) followed by manual reconstruction of confocal images, using the Neurolucida software. In physiological conditions, microglia morphology is different between the dHIP and the PFC: hippocampal cells are less complex than cortical cells, with lower number of ramifications, which are also shorter. These differences are already observed at PND7 and persist until PND90. Upon iuDEX, the total length (but not the number) of cellular processes decreases in hippocampal cells at PND7, but this effect is shifted into a hyper-ramification, observed at PND90, with an increase in the number of ramifications (although their total length remains unaffected). This is in clear contrast to the observations done in the PFC, where iuDEX caused a long-lasting atrophy of microglia. Our data reveal a physiological difference in the three dimensional morphology of microglia between brain regions, as well as regional differences in the morphological remodelling of these cells in response to changes in the immune environment during the uterine life. Further studies are needed to clarify if these regional differences may have implications for different components of mood disorders, namely anxiety and cognition.

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Poster: P.129 | Chrysoula Dioli
Tau-dependent suppression of neurogenesis in the stressed hippocampus

Presenter: Chrysoula Dioli | ICVS, University of Minho
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Stress, a well-known sculptor of brain plasticity, is shown to suppress hippocampal neurogenesis in the adult brain; yet, the underlying cellular mechanisms are poorly investigated. Previous studies have shown that chronic stress triggers hyperphosphorylation of the cytoskeletal protein Tau, a process that impairs the cytoskeleton-regulating role(s) of this protein with impact on neuronal function. Here, we analyzed the role of Tau on stress-driven suppression of neurogenesis in the adult dentate gyrus (DG) using animals lacking Tau (Tau-KO) and wild-type (WT) littermates. Unlike WTs, Tau-KO animals exposed to chronic stress did not exhibit reduction in DG proliferating cells, neuroblasts and newborn neurons; however, newborn astrocytes were similarly decreased in both Tau-KO and WT mice. In addition, chronic stress reduced PI3K/mTOR/GSK3β/β-catenin signaling in the DG, known to regulate cell survival and proliferation, in WT, but not in Tau-KO. These data establish Tau as a critical regulator of the cellular cascades underlying stress deficits on hippocampal neurogenesis in the adult brain.

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Poster: P.130 | Daniela Mesquita Moutinho

Human VGF-derived anti-depressive neuropeptide TLQP-62 binds to the heat shocks proteins HSPA8 and HSPD1 on the SH-SY5Y surface cells

Presenter: Daniela Moutinho | Uni. Santiago de Compostela
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Chronic mental disorders (CMD), as schizophrenia, bipolar disease or major depression, affect about 10% of the world population. VGF, a neuropeptide precursor, was found to be downregulated in patients suffering from CMD. This peptide is synthesized in neuronal cells of the olfactory bulb, cortex, hypothalamus, hippocampus and cerebellum and is the precursor for multiple peptides with different activities in synaptic plasticity, neurogenesis, glucose, energy balance, food intake, among others. The VGF-derived 62 amino acid peptide TLQP-62 shows an acute antidepressant effect in behavioural mice models of depression enhancing neurogenesis at the hippocampus[1][2][3]. To search for a TLQP-62 receptor in the human brain, a crosslinker was used to link biotinylated TLQP-62 to a possible receptor present in the human neuroblastoma derived cells SH-SY5Y. The putative receptor is trapped in an avidin column and identified by separation in SDS-PAGE, followed by mass spectrometry and confirmed by western blotting. We found TLQP-62 to bind heat shock proteins HSPA8 and HSPD1 on the surface of SH-5Y5Y cells. Heat shock proteins are responsible for the proper folding and transport of proteins, among other important cell functions contributing to signal transduction, apoptosis, protein homeostasis, and cell growth and differentiation. HSP70 family members, as HSPA8, pass unfolded proteins to the ones of HSP60 family, as HSPD1, that will lead to folded proteins. Small peptides hardly remain their structure once excised from theirs targets, and TLQP-62 shows a mainly disordered structure with a tendency to form an alpha-helix in solution. HSPs are also involved in the regulation of signaling pathways via G protein-coupled receptors, by acting as activators or inhibitors. Although HSPA8 is mainly present in the cytoplasm and HSPD1 in the mitochondria, it is known they also localize in the plasma cell membrane. Moreover, HSPA8 and HSPD1 are constitutively expressed chaperones critical for cell survival and are downregulated in patients suffering from depression and schizophrenia[4]. We propose these chaperones participate in the transport and folding of the TLQP-62 neuropeptide, maybe being part of the receptor complex, facilitating its binding, endocytosis or signal transduction, once they can localize in the plasma membrane. Thus, these proteins seems to be an interesting target for further studies concerning chronic mental disorders molecular mechanisms.

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Poster: P.131 | Isabel Maria Sousa Castanho
Transcriptional and epigenomic profiling in the entorhinal cortex in amyloid and tau mouse models of Alzheimer's disease

Presenter: Isabel Castanho | University of Exeter
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Although the neuropathological signatures of Alzheimer's disease (AD) have been well characterized, the specific mechanisms involved in the onset and progression of the disease are still unknown. The onset of neuropathology is hypothesized to involve transcriptomic and epigenomic changes in specific regions of the brain, with certain brain regions affected earlier and more severely than others. The entorhinal cortex (ECX), located in the medial temporal lobe and involved in memory formation and recall, shows signs of pathology in the very early stages of AD, including the deposition of beta amyloid plaques and neurofibrillary tangles. In this study we investigated the transcriptional and epigenomic effects of amyloid and tau pathology in the ECX using two well-characterised rodent models of AD neuropathology: the J20 and the rTg4510 mouse lines. The J20 mouse model overexpresses human amyloid precursor protein (APP) with both the Swedish and Indiana mutations, and the rTg4510 mouse model overexpresses a human mutant (P301L) form of the microtubule-associated protein tau (MAPT). We used highly parallel RNA-seq to measure changes in gene expression in transgenic mice compared to wild-type littermate controls at four different ages. Longitudinal changes in gene expression associated with the progression of neuropathology were identified in mutant mice, including at loci previously implicated in amyloid pathology and the immune response. Transcriptional data is currently being integrated with epigenetic and histopathological data in the same animals. These data will identify novel pathways involved in the development of AD-associated neuropathology that will uncover mechanisms associated with the progression of disease. Ultimately, changes identified in mouse models of neuropathology will be compared with data generated with ongoing studies of human AD using extensive datasets generated by our group.

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Poster: P.132 | Joana Vanessa Santos dos Reis
Identification of electroencephalogram predictors of functional magnetic resonance activations in an emotional regulation task

Presenter: Joana Reis | ICVS, University of Minho
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Electroencephalogram (EEG) and functional magnetic resonance (fMRI) are two brain mapping techniques with outstanding complementarity between temporal and spatial resolution. Technological advances have made possible multimodal recordings of EEG and fMRI that are useful for both clinical and research proposes [1]. The integration between these two modalities was motivated, at first, in the context of epilepsy (to identify the origin of epileptic discharges) [2] but in the past decades has been used in neuroscience research to study brain dynamics and its relationship with various psychiatric disorders. Such integration allows the increase of the spatial resolution of the EEG data based on the fMRI collected simultaneously. In this study, we aim to investigate the electrodes and frequencies that represent the electrophysiological signature of the fMRI data using simultaneously recorded data during an emotional regulation task. In fact, evidence published by Meir-Hasson et al. in 2014 suggests that activations in deep regions of the brain produce electrophysiological signatures that could be detected using features of the scalp EEG [3]. The electrophysiological signatures of deep brain regions (like the amygdala) may be used as a basis for brain-training in therapeutic approaches like EEG-based Neurofeedback [5]. With this study we aim to explore a framework for producing the EEG predictors of deep regional fMRI activations. In order to construct the EEG predictor, this framework uses advanced signal processing and machine learning methods. The proposed framework was tested in 8 healthy volunteers who were submitted to a simultaneous EEG and fMRI acquisition during an emotional regulation task. The International Affective Picture System (IAPS) was applied to emotionally stimulate subjects and thus, identify EEG predictors of sub-cortical regions activity. The target region of interest was set to be the amygdala because it is a major node in the limbic network and play an important role in emotional processing [6].

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Poster: P.133 | João Pedro Oliveira da Silva Paulino Lopes
The blockade of neuronal A2A adenosine receptors prevents impairment by beta-amyloid peptides of hippocampal synaptic plasticity

Presenter: João Pedro Lopes | CNC
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Caffeine, an antagonist of adenosine receptors, has been shown to attenuate memory deterioration upon aging or Alzheimer's disease (AD). Proposed as culprits of AD, amyloid-β peptides (Aβ) formation is increased in this pathology and can trigger synaptic and memory dysfunction. Accordingly, Aβ affect synaptic plasticity, namely impairing long-term potentiation (LTP). Adenosine A2A receptors (A2AR) control synaptic plasticity and their blockade attenuates Aβ-induced synaptotoxicity and memory deficits. Albeit A2AR control synaptic plasticity in the hippocampus, it remains to be shown if the modulation of A2AR activity can abrogate the Aβ-induced impairment of LTP. Applying high frequency stimulation (100 Hz, 1 s) to Schaffer fiber-CA1 pyramid synapses in hippocampal slices from 10 weeks-old male C57/BL6 or A2AR-knockout mice to trigger LTP, we aimed at assessing if A2AR blockade controls hippocampal LTP deficits induced by Aβ. Acute exposure to Aβ (50 nM) decreased LTP, an effect not altered by the blockade of adenosine A1R (DPCPX, 100 nM) but prevented when the slices were superfused with the selective antagonist of A2AR (SCH58261, 50 nM). Aβ was also devoid of effects on LTP amplitude in global A2AR-KO mice and in forebrain selective A2AR-KO (genetic deletion of A2AR only in forebrain neurons), in contrast with the respective WT littermates. These results indicate that the deficits in hippocampal synaptic plasticity triggered by Aβ are strictly dependent on the activation of neuronal A2A receptors.

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Poster: P.134 | Marton Mayer

New aspect of the glutamatergic nature and projection patterns of the median raphe cells

Presenter: Marton Mayer | IEM HAS
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Subcortical monoaminergic pathways, like that originating from the serotonergic median raphe region (MRR), are fundamental for the modulation of forebrain neuronal networks.
MRR neurons are classically identified based on serotonin (5-HT), vesicular glutamate transporter type 3 (VGLUT3) and gamma-aminobutyric acid (GABA) content, however, the exact cellular composition of MRR is still unknown. Using unbiased stereological method, we found that 2.1% of the MRR neurons were only serotonergic, 7% contained only VGLUT3, about 3.6% were double positive (5-HT/VGLUT3), whereas 61% expressed vesicular GABA transporter (VGAT). Surprisingly, about 25% of the neurons were only positive for the neuronal marker NeuN, and negative for all other examined markers. MRR cells can also express the PET-1 transcription factor enhancer region ePET that is thought to be present only in serotonergic neurons. Interestingly, however, we found that far more VGLUT3 cells expressed ePET than 5-HT cells and about 38% of the ePET cells contain only VGLUT3, while about one third of 5-HT cells are ePET negative. Previously, we have shown that the MRR can establish fast glutamatergic excitatory synapses in the hippocampus (HIPP). Using retrograde tracing, we found that about 91, 84 and 82% of the MRR cells projecting to the HIPP, medial septum (MS) and medial prefrontal cortex (mPFC) are glutamatergic. Furthermore, using double retrograde tracing, we show that a large portion of MRR neurons can innervate the HIPP and mPFC or the MS and mPFC simultaneously and about 90% of these double-projecting cells are also glutamatergic. Our results show that although the serotonin content of the projecting MRR cells is indeed typical, the vast majority of the projecting neurons are at least partly glutamatergic. These neurons can also innervate more brain areas simultaneously; therefore, these cells could precisely synchronize these brain areas. In addition, we show that the quarter of MMR neurons belong to an unknown population. While the MRR was previously thought to be a tonic modulatory serotonergic nucleus, these observations put this nucleus into a new perspective and can help us understand its real functions.

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Poster: P.135 | Patrícia Inês Pires dos Santos
Alterations of excitatory/inhibitory balance in different brain regions of suicide completers

Presenter: Patrícia Santos | CNC, University of Coimbra
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Depression involves an altered connectivity and dysfunctional alterations in brain regions that are essential in regulating emotion, reward and executive function (Russo, and Nestler 2013). Moreover, it was described a synaptic dysfunction (Duman and Aghajanian, 2012) and a loss of spine number and dendritic arborization in frontocortical regions (Russo, and Nestler 2013). Keeping a balanced inhibitory/excitatory neurotransmission is essential for the normal functioning brain; two main neurotransmitters essentially control this balance - glutamate and GABA. Since depression is prevalent in suicide completers, we now aim to indirectly gauge if there is a shift in the excitatory/inhibitory balance in different human brain regions. We measured proteins that are important in the post-synaptic compartment for synaptic dynamics postsynaptic density 95 kDa (PSD-95) for excitatory synapses (Doucet et al., 2012) and gephyrin for GABAergic synapses (Tyagarajan and Fritschy, 2014). Samples were collected from the cingulate cortex (Brodmann area 25, BA25), hippocampus, amygdala, medial and posterior caudate nucleus of brains from male suicide completers and age-matched controls (age range: 20-80 years). We prepared nerve terminals to evaluate by Western Blot the densities of PSD-95 and gephyrin, which were normalized by reprobing with GAPDH. Compared with age-matched controls, suicide completers displayed a selective decrease of PSD-95 density in nerve terminals of amygdala (53.6±13.3%, n=8 $p<0.05$) and posterior caudate (35.6±10.4%, n=6 $p<0.05$). Additionally we observed a decrease in gephyrin density in hippocampus (55.4±11.3%, n=7 $p<0.05$) and gephyrin breakdown products in BA25 (42.3±9.9%, n=6 $p<0.05$) of suicide completers compared with age-matched controls. Overall, these findings suggest asymmetric alterations of excitatory/inhibitory balance and control of excitability and plasticity in different brain regions of suicide completers that are fundamental to develop novel anti-depressant therapies.

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Poster: P.136 | Pedro Miguel Silva Moreira
The neural correlates of Obsessive Compulsive Disorder: a multimodal perspective

Presenter: Pedro Silva Moreira | ICVS, University of Minho
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BACKGROUND: Obsessive Compulsive Disorder (OCD) is one of the most debilitating psychiatric conditions, having a dramatic impact on patients' daily living. An extensive body of neuroimaging literature has described some of the neurobiological mechanisms underlying the core manifestations of the disorder. Nevertheless, most reports have focused on individual modalities of structural/functional brain alterations, mainly through targeted approaches, thus possibly precluding the power of unbiased exploratory approaches. In this study, we conducted a multimodal magnetic resonance imaging (MRI) investigation of OCD, integrating structural and functional analyses. METHODS: A cohort of 40 OCD patients (mean age, 26.28, standard deviation, 6.62) and 40 healthy individuals (mean age, 26.45; standard deviation, 5.39) was assessed using structural and resting-state functional MRI protocols. A Voxel-Based Morphometry analysis was conducted to compare between-group volumetric differences. The whole-brain functional connectome, derived from resting-state functional connectivity (FC), was analyzed with the Network-Based Statistic methodology. Results from structural and functional analysis were integrated in mediation models. RESULTS: OCD patients revealed volumetric reductions in the right superior temporal sulcus. Patients had significantly decreased FC in two distinct subnetworks: the first, involving the OFC, temporal poles and the subgenual anterior cingulate cortex; the second comprising the lingual and postcentral gyri. On the opposite, a network formed by connections between thalamic and occipital regions had significantly increased FC in patients. Integrative models revealed direct and indirect associations between volumetric alterations and FC networks. CONCLUSIONS: Altogether, this study suggests that OCD patients display alterations in brain structure and FC, involving complex networks of brain regions. Furthermore, we provided evidence for direct and indirect associations between structural and functional alterations representing complex patterns of interactions between separate brain regions which may be of upmost relevance for explaining the pathophysiology of the disorder.

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Poster: P.137 | Vanessa Filipa Coelho Santos

Effects of early developmental chronic exposure to methylphenidate on both brain´s immune privilege and anxiety-like behavior: Control vs ADHD rats

Presenter: Vanessa Coelho-Santos | FMUC, IBILI

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Attention deficit hyperactivity disorder (ADHD) is a highly prevalent neuropsychiatric disorder in children, and the psychostimulant methylphenidate (MPH) is the first line medication prescribed for its treatment. Concerns have been raised about ADHD overdiagnosed and the unknown long-term neurological consequences of MPH treatment. Thus, to clarify the chronic effects of MPH on anxiety and cortical brain immune response, we used a rat model of ADHD, the Spontaneously Hypertensive (SHR) rats, and its control strain, the Wistar Kyoto (WKY) rat to simulate a therapeutic use and a misused condition, respectively. Rats were administered for Monday-Friday with vehicle or MPH (1.5 mg/kg/day or 5 mg/kg/day, per os) from P28-P55 (equivalent to late-childhood through late-adolescence in humans). MPH (5 mg/kg/day) elicited anxious-like behavior in the open-field test and increased blood-brain barrier (BBB) permeability in both WKY and SHR animals. Interestingly, BBB dysfunction was more prominent in WKY rats. This effect can be explained by the downregulation of the tight junction protein, claudin-5, and by the significant degradation of cerebrovascular basal lamina protein, collagen-IV. Also, WKY animals showed an increase in the protein levels of caveolin-1 and both vascular cell and intercelular adhesion molecules (VCAM-1 and ICAM-1, respectively). Noteworthy, these BBB alterations led to the infiltration of peripheral immune cells, including CD169+ macrophages and CD4+ T-cells. Moreover, both doses of MPH triggered a robust oxidative and neuroinflammatory response in control rats. Curiously, the lower dose of MPH had the opposite effect in the ADHD model, characterized by a decreased glial activation and production of anti-inflammatory mediators. Overall, our results show that chronic exposure to MPH promotes neuroinflammatory events and brain vascular alterations particularly under physiological conditions. In fact, in ADHD animal model MPH has an anti-inflammatory effect at the lower dose. These contrasting effects observed between control and ADHD model support the importance of an appropriate MPH dose regimen for ADHD, and also highlights the problems raised by an inadequate ADHD diagnosis or MPH misuse.

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SESSION: REGENERATION AND THERAPIES

Poster: P.138 | Luís António Ferreira Rocha
Role of ECM-derived Peptides on Cellular Adhesion and Neurite Outgrowth for Spinal Cord Injury Repair

Presenter: Luís Rocha | ICVS, University of Minho
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Spinal Cord Injury (SCI) is a drastic condition that currently has no cure. Some of the recent therapeutic strategies have been focused on the development of biomaterials that could serve both as a matrix for neuronal regeneration and also as a vehicle for the transplantation of cells with therapeutic potential. Our group has previously shown the capacity of a Gellan Gum-based hydrogel (GG) modified with a fibronectin-derived peptide (GRGDS) for the functional recovery and axonal regeneration of a rat model of SCI [1]. In this work we try to improve the properties of this hydrogel, by combining three different peptides: 1) GRGDS (G) for inducing cellular adhesion; 2) YIGSR (Y) for promoting neurite outgrowth and extension; and 3) IKVAV (K) for neuronal differentiation. The hydrogels were prepared at 1% (w/v) concentration and we mixed the three peptides in all the possible combinations: 1- G; 2- Y; 3- K; 4- GK; 5- GY; 6- KY; 7- GKY. Then we used these hydrogels for the culture of Adipose tissue-derived Stem Cells (ASCs) and Olfactory Ensheathing Cells (OECs), two populations of cells already used by our group in SCI repair strategies. Moreover, we used the same hydrogels for the growth of Dorsal Root Ganglia (DRG) explants, as an in vitro model of axonal regeneration. The results show that the presence of GRGDS peptide is essential for the adhesion and morphology of both ASCs and OECs. Whenever this peptide is absent, cells present an atypical round shape and in general less number of cells. Similar results were observed in DRG explants. Hydrogels with GRGDS induced higher areas of neurites and increased neurite extension and ramification, indicating the relevance of this peptide for neurite outgrowth. These results highlight the importance of adhesion molecules such as RGD peptides for neurite formation and extension, which are crucial for SCI regeneration strategies.

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Link between abstracts: *Edeotas*

Poster: P.139 | Rita Catarina Assunção Ribeiro Silva
Exploring the impact of different tissue-sources MSC Secretome on neuronal differentiation and axonal growth: A comparative study

Presenter: Rita Assunção Silva | ICVS, University of Minho
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Human mesenchymal stem cells (hMSC) have been proposed as possible therapeutic agents for central nervous system (CNS) disorders (1,2). Their effects are believed to be mostly mediated through their secretome, which contains several neuroregulatory molecules and vesicles known by neuroprotective and regenerative capacity (3,4). In fact, studies show that MSC secretome is able to increase the survival, proliferation, and differentiation of neural populations. More recent evidences have shown that it composition may vary according to the different tissue sources from which that are obtained (5). In line with this, we hypothesize if MSC conditioned media (CM) derived from adipose-tissue (ASCs), bone-marrow (BMSCs) and umbilical cord perivascular (HUPVCs) might have distinct roles in nerve repair and regeneration. To clarify this, the present study aims to evaluate the effect of ASC, BM and HUCPVCs CM on the neuronal differentiation of human neural precursor cells (NPCs) and on axonal growth of dorsal root ganglion (DRG) explants. Results revealed that all the collected MSC CM promoted neuronal differentiation, though no statistical differences were found between them. In addition, the different MSC CM supported DRG axonal growth as well, with the extent of axonal growth being clearly higher in contact with ASC CM when compared to BMSC and HUCPVC CM. In summary, our work showed that ASC, BMSC, and HUCPVC CM promoted both neuronal differentiation and axonal growth, with differences being observed only for DRG cultured with ASC CM, which present higher neurite growth then the other conditions.

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Link between abstracts: *Edeotas*

Poster: P.140 | Rui Augusto Ribeiro Lima
Effect of Systemic Administration of Interleukin-4 After Spinal Cord Injury

Presenter: Rui Lima | ICVS, University of Minho
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Traumatic spinal cord injury (SCI) provokes dramatic disability and dysfunction in the motor, sensory and autonomic systems (1). The inflammatory reaction that occurs in SCI has been strongly associated with further tissue damage. However, this inflammatory profile can be modulated into a protective response associated with tissue repair. Intraspinal treatment with IL-4 has been shown to reduce the numbers of macrophages displaying the proinflammatory M1-phenotype and to boost the M2-phenotype associated with neuroprotection and tissue repair (2). To further evaluate the impact of IL-4 on spinal cord injury, we tested the systemic administration of this cytokine for a 7-days period and assessed both histological and motor recovery. IL-4 treatment induced an elevation of the cytokine IL-10 in the serum both at 24h and 7 days after injury. Interestingly, IL-4 treatment also led to a reduction on local inflammatory markers, namely to a reduction on the microglia and on iNOS-positive cells. Importantly, in the spinal cord of injured animals that received IL-4, an increase of βIII-tubulin and NeuN staining was observed suggesting an increased neuronal protection. An increase of oligodendrocytes was also observed in the group treated with IL-4. Moreover, 100% of the animals treated with IL-4 were able to recovery a clinically relevant feature of locomotion (weight support) against only 33% of saline treated animals. Overall, these results show that the systemic administration of IL-4 positively impacts different aspects of spinal cord injury, creating a more favorable environment for recovery to take place.

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***Link between abstracts**: Edeotas*

Poster: P.141 | Susana Isabel Gonçalves Monteiro
Exploring doublecortin (DCX)+ cells after spinal cord injury

Presenter: Susana Monteiro | ICVS, University of Minho
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Spinal cord injury (SCI) leads to a broad spectrum of permanent neurological deficits and currently there is no fully effective treatment available (1).
A central challenge following SCI is to promote re-growth of injured axons and replacement of loss neurons in order to re-establish synaptic connections and achieve functional recovery. However, contrarily to what happens in some invertebrates, mammals present reduced neurogenic activity at the spinal cord (2) therefore strategies to boost regenerative cell plasticity are needed. Doublecortin (DCX) is a cytoskeleton protein associated with neuronal migration and axonal outgrowth (3). Although mostly known for its expression by neuroblasts in the subgranular zone of the dentate gyrus and in the subventricular zone, its expression has also been observed in non-neurogenic places such as the piriform cortex (4) and others following injury (5). Nevertheless, its exact contribution for injury recovery is not yet understood. Therefore we aimed at assessing the impact of a drug known to modulate DCX+ cells on neurogenic niches, Citalopram, on the injured spinal cord. Rats underwent a contusive spinal cord injury at T8 and were chronically treated with Citalopram for 8 weeks. DCX expression was assessed throughout the spinal cord and correlated with histological and behavioural indicators of recovery. Although described as being absent in the adult intact spinal cord (2), we found that DCX is expressed after SCI. Moreover, similarly to what was already described for the brain, treatment with Citalopram increased DCX+ cells in the spinal cord after injury. Importantly these DCX+ cells were observed in the corticospinal tract, one of the most important motor systems. The effect of citalopram on DCX+ cells was only observed at the epicenter of the lesion and not rostral or caudally to the lesion. DCX+ cells may represent an interesting therapeutical target for regeneration after SCI.

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***Funding**: Prémios Santa Casa Neurociências - Prize Melo e Castro for Spinal Cord Injury Research; Portuguese Foundation for Science and Technology (Financiado no âmbito do Projecto 3599 – Promover a Produção Científica e Desenvolvimento Tecnológico e a Constituição de Redes Temáticas (3599-PPCDT), project: PTDC/DTP-FTO/5109/2014; Post-Doctoral fellowship - SFRH/BPD/97701/2013 - to N.A. Silva; IF Development Grant to A. J. Salgado; PhD fellowships - PD/BDE/127836/2016 – to R. Lima; SFRH/BD/103075/2014 - to E. Gomes and PDE/BDE/113596/2015 - to R. Silva. This work is a result of the project (NORTE-01-0145-FEDER-000013), supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF); Cofinanciado pelo Programa Operacional Regional do Norte (ON.2 SR&TD Integrated Program – NORTE-07-0124-FEDER-000021), ao abrigo do Quadro de Referência Estratégico Nacional (QREN), através do Fundo Europeu de Desenvolvimento Regional (FEDER); Projeto Estratégico – LA 26 – 2011-2012 and Projeto Estratégico – LA 26 – 2013-2014 cofinanciado por fundos nacionais, através da Fundação para a Ciência e a Tecnologia (PEst-C/SAU/LA0026/2011; PEst-C/SAU/LA0026/2013), e pelo Fundo Europeu de Desenvolvimento Regional (FEDER), através do COMPETE (FCOMP-01-0124-FEDER-022724; FCOMP-01-0124-FEDER-037298).*
***Link between abstracts**: Edeotas*

Poster: P.142 | Elisabete Apolinário da Costa

Baccharis dracunculifolia decreases nociception, depressive-like behaviour and supraspinal activated microglia in rats with experimental monoarthritis

Presenter: Elisabete Apolinário da Costa | CITAB

Laranjeira, I.M.(1,2,3); Apolinário, E.(1,2,3); Amorim, D.(2,3); Silva-Filho, A.A.(4), Pinto-Ribeiro, F.(2,3); Dias, A.C.P.(1)

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In arthritic disorders both inflammation and the progressive degeneration of joints persistently activate nociceptors, in periarticular structures, leading to the development of persistent pain and comorbid emotional impairments. Arthritis-induced peripheral sensitization leads to increased release of nociceptive molecules by primary afferents that activate neurones e glial cells in the spinal cord and supraspinal pain modulatory areas such as the amygdala (AMY) and the periaqueductal grey matter (PAG). Baccharis dracunculifolia DC (Asteraceae) (Bd) is a medicinal shrub from the brazilian flora, popularly known as "Alecrim do Campo", considered to be an important source of active anti-inflammatory and antinociceptive compounds. Adult 8 weeks old ovariectomized female rats (Rattus norvegicus, vr. Albinus, Wistar) weighting 210±17g were divided in four groups (n=6 per group): (i) SHAM, (ii) ARTH, (iii) ARTH treated with B. dracunculifolia (50mg/kg), and (iv) ARTH treated with B. dracunculifolia (100 mg/kg). Mechanical hyperalgesia in ARTH animals was assessed using the pressure application measurement apparatus, anhedonia using the sucrose preference test and learned helplessness using the forced swimming test. Activated microglia was stained with IBA-I and quantified in a subset of brain slides containing the target areas, the amygdala and the periaqueductal gray matter. A three-week oral treatment with Bd extract reversed ARTH-induced mechanical hyperalgesia and partly reserved depressive-like behaviour. Concomitantly, Bd treatment decreased the number of activated microglia in the AMY and PAG of ARTH animals.

***References**: BONNET, C. S.; WALSH, D. A. Osteoarthritis, angiogenesis and inflammation. Rheumatology, 2005, 44.1: 7-16; JI, Ru-Rong; BERTA, Temugin; NEDERGAARD, Maiken. Glia and pain: is chronic pain a gliopathy?. PAIN®, 2013, 154: S10-S28; LOGGIA, Marco L., et al. Evidence for brain glial activation in chronic pain patients. Brain, 2015, 138.3: 604-615; DOS SANTOS, Diogo A., et al. Anti-inflammatory and antinociceptive effects of Baccharis dracunculifolia DC (Asteraceae) in different experimental models. Journal of ethnopharmacology, 2010, 127.2: 543-550.*

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***Link between abstracts**: elephant*

Poster: P.143 | Inês Martins Laranjeira

Baccharis dracunculifolia decreases nociception, depressive-like behaviour and supraspinal activated microglia in rats with experimental monoarthritis

Presenter: Inês Laranjeira | ICVS, University of Minho

Laranjeira, I.M. (1,2,3) ; Apolinário, E. (1,2,3); Amorim, D. (2,3) ; Silva-Filho, A.A. (4) , Pinto-Ribeiro, F. (2,3) ; Dias, A.C.P. (1)

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In arthritic disorders both inflammation and the progressive degeneration of joints persistently activate nociceptors, in periarticular structures, leading to the development of persistent pain and comorbid emotional impairments. Arthritis-induced peripheral sensitization leads to increased release of nociceptive molecules by primary afferents that activate neurones e glial cells in the spinal cord and supraspinal pain modulatory areas such as the amygdala (AMY) and the periaqueductal grey matter (PAG). Baccharis dracunculifolia DC (Asteraceae) (Bd) is a medicinal shrub from the brazilian flora, popularly known as "Alecrim do Campo", considered to be an important source of active anti-inflammatory and antinociceptive compounds. Adult 8 weeks old ovariectomized female rats (Rattus norvegicus, vr. Albinus, Wistar) weighting 210±17g were divided in four groups (n=6 per group): (i) SHAM, (ii) ARTH, (iii) ARTH treated with B. dracunculifolia (50mg/kg), and (iv) ARTH treated with B. dracunculifolia (100 mg/kg). Mechanical hyperalgesia in ARTH animals was assessed using the pressure application measurement apparatus, anhedonia using the sucrose preference test and learned helplessness using the forced swimming test. Activated microglia was stained with IBA-I and quantified in a subset of brain slides containing the target areas, the amygdala and the periaqueductal gray matter. A three-week oral treatment with Bd extract reversed ARTH-induced mechanical hyperalgesia and partly reserved depressive-like behaviour. Concomitantly, Bd treatment decreased the number of activated microglia in the AMY and PAG of ARTH animals.

***References**: BONNET, C. S.; WALSH, D. A. Osteoarthritis, angiogenesis and inflammation. Rheumatology, 2005, 44.1: 7-16; JI, Ru-Rong; BERTA, Temugin; NEDERGAARD, Maiken. Glia and pain: is chronic pain a gliopathy?. PAIN®, 2013, 154: S10-S28; LOGGIA, Marco L., et al. Evidence for brain glial activation in chronic pain patients. Brain, 2015, 138.3: 604-615.; DOS SANTOS, Diogo A., et al. Anti-inflammatory and antinociceptive effects of Baccharis dracunculifolia DC (Asteraceae) in different experimental models. Journal of ethnopharmacology, 2010, 127.2: 543-550.*

***Funding**: This study was supported by grants from INTERACT project – "Integrative Research in Environment, Agro-Chains and Technology", no. NORTE-01-0145-FEDER-000017 and the Portuguese North Regional Operational Program (ON.2 - O Novo Norte) under the National Strategic Reference Framework (QREN), through the European Regional Development Fund (FEDER), in its line of research entitled ISAC*

***Link between abstracts**: elephant*

Poster: P.144 | Raquel Leal Monteiro Mano de Oliveira
Development of neurogenic detrusor overactivity is prevented by early bladder afferent desensitization in spinal cord injured rats

Presenter: Raquel Oliveira | FMUP; i3S, Porto
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Introduction & Objectives: In the current management of spinal cord injury, treatment of neurogenic detrusor overactivity (NDO) is a key aspect, aiming to reduce periods of high intravesical pressure and promote urinary continence in order to render the urinary bladder a low-pressure, high volume, continent urine reservoir. Presently, NDO treatment is initiated once SCI patients emerge from spinal shock. Here, we tested if an early intervention, during the spinal shock, would be able to attenuate or prevent NDO development. We focused on resiniferatoxin (RTX), a TRPV1 agonist, as this is a relevant neurotoxin already tested in chronic NDO patients that can be easily delivered via bladder instillation. Material & Methods: Female rats were submitted to T8/T9 complete spinal cord transection (SCT) and received RTX 50 nM (n=8) or vehicle (n=8) 3 and 9 days after lesion. Four weeks post-SCT, animals underwent 1 hour cystometry, followed by collection of bladder and L5/L6 spinal cord segments and associated dorsal root ganglia (DRG). Neuronal tissue was processed for immunohistochemical detection of a) in the spinal cord: CGRP (marker of peptidergic neurons), GAP43 (marker of axonal sprouting), phosphoERK (pERK, marker of neuronal activation) and BDNF (neurotrophin associated with spinal tissue); and b) in the DRG: ATF3 (lesion marker and associated with axon elongation). DRG were also used for primary cultures (n=2/group). Bladders were processed for Western blotting analysis of TRPV1, CGRP and GAP43 relative expressions. Results: Four weeks after SCT, vehicle-treated animals presented a typical pattern of NDO, with increased peak pressure (47.8±4.48 cm H2O), amplitude (35.8±4.0 cm H2O) and frequency (0.7±0.1 cm H2O) of detrusor contractions. Early RTX significantly reduced the peak pressure (34.6±2.4 cm H2O, $p=0.01$) and amplitude (17.4±1.5 cm H2O $p<0.0001$) of bladder contractions. This was accompanied by decreased bladder expression of TRPV1, CGRP and GAP43 ($p<0.01$). In L5/L6 DRG, RTX-treated animals presented a modest increase in ATF3 expression although no changes in intrinsic growth ability were observed in in vitro conditions. At the lumbosacral spinal cord, no significant variations in CGRP, GAP43, pERK and BDNF expression were observed between controls and RTX-treated rats. Conclusions: Early RTX instillation markedly improved bladder function, by effectively reducing maximal detrusor pressures. These beneficial urodynamic effects were due to a restricted action on the peripheral branches of bladder sensory afferents, as no significant alterations were found in the DRG and lumbosacral spinal cord. These observations support that early administration of RTX, due to its simplicity and safety may become an attractive therapeutic tool to prevent the emergence of NDO in SCI patients. Future studies will define the best concentration and time-point for RTX administration.

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***Link between abstracts**: SpinalCordInjury*

Poster: P.145 | Bruno MDC Godinho
Polyunsaturated Fatty Acid hsiRNA Conjugates Display Enhanced Distribution and Robust Huntingtin Gene Silencing in the Mouse Brain

Presenter: Bruno MDC Godinho | Uni. Massachusetts Med. School
Bruno M. D. C. Godinho (1,2), Mehran Nikan (1,2), Andrew H. Coles (1,2), Maire F. Osborn (1,2), Reka A. Haraszti (1,2), Annabelle Biscans (1,2), Dimas Echeverria (1,2), Neil Aronin (1,3), Anastasia Khvorova (1,2)
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Therapeutic gene silencing using RNA interference (RNAi) technology holds great promise as a transformative clinical strategy for incurable, genetically-defined diseases, such as Huntington's Disease (HD)1. However, and despite recent clinical success in liver indications, delivery of synthetic RNAi-based drugs to the central nervous system (CNS) remains a primary challenge in its application to neurodegenerative diseases (2,3). In order to enable non-toxic and efficient delivery to the CNS, we have recently synthesized and validated the utility of a hydrophobic short interfering RNA (hsiRNA) conjugated to docosahexanoic acid (DHA) – a predominant omega-3 polyunsaturated fatty acid in the brain4. Indeed, conjugation of DHA to a chemically-modified hsiRNA allowed for widespread distribution and retention, as well as efficient Htt gene silencing with safe toxicity profile in the mouse brain (4).To further enhance delivery and gene silencing efficacy of first generation DHA-hsiRNA conjugates, here we sought to adapt the existing scaffold to resemble naturally occurring phosphatidylcholines and sphingomyelins. Thus, second generation DHA conjugates contain a phosphocholine (PC) group and were designed to target the HTT gene, and/or containing a Cy3 moiety on the 5'-end to enable visualization of biodistribution. In primary cultures of cortical neurons, PC-DHA-hsiRNAs were effectively internalized and primarily localized around the perinuclear area – main site of action for siRNAs, as determined by fluorescent microscopy. Furthermore, PC-DHA-hsiRNA conjugates significantly reduce the expression of the Htt gene to a greater extent (~68%) than first generation DHA-hsiRNA conjugates (~55%) as determined by Quantigene® b-DNA assay. In vivo, after intrastriatal and intracerebroventricular injections, PC-DHA-hsiRNA conjugates present a broader distribution and tissue retention than former conjugates. Most importantly, PC-DHA-hsiRNA conjugates enable potent gene silencing of the Htt target (~78%) both in the ipsilateral striatum and cortex of wild-type FVB/NJ mice after single intrastriatal injection. Furthermore, significant gene silencing of the human mutant HTT (~36%) was also achieved in the striatum of YAC128 mice, rodent model of HD. Together with its favorable toxicity profile, these data validate the utility of PC-DHA-hsiRNA conjugates for gene silencing in the brain, and greatly contributes to the development of RNAi-based therapeutics for neurogenetic disorders.

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***Funding**: CHDI Foundation (Research Agreement A-6119 and JSC A6367), NIH RO1GM10880302, NIH RO1NS03819415, and NIH UH3TR00088803*

Poster: P.146 | Oral presentation: O.21 | Sofia Cristina Soares de Morais Grade
Local environment determines integration of transplanted neurons

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Cell transplantation aiming at replacing neurons lost upon brain injury or disease has been pursued, however, it remained elusive whether transplanted neurons can faithfully wire into mature circuits. We induced selective cell death in the primary visual cortex (V1) of adult mice, a confined and non-inflammatory injury, and transplanted neurons from the embryonic mouse neocortex. We demonstrate that neurons acquire mature morphologies, extend axons to correct targets through the host parenchyma, and develop synaptic specializations. Using rabies virus-based monosynaptic tracing we show that they receive area-specific, afferent projections matching those of endogenous V1 neurons, including topographically organized geniculate-cortical (graft) connections. Next, we compared neuronal integration in different environments: a traumatic invasive brain injury and the intact cerebral cortex. Transsynaptic tracing after transplantation into the stab wound injured cerebral cortex reveals a V1-specific connectome with no aberrant afferent areas, but an excess of local input. Conversely, neurons transplanted in the intact brain receive correct but considerably fewer connections per cell. Altogether our data indicate that neurons can integrate with great specificity into adult neocortical circuits, a central question for functional reconstruction of the brain, and pinpoint a key role for the local environment critically affecting the synaptic integration into host neuronal networks.

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Poster: P.147 | Ana Iolanda d'Armada Moreira
Astrosomes: artificial astrocytes as a novel approach to regulate neuronal communication

Presenter: Ana Armada-Moreira | iMM–ULisboa; iNANO–Aarhus Univ
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Neuronal excitotoxicity is a common phenomenon in several neurological diseases (1), as well as in cerebral ischemia and traumatic brain injury (2). This phenomenon is generally associated with an impaired clearance of synaptically-released glutamate by astrocytes, which leads to an over-activation of post-synaptic glutamate receptors (1,2) and can be accompanied by oxidative stress and a rise in hydrogen peroxide (H2O2) levels (3). Several therapeutic approaches regarding excitotoxicity focus on limiting glutamate release or blocking post-synaptic glutamate receptors (2). However, this type of permanent blockage interferes with physiological glutamate functions as well as brain functions (2). Therapeutic cell mimicry aims at substituting for missing or lost cellular function (4),through artificial cells envisioned as micron-sized entities which can mimic a specific cellular structure (5) and/or function (6). Thus, the main aim of our study was to design and develop an artificial astrocyte (an astrosome) capable of removing excess glutamate, ammonia (NH3), and H2O2 on synaptic clefts during excitotoxic events. By using droplet-microfluidics we generated alginate particles containing the enzyme glutamate dehydrogenase (GDH) and platinum nanoparticles (Pt-NP) entrapped in liposomal subunits. GDH converts glutamate into NH3 and a-ketoglutarate (7), and Pt-NP catalyses both NH3 and H2O2 degradation at an industrial level (8). First, we have confirmed that GDH and Pt-NP maintain their catalyst activities in vitro. GDH encapsulated in DOPC liposomes presented 28±8.5% of the activity of free GDH 10 U/mL (n=3). By exposing different concentrations of NH3 and H2O2 to Pt-NP throughout time, it was observed that PtNP degrade NH3, with a reduction of the initial concentration (100%) after 24 hours to 61±28%, 63±18%, 73±16% and 84±7.3% for Pt-NP incubated with 0.25 mM, 0.5 mM, 1 mM and 2 mM NH3, respectively (n=5 for all NH3 concentrations, $p<0.001$-0.0001 compared to control). Regarding H2O2, within 2h, Pt-NP were able of drastically reducing the concentration of H2O2 in solution, with a decrease to 3.9±1.2%, to 2.1 ± 0.7% and to 1.180 ± 0.3887% of the original H2O2 concentration for Pt-NP incubated with 0.25 mM, 0.5 mM and 1 mM H2O2, respectively (n=3 for all concentrations, $p<0.0001$ compared to control). Another key feature of the astrosome is its surface coating, which is essential for cell interaction and compatibility. Alginate particles coated with PLL (1 and 2 mg/mL) were tested with SH-SY5Y human neuroblastoma cells towards the astrosome ability to integrate with the proliferating cells. We observed by immunocytochemistry that only coated particles are able to interact with SH-SY5Y cells. The present results show for the first time the assembling and the characterization of the first artificial particle with relevance in neuroscience. We anticipate ameliorating the effects of excitotoxicity, thus providing a therapeutic approach for several neurological diseases.

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***Funding**: Fundação para a Ciência e Tecnologia (FCT) – PD/BD/114278/2016*

Poster: P.148 | Ana Paula Pêgo
BDNF gene therapy vectorized by neuron-targeted nanoparticle is neuroprotective in peripheral nerve injury

Presenter: Ana Paula Pêgo | INEB / i3S
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Peripheral neuropathies are common and still lack an effective treatment option. Neuron-targeted gene delivery is a promising strategy to treat peripheral neuropathies. Here we propose the use of polymeric nanoparticles based on thiolated trimethyl chitosan (TMCSH) to mediate targeted gene delivery to peripheral neurons upon a peripheral and minimally invasive intramuscular administration (1). Nanoparticles were grafted with the non-toxic carboxylic fragment of the tetanus neurotoxin (HC) to allow neuron targeting and retrograde transport, as confirmed using compartmentalized primary neuron cultures and taking advantage of (quantitative) bioimaging tools (2). Subsequently, we explored the delivery of a plasmid DNA encoding for the brain-derived neurotrophic factor (BDNF) in a peripheral nerve injury model. The TMCSH-HC/BDNF nanoparticle treatment promoted the release and significant expression of BDNF in neural tissues, which resulted in an enhanced functional recovery after injury as compared to control treatments (vehicle and non-targeted nanoparticles), associated with an improvement in key pro-regenerative events, namely, the increased expression of neurofilament and growth-associated protein GAP-43 in the injured nerves. Moreover, the targeted nanoparticle treatment was correlated with a significantly higher density of myelinated axons in the distal stump of injured nerves, as well as with preservation of unmyelinated axon density as compared with controls and a protective role in injury-denervated muscles, preventing them from denervation. These results highlight the potential of TMCSH-HC nanoparticles as non-viral gene carriers to deliver therapeutic genes into peripheral neurons and thus, pave the way for their use as an effective therapeutic intervention for peripheral neuropathies.

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***Funding**: Project NanoProtect (INFARMED, Portugal) and BAITS (FCT, Portugal).*

Poster: P.149 | Ana Rita Leitão Costa
Actin Rings: its assembly and physiological relevance

Presenter: Ana Rita Costa | IBMC
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Neurons are highly polarized cells that depend on cytoskeleton organization to establish their shape and function. Advances on super resolution microscopy allowed the identification of a membrane periodic skeleton composed by actin rings interconnected by spectrin tetramers. However, the assembly and function of this structure remains largely elusive. Recent findings support the relevance of additional components of the membrane periodic skeleton apart from actin and spectrin. In this respect, our previous data suggest that alpha-adducin is essential to control actin ring diameter. We are currently dissecting, by STED nanoscopy, how actin rings are nucleated, elongated and organized using specific inhibitors and knocking down relevant actin-binding proteins. Additionally, we are modeling the contribution of the membrane periodic skeleton to the electrophysiological properties of axons.
We expect that our findings open new perspectives on the study of the physiological roles of axonal actin.

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Poster: P.150 | Catarina Sofia Oliveira Miranda

Repeated systemic injection of mesenchymal stem cells sustainably alleviates motor impairments and mitigates neuropathology in Machado-Joseph disease

Presenter: Catarina Oliveira Miranda | CNC

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Machado-Joseph disease (MJD) or Spinocerebellar ataxia type 3 (SCA-3) is the most common SCA worldwide, caused by an expanded CAG repeat in the MJD1/ATXN3 gene, which translates into a polyQ tract within the ataxin-3 protein. Currently, there is no therapy able to modify or delay disease progression. Mesenchymal stem cells (MSC) are promising tools for therapy of neurodegenerative disorders and have recently received considerable interest with respect to SCAs. Aligned with these results, clinical trials with MSC have been reporting to be safe and to delay disease progression in some SCAs, including MJD. However, recent studies reported that some patients had regressed to the status prior to the treatment [1-3]. As such, the objective of this work was to investigate whether repeated intravenous (iv) transplantation of MSC can be used as a sustainable therapy for MJD. MSC were isolated from the bone marrow of adult wild type mice with C57BL/6 background, selected negatively for CD45 and ex vivo expanded. Phenotypical and neuropathological evaluations were performed in MSC-treated and non-treated MJD transgenic mice (Tg-ATXN3-69Q MJD model) [4] after transplantation of MSC in the parenchyma of the cerebellum (single injection of 3x105 MSC), icv (single injection of 3x105 MSC) or in the tail vein (four injections of ~7x108 MSC/kg, separated from each other by 2 weeks). A single intracerebral injection of MSCs (either in the lateral ventricles or in the cerebellum) could promote neuronal protection in Tg-ATXN3-69Q MJD mice, as revealed by a preservation of Purkinje cell number and of the volume of cerebellar lobules. Phenotypically, a highly beneficial but transient effect was registered 2-4 weeks after transplantation, disappearing later on. Moreover, icv longitudinal assessment by MRI of MSCs pre-labelled with superparamagnetic oxide nanoparticles (SPION) disclosed that MSC's volume inside the ventricles decreased 57% from 1 to 4 weeks after transplantation. In order to assess whether repeated injections of MSCs could overcome the ephemeral effect of MSC's therapies in this pathology, we performed four intravenous injections every 2-3 other weeks. MSC-treated MJD transgenic mice showed better performances in rotarod, beam walking and footprint tests, four and eight weeks after the treatment, whereas neuropathological mitigation was preserved, suggesting that this approach could sustainably alleviate MJD phenotype. The present study provides evidence that icv transplantation of MSCs transiently alleviates MJD phenotype, while repeated intravenous transplantation of MSC can sustainably alleviate MJD and become an effective candidate for disease-modifying MJD therapies, so far inexistent.

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Poster: P.151 | Cláudia Filipa Martins Afonso
Adenosine a2a receptors modulate rat in vivo oligodendrogenesis

Presenter: Cláudia Afonso | Fac. de Medicina, Univ. Lisboa
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Oligodendrocytes (OLGs) are the cells responsible for myelinating the neuronal axons of the Central Nervous System (CNS). The emergence of the myelin sheath was an important event in vertebrate development, allowing the rapid propagation of action potentials while preserving axonal diameter and reducing metabolic costs of neuronal activity. During a demyelinating disorder, such as multiple sclerosis (MS), myelin disruption and oligodendrocyte death is observed. In MS, remyelination occurs with new oligodendrocytes being generated from brain parenchymal oligodendrocyte progenitor cells (OPCs) and a minority from neural stem cells present in the subventricular zone (SVZ) along the lateral ventricles. Interestingly, previous data from our group showed that adenosine A2A receptor (A2AR) activation stimulates SVZ oligodendrogenesis in the context of the neurosphere assay. Therefore, our aim was to evaluate the role of A2ARs in rat oligodendrogenesis in vivo. A cannula was inserted in the right lateral ventricle of 6-week old Wistar rats and connected to an osmotic minipump, from which the A2AR agonist (CGS21680 100 nM) or the vehicle was delivered continuously for 28 days. Cell proliferation was evaluated with two intraperitoneal injections of BrdU at the end of drug administration, separated by 2-hour intervals. Cell migration was studied by injecting animals with BrdU twice a day at 12-hour intervals in the first three days of treatment. Immunohistochemical processing was performed for Olig2 and BrdU. Our results unexpectedly showed that using the proliferation protocol, activation of A2ARs in the SVZ decreases the proliferation of OPCs, leading to a reduction in the percentage of double-labeled cells for BrdU and Olig2 per volume (control: 100.0%±16.0, CGS21680: 34.0%±8.6, N=3, *p=0.0222) and per total BrdU-positive cells (control: 8.3%±1.2, CGS21680: 4.2%±1.2, N=3, ns, p=0.0715). Furthermore, the percentage of BrdU-positive cells per volume of SVZ is also diminished following A2AR activation (control: 100%±13.3, CGS21680: 69.8±7.9, N=3, ns, p=0.1236). Most of these double-labeled Olig2 and BrdU cells migrate to the OB via the rostral migratory stream (RMS). However, using the differentiation protocol, we observed that A2AR activation slows the migration of Olig2-positive cells, since a lower percentage of double-labeled cells, both per volume (control: 100.0%±8.7, CGS21680: 22.5%±9.8, N=3, **p=0.0060) and per total BrdU-positive cells (control: 17.2%±2.6, CGS21680: 5.0%±0.5, N=3, *p=0.0105) is observed in the RMS following administration with CGS21680. A2AR activation appears to negatively modulate SVZ oligodendrogenesis in vivo. However, further immunohistochemical analysis of cells in the later stages of oligodendrocyte development will be necessary to determine the precise role of A2ARs in this process.

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Poster: P.152 | Diana Alves Afonso

The impact of choroid plexus derived factors in the modulation of the sub ventricular zone neurogenic niche

Presenter: Diana Afonso | ICVS, University of Minho

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The subventricular zone (SVZ) neurogenic niche, is a source of new neurons in the adult brain regulated by a tight balance between intrinsic and extrinsic signals. One source of extrinsic factors is the choroid plexus (CP), located inside the cerebral ventricles. The CP plays active roles in barrier function, secretion and as the major regulator of the cerebrospinal fluid (CSF) composition. Of notice, the CSF composition is significantly altered in response to inflammation. As so, it is plausible to assume that alterations in CP transcriptome can impact the SVZ neurogenic niche. In this work we investigated the impact of peripheral inflammation in CP gene expression and in cell proliferation at the SVZ. We report a rapid and transient CP gene expression alteration of SVZ modulators, such as growth factors. We show an increase in cell proliferation at the SVZ, upon LPS stimulus. Amphiregulin (AREG) expression is altered the CP upon a peripheral inflammation. This gene, which codes for a protein that belongs to the EGF family, is described to modulate cell proliferation and migration. We next investigated if this protein alone could have an impact in the SVZ niche. Here, we show that AREG has an impact on cell proliferation and the number of neuroblasts at the SVZ.

Poster: P.153 | Isabel Freitas Amaral

Fibrin hydrogels functionalized with integrin-specific adhesive cues for therapeutic vascularization in the central nervous system

Presenter: Isabel F. Amaral | INEB, I3S, University of Porto.
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INTRODUCTION: The formation of new blood vessels has been shown to correlate with regeneration and functional recovery in a number of animal models of spinal cord injury (SCI), regardless of the therapeutic regenerative strategy explored [1]. As such, therapeutic approaches improving vessel density and restoring blood flow and the blood-spinal cord barrier are desirable following SCI. Apart from providing trophic support to the damaged tissue, angiogenesis is also of major importance in regenerative therapies involving cell delivery, namely to assure an adequate supply of oxygen and nutrients to the transplanted cells. In this sense, we aimed at engineering a fibrin-based hydrogel matrix for application in the injured spinal cord, capable of encouraging the invasion of capillary-like sprouts from the surrounding host vasculature and from endothelial progenitors eventually embedded within the hydrogel matrix prior to implantation. EXPERIMENTAL METHODS: Fibrin (Fb) hydrogel was functionalized with the integrin α6β1 binding sequence of the angiogenic inducer CCN1 (T1 peptide) [2] at different concentrations (20 to 60 μM). Covalent immobilization of T1 was performed using the enzymatic cross-linking action of factor XIIIa, which results in average peptide binding efficiencies of 32% (radiolabelling). Functionalized gels were characterized in terms of viscoelastic properties and ability to promote endothelial cell (EC) sprouting, using a microcarrier-based angiogenesis assay and high throughput fluorescence microscopy. For in vitro studies, human brain microvascular ECs (hCMEC/D3 cell line) and a clinically relevant source of endothelial progenitors (outgrowth endothelial cells from human umbilical cord blood - OECs) were used, after characterized in terms of expression of α6 and β1 integrin subunits. EC sprouting (number of sprouts per bead, sprouting area, and maximal sprouting length in 3D) was determined in z-stacks of fluorescent images of samples stained for F-actin/DNA. Finally, to evaluate the in vivo angiogenic potential of T1-functionalized Fb, the Chorioallantoic Membrane (CAM) assay was performed. RESULTS AND DISCUSSION: Functionalization with 40 μM of T1 peptide was efficient in promoting EC sprouting of hCMEC/D3 cells (1.4-fold increase in all EC sprouting parameters vs. unm Fb). Interestingly, in the presence of VEGF, the pro-angiogenic ability of T1-functionalized Fb hydrogels was significantly enhanced (1.9-fold increase in the number of sprouts per bead and 2-fold increase in the sprouting area vs. unm Fb). EC sprouting enhancement was not associated to changes in Fb network structure, since peptide immobilization did not significantly alter fibrin viscoelastic properties. In the case of OECs, 60 μM of T1 peptide were required to elicit EC sprouting, suggesting lower α6β1 integrin expression levels in these cells. In line with this hypothesis, immunocytometry analysis revealed significantly lower levels of α6 integrin subunit in OECs when compared to hCMEC/D3 cells. Finally, results from three independent in ovo CAM assays evidenced a reproducible and significant increase in the number of newly formed vessels in the presence of immobilized T1, validating the angiogenic potential of T1-functionalized Fb gels. CONCLUSION: Taken together, these results suggest that T1-functionalized fibrin gels may be of interest to induce locally angiogenesis in a SCI clinical scenario, apart being potentially useful for transplantation of OECs.

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Poster: P.154 | Maria Helena Bica Madeira
Modulation of the complement cascade and inflammasome pathways in human microglial cells by adenosine A2A receptor blockade

Presenter: Maria H. Madeira | IBILI - FMUC

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It is well accepted that inflammation has a key role in the progression of chronic neurodegenerative diseases. Accumulating evidence points to reactive microglia as a source of inflammatory mediators such as cytokines and complement proteins, which might lead to neurotoxic effects and influence pathological processes. Particularly, the complement system (CS) and inflammasome pathways, key innate immune defenses against inflammation, orchestrate critical responses in the central nervous system. In age-related macular degeneration (AMD) evidence point to a critical involvement of microglial cell recruitment, accumulation CS components and the activation of inflammasome pathways. Data collected by us and others show that adenosine A2A receptor (A2AR) blockade controls microglia-mediated neuroinflammatory processes, thus facilitating protective effects. To date, the effects of microglial reactivity and modulation by A2AR blockade on the CS and inflammasome pathways remain to be elucidated. In this work immortalized human microglial cells (SV40) were pretreated with 50 nM SCH 58261 (A2AR antagonist) and challenged with Zymosan (50 µg/mL) and phorbol 12-myristate 13-acetate (PMA; 100 nM) for 6h. Challenge with Zymosan and PMA up-regulated the expression of A2AR and activated immune response in immortalized human microglial cells. A2AR antagonist treatment reduces the challenge-induced up-regulation of mRNA expression levels of CCL2 and tumor necrosis factor (TNF), and in nitric oxide (NO) levels. Further, the phagocytic efficiency of reactive human microglia is reduced by A2AR blockade. Notably, A2AR blockade was also able to prevent the up-regulation of CS components, namely in the mRNA levels of complement component 3 (C3) and complement factor B (CFB). Moreover, SCH58261 treatment prevented the up-regulation in the mRNA levels of IL-18 in stimulated human microglial cells, suggesting a possible impact of A2AR modulation on the microglial inflammasome pathway. We here show that A2AR blockade impacts human microglial cell reactivity, being able to prevent their activation, and the expression of inflammatory markers and CS components. Further studies are still necessary for a better understanding of A2AR role in the CS. Yet, these results indicate the use of A2AR antagonists as potential therapeutic strategies for degenerative diseases, whose pathogenesis include microglia reactivity and CS alterations, such as AMD.

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Poster: P.155 | Marília Judite Falcão Torrado

The LAL (levator auris longus) muscle is an accessible system to study the effects of Botulinum Toxins in vivo

Presenter: Marília Torrado | FMUP
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Botulinum neurotoxins (BoNTs), the most potent toxins known, are produced by anaerobic Clostridium bacteria and can cause persistent and, in some cases, lethal muscle paralysis. Seven distinct serotypes (BoNT/A–G) have been identified, all producing neuronal inactivation by blockade of SNAREs-dependent neurotransmission (soluble N-ethylmaleimide-sensitive factor attachment protein receptors; proteins involved in exocytosis of synaptic vesicles). The high potency and neurospecificity of BoNTs puts them among the therapeutics of choice for a variety of human conditions that affect not only neuromuscular junctions, but also autonomic cholinergic synapses, such as dystonias, hyperhidrosis, gastrointestinal and urinary disorders. Surprisingly, despite their well-established successful use, the mechanisms of action of BoNTs is still not fully understood. In fact, binding, internalization and duration of action still require investigation. Thus, there is an emergent need of new and accurate models to study the effects of BoNTs. Considering their potential lethality, it is challenging to find reproducible models to study the local application of BoNTs in living animals that allow a widespread visualization of the intoxicated muscle nerve terminals. In the present work, we studied the innervation pattern and the effect of BoNTs in a group of small subcutaneous cranial muscles that are responsible for moving the pinna in rats and mice, comprising the levator auris longus (LAL), the interscutularis (IS), the auricularis superior (AS) and the abductor auris longus (AAL) muscles. Although all are easily accessible and manipulated in ex vivo preparations, we focused on LAL. After injection of BoNT/C or BoNT/A, muscles were dissected and prepared for wholemount staining for Synapsin-I and β3-tubulin. We found no effects of BoNT/C delivery. In contrast, detection of cleaved SNAP-25 (synaptosome-associated protein of 25 kDa), the end-product of the catalytic action of BoNT/A, was possible even after injection of as little as 0.1 ng, indicating the action of this toxin and a good sensibility of the model. Mapping of the injected muscle showed the effect of BoNT/A in the majority of the endplate population. This indicates that BoNTs delivery to the LAL is a sensitive, simple and reproducible model to study the mechanisms of action of these toxins as it allowed the evaluation of BoNT/A effects throughout the entire muscle, without sampling bias. It should be noted that current observations could be easily expanded using additional analytical tools (Western blot, gene expression assays). Thus, we forward that this LAL manipulation may constitute an excellent model to clarify the mechanisms of action of BoNTs in the neuromuscular system.

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Poster: P.156 | Nuno Sérgio Mendes Dias
Working memory assessment: A computerized test battery for the older adult

Presenter: Nuno Dias | ICVS, University of Minho
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Working memory is a limited capacity system that allows the temporary storage and manipulation of information (Conway et al., 2005). Working memory changes with age progression. The development of appropriate measures for its assessment on scale is paramount for better understanding these changes. This project aimed at creating a computerized working memory test battery for the assessment of the older adult (Wild et al., 2008). The test battery reveals an attempt to associate tasks and stimuli of everyday life with paradigms that have been proved to be valid in the assessment of working memory skills. These tests were conducted on a sample of 106 Portuguese subjects, between 54 and 86 years. The statistical analysis included classical statistical procedures and procedures based on item-response theory (Rasch model). Results pointed out a battery with a unidimensional structure, composed of five tasks. The battery revealed good internal consistency (α=0.88), as well as positive and strong correlations with measures of cognitive function (Baddeley, 1992), which supports the use of ecologically-oriented tasks for cognitive assessment. In the future, we aim to validate the working memory battery for the Portuguese population and to study its capacity in the assessment of clinical samples (e.g., addiction disorder, mild cognitive impairment, etc .)

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Poster: P.157 | Raquel Marinho do Nascimento Alonso
Effect of SPRR1A overexpression on the nociceptive behaviour of OA animals

Presenter: Raquel Alonso | FMUP
Raquel Alonso, Diana Nascimento, José Manuel Castro-Lopes, Fani Neto e Joana Ferreira-Gomes
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Osteoarthritis (OA) is an articular disease characterized by the presence of chronic pain. In fact, pain control in OA patients remains the main clinical concern in its treatment. Although OA has been considered mostly as an inflammatory disease, a neuropathic component has been suggested lately. Indeed, studies from our group using both the monoiodoacetate- (MIA) and the collagenase-induced models of OA have found an increase in the number of primary afferent neurons expressing markers of neuronal damage, in ipsilateral dorsal root ganglia (DRGs), which may be responsible for the ineffectiveness of treatments based on inflammatory drugs. In both models an increased expression of regeneration associated- genes (RAGs) was also observed. In the collagenase-induced OA model a significant increase in the expression of the RAG small proline rich protein 1A (SPRR1A) was found, which is believed to be related with axonal growth. So, in the present study we aimed at clarifying the role of SPRR1A in OA pain mechanisms at the primary afferent neurons level. For this purpose, a viral vector engineered to express SPRR1A was injected intra-ganglionically in OA and control animals and the effect of this overexpression on the nociceptive behaviour was evaluated through the Von Frey, CatWalk and Knee-Bend tests. The preliminary data suggests that there is an attenuation of mechanical allodynia and movement- and loading-induced nociception in OA animals injected with the virus. RT-PCR analysis confirmed an overexpression of SPRR1A mRNA levels in L4/L5 ipsilateral dorsal root ganglia of virus injected animals and showed changes in the levels of other associated genes.

Poster: P.158 | Rita Catarina Gonçalves Perfeito
Alpha-synuclein secretion in in vitro models of Parkinson's disease – a role for autophagy

Presenter: Rita Perfeito | CNC, University of Coimbra

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by the accumulation of misfolded protein aggregates of alpha-synuclein (α-syn). α-Syn has been described to be transmitted from neuron to neuron through different mechanisms, including exosomes, propagating aggregate pathology to different brain areas and contributing to PD progression. α-Syn accumulation in neurons has also been linked to an ineffective clearance of this protein by autophagy. The main goal of this work was to clarify a possible role for the interaction between α-syn exosome secretion and autophagy in PD and to investigate whether pharmacological activation of autophagy would prevent secretion of α-syn in exosomes. For this purpose two in vitro models were used: mouse neuroblastoma (N2a) cells expressing human WT α-syn and human neuroepithelial stem cells (hNESCs) derived from fibroblasts of PD patients. Results show that upon incubation of exosomes isolated from N2a cells expressing human α-syn with non-transfected N2a cells, α-syn was detected in the latter, suggesting that α-syn was transferred from cell-to-cell via exosomes. Pharmacological activation of autophagy in N2a cells overexpressing human WT α-syn, effectively reduced the levels of this protein. Importantly, we found that in exosomes isolated from transfected N2a cells treated with an autophagy activator during 12 h, α-syn levels were significantly decreased, suggesting that this strategy of autophagy activation prevented the secretion of α-syn via exosomes. Preliminary data with hNESCs derived from PD patients show a decrease in the autophagic flux in PD cells compared to control cells. Furthermore, an increased concentration of extracellular vesicles staining for exosomal markers was detected in PD cells in comparison with control hNESCs. Our data strengthens previous evidence that the autophagy pathway plays an important role in the clearance of α-syn levels and provides new information on a possible interaction between the autophagic process and the spreading of α-syn via exosomes. We propose that inhibiting α-syn release in exosomes and inducing degradation of α-syn and/or aggregates by autophagy may constitute a novel pharmacological approach for treatment of synucleinopathies such as PD.

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Poster: P.159 | Sandra Marisa de Jesus Oliveira

Therapeutic effects of a novel mesenchymal stem cell population in a rat model of diabetic neuropathy: Is there a role for soluble adhesion molecules?

Presenter: Sandra Marisa Oliveira | Dept Biomedicina FMUP/IBMC/i3S

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Diabetic neuropathy (DN) is one of the most common diabetes complications, with several clinical manifestations. Patients with DN may develop painful symptoms and/or present abnormal pain responses. Such conditions most often have enormous physical and psychological impacts. Moreover, current treatment strategies are still very limited, and most of them present undesired side effects. Under the REDDSTAR project (Repair of Diabetic Damage by Stromal Cell Administration, http://www.reddstar.eu/), we used a streptozotocin (STZ)-induced rat model of DN with associated altered pain responses and showed that one intravenous injection of human bone marrow CD362+ mesenchymal stem cells (MSCs) to STZ-diabetic rats was effective in preventing the development of behavioural signs of DN, without affecting metabolic parameters typical of this disease model (impaired weight gain, hyperglycemia, and elevated HbA1C levels). Further, as to the neurobiological mechanisms underlying the protective effects of this novel MSC population, we showed the existence of a potential peripheral effect through the modulation of sciatic nerve inflammation. However, given the observed protective effects of intravenously delivered CD362+ MSCs in not only our animal model of DN but also in animal models of other diabetes complications, namely diabetic kidney disease and diabetic retinopathy, a common mechanism of action relying on a systemic effect emerges as being also a plausible hypothesis. In this context, we decided to evaluate the paracrine effects over time of systemically administered CD362+ to STZ-diabetic rats through the analysis of a panel of circulating cytokines/chemokines/trophic factors/adhesion molecules using state-of-the-heart Luminex Multiplex Array analyses. Briefly, control, STZ-diabetic, and STZ-diabetic rats administered CD362+ or CD362- MSCs one week after the induction of diabetes were used for this study. Always allowing a 5-hour fast, blood samples were collected by tail vein puncture before (0h), and 24h, 48h, 1 week, and 10 weeks after intravenous MSCs or vehicle solution (PBS) administration. We show that, at baseline (0h), STZ-diabetic rats exhibited elevated levels of soluble ICAM-1 and L-selectin adhesion molecules as compared to controls. After the administration of MSCs (or vehicle solution), only STZ-diabetic rats that received CD362+ MSCs maintained elevated levels of these soluble adhesion molecules at 10 weeks post-administration. These results, in light of a body of literature that describes protective immunomodulatory actions of soluble forms of ICAM-1 in the context of diabetes, suggest that CD362+ MSCs may, indeed, exert systemic protective actions in our rat model of DN through the maintenance of elevated levels of immunoprotective circulating adhesion molecules.

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Poster: P.160 | Sara Xapelli
Postnatal hippocampal neurogenesis is regulated by adenosine A2A receptor

Presenter: Sara Xapelli | iMM, FMUL
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Constitutive neurogenesis takes place in both adult mammalian subventricular zone of the lateral ventricle and in the subgranular zone of the dentate gyrus (DG) in the hippocampus. Adenosine, through A2AR activation, is a known modulator of synaptic transmission, however its influence in neurogenesis has been much less investigated. This study evaluated whether adenosine A2ARs have a role in postnatal neurogenesis, namely in cell proliferation and neuronal differentiation, and in the capacity of progenitor cells to divide and self-renew within the DG. Results from in vitro experiments, using DG stem/progenitor cells from early postnatal (P1-3) Sprague-Dawley rats, demonstrated that A2AR agonist/antagonist did not alter cell viability (measured by propidium iodide staining) nor cell proliferation (measured by BrdU staining). However, A2AR activation promoted DG neuronal differentiation (measured by NeuN staining), which was prevented by A2AR blockade. Moreover, a cell-fate study was performed using an immunocytochemistry against Sox2 (a marker of neural stem cells with the ability to self-renew) to study cell-type division. A2AR activation promoted an increase in the number of Sox2+/+ DG cell-pairs derived from a progenitor cell division, with a concomitant decrease in Sox2-/- cell-pairs. These results were corroborated by the increase in the self-renewal capacity of progenitor cells by A2AR activation. Importantly we evaluated the role of A2AR exogenous activation in the SGZ derived neurogenesis in vivo, namely in progenitor cell proliferation and in the number of new neurons. For that, a cannula was inserted in the lateral ventricle of 6 week-old rats and connected to an osmotic mini pump, from where A2AR agonist (CGS 21680) or the vehicle (aCSF) were delivered continuously for 28 days. To study neural stem/progenitor cell proliferation, BrdU was administered intraperitoneally (ip) twice at the end of 28 days with 2h interval, with the last injection 2h before perfusion. To study neuronal differentiation, BrdU was administered ip twice a day, with 12h interval, for the first 3 days after surgery. Data show that A2AR activation does not affect SGZ-derived progenitor cell proliferation, BrdU+ cells, but promotes an increase in the number of neuroblasts, DCX+ cells, and new neurons, BrdU+/NeuN+ cells, in the DG. Accordingly, with the previously obtained in vitro results, the in vivo data shows that the percentage of new neurons originated from the DG are enhanced in the presence of the A2AR agonist. Taken together, A2AR activation is required for postnatal hippocampal neurogenesis both in vitro and in vivo.

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Poster: P.161 | Sofia Alexuandra Duque Santos
PEGylated PAMAM dendrimers can effectively reach the injured ischemic brain

Presenter: Sofia Duque Santos | i3S
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Introduction: Dendrimers are special three-dimension macromolecules with a nano-scale dimension and a highly branched structure, which have a tremendous potential as delivery systems. In central nervous system pathologies, dendrimers have been applied with therapeutic purposes, being PAMAM the most extensively described. Materials and Methods: We have investigated PEGylated G4 PAMAM dendrimers functionalized with rhodamine (PEG-PAMAM-Rhod) in a mice model of ischemic stroke. The fate of the dendrimers was investigated, focusing on the brain and some peripheral organs, by following the rhodamine signal after systemic administration at different time points post-injury. Blood and urine was analyzed to assess blood circulation and excretion. Moreover, hemolysis and clotting assays were performed to assure biocompatibility after intravenous administrations. Results: The presence of PEG-PAMAM-Rhod dendrimers was significantly increased in the brain cortex after ischemia when compared to unlesioned tissue. Moreover, this increase was not observed upon free rhodamine administration (control). In contrast, the inspection of the choroid plexus of the brain showed a high accumulation of dendrimers in unleasioned animals while it was decreased when administered in the post-lesion period. Nonetheless, similar to what was observed in the brain cortex, the presence of free rhodamine is minor when compared to PEG-PAMAM-Rhod dendrimers. Besides the brain, these dendrimers were also detected in the liver and kidney as a result of the selected route of administration. The detection of rhodamine (free and bound) in both blood and urine one day after administration was a reflection of the prolonged circulation and filtration processes. Moreover, the contact of the PEGylated dendrimers with red blood cells was safe as no hemolysis was detected contrary to the unPEGylated parent dendrimer. Additionally, the PEGylated dendrimers behaved as anti-coagulation factor since the clotting period was substantially delayed. Conclusion and discussion: Upon injury such as in ischemic stroke, the blood-brain barrier is compromised which allows for the entrance of molecules that otherwise would be excluded and also for the increase of the flux and entry of substances and cells. Our data reflects this response with a much higher presence of PEGylated dendrimers bound to rhodamine in the ischemic brain, which was still detected 24 hours after administration. Such a result points to the dendrimers being an effective delivery vehicle for therapeutic drugs towards the injured central nervous system.

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Poster: P.162 | Sónia Patrícia Dias Duarte

Autophagy activation mediated by let-7 alleviates motor and neuropathological deficits in pre- and post-symptomatic Machado-Joseph disease mouse models

Presenter: Sónia Duarte | CNC, University of Coimbra

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Machado-Joseph disease or spinocerebellar ataxia type 3 (MJD/SCA3) is a genetic neurodegenerative disorder associated with expansion of the number of CAGs within the coding region of the MJD1/ATXN3 gene, which translates into an expanded polyglutamine tract within ataxin-3 protein. MJD patients have severe clinical manifestations and premature death and there is no treatment available to modify disease progression. We and others provided evidence that autophagy impairments contribute to MJD pathogenesis with autophagosome accumulation, reduction of autophagy-associated protein levels, accumulation of mutant ataxin-3 and neurodegeneration. Recently, we also brought evidence that the let-7 microRNA is a key regulator of autophagy with particular relevance in polyglutamine disorders. In this work we aimed at investigating let-7 potential as a new therapeutic approach in a lentiviral-based and in a transgenic mouse model of MJD. Let-7 levels showed a tendency to be reduced in a lentiviral MJD mouse model, which we attempted to overcome by injection of lentiviral vectors encoding for let-7. A 20% increase of let-7 levels was observed in mice striata. LC3 immunoblot analysis revealed increased levels of LC3-II relative to actin upon let-7 treatment. A significant let-7-mediated reduction of ubiquitin-positive inclusions and neuronal dysfunction was observed in the lentiviral-based model at 4 weeks, while in transgenic MJD mice, let-7 significantly reduced motor incoordination and imbalance. Hence, let-7 activates autophagy in the mammalian brain, promotes increased turnover of mutant ataxin-3 protein in mouse CNS, reduces neuronal dysfunction and ameliorates motor deficits. Therefore, autophagy activation mediated by let-7 may represent a new therapeutic approach for MJD.

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Poster: P.163 | Ana Rita Pinto Costa

Profilin-1 is a key regulator of actin and microtubule dynamics required for optimal axon growth and regeneration

Presenter: Rita Costa | i3S/IBMC

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Although actin is well recognized as a key player in axon growth, how different actin-binding proteins control its dynamics is not fully understood. Here we investigated the role of profilin-1 (Pfn1) in axon growth and regeneration. Profilins provide the pool of competent ATP-actin monomers to be added to F-actin ends to support their polymerization. In vitro, Pfn1 knockdown severely impaired actin retrograde flow, microtubule growth speed, and axon formation and growth. In vivo, mice with an inducible neuronal deletion of Pfn1 had decreased axon regeneration. In a model with increased regeneration capacity, Pfn1 activity was increased in the growth cone of regenerating axons. In line with these findings, overexpression of constitutively active Pfn1 strongly enhanced actin and MT dynamics, and axon growth. In summary, we show that Pfn1 is a determinant of axon growth and regeneration acting as a key regulator of both actin and MT dynamics.

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***Link between abstracts**: actin, profilin-1*

SESSION: NEURODEVELOPMENT AND CELL BIOLOGY

Poster: P.164 | Márcio Gabriel Silva Costa
Structural functional relationships of the Alzheimer's Amyloid Precursor Protein

Presenter: Márcio Costa | iBiMED - University of Aveiro
Márcio Costa, Steven Alves, Sandra Rebelo, Ana Gabriela Henriques and Odete A. B. da Cruz e Silva
(1) iBiMED - Institute of Biomedicine, Department of Medical Sciences, University of Aveiro

The Amyloid Precursor Protein (APP) is a ubiquitously expressed type I transmembrane glycoprotein .[1] The precise function of APP is not clear, but several biological functions such regulation of neurite extension, blood coagulation, wound-healing, growth regulation, modulation of neuronal excitability, synaptic plasticity, and cell survival have been attributed to this protein.[2] Post-translational modifications like protein phosphorylation act as the gatekeepers of cellular processes like neuronal plasticity, neurotransmission, and consequently compromise memory and learning [3-5]. In neuronal cells, APP isoform AβPP695 can be phosphorylated at serine, threonine and tyrosine residues. In the intracellular domain 8 putative phosphorylation residues have been described being Y653, T654, S655, T668, S675, Y682, T686 and Y687 [6-8]. In the extracellular domain two phosphorylatable residues have been identified, S198 and S206 [9]. The consequence and physiological relevance of phosphorylation events at each of these residues is not clearly understood. Another important APP functional domain is the RERMS sequence, in the ectodomain two (E2), which appears to be important for dimerization. In fact, a recent study where the x-ray structure of APP E2 domain was present, revealed that the RERMS sequence directly participates in the dimerization of APP [10]. RERMS has also been reported to play a role in neurite outgrowth [11], to induce neurite outgrowth in a neuronal cell line from rat central nervous system (CNS), B103 cells [12], and RERMS-containing APP peptides were demonstrated to promote neuronal survival in primary rat cortical cells [13]. Our previous results demonstrate that RERMS can modulate cell morphology, and although the precise mechanism has to be further investigated; the process involves cytoskeletal reorganization and Focal Adhesion Kinase (FAK). This was shown for differentiated SH-SY5Y cells thus pointing to the potential relevance of RERMS - ECM mediated interactions in neuronal systems. Ongoing work aims to better describe the mechanism behind RERMS function on neuronal plasticity and neurite outgrowth, and how protein phosphorylation may contribute to this process.

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***Link between abstracts**: Bicicleta*

Poster: P.165 | Tiago José Carvalho Ferreira da Silva
Plasmalogen deficiency affects muscle innervation and synapse formation

Presenter: Tiago Silva | I3S
Tiago Silva1, 2, 3, Mónica Sousa1, 2, Pedro Brites1, 2
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Plasmalogens (PLS) are a special class of phospholipids characterized by a vinyl-ether bound at the sn-1 position of the glycerol backbone. PLS make up for 18% of the total phospholipid content and are the main ether-phospholipid synthesized by mammals, and are highly enriched in the nervous tissue and muscle. The importance of PLS for normal development is highlighted by the severe clinical presentation of rizhomelic chondrodysplasia puntacta (RCDP), a lethal disorder caused by an impairment in the biosynthesis of PLS. Clinically RCDP patients display bone abnormalities, contractures, congenital cataracts, hypotonia, and profound growth and mental retardation. To investigate the role of PLS in nervous tissue and investigate how a PLS deficiency causes hypotonia, we studied muscle innervation and synapse formation in the peripheral nervous system. Using the Pex7 and Gnpat knockout (KO) mice as models for RCDP, we found an abnormal innervation of several skeletal muscles. In the diaphragm of wild type (WT) mice at several development ages, we found a well defined and narrowed band of acetylcholine receptor (AChR) clusters, whereas in the diaphragms from KO mice, these AChR clusters are spread throughout the muscle. Moreover, the disorganization of the neuromuscular junctions (NMJs) innervating the KO muscles increased with development. The morphological and morphometric assessment of the NMJs in diaphragms revealed an overall increase in size and volume of the abnormally disorganized NMJs of KO mice when compared to the WT NMJs. Given that we have also found an increased sprouting and branching of motor axons from the phrenic nerve, our findings suggest that the NMJ disorganization could be a consequence of an abnormal motor axon development. Moreover, our results indicate that the muscle weakness and hypotonia characteristic of these KO models is caused by an abnormal innervation and NMJ formation. These findings may contribute to a better understanding of the human disease and current models of muscle and nerve biology.

Poster: P.166 | Oral presentation: O.26 | Céline Saraiva Gonçalves
WNT6 regulation in glioblastoma: mechanistic, functional and clinical implications

Presenter: Céline S. Gonçalves | ICVS, University of Minho
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Background: Glioblastoma (GBM) is the most common and most malignant type of glioma, a heterogeneous group of primary brain tumors. While the clinical outcome of GBM patients is unpredictable, patients are equally treated with a standardized approach. Thus, the identification of new biomarkers is crucial. HOXA9 overexpression in GBM is associated with poor prognosis and a more aggressive tumor phenotype. We recently found that HOXA9 transcriptionally activates the WNT pathway; here, we explore how WNT6, a WNT ligand/activator, may contribute to the malignant behavior of GBM. Material and Methods: Gene set enrichment analysis (GSEA) was used to query the HOXA9 transcriptome. Quantitative PCR, Western blot, chromatin immunoprecipitation (ChIP), methylation-specific PCR (MSP), and immunohistochemistry were performed in GBM cell lines, in vivo xenografts, or in patient samples to study WNT6 at various molecular levels. The functional effects of WNT6 in cell viability (MTT/Trypan blue), proliferation (BrdU), invasion (transwell matrigel), migration (ibidi inserts), angiogenesis (Chick Chorioallantoic Membrane), cell death after treatment with temozolomide (TMZ; Annexin/PI staining) and stemness capacity (limiting dilution assay) were assessed after silencing WNT6 with shRNA. U373+/-WNT6 cells were intra-cranially implanted in NSG mice to evaluate implications in survival. TCGA dataset was assessed for WNT6 status and clinicopathological correlations. Results: We found that the Wnt pathway is over-activated in HOXA9-positive GBM cells. Specifically, WNT6 is a direct transcriptional target of HOXA9 and is overexpressed in a subset of GBM patients. Additionally, we observed that WNT6 expression correlates with higher glioma grades and with the GBM proneural subtype, whose patients do not benefit from more intensive therapies. Interestingly, we demonstrated that WNT6 expression is also regulated by DNA methylation in GBM patients. In vitro, WNT6-positive cells showed increased viability, migration, invasion and resistance to TMZ, and decreased cell death, when comparing to their negative counterparts. When cultured in stem-cell conditions, WNT6-positive cells show increased viability and capacity to form neurospheres than WNT6-negative cells. In addition, mice bearing WNT6-positive tumors presented faster glioma-related symptomatology and a significantly shorter overall survival (p=0.0042). Importantly, we provide the first evidence of the clinical prognostic value of WNT6 in GBM patients from TCGA and at the protein level in a cohort of Brazilians patients, implicating high levels of WNT6 as a novel independent negative prognostic marker. Conclusion: Together, our findings provide mechanistic, functional and prognostic insights into the role of WNT6 in GBM, creating opportunities to novel therapeutic approaches to treat this highly-aggressive cancer.

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***Link between abstracts**: qCEJA*

Poster: P.167 | Ana Xavier Silva de Magalhães
Reversal of tumor cell replicative immortality in a TERT promoter mutation dependent manner

Presenter: Ana Xavier-Magalhães | ICVS, University of Minho

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Reactivation of telomerase reverse transcriptase (TERT) expression enables cells to overcome replicative senescence and escape telomere dysfunction-induced cell death [1, 2], fundamental steps in the initiation of human cancer. Over fifty cancer types, including up to 83% of glioblastomas (GBM), 75% of oligodendrogliomas, and 30% of medulloblastomas, harbor activating TERT promoter mutations that are sufficient to enable replicative immortality [3-5]. Both the C228T and C250T TERT promoter mutations create a binding site that recruits the multimeric ETS factor GABP to the mutant TERT promoter to activate TERT transcription [6, 7]. GABP recruitment occurs across multiple types of cancer with TERT promoter mutations, suggesting a shared mechanism. While GABP can act as a heterodimer or as a heterotetramer, depending on the number of GABP binding sites at the specific locus, only the heterodimer is necessary for normal cell function [8-10]. Due to the presence of an endogenous GABP binding site in the TERT promoter, we hypothesized that the mutant TERT promoter recruits the GABP heterotetramer to activate TERT transcription in GBM [6]. We further hypothesized that targeting the GABP heterotetramer may be a viable strategy to reverse replicative immortality in GBM. Using CRISPR/Cas9 editing to heterozygously knock out GABPβ1L, the tetramer-forming isoform of GABP, we identify the GABP tetramer as necessary for the activation of the mutant TERT promoter. We demonstrate that GABPβ1L haploinsufficiency reverses replicative immortality in GBM, leading to widespread telomere dysfunction and loss of cellular viability only in TERT promoter mutant cancer cells. Our data suggest inhibition of the GABP heterotetramer as a viable therapeutic strategy in TERT promoter mutant GBM and possibly many other cancers.

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***Link between abstracts**: qCEJA*

Poster: P.168 | Eduarda Pereira Martins

CDH3/P-cadherin as a putative HOXA9 target in gliomas: functional and clinical relevance

Presenter: Eduarda Martins | ICVS, University of Minho

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Glioblastoma (GBM) is the most malignant and frequent primary brain tumor in adults [1], but the mechanisms underlying its aggressiveness are still not fully understood. Previous studies of our group demonstrated that HOXA9 has an important role in GBM aggressiveness, influencing cell viability, cell death and resistance to treatment [2,3]. In ovarian cancer, it was already shown that HOXA9 contributes to the tumor's aggressiveness by inducing the expression of P-cadherin – a cell-to-cell adhesion molecule encoded by CDH3 [4]. In this study, we investigate if CDH3 is a HOXA9 target in GBM, evaluate the functional roles of P-cadherin, and assess its prognostic value. HOXA9 and CDH3 mRNA levels were evaluated using qPCR in GBM cell lines and in glioma tumor samples. P-cadherin silencing in GBM cell lines was obtained using specific siRNAs; P-cadherin overexpression was performed using the LZRS-IRES-EGFP vector with cDNA encoding full-length P-cadherin. P-cadherin functional roles were assessed in cell viability (Trypan Blue and MTS), migration (wound healing assay), invasion (Matrigel chamber assay), stemness (neurosphere formation assay and differentiation ability) and adhesion (cell substrate adhesion assay). In vivo experiments were performed through the subcutaneous injection of P-cadherin-overexpressing GBM cells in NOD-scid gamma (NSG) mice to evaluate the effect in tumor growth and volume, and orthotopically to evaluate the effect in mice survival. Additionally, the relevance of CDH3 in patients' overall survival and recurrence-free survival was evaluated through the generation of Kaplan-Meier curves and differences were assessed using Log-rank tests. We found CDH3 and HOXA9 are co-expressed in gliomas, suggesting that this cadherin might be a HOXA9 target relevant to GBM aggressiveness. In vitro studies demonstrated that P-cadherin silencing is associated with a decreased GBM viability, adhesion, migration, invasion and stemness. Furthermore, P-cadherin-overexpressing GBM cells have an increased cell viability, and generate in vivo tumors with increased volumes when injected subcutaneously. Additionally, the injection of P-cadherin-overexpressing GBM cells in a mice orthotopic model was associated with shorter survivals. Concordantly, higher CDH3 expression in GBM patients was associated with shorter survival and recurrence-free survivals. Our data shows for the first time that P-cadherin is associated with GBM aggressiveness, suggesting it may be an attractive therapeutic target. Since antibodies targeting P-Cadherin have already been developed, it will be interesting to assess their therapeutic value in GBM.

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***Link between abstracts**: qCEJA*

Poster: P.169 | Joana Isabel Martins Cosme Vieira de Castro
Autofluorescence: a new biomarker for Glioblastoma Stem Cells' identification

Presenter: Joana Vieira de Castro | ICVS, University of Minho
Joana Vieira de Castro (1, 2); Irene Miranda-Lorenzo (3); Céline S. Gonçalves (1, 2), Mariana T. Cerqueira (2, 4); Afonso A. Pinto (5); Rui L. Reis (2, 4), Nuno Sousa (1, 2), Christopher Heeschen (3, 6); Bruno M. Costa (1, 2)
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Introduction: Cancer stem cells (CSCs) have paramount roles in tumor initiation, progression, recurrence, and therapy resistance. Thus, CSCs, including Glioblastoma Stem Cells (GSCs), must be specifically targeted to achieve improved clinical responses. Several stem cell markers have been historically used to isolate GSCs, but they lack full specificity and sensitivity, highlighting the need for better biomarkers to isolate/identify GSCs. In the context of the recent identification of a subpopulation of cells with CSCs features presenting an autofluorescent subcellular compartment in a variety of epithelial cancers, we proposed to evaluate whether this phenotype is also present in glioblastoma, and if it associates with GSCs. Materials and Methods: Autofluorescent (Fluo+) cells were detected in human primary and established glioblastoma cell lines by flow cytometry. Neurospheres were generated in Neurobasal medium supplemented with B27, 20 ng/mL of b-FGF and EGF. The expression of pluripotency-associated genes and stem cells markers were performed by qPCR and flow cytometry, respectively. The percentage of Fluo+ cells in GBM cell lines was evaluated after temozolomide or radiation treatment, as well as after riboflavin, FTC and basal media treatment. U373 Fluo+/- were intracranially implanted in NSG mice to evaluate implications in survival. Results: We identified a subpopulation of autofluorescent (Fluo+) GBM cells, both in established and primary GBM cells. Functionally, these Fluo+ cells present typical features of GSCs, including higher capacity to grow and long-term self-renewal ability as 3D neurospheres, and increased expression of several stem cell and pluripotency-associated genes. In addition, exposure of GBM cells to temozolomide (TMZ) chemotherapy or to radiation treatment led to a significant enrichment of the autofluorescent cells' population in all tested models. Importantly, in vivo orthotopic models showed that mice with intracranial tumors derived from Fluo+ GBM cells have a significantly shorter overall survival than those with non-autofluorescent cells (Fluo-) GBM cells, further highlighting the GSC-associated malignant/aggressive phenotype of Fluo+ cells. Mechanistically, the autofluorescent phenotype of GSCs was due to the accumulation of riboflavin in cytoplasmic vesicles bearing ATP-dependent ABCG2 transporters. Conclusions: Together, we identified an intrinsic autofluorescent phenotype present in GBM cells with GSCs features, which can be straightforwardly used as a novel biomarker in this highly-malignant and therapy-insensitive tumors.

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***Link between abstracts**: qCEJA*

Poster: P.170 | Oral presentation: O.24 | Diogo Pinto da Cruz Sampaio e Castro
The transcription factor MyT1 counteracts the neural progenitor program to promote vertebrate neurogenesis

Presenter: Diogo S. Castro | Instituto Gulbenkian de Ciência
Francisca F. Vasconcelos(1), Alessandro Sessa(2), Cátia laranjeira(1), Alexandre A.S.F. Raposo (1), Daniel W. Hagey(3), Jonas Muhr(3), Vania Broccoli(2) and Diogo S. Castro(1)
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In the developing vertebrate embryo, generation of neurons at the correct time and location requires a fine balance between gene expression programs that regulate differentiation and maintenance of neural stem cells. This is to large extent regulated by the opposing forces of the Proneural and Notch pathways. While recent studies have focused on characterizing the differentiation genes activated by proneural factors such as Ascl1, less is known on the mechanisms that suppress progenitor cell identity. Here, we show that Ascl1 induces the transcription factor MyT1 at the onset of neuronal differentiation. We investigate the function of MyT1 at this critical stage by combining acute functional experiments in the mouse telencephalon, with the characterization of its transcriptional program. We found that MyT1 binding occurs mostly at active regulatory regions in undifferentiated neural stem/progenitor cells and is associated with transcriptional repression genome-wide. We further show that MyT1 acts at multiple levels to antagonize the inhibitory activity of Notch signaling, targeting both Notch pathway components and downstream targets. Notably, MyT1 promotes the downregulation of Hes1, a determinant step for the onset of neurogenesis, by competing with Rbpj for binding to the Hes1 promoter. Our results reveal a function of Ascl1 in inhibiting Notch signaling cell-autonomously, showing how activation of neuronal differentiation is tightly coordinated with repression of the progenitor program.

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Poster: P.171 | Oral presentation: O.25 | Patrícia Sofia Alçada Tomás de Morais
Cortical neuronal migration entails a2a receptor-driven neuronal polarization and axon formation

Presenter: Sofia Alçada-Morais | CNC, University of Coimbra
Sofia Alçada-Morais (1,2), Veronica Moreno-Juan (3), Nélio Gonçalves (1), Belén Andres (3), Sofia Ferreira (1,2), Joana M. Marques (1), Xinli Xu (1,2), Rodrigo A. Cunha (1,4), Guillermina López-Bendito (3), Ricardo J. Rodrigues (1)
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Neuronal migration is a fundamental process in brain development. Indeed, impairment in neuronal migration is one of the major causes of cortical malformation, which has been associated to several neurological and psychiatric disorders [1]. Hence, it is of upmost importance to unravel the mechanisms driving neuronal migration. In this regard, it was recently shown that adenosine A2AR controls interneurons migration [2]. We now aimed to evaluate if A2AR is also involved in the migration of cortical principal neurons. For that purpose, we first evaluated the impact of the genetic deletion (A2AR KO) or the pharmacological blockade of A2AR on mice cortical neurons migration during embryonic development. In comparison to their wild-type littermates, embryos lacking the A2AR showed a delayed migration of cortical principal neurons at embryonic day 17 (E17). Similarly, embryos exposed to the A2AR antagonist SCH58261 (daily 0.1mg/kg i.p. injection in pregnant females from E13 to E16) have shown delayed migration, when compared with embryos exposed to vehicle. This should be due to A2ARs expressed by migratory neurons since in utero electroporation of a plasmid encoding shRNA specific for A2AR (E14-E17) also delays migration. The neuronal migration delay occurs mostly in the intermediate zone, where it was observed an accumulation of neurons. It is well-known that it is required a transition from a multipolar to a bipolar shape at the intermediate zone and the establishment of an axon-like leading process in order to the neurons to proceed their migration into the cortical plate [3]. Accordingly, we found in mice cortical neurons that the pharmacological blockade of A2AR with the selective antagonist SCH58261 (50 nM) leads to a reduction in the number of axons (SMI-31 positive neurites) and in their length (DIV 0-3), and we could observe that the knockdown of A2ARs leads to an impairment both in neuronal polarization and axon formation in the migratory neurons. Finally, the observation of a similar delayed cortical principal neurons migration in the CD73-KO mice, which lacks the ecto-5'-nucleotidase that converts AMP into adenosine, indicates that the adenosine that is activating the A2ARs derives from the extracellular catabolism of ATP. This is further heralded by the observation of immunoreactivity for the vesicular nucleotide transporter in the developing mice cortex at E13-E17. Altogether, these results show that A2ARs activated by ATP-derived adenosine are required for cortical principal neuronal migration, in particular for the transition from the intermediate zone into the cortical plate by controlling the establishment of neuronal polarity and axon formation.

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Poster: P.172 | Ana Catarina da Silva Almeida
A novel system to study the spontaneous dimerization of inactive STAT3

Presenter: Catarina Almeida | ITQB
Catarina Almeida, Joana Silvestre-Ferreira, Ricardo Letra-Vilela, Joana Branco-Santos, Federico Herrera
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The canonical pathway of astrogliogenesis is the JAK/STAT3 phosphorylation pathway. It is frequently pictured as a relatively simple pathway, with only 4-5 key phosphorylation events and 4 rate-limiting protein-protein interactions. However, the apparent simplicity of this astrogliogenic axis is misleading, and probably biased by the current lack of knowledge. Several pathways converge at various points into the JAK/STAT canonical pathway and control the final outcome. The actual nature of the interplay between these pathways is still poorly understood, but it seems to involve multiple protein-protein interactions. We are creating a series of new cellular models to visualize and study, in living cells, the rate-limiting protein-protein interactions within the astrogliogenic pathway, including gp130 receptor and STAT3. These systems are based on bimolecular fluorescence complementation (BiFC) technology. Briefly, STAT3 was fused to two non-fluorescent halves of Venus, a third-generation fluorescent reporter. When STAT3 formed a homodimer, the complementary halves are brought together and reconstitute Venus fluorescence. Fluorescence is therefore proportional to the amount of protein dimers and can be readily measured in live cells by conventional methods, such as flow cytometry or quantitative microscopy. Using this system, we analyzed the effect of cytokine stimulation (LIF) and STAT3 inhibitors (Stattic and some molecules produced in-house through our collaborators at ITQB-NOVA), as well as a number of selected kinase inhibitors, on the dimerization of the STAT3 protein. Our findings confirm previous observations by other authors indicating that STAT3 exists as non-phosphorylated, inactive dimers in living cells, which become active when they are phosphorylated by a simple change of conformation. Our BiFC system could provide relevant information about the pathways that regulate the dimerization of inactive, non-phosphorylated forms of STAT3.

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Poster: P.173 | Ana Cristina Costa Veloso
The role of N-glycans in the structure and function of the C. elegans nervous system

Presenter: Ana Veloso | ICVS, University of Minho
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Glycans are the most diverse and complex organic compounds synthetized by living organisms. This diversity makes them complex regulatory and signaling molecules due to their high specificity. Their synthesis, generally termed glycosylation, is highly conserved throughout evolution in terms of enzymatic machinery; also the role of glycans is conserved, which stands for the relevance of these molecules for general cellular processes. Particularly in the case of the nervous system, a temporal expression of specific glycans that have an impact in neuronal development has been described, with abnormal composition often leading to lethal phenotypes. Furthermore, glycans are key intermediates in maintaining homeostasis, modulating processes such as axon guidance, transporter localization at the synapse and vesicular release. One particularly interesting group of glycans, in the context of the nervous system, are the N-glycans class that were shown to impact both nervous system development and function, from structural stability to synaptic transmission. Taking this into consideration, in this thesis we studied the role of N-glycans, focusing in the structure and function of the nervous system, through the elimination of the GNT-I enzyme in C. elegans. This targeted approach allowed us to find that disturbing N-glycans synthesis impacts the nervous system leading to structural and functional alterations. While motor neurons show no visible structural abnormalities, subtle changes in motor function were observed, namely an increase in average speed when the N-glycosylation enzymatic machinery is disturbed. Most interestingly, we observed that sensorial neurons present foci along their dendritic projections and that this is translated into deficits in assays that target the function of these specific neurons. Specifically, eliminating GNT-I enzymes elicited different behavioral outcomes in attractive and repulsive chemotaxis assays. Furthermore, it changes the response to a repulsive stimulus. This study is the first to address the role of N-glycans in the nervous system of the C. elegans model and reinforces the role of this class of glycans in nervous system function. Furthermore, the approach used and the data collected bring a novel perspective on the potential of using an invertebrate species to measure in vivo, and in adult animals with fully assembled nervous system, how glycosylation affects neuronal function; this may later be translated to mammalian models where the impact of disturbing glycosylation is often lethal.

Poster: P.174 | Ana Filipa Martins Dias
A role for Hipk2 in Prrxl1 phosphorylation and activity during the development of nociceptive neurons

Presenter: Ana Filipa Dias | I3S/IBMC/FMUP
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The dorsal spinal cord is one of the main relay sensory centers for neurons who integrate somatic sensory information. Noxious information arrives the spinal cord, brought by primary afferent neurons with their cell bodies located in dorsal root ganglia (DRG). This circuitry is the ground level for the transmission of information from periphery to central nervous system. How neuronal subtypes are specified from progenitor cells during DRG and spinal cord development are far to be understood. Prrxl1, gene encodes for a transcription factor that is key for the adequate development of the DRG/spinal cord neuronal circuitry and that cooperates along other transcription factors in the terminal differentiation of nociceptive neuron population. Prrxl1 changes its phosphorylation states along development, which is accompanied with conformational changes. Here, we present evidences that the kinase Hipk2 phosphorylates Prrxl1, modulating its transcriptional activity. We evaluated which Prrxl1 phosphorylation sites are associated to this modification and also ascertained Prrxl1 stability in the presence of Hipk2. This regulatory mechanism was further explored and its role in the development of nociceptive neurons addressed.

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Poster: P.175 | Ana Filipa Santos Pinto
Brain metastases from testicular cancer: Imaging features

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Background and Purpose: Approximately 95% of testicular tumors are testicular germ cell tumors (TGCT) [1]. The incidence of these tumors has been increasing, being the most frequent malignancy diagnosed in males between the ages of 20 and 39 [2-4]. Sertoli cell tumors are rare non-germ cell origin tumors and account for less than 1% of testicular cancer [5]. Brain metastases from germ cell tumors are very uncommon, occurring in less than 2-3% of patients [6]. In testicular cell cancer, it is estimated that the incidence of brain metastases is 1-2% in all TGCT, whereas in advanced stages of TGCT the incidence rises to about 10-15% [7-12]. There are not enough studies that analyze the incidence of brain metastases regarding testicular non germ cell tumors. The main objectives of this study were to evaluate the incidence, imaging characteristics, and prognosis of parenchymal brain metastases originating from testicular tumors. Methods: Review of case records of testicular tumors patients within the IPO Porto data base from 2006 to 2015 was conducted in order to identify the patients with testicular tumors and evidence of brain metastases. Results: We identified 368 patients with testicular tumors, with only four having evidence of brain metastases. Prior to the diagnosis of brain metastases, 75% of the patients had pulmonary metastases, followed by 25% with liver metastases or lymph node metastases or cutaneous metastases. Moreover, 25% had concomitant liver and lung metastases and one had no extra-cerebral metastases. The overall survival rate after brain metastases detection in the three patients who died was 8,9 months. The mean time between testicular cancer diagnosis and the appearance of brain metastases was 14,2 months. In terms of histopathology, one of the patients had a non-germ cell tumor, in this case, a Sertoli cell tumor, while the others had mixed germ cell tumors. All patients had supratentorial metastases. Half of them had only a single lesion in the right frontoparietal (21mm) or the right occipital (42mm) regions, both were heterogeneous in T1WI and T2WI, and with intense and heterogeneous enhancement with gadolinium. The other two patients had multiple lesions. One of them had left frontoparietal (2.2mm, hyperintense in T1) and right occipital (1.8mm, hypointense in T1) lesions, both heterogeneous and predominantly hypointense in T2 and T1WI, and showed no enhancement. The other had right temporal (5mm) and left occipital (11mm) lesions, both isointense in T1W1 and T2WI, and with intense and homogeneous enhancement. There was no diffusion restriction in all three cases in which it was studied and all four cases were hypointense in T2*. All patients continued chemotherapy and initiated holocranial radiotherapy after brain metastases diagnosis. Conclusion: In our study, although the imaging features of brain metastases differ in some aspects, they all have a hemorrhagic component and a very low survival rate after diagnosis.

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Poster: P.176 | Ana Isabel Delgado Cravo Lopes Nascimento
Dissecting the establishment and maintenance of the central-peripheral polarity in dorsal root ganglia neurons: The proximal segment in health and disease

Presenter: Ana Isabel Nascimento | IBMC, I3s, University of Porto
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During early embryonic development, sensory dorsal root ganglia (DRG) neurons are bipolar and then undergo a unique morphological change - pseudo-unipolarization - to attain their mature shape where a single stem axon bifurcates in one peripheral and one central axon. The DRG central and peripheral branches have different functional characteristics and cellular properties. In multipolar neurons, the axon initial segment (AIS) is a specialized structure that maintains neuronal polarity by separating the somatodendritic from the axonal compartment. In DRG neurons, the structural and molecular features that may underlie the central-peripheral asymmetry are largely unknown. The aim of our project is to understand the establishment and maintenance of the central-peripheral polarity in DRG neurons. For that, we have set an in vitro model of DRG pseudo-unipolarization. In this model, we show that AIS-specific proteins, including AnkyrinG and βIV-spectrin, are accumulated proximally to the cell body in the neurites of developing bipolar DRG neurons. This molecularly-defined compartment- proximal segment- is maintained later in the stem axon of pseudo-unipolar DRG neurons. Whereas the assembly of the proximal segment is cell-autonomous, the efficiency of pseudo-unipolarization is dependent on the presence of glial cells. We are currently characterizing the assembly, positioning and molecular composition of the proximal segment in vivo using Thy1-GFP mice that allow imaging single neurons. We also aim at testing whether similarly to the AIS, that under several pathological conditions, changes in length or spatial location, the proximal segment of DRG neurons may also suffer rearrangements. This will be tested in a model of neuropathic pain, where this plasticity may contribute to alterations in the transmission of nociceptive signals. This study will allow identifying the mechanisms underlying central-peripheral polarity in DRG neurons and may identify novel mechanisms that underlie neuropathic pain.

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Poster: P.177 | Ana Luisa Garcias Mestre
Electrical detection of extra-cellular long-lasting potentials in astrocytes using polymer based devices

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Long-lasting or slow electrical fluctuations are normally found in the extracellular milieu. These are caused by ions and charged molecules that can move from cell to cell by gap junctions. These fluctuations are steady or slowly changing gradients and they progress thousands of times more slowly than action potentials. This slow activity does not show spikes but smooth potentials that can change over a period of time from several seconds to minutes. Astrocytes are neural cells that generate this type of slow signals. Indeed, astrocytes cells exhibit a form of excitability that modulate synaptic activity. This process has been named “gliotransmission” introducing the notion that information processing is not a unique feature of neurons. Until now these slow ionic fluctuations have been recorded using optical techniques. Here we show that these electrical fluctuations can also be recorded using extracellular conducting polymer electrodes in cell cultures in a totally non-invasive way and over extended periods of time. The detection limit of extracellular electrodes was brought down to the low nanovolt range. Bioelectrical signals with amplitudes of 150 nanovolts in a noise level of 20 nanovolts (peak-to-peak) were recorded. The strategy behind this ultra-high sensitivity is the use of high capacitive polymer based electrodes and observation windows below 10 Hz. The electrodes and the methodology are demonstrated by recording ultra-weak signals produced by primary cultures of astrocytes and in C6 cell line (astroglioma). Our electrical recordings are comparable with optical fluorescence recordings reported in literature. We propose that polymer based devices are a powerful tool to unravel the dynamics of neuroglial ionic signalling. Keywords: Astrocytes, cooperative bioelectrical activity, polymer-based electrodes.

Poster: P.178 | André Emanuel Pinheiro Bastos
Effect of feeding cycle on excitability of rat hippocampal neurons – the role of voltage-gated Na+ channels

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Feeding cycle influences excitability in acutely isolated rat hippocampal CA1 neurons [1], namely through the involvement of ion channels. In view of the functional implications of the feeding cycle, it is important to investigate further the mechanisms underlying the adaptation of intrinsic neuronal membrane properties to such metabolic conditions. In this work, electrophysiological techniques, namely whole-cell voltage clamp and excised inside-out patches, were used to study the biophysics of voltage-gated sodium (Na+) channels in acutely isolated CA1 hippocampal neurons, from Wistar rats (P21-29). These were decapitated after being subjected to overnight fasting and subsequently either fed for 45min ('fed neurons') or not fed ('Fasted neurons'). The results indicate a conspicuous effect of the physiological feeding cycle over the activation of Na+ channels, as we detected higher current amplitude upon feeding. Whole-cell Na+ currents depicted the following values of mean maximum current density: 1.537mA.cm-2 ± 0.121 (fed neurons, n=18) and 0.995mA.cm-2±0.097 (fasted neurons, n=28). The hyperpolarizing shift observed between the mean half maximum potential (Vh) values of fed (-19.06 ± 0.867mV) and fasted neurons (-15.21 ± 0.887mV) clear indicates an influence of the physiological feeding cycle on the conductance of Na+ channels. The inactivation process was also affected. The average fitting parameters of steady-state of inactivation (-51.23 ± 1.4mV, fed neurons, n=24, and -58.41 ± 1,8mV, fasted neurons, n=27) point to a greater channel availability of fed neurons to respond to activation. The corresponding shift in the time-constant of inactivation (τh(ms)), namely on its slow component, suggests a conformational rearrangement of Na+ channels after feeding, most likely due to biophysical alterations of the plasma membrane. Na+ currents were also recorded in excised inside-out configuration. Interesting differences were observed in single channel conductance: 16.7 ± 0.76pS (fed neurons, n=8) and 12.6 ± 1.30pS (fasted neurons, n=8), which corroborate the results of macroscopic recordings but do not exclude a change in the channel expression at the surface membrane. The expression of Na+ channels was surveyed on plasma membrane-enriched fractions of hippocampal neurons. Western blotting experiments were performed using an antibody that recognizes the most common Na+ channel α-subunit in rat brain, Nav1.2 [2]. Results demonstrated that hippocampal neuronal membranes from fed rats present a higher concentration of this α-subunit, explaining, at some extent, the different electrophysiological properties brought up by the feeding cycle. This work gives new insights into the comprehension of the influence of feeding on brain function. Overall, the results indicate an influence of feeding cycle in the expression and biophysics of voltage gated Na+ channels present in rat hippocampal CA1 neurons, phenomena that account for a change in neuroactivity.

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Poster: P.179 | Carlos Jorge Pereira Bessa
Functional analysis of UPS genes linked to developmental disorders, in C. elegans

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Neurodevelopmental disorders such as intellectual disability (ID) and autism spectrum disorders (ASDs) can be caused by diverse factors. From a genetic perspective, disease-causing mutations have been identified in genes involved in various cellular processes, namely impacting synaptic transmission [1]. Specifically, is has been suggested that a dysfunction of the (development) GABAergic circuitry may be a common pathological mechanism underlying these disorders [2]. A key mechanism in maintaining cellular homeostasis and function is ubiquitin signaling and the ubiquitin proteosome pathway (UPS). Moreover, it is also critical for the regulation of several neuron specific processes, from development to connectivity [3]. Here, we have used the nematode C. elegans's neuromuscular junction (NMJ) paradigm to study the effect of the silencing of a set of orthologues for 10 human disease-associated genes that encode components of the ubuquitin signaling pathway. Using RNAi and compounds that interfere with GABAergic/cholinergic synaptic transmission at the worm NMJ (aldicarb, pentilenetetrazole/PTZ), we have evaluated the rate of paralysis upon the knock-down of each target gene. Our findings suggest that 5 of the C. elegans target genes seem to be required for a normal response to PTZ (math-33, ubc-1), aldicarb (y38f1a.2) or both compounds (cul-4, uba-1). Whereas uba-1 encodes the C. elegans only E1 ubiquitin activating enzyme and is critical for worm development [4], the involvement of math-33, ubc-1, y38f1a.2 or cul-4 in neuronal function has not been described so far. To further understand how these genes may be required for neurotransmission or neuronal development, these findings will be followed by GABAergic neuron-specific gene silencing and analysis of neuron morphology, as well as the use of GABA-dependent worm behavior to evaluate neuronal dysfunction.

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Poster: P.180 | Catarina Miranda Lourenço
Characterization of BDNF signalling and adenosinergic system in Rett Syndrome

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Rett Syndrome (RTT) is a neurodevelopmental disorder primarily caused by mutations in the methyl-CpG binding protein 2 (MECP2) gene [1]. MeCP2 is known to modulate the expression of brain-derived neurotrophic factor (BDNF). BDNF is a neurotrophin with central role on neuronal differentiation, survival and synaptic plasticity [2]. BDNF signalling is significantly impaired in RTT. However, until now, strategies to potentiate its actions have been a challenge. Adenosine is a neuromodulator that mainly acts through A1 and A2A receptors (R). Recently, we suggested adenosine augmentation therapy as a new pharmacological strategy for RTT. This proposal was based on the knowledge that: 1) the activation of A2AR potentiates/facilitates BDNF actions [3] and that 2) the A1R activation, by providing seizure control, could be important to treat epilepsy [4], present in the majority of RTT patients. Remarkably, our recent data clearly showed that, in the well stablished animal model, Mecp2 knockout (KO) mouse [5] at symptomatic stage (6 weeks old), BDNF levels are decreased as well as its TrkB-FL receptor and that adenosinergic system is drastically affected (endogenous adenosine levels decreased; A1R expression levels increased and A2AR expression levels decreased [6,7]). In the present study, we evaluated, from the presymptomatic (1 and 3 weeks old) to the symptomatic (6 weeks old) stage, the changes on one of the central actors of adenosinergic system, the adenosine kinase (ADK), the most relevant enzyme responsible to the maintenance of adenosine levels. Moreover, the levels of BDNF and its receptors were also evaluated in the same time points. The protein levels of ADK and BDNF receptor were evaluated in hippocampus and cortex homogenates taken from KO and wild-type (WT) mice (1, 3 and 6 weeks of age) by Western Blot technique and BDNF protein concentration was evaluated by ELISA. The statistical significance of results was evaluated by Student's t test. The results obtained revealed that ADK protein expression levels are increased in the cortex of 6 week old (w/o) KO animals (KO6w/o: 128.9±36.1%, n=10 and WT6w/o: 100%, n=11, $p<0.05$). TrkB-FL receptor protein expression levels are decreased in hippocampus of 3 and 6 w/o KO mice (KO3w/o: 37.5±8.2% and WT3w/o:100%, n=3, $p<0.05$; KO6w/o: 43.2±12.5% and WT6w/o:100%, n=3, $p<0.05$). The changes in ADK expression could be a new clue for understanding RTT pathophysiology. This alteration could explain the diminished endogenous adenosine levels at symptomatic stage and could also be implicated in the loss of BDNF signalling detected in RTT mice model.

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Poster: P.181 | Celso Henrique Freitas Alves

Reduction of CRB2 Specifically in Mouse Photoreceptors with concomitant loss of CRB1 in Müller Glial Cells Mimics early-onset Retinitis Pigmentosa

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Mutations in the Crumbs homologue-1 (CRB1) gene cause early-onset Retinitis pigmentosa (RP) in children. Mice lacking CRB1 show moderate retinal degeneration limited to one quadrant of the retina while in retinas lacking CRB2 the entire retina is affected. In the mouse retina, CRB1 is localized in Müller glial cells and CRB2 in Müller glial and photoreceptor cells, at a subapical region at the outer limiting membrane,. We generated and analysed the phenotype of a new CRB1-RP mouse model with reduced levels of Crb2 in photoreceptors and complete ablation of Crb1 in Müller glial cells. These can be used to test the efficacy of adeno-associated-viral (AAV) CRB1 and CRB2 gene augmentation vectors. Crb1-/-Crb2flox/+Crx-Cre mice were generated by crossing Crb1Crb2 with Crx-Cre mice driving the expressing of Cre in photoreceptors. Animals from embryonic day (E)15.5 till 8 months of age were analysed. To investigate the possible ectopic localization of proteins and cell types immunohistochemistry (IHC) was performed. Morphology of the retina was analysed using toluidine stained ultra-thin sections. In vivo analysis of retinal function was performed using electroretinography (ERG). Retinal disorganization was observed as early as embryonic day 17.5. Progressive retinal disorganization was detected at early post-natal days (10 and 14), characterized by gaps at the outer limiting membrane, photoreceptor rosettes and many ectopic photoreceptor cell nuclei in the subretinal space. At a later stage (1M), retinal disorganization and degeneration affected mainly the ventral area of the retina and the outer retina. IHC experiments showed loss of localization of members of the Crumbs complex at regions with disrupted OLM. At 3 month of age, severe degeneration was observed in the ventral area of the retina, where only very few photoreceptors remained. Increased levels of GFAP and CD11b were also observed suggesting gliosis and activated microglia. ERG performed at 1 month of age showed an attenuation of the a-wave, while no effect was observed in b-wave and flicker responses. Reduction of CRB2 levels in photoreceptors with concomitant loss of CRB1 from Müller glial cells leads to an exacerbation of the late Crb1 retinal phenotype. The retinal phenotype of these mice mimics early-onset RP. This new mouse retinal degeneration model has a suitable therapeutic window to test efficacy of new CRB1 and CRB2 gene therapy vectors in photoreceptors.

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Poster: P.182 | Filipa de Sá Martins
BRI2 is a novel player in neuritogenesis

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BRI2 is a ubiquitously expressed type-II transmembrane protein that is abundant in the brain, particularly in the hippocampus and cerebellum, when compared with cerebral cortex 1,2. BRI2 undergoes regulated intramembrane proteolysis in the cis- or medial-Golgi resulting in the formation of several secreted peptides 3. Moreover, during the maturation process it suffers post-translational modifications leading to various forms of BRI2 that may have different cellular locations and physiological functions 3,4. BRI2's precise physiological function remains elusive, although some BRI2 interacting proteins have been identified, including protein phosphatase 1 (PP1) 4. In the work here presented using BRI2 PP1-binding motif mutant constructs, the importance of the RVxF motif (3KVTF6) for BRI2:PP1 complex formation was determined. In fact, using BRI2 KATA mutant, the interaction between BRI2 and PP1 was completely abolished and consequently BRI2 becomes irreversibly phosphorylated (PP1 is no longer able to dephosphorylate it). Furthermore, it was possible to show that in SH-SY5Y cells BRI2 phosphorylation seems to be of paramount importance since it appears to be responsible for the regulation of its processing and neuritogenic role. Further, by modulating BRI2 processing we observed that phosphorylated full-length BRI2 appears to be important for the establishment of neuritic processes, while the BRI2 NTF promotes neurite elongation 5. In order to understand the trafficking and signalling effecs of BRI2 and to unravel the mechanisms underlying the BRI2 neuritogenic role, rat brain was processed for BRI2 immunoprecipitation followed by Nano-HPLC-MS/MS and in silico analysis. Functional enrichment analysis of the BRI2 interactome suggests a subcellular localization for the BRI2 protein in neuron projections, synapses, both presynapse, and postsynapse, and associated with synaptic vesicles. These results together with the fact that the majority of these BRI2 interactors are cytoskeletal proteins, transporters and membrane traffic proteins are consistent with the concept that BRI2 in the CNS has a role in nerve terminals and neuronal differentiation3–6. In line with this observation, functional enrichment pathway analysis of the interactome associated BRI2 to signalling pathways involved in several neuronal differentiation related events, namely adherens junction, axon guidance, ErbB signalling pathway, FoxO signalling pathway, neurotrophin signalling pathway and estrogen signalling pathway. Therefore, the results strengthen the proposed role for BRI2 in neuronal differentiation and neurite outgrowth and an association of BRI2 with these signalling pathways deserves further investigation. Moreover, a complex interaction of BRI2 with proteins related to synaptic signalling and plasticity was observed. In essence, this work significantly contributed to the understanding of the physiological function of BRI2 that could be valuable for the understanding of its associated pathologies (FBD, FDD, and AD).

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Poster: P.183 | Filipe Almeida Monteiro

TIx3 is a master gene in generating excitatory over inhibitory neurons in the dorsal spinal cord

Presenter: Filipe Monteiro | FMUP, IBMC & I3S

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The production of excitatory and inhibitory neurons relies on combinatorial expression of transcription factors to promote specific neuronal differentiation programs. The Tlx-class homeobox gene Tlx3 works as post-mitotic selector gene in embryonic dorsal spinal cord determining excitatory at the expenses of inhibitory neuronal fate, as revealed by mouse genetics. Yet, it is unclear whether Tlx3 acts directly or indirectly to promote excitatory differentiation, as very little is known on the identity of its target genes. Here we used chromatin immunoprecipitation followed by next generation sequencing to map genome-wide Tlx3 binding sites in mouse embryonic dorsal spinal cord. Strikingly, we find that Tlx3 directly regulates a wide range of genes, including transcription factors with an established role in cell fate specification, as well as terminal differentiation genes. Tlx3 targets include genes associated with both an excitatory and inhibitory cell fate. Our data supports a role for Tlx3 as a major regulator in dorsal spinal cord development, where it directly activates a diverse transcriptional program associated with an excitatory neuronal sub-type, while simultaneously repressing target genes characteristic of an inhibitory cell fate.

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Poster: P.184 | Filippo Calzolari
Changes in clonal dynamics during aging of the mouse subependymal zone

Presenter: Filippo Calzolari | JGU, Mainz; LMU Munich
Filippo Calzolari (1,2,3), Lisa Bast (4), Michael Strasser (4), Magdalena Götz (1,2), Carsten Marr (4), Jovica Ninkovic (1,2)
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The population of adult neural stem cells (NSCs) in the mammalian subependymal zone (SEZ) is responsible for life-long generation of olfactory bulb neurons. While total NSC numbers and neurogenesis decline with age, how the behavior of actively proliferating NSC changes in vivo during the animal lifespan is unknown. This prompted us to compare the clonal output of NSCs in young and aged mice to infer the population dynamics of lineage differentiation from NSCs. By means of in vivo clonal lineage-tracing, we have recently revealed that actively neurogenic NSCs in the SEZ of young mice (8-10wks) can rapidly generate significant numbers of cells, but have limited long-term self-renewing ability and rapidly become exhausted (1). These observations suggested that the resulting reduction in NSC numbers could contribute to the age-related decline in neurogenesis. We have now tested the neurogenic potential of NSCs remaining in the aged SEZ by clonally tracing the progeny of NSCs in the 12-14 months-old SEZ, when neurogenesis is markedly decreased. Unexpectedly we observed that, similarly to our observations in young animals, NSCs can repeatedly divide, producing multiple waves of rapidly expanding progeny, generating clones containing more than 100 cells in 3 weeks. Like in the young SEZ, however, most NSCs become exhausted a few weeks after activation. These observations challenge the simple view that major differences in NSC behavior and lineage progression dynamics underlie aging-associated defects in adult neurogenesis. To clarify this issue, we studied the population dynamics of the system computationally, fitting over 4000 mathematical models of adult neurogenesis to population-level (2-4) and clonal data of young (1) and aged mice. In our models, we allow for different division strategies (symmetric vs. asymmetric, proliferation vs. differentiation) and differentiation rates of NSCs and their progeny. We find that the models need a considerable amount of asymmetric NSC divisions to explain the observed data, and that this changes during aging. Moreover, our approach indicates that more rapid activation/inactivation rates in the stem cell compartment shape the loss rate of NSCs during aging. We therefore propose that progressive but subtle changes in NSC dynamics can explain clonal and population-level observations performed in the adult SEZ.

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Poster: P.185 | Joana Grand-Guillaume-Perrenoud Silvestre Ferreira
A possible role for Polo-like kinase 1 in the spontaneous dimerization of STAT3

Presenter: Joana Ferreira | ITQB-NOVA
Joana Silvestre-Ferreira, Catarina Almeida, Ricardo Letra-Vilela, Joana Branco-Santos, Federico Herrera
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The JAK/STAT3 pathway is involved in multiple biological phenomena, mostly related to stress or tissue damage, but also to development and cancer. A rate-limiting step of the pathway involves the phosphorylation, dimerization and translocation to the nucleus of STAT3. Originally, it was thought that STAT3 dimerized upon phosphorylation by JAK kinases on Tyr705. However, current evidence indicates that STAT3 exist as a dimer prior phosphorylation and activation, and that phosphorylation only induces a change in the conformation of the dimer. Here, we explored whether the dimerization of non-phosphorylated STAT3 is actually spontaneous or regulated by intracellular pathways. We developed a system to visualize and study STAT3 dimers in living cells. This system is based on bimolecular fluorescence complementation assays (BiFC), where STAT3 was fused to two non-fluorescent fragments of the Venus yellow fluorescent protein. When STAT3 dimerizes, the two fragments are brought together and reconstitute the fluorescence. Fluorescence is therefore proportional to the amount of dimers in cells. We used this system to carry out a pharmacological screening with more 100 kinase inhibitors and to study the role of particular post-translational modifications on STAT3 dimerization. Our results indicate that Polo-like kinase 1 could regulate the dimerization of non-phosphorylated STAT3. The relevance of our findings stems from the fact that STAT3 is an important molecule in developmental astrogliogenesis, reactive gliosis during neurodegeneration and brain or spinal cord injury, and brain cancer, for example.

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Poster: P.186 | José Ricardo da Cruz Vieira
Effect of TTR in the blood brain barrier – Implications in physiology and pathology

Presenter: José Ricardo Vieira | I3S/FMUP
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Background: Transthyretin (TTR) is a 55kDa protein that is synthesized mainly in the liver and in the choroid plexus of the brain. For decades, TTR was known as a transporter of the thyroid hormone thyroxine (T4) [1] as well as of retinol (vitamin A) that is bound to retinol binding protein (RBP) [2]. TTR is also the key protein in Familial Amyloid Polyneuropathy (FAP), a neurodegenerative disease caused by the accumulation of mutant TTR in several organs and tissues, with a special involvement of the peripheral nervous system (PNS) [3]. More recently, wild-type TTR was shown to be neuroprotective in the central nervous system (CNS), namely in ischemia [4], regeneration [5] and memory [6]. TTR has also been implicated as a neuroprotective molecule in Alzheimer's disease (AD): TTR was shown to participate in Abeta peptide efflux at the blood-brain barrier (BBB) and to regulate the expression of the low-density lipoprotein receptor-related protein 1 (LRP1) [7]. Thus, our lab is interested in unraveling the mechanisms underlying TTR protection in the CNS, namely its effects in blood-brain barrier-related genes and in brain vasculature. We also aim at studying the impact of the presence of TTR mutations in these possible TTR functions. Methods: In this work we investigated the effect of TTR in different tight junction-related genes, including occludin, using a cellular model of the BBB (the hCMEC/D3 cell line), at the mRNA and protein levels, by qRT-PCR and immunocytochemistry, respectively. Next, we performed a wound healing assay, using the same cell line, to assess the influence of TTR in angiogenesis. Results: Our results indicated that TTR did not influence the expression of the tested tight junction-related genes, as indicated by the qRT-PCR results. For occludin, which was also studied at the protein level, no differences were detected between cells incubated with and without TTR. Interestingly, hCMEC/D3 incubated with Abeta peptide showed decreased levels of occludin, which were restored when cells were co-incubated with TTR. This observation might underlie Abeta cytotoxicity which was inhibited by TTR, as previously shown for other cells. The preliminary results from the wound healing assay suggested that TTR enhanced the angiogenic capacity of cells, promoting migration and closing the wound faster when incubated with TTR comparing with cells without TTR incubation. Conclusions: TTR protective effect in the CNS might be exerted through its ability to regulate angiogenesis but not by influencing tight junction proteins.

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Poster: P.187 | Juliana Pereira Macedo
Rare Brain Metastases from Prostate Cancer: Neuroimaging Analysis

Presenter: Juliana Macedo | Depart. of Biomedicine, FMUP
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Background and Purpose: Prostate cancer is considered the second most commonly diagnosed cancer [1]. In addition, it is considered the fifth leading cause of cancer death amongst males [2]. A small percentage (2%) of patients with prostate cancer are found to be castrate-resistant and to develop brain metastasis, a rare complication which is associated to an advanced systemic state of the disease when the tumor has already spread to other sites [3]. Although, there is not much evidence on optimal management of these patients [4]. The main objectives of this study were to evaluate the incidence, imaging characteristics, and prognosis of parenchymal brain metastases originating from prostatic tumors. A review of relevant literature is also discussed. Methods: A review of case records of prostate cancer patients within the IPO Porto data base from 2013 to 2015 was conducted in order to identify the patients with prostate cancer and evidence of brain metastases. As criteria of exclusion, cases transferred to other hospitals without follow up and cases that were incorrectly categorized were excluded. Results: We screened 2194 patients with prostate cancer, with only one having evidence of brain metastasis. Additionally, one case was identified with bilateral orbital metastatic lesions. The patient with evidence of brain metastasis aged 48 years old. Magnetic resonance imaging showed six metastatic lesions, three infratentorial and three supratentorial. The largest lesion was found in the parieto-occipital region. These brain metastasis were detected 42 months after the initial diagnosis of prostate adenocarcinoma. In addition, by the time of brain metastasis detection, the patient already had bone metastatic lesion. Conclusion: Brain metastases from prostate cancer are rare, with only a few cases described in the literature. Variable magnetic resonance imaging characteristics are described. Brain metastases are also associated with a poor prognosis, with a mean survival of 1 to 7.6 months.

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Poster: P.188 | Mafalda Maria Pais Neto Sampaio Alves
Brain Metastases from Thyroid Cancer: A 10-Year Analysis with Emphasis on Imaging Characteristics, Incidence, and Prognosis

Presenter: Mafalda Sampaio Alves | Depart. of Biomedicine, FMUP

Mafalda Sampaio Alves(1), Eduarda Carneiro(4), Diana Ferreira(4), Isabel Torres(5), Susana Maria Silva(2,3), Mavilde Arantes(2,3,4)

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Background and Purpose: While thyroid cancer is a relatively common type of cancer, it is usually highly curable [1]. Brain metastases from thyroid cancer are rare and their imaging appearance has not been well defined [2]. The main objectives of this study were to evaluate the incidence, imaging characteristics, and prognosis of parenchymal brain metastases originating in thyroid cancer. Methods: Review of case records of thyroid cancer patients within the IPO Porto data base from 2005 to 2015 was conducted in order to identify the patients with thyroid cancer and evidence of brain metastases. Results: We identified 3175 patients with thyroid cancer, with only five having evidence of brain metastases (two from papillary thyroid cancer, two from follicular thyroid cancer and one from poorly differentiated thyroid cancer). At the time of brain metastases detection, 100% of the patients had concurrent lymph node metastases, 80% lung metastases and 60% osseous metastases. Of those brain metastases, 60% were multifocal and 40% presented as partially cystic/necrotic. Of the two cases in which the patients died, the median overall survival after brain metastasis detection was less than one year. Conclusion: Brain metastasis from thyroid cancer remains a rare phenomenon that most frequently occurs in the setting of widely disseminated lymph node disease. The imaging appearance is highly variable and the prognosis is poor.

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Poster: P.189 | Mário António Fonseca Soares
Mitotic inheritance of the neural stem cell network

Presenter: Mário Soares | Instituto Gulbenkian de Ciência
Mário Soares, Raquel Oliveira, Diogo Castro
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During mitosis, DNA becomes highly condensed and nuclear transcription is globally arrested, while most of the transcriptional machinery disengages from chromatin. Nevertheless, following chromosome segregation and re-formation of the nuclear envelope, bulk transcription is reset in daughter cells and the transcriptional program that confers the cell its identity is reestablished. Our current knowledge of the mechanisms underlying the mitotic inheritance of gene expression is mostly based on so called “bookmarking” of genomic regions by chromatin or DNA modifications that can be inherited throughout cell division. For many years, condensed chromosomes appeared to be refractory to transcription factor (TF) binding. However, an increasing number of studies suggest that some transcriptional regulators can resist chromatin condensation and remain bound to mitotic chromatin. One possibility is that binding of TFs to mitotic chromatin contributes to the quick reactivation of genes important for the maintenance of cell identity, soon after exit from mitosis. Several studies have explored this paradigm in dividing hematopoietic (e.g. Gata1) and hepatic (e.g. FoxA1) cells as well as in embryonic stem cells (e.g. Esrrb and Sox2). We are seeking to understand the importance of mitotic bookmarking by TFs in vertebrate neurogenesis. Here we report ongoing work at identifying TFs with mitotic bookmarking activity in neural stem/progenitor cells. Using live-imaging of GFP fusion proteins, we have identified the important neural progenitor factors Sox2 and Brn2 as potential mitotic bookmarkers. We are combining functional studies, with the genome-wide mapping of binding sites in both interphase and mitotic chromatin, in order to address the role of mitotic bookmarking of these TFs in neural stem/progenitor cell maintenance.

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Poster: P.190 | Miguel Maria Restolho Mateus Pinheiro
Portraying Gender Differences in Microglia Morphology Across Brain Regions

Presenter: Miguel Pinheiro | IBILI - FMUC
Miguel Pinheiro (1,2), Rita Gaspar (1,2), Helena Pinheiro (1,2), Carla Henriques (1,2), Joana Mendes Duarte (1,2), Rodrigo A. Cunha (2,3,4), António F. Ambrósio (1,2,4), Catarina A. Gomes (1,2,4)
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In the past few decades, there has been an emerging effort in understanding brain differences among genders in terms of anatomy, chemistry and function. Documenting this dissimilarities might be crucial in order to unravel the distinct susceptibility between genders towards neuropathology development. Since the disruption of our brain homeostasis is intrinsically coupled with changes in microglia morphology [1], we ought to address these morphological features between genders under physiological conditions. Our group recently described gender differences in microglia morphology in the prefrontal cortex (PFC) of rats [2]. These differences were also observed between brain regions, namely between the PFC and the dorsal hippocampus (dHIP) [3]. Additionally, we conducted the same study in adenosine A2A receptor (A2AR) knockout (A2AKO) mice, since it modulates microglia morphology [2, 4] . We thus performed a comparative analysis of microglia morphology in young adult (3 month's age) male and female mice in the PFC and in the dHIP. We found that microglia from males in the dHIP have a higher length and number of ramifications than microglia from female. We also detected interregional differences in the PFC and dHIP of male mice, in which dHIP microglia have a more ramified architecture. These differences strengthen what we have covered concerning microglia morphological differences between gender and across different brain regions and in different rodent species, further validating our previous results. Surprisingly, we did not observe any significant changes in microglia morphology in A2AKO mice and control ones between both regions for each sex, indicating a preponderant gender effect over the A2aR in defining microglia identity. These results consolidate our latest reports while introducing new insights towards the activity of the A2AR in modulating microglia morphology in a physiological background.

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Poster: P.191 | Pedro Mota Prego Rosmaninho
Zeb1 potentiates genome-wide gene transcription in glioblastoma cancer stem cells via a novel Lef1-dependent mechanism

Presenter: Pedro Rosmaninho | Instituto Gulbenkian de Ciência
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Glioblastoma Multiforme (GBM) is the most prevalent and lethal type of brain and CNS tumor. Tumor recurrence after surgical resection and radiation invariably occurs, regardless of aggressive chemotherapy and there are several contributors to the poor responsiveness of GBM tumors to treatment. GBM tumors harbor a cancer stem-like cell (CSC) population crucial for driving tumor growth and relapse, due to their potential to proliferate and infiltrate the surrounding brain tissue. ZEB1 is a zinc-finger transcription factor known for its ability to induce an epithelial to mesenchymal transition (EMT), a complex genetic program associated with loss of cell polarity, extensive extracellular matrix remodeling and acquisition of a migratory behavior. Although a proper EMT is not at work in GBM, the contribution of EMT regulators to the highly invasive nature of this cancer type has been the focus of recent attention. Here we show that Zeb1 is expressed in Glioblastoma derived CSCs, and investigate its function by characterizing its transcriptional program. Although ZEB1 has been widely viewed as a transcriptional repressor in a carcinoma context due to its capacity to trigger EMT by repressing expression of epithelial genes, we found that genome-wide binding of Zeb1 associates with both gene repression and activation, resulting from two distinct modes of recruitment to regulatory regions. Transcriptional repression requires direct Zeb1 binding to its consensus sites, while indirect recruitment by the downstream effector of the Wnt pathway Lef1 results in gene activation, in the absence of active Wnt signaling. Genes activated by Zeb1 include mediators of tumor cell migration and invasion, many of which show a correlative expression with Zeb1 in GBM tumor samples, and include the guanine nucleotide exchange factor Prex1. We further show that Prex1 expression promotes migration of GBM cells and is predictive of low patient survival, highlighting the importance of the novel Zeb1/Lef1 gene regulatory mechanism in GBM.

Poster: P.192 | Rafael Mendes Miranda
Insight into transcriptional regulation and expression of Neph1 in the establishment of dorsal root ganglion-spinal cord circuitry

Presenter: Rafael Miranda | FMUP, I3S, IBMC
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Dorsal root ganglion (DRG) sensory neurons are responsible for receiving mechanical, proprioceptive, thermal and nociceptive stimuli and to transmit it to second order neurons present in the spinal cord. Therefore, the correct processing of the somatic sensory stimuli is dependent on the correct connectivity between DRG and spinal cord neurons. However, it is still unclear how this connectivity is established. The homeodomain transcription factor Prrxl1 was shown to be crucial for the development of the DRG-dorsal spinal cord nociceptive circuit. Through a genomic location analysis using chromatin immunoprecipitation (ChIP) combined with gene expression profiling , we identified Neph1 as a target gene of Prrxl1. Our microarray data indicate that Neph1 expression is upregulated in the dorsal spinal cord of Prrxl1-null embryos, indicating that Neph1 expression is repressed by Prrxl1 in this structure. Also, our binding results, validated through ChIP-qPCR, show several binding sites for Prrxl1 at Neph1 locus suggesting that Prrxl1 directly repress Neph1 transcription. In addition, we performed a detailed spatiotemporal characterization of Neph1 expression in the mouse DRG by immunochemistry. The same analysis for the dorsal spinal cord is ongoing. Altogether, our results indicate that Neph1 as a potential player downstream of Prrxl1 in the establishment of the correct connectivity between DRG and dorsal spinal cord nociceptive neurons.

Poster: P.193 | Ricardo José Letra Vilela Ribeiro
New protein complementation systems of optogenetic proteins

Presenter: Ricardo Letra Vilela | ITQB
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Protein complementation is a property of at least some proteins that allows to split them in two or more non-functional fragments that will reconstitute the protein function when they are brought together. This property has been observed in a wide number of proteins with technological potential, such as the green fluorescent protein family, luciferases, thymidine kinase, beta-galactosidase, or ubiquitin. Protein complementation systems have been used for various technological applications, such as the study of specific protein-protein, protein-DNA and protein-RNA interactions. Here, we are trying to determine if optogenetic proteins –i.e. proteins activated by light- display the protein complementation property and can therefore be used for particular applications. Halorrodhopsins and the miniSOG tag are our first target proteins. Halorhodopsins are channels that allow the transport of chloride anions (Cl-) in response to light, thus allowing the inhibition of the neurons where they are expressed. The miniSOG protein is also extremely interesting because it is able to 1) emit green fluorescence and 2) produce superoxide radicals when excited with particular wavelengths. The generation of superoxide radicals in combination with a diaminobenzidine reaction allows the visualization of the tagged protein by electron microscopy at an ultrastructural level. Moreover, the miniSOG tag allows to kill specific cells by means of light, as it produces oxidative stress either in whole cells or specific parts of the cell, such as the membrane, the mitochondria or specific DNA regions. Our results indicate that these proteins can be actually split in non-functional fragments that become functional when they are brought back together. By means of the systems we are developing, we believe we will soon be able to visualize protein-protein interactions at an ultrastructural level, kill or inactivate specific subtypes of neural cells, and produce mutations in specific sites of the genome, for example.

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Poster: P.194 | Sandra Filipa Ferreira Gomes
Striatal functions of Foxp1 in motor-sequence learning, automatisation of behaviour and social interaction

Presenter: Sandra F. Gomes | Champalimaud Foundation
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Disruptions of the Foxp1 gene have been linked to autism spectrum disorder, intellectual disability and language impairment. FOXP1 is a member of the Forkhead Box P (FOXP) family of transcriptions factors and is conserved in mice where it is highly expressed in the cortex, hippocampus and striatum. Mice with brain-specific loss of Foxp1 show deficits in social behaviour, altered hippocampal electrophysiology and gross changes in striatal morphology. Global heterozygous knockouts display defects in ultrasonic vocalisations and increased excitability of striatal Drd2-expressing medium spiny neurons (MSNs). The striatum is important for motor-skill learning and automatisation of behaviour (dorsal striatum) and social behaviour (ventral striatum), which are relevant for neurodevelopmental disorders. Foxp1 functions in this brain region are still relatively unexplored. Interestingly, it is also an area where Foxp2 is co-expressed with a closely related protein Foxp2, which in humans is implicated in a speech and language disorder, and the two proteins may potentially act cooperatively. To study the striatal functions of Foxp1 in motor-sequence learning, automatisation of behaviour and social interaction we generated mice with Foxp1 disrupted specifically in the striatum by crossing mice with a Foxp1 conditional allele with mice expressing Cre recombinase under control of the Rgs9 promoter (specific for striatal MSNs). We validated Foxp1 knockdown in these striatal-specific mutants by immunohistochemistry and western blotting. We also assessed the performance of mutant and control mice on behavioural tasks designed to assess motor-sequence learning and automatisation of behaviour (rotarod task, operant lever-pressing task) as well as social interaction and preference for social novelty (3-chamber task). Foxp1 knockdown was substantial in the dorsal striatum but modest in the ventral striatum. We did not see any deficits in social interaction or preference for social novelty in Foxp1 striatal mutants. However, we cannot rule out a role for Foxp1 in social behaviour because knockdown in the ventral striatum was incomplete. There was also no significant difference in performance on the accelerating rotarod between Foxp1 striatal mutants and controls, although some subtle changes were observed on the operant lever-pressing task. This could indicate that Foxp1 is not important for motor function, but could also be explained by a developmental or functional compensation mechanism (Foxp2 can compensate for Foxp1 function).Our future studies include the interaction between Foxp1 and Foxp2 to evaluate possible functional compensation and also Cre virus injections into ventral striatum to knockdown Foxp1 in this region.

Poster: P.195 | Ana Rita Pereira
Effect of Seizures on the Severity of Epileptic and Cognitive Phenotypes in Mouse Models of Scn1a Mutations

Presenter: Ana Rita Pereira | IPMC CNRS France
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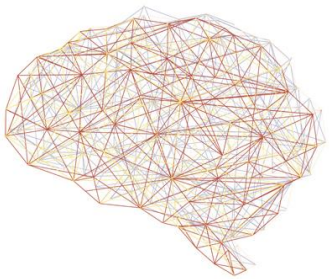
Sodium voltage-gated channel alpha subunit 1 (SCN1A) gene code for type-I voltage-gated sodium channels (NaV1.1) in human and rodent central nervous systems. SCN1A mutations cause genetic epilepsies, as Generalized Epilepsy with Febrile Seizures plus (GEFS+), a mild epileptic syndrome, or Dravet Syndrome (DS), a rare, severe and drug-resistant epileptic encephalopathy (EE). DS patients show severe cognitive/behavioral impairments that, according to the definition of EE, should be caused by the recurrent epileptic activity. Yet, this causal relationship has never been proved and it is been challenged by studies in mouse models showing that the genetic mutation itself, which causes a decrease in GABAergic activity, can be responsible for DS cognitive outcome. Identification of the pathomechanisms responsible for disease progression is crucial for the development of efficient treatments.

Identification of the pathomechanisms responsible for disease progression is crucial for the development of efficient treatments. We studied the implication of repeated seizures during childhood to the later long-term modifications on cognitive/behavioral and epileptic phenotypes by submitting the Scn1a mouse model carrying the R1648H missense mutation and presenting mild phenotype to a protocol of repeated seizures induction by hyperthermia (10 days/one seizure per day). We observed that early life seizures can worsen the epileptic phenotype and induce cognitive/behavioral defects notably by inducing hyperactivity, sociability deficits and hippocampus- and prefrontal cortex-dependent memory deficits. These deficits are correlated with changes in the intrinsic neuronal excitability in the hippocampus without major cytoarchitecture changes or neuronal death.

Although the effect of NaV1.1 dysfuntion in altering brain synchrony and the effect of repeated seizure activity in the young brain are not mutually exclusive, we thus conclude that epileptic seizures are sufficient to convert a Scn1a mouse model carrying a mild phenotype into a severe phenotype. This work points the necessity of treating the epileptic seizures for a better long-term outcome in DS patients.

THANK YOU



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