XIV MEETING PORTUGUESE SOCIETY FOR NEUROSCIENCES 4-5 JUNE 2015 I PÓVOA DE VARZIM

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WELCOME ADDRESS

Dear Colleagues,

It is a great pleasure to welcome you for the XIV Meeting of the Portuguese Society for Neurosciences, from 4-5 June 2015, which will take place in Póvoa de Varzim and held at the Axis Vermar Conference & Beach Hotel.

In line with previous events, the 2015 meeting will bring together the Portuguese neuroscience community for the purpose of reviewing and discussing the most recent data, exchanging information and new ideas, and also, of course, get together, meet friends and have a good time.

We hope that through your active involvement in the lectures and poster sessions, we can build a great 2015 meeting.

Porto, 06 de maio de 2015

João Bettencourt Relvas

João Bettencout Relvas
SPN / i3S - IBMC / FCUP

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IMPORTANT INFORMATION

REGISTRATION DESK

The Registration desk will open at 8:45 on June 4 and at 8:30 on June 5.

NAME BADGES

For identification and security purposes, participants must wear their name badges when in the venue. The use of the badge is mandatory during coffee breaks, lunches and dinner.
The distribution of badges will be done in alphabetical order by first name.

PRESENTATION INSTRUCTIONS

The Plenary Lectures should last up to 60 minutes. The recommended length of the talk is 45-50 minutes, plus 10-15 minutes for discussion. The invited oral communications last 30 minutes. The recommended length of the talk is 20 minutes, plus 10 minutes for discussion. The selected oral communications last 15 minutes. The recommended length of the talk is 10 minutes, plus 5 minutes for discussion.
Session chairs are responsible for leading the discussion. Session chairs and speakers are requested to respect the allocated time for each individual lecture or oral communication.
Speakers presenting in the morning of June 4 should hand in their presentations in the auditorium until 9:30. Those having their presentations in the morning session of June 5 should hand in the presentation in the auditorium at 8:50. Speakers presenting in the afternoon sessions, should hand in their presentations during lunch break.
A data show and personal computer will be at the presenters' disposal. Technicians will be available to make sure that you have successfully uploaded your presentation.
You will be requested to provide your presentation in a USB key. If strictly necessary, you may use your own computer, which should be installed and properly tested prior to your presentation at the indicated times.

POSTER PRESENTATIONS

Posters should have 1.20m high and 0.90m wide, and will be displayed on the designated poster areas:

POSTERS OF SYMPOSIUM I - COGNITION AND NEURAL SYSTEMS - EÇA DE QUEIRÓS ROOM
POSTERS OF SYMPOSIUM II - NEURODEGENERATION - GOMES DE AMORIM ROOM
POSTERS OF SYMPOSIUM III - NEURODEVELOPMENT / REGENERATION - ESTELA ROOM
POSTERS OF SYMPOSIUM IV - GLIAL CELL BIOLOGY - PÓVOA DE VARZIM ROOM

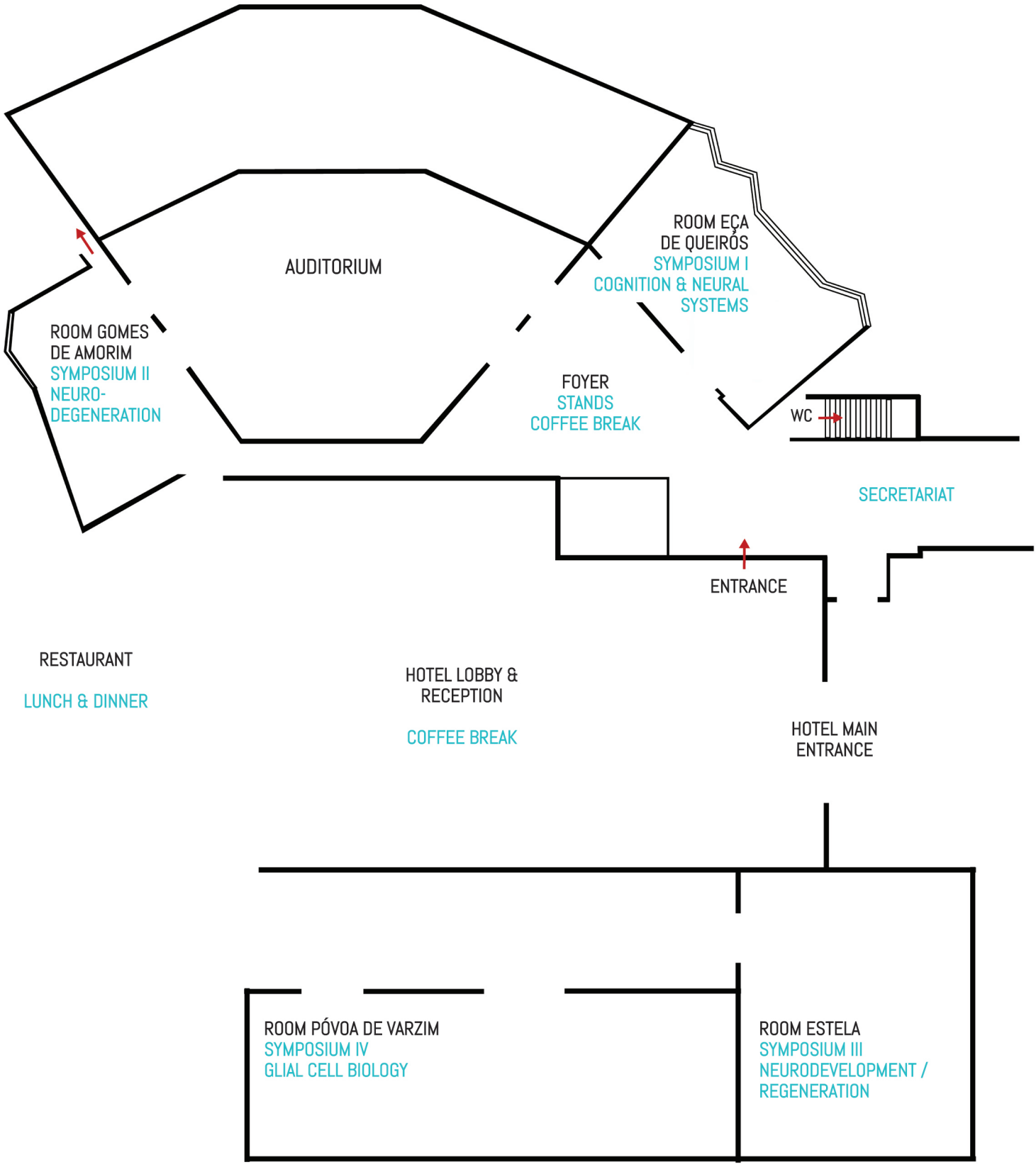
Please consult your poster number in the abstract book.
Conference staff will be present to provide assistance. Authors should remain next to their poster during their poster sessions.
All posters must be placed until 14:00 on June 4 and they should be removed until 16:30 on June 5.

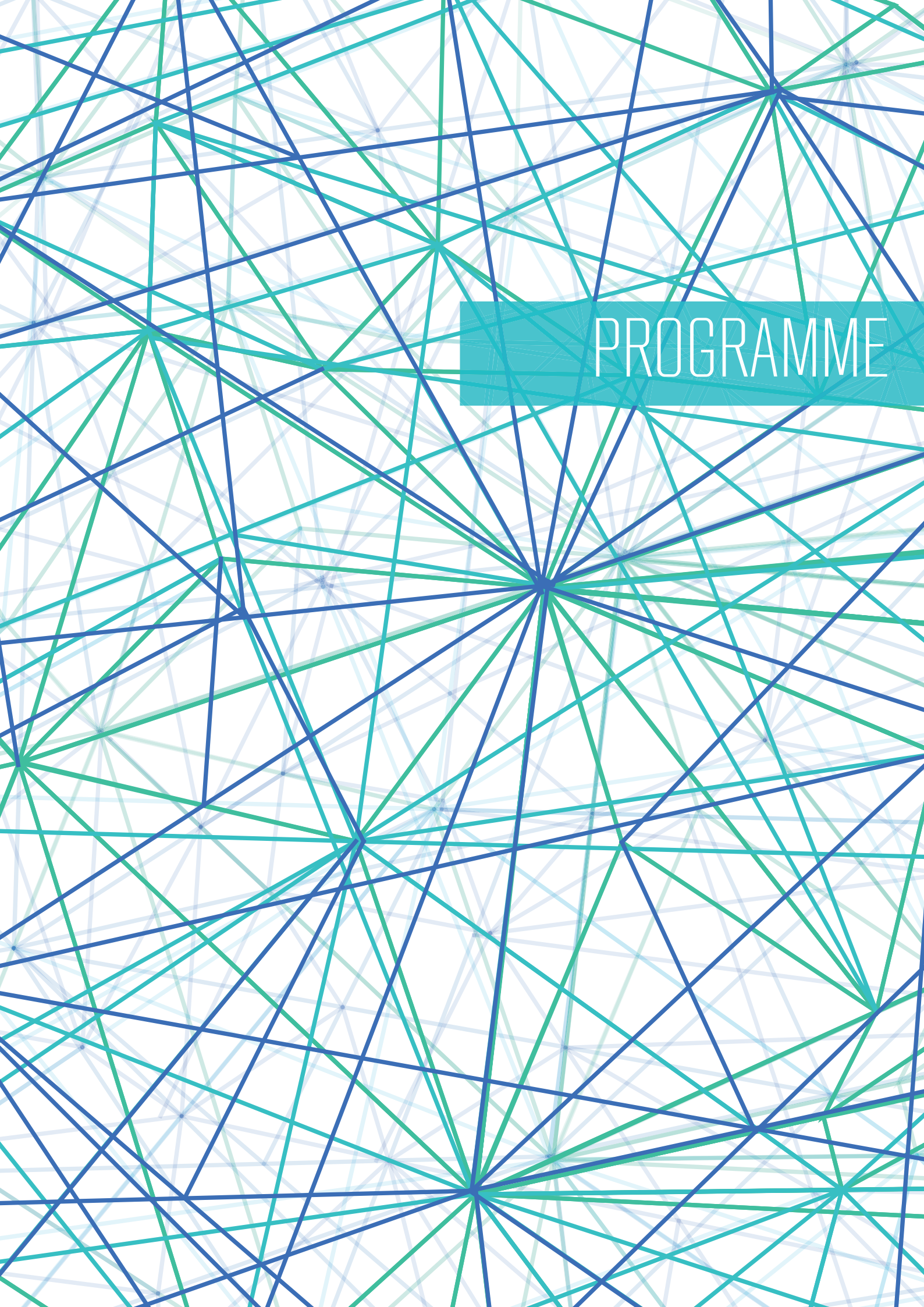
INTERNET ACCESS

Wireless Internet is available for free in the venue.

VENUE

AXIS VERMAR CONFERENCE & BEACH HOTEL




PROGRAMME

PROGRAMME

THURSDAY, JUNE 4

09.00 - 09. 45 REGISTRATION

09. 45 - 10.00 OPENING SESSION

SYMPOSIUM I – COGNITION AND NEURAL SYSTEMS

José Castro-Lopes & Teresa Summavielle

10.00 - 11.00 **Involvement of the endocannabinoid system in chronic pain / TALK SPONSORED BY SICAD**
Rafael Maldonado, Univ. Pompeu Fabra, Barcelona, Spain

11.00 - 11.30 **Imaging whole-brain neural activity dynamics in zebrafish**
Michael Orger, Fundação Champalimaud, Lisboa, Portugal

11.30 - 12.30 **The neural correlates of perceptual decision-making and high-level cognitive control: a multimodal approach**
Miguel Castelo-Branco, IBILI, Coimbra, Portugal

12.30 - 12. 45 **The triad of behavioural modifications characteristic of depression is abrogated by the selective amygdala neuronal down-regulation of adenosine A_{2A} receptors**
Nélio Gonçalves, CNC – Center for Neuroscience and Cell Biology, University of Coimbra, Portugal

12. 45 - 13.00 **Neuronal adenosine A_{2A} receptor overexpression affects AMPA and NMDA currents in CA1 hippocampal neurons**
Mariana Temido-Ferreira, IMM, Faculty of Medicine, Lisbon, Portugal

13.00- 14.00 LUNCH

POSTER SESSION & COFFE BREAK

14.00 - 15.00 - EVALUATION OF COGNITION AND NEURAL SYSTEMS POSTERS

15.00 - 16.00 - EVALUATION OF NEURODEGENERATION POSTERS

SYMPOSIUM II –NEURODEGENERATION

Isabel Cardoso & Márcia Liz

16.00 - 17.00	**The Ca^{2+}-dependent repressor DREAM regulates the onset and progression of Huntington disease** *José Naranjo, Centro Nacional de Biotecnología - CSIC, Madrid, Spain*
17.00 - 17.30	**Impact of modulating lysine deacetylases on mitochondrial function in Huntington's disease** *Ana Cristina Rego, CNC, FMUC, Coimbra, Portugal*
17.30 - 18.00	**Ataxin-3 gain and loss of function in neurodegenerative diseases** *Patricia Maciel, ICVS, UM, Braga, Portugal*
18.00 - 18.15	**Neuroprotective role of Transthyretin through Megalin in Ischemic Brain Injury** *João Gomes, i3S - IBMC, Universidade do Porto, Portugal*
18.15 - 18.30	**Caffeine administration modulates neuroinflammation and prevents rat retinal ganglion cell loss induced by ocular hypertension** *Maria Helena Madeira, CNC-IBILI, Coimbra, Portugal*
18.30 - 18.50	**Experience the next level of multiphoton microscopy with Olympus FVMPE-RS** Igor Del Vecchio, OLYMPUS ITALIA SRL - Societa' Unipersonale, Italy
19.00 - 20.00	**SPN GENERAL ASSEMBLY**
20.00	DINNER

FRIDAY, JUNE 5

SYMPOSIUM III - NEURODEVELOPMENT / REGENERATION

Ana Paula Pêgo, Meriem Lamghari & Carla Lopes

09.00 - 10.00	**Controlling the expansion of mammalian neural stem cells *in vivo*** *Federico Calegari, CRTD, Dresden, Germany*
10.00 - 11.00	**Regenerative Strategies in Spinal Diseases** *Hans Meisel, Director Centre of Neurosciences, BG-Clinic Bergmannstrost, Germany*
11.00 - 11.30	COFFEE BREAK
11.30 - 12.00	**Transcriptional control of vertebrate neurogenesis by the proneural factor Ascl1/Mash1** *Diogo Castro, IGC, Lisboa Portugal*
12.00 - 12.30	**Integrating Cell and Biomaterial based Strategies in Spinal Cord Injury Regenerative Medicine** *António Salgado, ICVS, Braga, Portugal*

12.30 - 12. 45 **Transplanted embryonic neurons integrate into adult neocortical circuits**
Sofia Grade, Biomedical Center, Ludwig-Maximilians University, Munich, Germany; Institute for Stem Cell Research, Helmholtz Zentrum, Neuherberg, Germany

1245 - 13.00 **Functional Genomics of Human Brain Development and Evolution**
Andre M. M. Sousa, Department of Neurobiology and Kavli Institute for Neuroscience, Yale University School of Medicine, New Haven, USA

13.00 - 14.30 LUNCH

POSTER SESSION & COFFEE BREAK
14.30 - 15.30 - EVALUATION OF NEURODEVELOPMENT / REGENERATION POSTERS
15.30 - 16.00 - EVALUATION OF GLIAL CELL BIOLOGY POSTERS

SYMPOSIUM IV - GLIAL CELL BIOLOGY / SPONSORED BY THE PORTUGUESE BIOCHEMICAL SOCIETY (SPB)

Francisco Ambrósio & João Relvas

16.00 - 17.00 **Targeting transcription factor Nrf2 in preclinical models of Parkinson's disease**
Antonio Cuadrado, Univ. Autónoma de Madrid, Spain

17.00 - 17.30 **Role of microglia in CNS inflammation and pathology**
Dora Brites, FFUL, Lisboa, Portugal

17.30 - 18.00 **The impact of methamphetamine on blood-brain barrier function: What is the role of inflammatory mediators?**
Ana Paula Silva, IBILI, Coimbra, Portugal

18.00 - 18.15 **Increased levels of S100B in multiple sclerosis are implicated in demyelination and glia reactivity**
Adelaide Fernandes, iMed.ULisboa, FFUL, Lisboa, Portugal; Department of Biochemistry and Human Biology, FFUL, Lisboa, Portugal

18.15 - 18.30 **Zeb1 potentiates gene transcription genome-wide in Glioblastoma Multiforme Cancer Stem Cells via a novel LEF1 dependent mechanism**
Pedro Rosmaninho, IGC, Oeiras, Portugal

18.30 **AWARDS AND CLOSING SESSION**

INVITED SPEAKERS

INVITED SPEAKERS

01 I INVITED SPEAKER

RAFAEL MALDONADO
UNIV. POMPEU FABRA, BARCELONA, SPAIN

Rafael Maldonado carried out his research for 11 years in France and the USA. Since 2000, he has been a professor of pharmacology at the University Pompeu Fabra, Barcelona, Spain, where he founded the Laboratory of Neuropharmacology, with 33 people under his direction. Dr Maldonado's research is focused on the study of the neurochemical basis of drug dependence and related disorders, including affective, pain, and eating disorders, with a particular focus on the development of novel behavioural models. He has published more than 235 scientific articles (H Index 52) in international journals and has been the principal investigator for 25 years of research grants funded by the main Spanish, European, and American agencies. Dr Maldonado is also a reviewer for or member of the editorial board of several scientific journals. He has collaborated with public authorities and private companies in the research policy and pharmaceuticals development on novel treatments for drug abuse and pain.

01 I INVITED SPEAKER

INVOLVEMENT OF THE ENDOCANNABINOID SYSTEM IN CHRONIC PAIN

RAFAEL MALDONADO
Univ. Pompeu Fabra, Barcelona, Spain

02 | INVITED SPEAKER

MICHAEL ORGER
FUNDAÇÃO CHAMPALIMAUD, LISBOA, PORTUGAL

Michael Orger received his PhD in Neuroscience in 2005 from the University of California, San Francisco, and subsequently conducted his postdoctoral research as a Helen Hay Whitney fellow at Harvard University. Since 2010, he is a Principal Investigator in the Champalimaud Neuroscience Programme in Lisbon. His lab's interest is in how the brain integrates sensory information and selects and executes appropriate actions, using innate visually guided behaviours in larval zebrafish as a model. These small transparent vertebrates are an ideal system to apply optical techniques to non-invasively record and manipulate neural activity. His lab's approach has three main components: quantitative analysis of behaviour; imaging of whole-brain neural activity dynamics at single-cell resolution in behaving animals using 2-photon and light-sheet microscopy and development of genetic tools that allow specific targeting and manipulation of identified cell types.

02 | INVITED SPEAKER

IMAGING WHOLE-BRAIN NEURAL ACTIVITY DYNAMICS IN ZEBRAFISH

MICHAEL ORGER
Fundação Champalimaud, Lisboa, Portugal

A challenge for studying the neural circuit basis of behavior is that even the simplest behaviors can involve networks of neurons that are distributed across many areas of the brain. Recently, developments in imaging technology have made it possible to record from thousands of neurons simultaneously, and, by applying them to small, transparent animals, we can even monitor activity throughout the entire brain, with single-neuron resolution. Zebrafish are an ideal model for this approach, since, at just one week old, they have a diverse array of behaviors, and a well-developed brain, which shows the typical vertebrate organization, but contains only about 100,000 neurons. We record activity, using two-photon and light-sheet imaging, from behaving zebrafish larvae, while presenting visual stimuli that elicit innate behavioral responses. While the fish's head is held still, its eyes and tail are free to move, and, by tracking these movements with a high-speed camera, we can both provide naturalistic visual feedback, and also associate neural responses with different sensory and motor signals. Whole-brain maps of activity dynamics, or correlations with behavioral variables, are registered to a reference brain anatomy, allowing comparison of the circuit organization across different fish, and also precise alignment with molecular and genetic markers. Sensory and motor circuits in the brainstem show a complex, fine-scale functional organization, with features that are highly stereotyped across individuals down to the micrometer scale. Cellular resolution, functional maps of a vertebrate brain, together with the ability to monitor activity dynamics comprehensively during behavior, provide a strong platform for dissecting the neural circuit mechanisms that underlie behavior.

03 I INVITED SPEAKER

MIGUEL CASTELO BRANCO
IBILI, COIMBRA, PORTUGAL

Miguel Castelo Branco (MD PhD) is the Scientific Coordinator of IBILI, a leading Vision Research Institute in Portugal and is the Scientific Coordinator of the National Functional Brain Imaging Scientific initiative. He is also the Director of ICNAS, the Medical Imaging Infrastructure at the University of Coimbra. Under his leadership IBILI was classified as an Excellent Research Unit by international evaluation panels. IBILI has now joined the CNC.IBILI consortium.

He obtained his PhD at the Max-Planck Institute for Brain research, Frankfurt, Germany and is now Associate Professor at the University of Coimbra. He has held a Professorship in Psychology in 2000 at the University of Maastricht, the Netherlands. Before, he was a Postdoctoral fellow at the Max-Planck-Institute for Brain Research, Germany where he had also performed his PhD work (1994-1999). His achievements are well reflected in publications in top General Journals, such as Nature and PNAS and Top Clinical Translational research journals such as Journal of Clinical Investigation, Brain, Human Molecular Genetics) as well as others in the field of Vision Research (Investigative Ophthalmology and Visual Sciences, Journal of Vision, Vision Research Archives of Ophthalmology), Human Neurophysiology and Neuroscience (The Journal of Neuroscience, The Journal of Neurophysiology, Human Brain Mapping, Neuroimage, Cerebral Cortex, Neuron and others).

Miguel Castelo Branco has made interdisciplinary contributions in the fields of Cognitive Neuroscience, Human and Animal Neurophysiology, Visual Neuroscience, Human Psychophysics, Functional Brain Imaging and translational research in Neurology (in particular Parkinson Disease). He has been awarded several National and International Prizes. Finally, he has been involved in major International grants such as EVIGENORET (Functional Genomics of the Retina), BACS (Bayesan Approach to cognitive Systems), EPILEPSIAE (on Epilepsia) and currently the FP7 Project BRAINTRAIN (Taking imaging into the therapeutic domain: Self-regulation of brain systems for mental disorders).

03 I INVITED SPEAKER

THE NEURAL CORRELATES OF PERCEPTUAL DECISION-MAKING AND HIGH-LEVEL COGNITIVE CONTROL: A MULTIMODAL APPROACH

MIGUEL CASTELO BRANCO

Visual Neuroscience Laboratory, IBILI, Institute for Biomedical Research on Light and Image, Faculty of Medicine, University of Coimbra; Institute for Nuclear Sciences Applied to Health, ICNAS, University of Coimbra

Decision-making is an important feature of brain function, and comprises several levels, from simple perceptual decisions to goal-oriented behavior under complex emotional and social contexts. Here we address the functional connectivity of core and extended neural architectures underlying choice behavior, by combining fMRI, EEG, EEG/fMRI and molecular imaging techniques. Understanding how people make choices under uncertainty is a timely topic in cognitive neuroscience. So far, the fields studying perceptual decision-making and high-level determinants of difficult choice, such as motivation and cognitive control have remained quite separate. It is important to study decision-making under uncertainty by generating a framework that includes self-relevant contexts and motivational/reward variables. This will help generate models of impaired decision making in diseases with both impaired/fragmented perception and/or behavioural control/motivation such as autism and schizophrenia. Moreover modulation of functional connectivity and neural synchrony are discussed as mechanisms underlying bistable perceptual decision and high-level control of decision. The combination of different modalities, including the nature and mechanism of GABAergic *vs* Glutamatergic signalling and their impact on cognitive function are relevant to the understanding of the physiological basis of behavior and its impairment in a set of neuropsychiatric conditions.

04 I INVITED SPEAKER

JOSE R. NARANJO
CENTRO NACIONAL DE BIOTECNOLOGÍA. CSIC. MADRID. SPAIN

Professor Jose R Naranjo, PhD in Medicinal Chemistry (1983), did his post-doctoral research in the USA (NIMH and Georgetown University) and France (IGBMC, Strasbourg) and was appointed as Staff Scientist at C.S.I.C. in 1986. EMBO Member since 2000, he was Director of the National Center for Biotechnology-C.S.I.C and Deputy Vice President for Scientific Programming at C.S.I.C. His research is focused on the molecular mechanisms controlling activity-dependent gene expression in physiological and pathological conditions. He is serving as Receiving Editor for JBC and is a member of several Advisory Committees and Reviewing Panels. He has published more than 100 pier-reviewed papers.

04 I INVITED SPEAKER

THE CA^{2+}-DEPENDENT REPRESSOR DREAM REGULATES THE ONSET AND PROGRESSION OF HUNTINGTON DISEASE

JOSE R. NARANJO

Centro Nacional de Biotecnología, CSIC, Madrid, Spain

Deregulated intracellular Ca^{2+} homeostasis underlies synaptic dysfunction and is a common feature in neurodegenerative processes. DREAM/calsenilin/ KChIP-3 is a multifunctional Ca^{2+} binding protein with specific functions in different subcellular compartments. In the nucleus, the Ca^{2+}-free form of DREAM binds tightly to DRE sequences in the DNA and controls the expression of several genes related to Ca^{2+} homeostasis, neuronal excitability and neuronal survival. DREAM mutants unable to respond to Ca^{2+} and/or cAMP will disturb gene regulation leading to changes in the physiology of the synapses that might be determinant for or predispose to neuronal damage and death. We have used transgenic mice over expressing dominant active DREAM mutants, i.e. insensitive to Ca^{2+}, and DREAM deficient mice to assess the role of DREAM in the onset of unbalanced motor coordination and neurodegenerative processes found in chemically- or genetically-induced mouse models of Huntington disease (HD). In addition, we have tested drugs able to bind to DREAM to investigate an effect on the onset and progression of motor dysfunction in the R6/2 mouse model of HD.

Funded by grants from Fundacion Reina Sofia y CIBERNED.

05 I INVITED SPEAKER

ANA CRISTINA REGO
CNC, FMUC, COIMBRA, PORTUGAL

Ana Cristina Rego (Ph.D.) is tenure Assistant Professor since 1999, lecturing classes of Biochemistry, Neuroscience and Neurobiology, at the Faculty of Medicine, University of Coimbra (UC), where she initiated as Teaching Assistant in 1997 and received the 'Agregação' degree in 2010. She is also head of the research group 'Mitochondrial Dysfunction and Signaling in Neurodegeneration' at the CNC-Center for Neuroscience and Cell Biology (CNC), at UC, since 2003. AC Rego received the Master in Cell Biology in 1994 and the Ph.D. in Cell Biology in 1999 at UC, under the supervision of Prof. Catarina R. Oliveira, and was postdoctoral researcher in the lab of Prof. David G. Nicholls, at the University of Dundee, Scotland, UK, and at the Buck Institute, Novato, CA, USA, from 1998-2000. In 2004 and 2005 AC Rego was the coordinator of the BEB PhD Programme at CNC. AC Rego investigates molecular mechanisms of familial and age-related neurodegenerative disorders, including Huntington's and Alzheimer's diseases, focusing on glutamatergic postsynaptic dysfunction, the role of lysine deacetylases on mitochondrial function, and modified neurogenesis. Funding has been garnered by HighQ Foundation (USA), Lundbeck Foundation, 'Instituto de Investigação Interdisciplinar' (IIIUC), Faculty of Medicine-UC, 'Fundação para a Ciência e a Tecnologia' (FCT), 'Santa Casa da Misericórdia de Lisboa' (SCML), and 'Fundação Luso Americana para o Desenvolvimento' (FLAD) projects or prizes. AC Rego holds an H factor of 32 and more than 3300 citations, published 94 original and review articles, 12 book chapters and more than 100 abstracts in research meetings, and is a member of the board of the 'Sociedade Portuguesa de Neurociências' (SPN) since 2007. AC Rego also supervised/co-supervised the work of several post-docs, 13 Ph.D. students and 20 Master students (concluded degrees).

05 I INVITED SPEAKER

IMPACT OF MODULATING LYSINE DEACETYLASES ON MITOCHONDRIAL FUNCTION IN HUNTINGTON'S DISEASE

A. CRISTINA REGO

CNC-Center for Neuroscience and Cell Biology _ 'Mitochondrial Dysfunction and Signaling in Neurodegeneration' group, University of Coimbra, and Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Altered mitochondrial function, dynamics and bioenergetics, and transcriptional deregulation have been described in Huntington's disease (HD), an autosomal dominant neurodegenerative disorder caused by CAG repeat expansion in the huntingtin gene (HTT). Modulators of lysine deacetylases (KDAC), which include classes I and II of histone deacetylases (HDAC) and the NAD+-dependent class III KDAC or sirtuins (SIRT), have been proposed as candidate neuroprotective targets to alleviate HD pathogenesis. In previous studies we showed that unselective HDAC inhibitors promote histone acetylation and positively influence mitochondrial function in cells expressing full-length mutant huntingtin subjected to excitotoxic stimuli. More recently, we found that HDAC inhibitors, namely sodium butyrate (SB), ameliorated the activity of pyruvate dehydrogenase complex (PDH) in HD striatal cells and YAC128 mice brain. Treatment with SB recovered cell viability and mitochondrial respiration, increased PDH E1alpha subunit levels and decreased PDH phosphorylation in HD cells. Importantly, SB decreased gene expression of the two most abundant PDK isoforms, PDK2 and PDK3, suggesting that SB may help to counteract HD-related deficits in mitochondrial bioenergetics. Considering the possible effects of modulators of class III KDACs we also tested resveratrol (RESV, a SIRT1 activator) *versus* nicotinamide (NAM, a SIRT inhibitor) in counteracting mitochondrial dysfunction in YAC128 mice primary neurons and HD human lymphoblasts and *in vivo*, in symptomatic YAC128 mice. In *in vitro* and *in vivo* models RESV decreased histone H3 acetylation (K9H3), whereas NAM increased K9H3 acetylation. Both RESV and NAM completely restored mitochondrial function in both HD cell models. Moreover, both compounds alleviated decreased PGC-1alpha and TFAM protein levels, and the reduction in mitochondrial DNA copies in HD lymphoblasts. In addition, RESV treatment enhanced mRNA levels of mitochondrial-encoded electron transport chain genes and reduced motor incoordination in YAC128 mice, establishing a possible link between mitochondrial function and the control of HD-related motor disturbances. More recently, we investigated the role of SIRT3 (mitochondrial SIRT) in HD cells. SIRT3 overexpression in striatal cells decreased Lys acetylation, cell viability and mitochondrial membrane potential, while increasing mitochondrial calcium accumulation and mitochondrial reactive oxygen species. Interestingly, untransfected HD striatal cells and human lymphoblasts exhibited increased SIRT3 protein and mRNA levels and SIRT3 enzymatic deacetylation activity, implicating that a rise in mitochondrial SIRT3 exert negative effects in HD cells. Overall, inhibiting unselective HDACs modulates transcription and promotes mitochondrial bioenergetics in HD, whereas enhanced mitochondrial function evoked by modulating SIRTs activity appears to depend on selective SIRTs.

HD research supported by FCT, Portugal (PTDC/SAU-FCF/108056/2008; EXPL/BIM-MEC/2220/2013), co-financed by COMPETE, supported by FEDER, GAI (funded by FMUC and Santander Totta Bank), 'Santa Casa da Misericórdia de Lisboa' (SCML) and 'Fundação Luso-Americana para o Desenvolvimento' (FLAD).

06 I INVITED SPEAKER

PATRICIA MACIEL
ICVS, UM, BRAGA, PORTUGAL

Patricia Maciel graduated in Biochemistry from the University of Porto in 1993 and completed her PhD in Biomedical Sciences (Genetics) in 1998, with work developed at McGill University, Canada. The goal of her research is to understand the genetic basis of nervous system function and dysfunction, with a strong focus on human neurological disease, using molecular genetics, genomics, transcriptomics, proteomics, cell biology and behavioral analysis. Her team at the University of Minho studies neurodegenerative disorders, such as Machado-Joseph disease, and neurodevelopmental disorders, including intellectual disability, autism spectrum disorders and epilepsy. The disease models they develop in *C. elegans* and mouse are used to not only to understand pathogenic mechanisms, but also to search for therapeutic targets and test the efficacy of therapeutic strategies. A strong focus of interest of the lab is the role of the ubiquitin proteasome system and of chromatin remodeling in neuronal function and dysfunction.

06 I INVITED SPEAKER

ATAXIN-3 FUNCTION AND DYSFUNCTION IN THE NERVOUS SYSTEM: A LINK TO RNA SPLICING

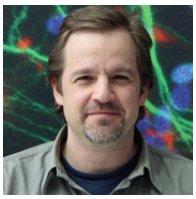
P. MACIEL [1,2]

[1]Life and Health Sciences Research Institute(ICVS), School of Health Sciences, University of Minho, Braga, Portugal; [2]ICVS-3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Ataxin-3 (ATXN3) is a 30kDa protein with deubiquitylase (DUB) activity which is mutated in Machado-Joseph disease (MJD), a late onset neurodegenerative disorder. The disease causing mutation is the expansion of a polyglutamine tract, thought to confer novel and (neuro)toxic properties to this protein. However, the normal role of ATXN3 in cells and organisms, particularly in the nervous system, remains mostly unknown, some links to protein quality control, DNA repair, transcription regulation, cell adhesion and cytoskeletal organization being established. We have used a functional genomics approach to the characterization of ATXN3 function in neuronal cells, and took advantage of animal models of MJD to assess the possible perturbation of this function in the disease context.

We found that ATXN3 is required for neuronal differentiation of SH-S5Y5 cells and for normal cellular morphology, cytoskeleton organization, proliferation and survival. This phenotype was associated with increased proteasomal degradation of alpha5-integrin subunit (ITGA5) and reduced activation of integrin signaling. Interestingly, we observed that silencing of ATXN3, overexpression of a catalytically inert version of the protein or a mutant protein bearing an expanded polyQ tract led to partially overlapping phenotypes, suggesting that a loss of the neuronal function of ATXN3 may be contributing to neurodegeneration.

In an attempt to identify candidate substrates of its DUB activity, we also characterized the ubiquitome of SH-S5Y5 cells lacking ATXN3. We found that a large proportion of the proteins showing altered ubiquitylation in the absence of ATXN3 were involved in RNA post-transcriptional modification. A set of these were splicing regulatory proteins that physically interacted with ATXN3 and, in addition to altered polyubiquitylation, also showed decreased levels in ATXN3-deficient cells, which were rescued by proteasome inhibition. This suggests they could be targets of the DUB activity of ATXN3. Considering this, we analyzed RNA splicing by transcriptomic analysis and using reporter minigenes in ATXN3-deficient neuronal cells and found that splicing was globally and markedly altered. These findings lead us to propose for the first time that ATXN3 plays a role in splicing regulation in neurons, a novel function for this protein. Intriguingly, many of deregulated target genes were linked to the cytoskeleton, and, more specifically, loss of function of ATXN3 lead to a deregulation of tau exon 10 splicing, resulting in a decreased 4R/3R tau ratio. The fact that similar alterations in the 4R/3R tau ratio were found in the brain of a mouse model of MJD expressing mutant human ATXN3 with an expanded polyglutamine tract, suggests that this mechanism might also be contributing for the pathogenesis of MJD, and establishes a link between two key proteins involved in different neurodegenerative disorders.



07 I INVITED SPEAKER

FEDERICO CALEGARI
CRTD, DRESDEN, GERMANY

Federico Calegari studied regulated exocytosis in hippocampal astrocytes in the laboratory of Patrizia Rosa, Milan. He then moved to the group of Wieland Huttner in the newly founded Max Planck Institute in Dresden. There he studied mammalian brain development, in particular the role of the cell cycle in neural stem cell commitment to neurogenesis. His studies continued as a group leader and then professor at the Center for Regenerative Therapies of the Technishe Universitat Dresden. His goal is to manipulate somatic stem cells as a means to control brain formation and function.

07 I INVITED SPEAKER

CONTROLLING THE EXPANSION OF MAMMALIAN NEURAL STEM CELLS *IN VIVO*

FEDERICO CALEGARI
CRTD, Dresden, Germany

The goal of our laboratory is to understand and manipulate the mechanisms controlling the expansion of neural stem cells (NSC) and the generation of neurons in the mammalian brain. We found that a transient manipulation of the length of the G1 phase of the cell cycle can be used as a means to increase neurogenesis in development and adulthood (Lange *et al.*, 2009; Artegiani *et al.*, 2011). Ongoing experiments aim to address whether or not this acute increase in neurogenesis positively contributes to brain function or recovery upon injury. In addition, we use a number of approaches to identify the mechanisms underlying physiological proliferation *vs* differentiation of NSC, including regulation by long non-coding RNAs (Aprea *et al.*, 2013; 2015).

08 I INVITED SPEAKER

HANS MEISEL
DIRECTOR CENTER OF NEUROSCIENCES
CHAIR DEPT. OF NEUROSURGERY, BG-CLINIC BERGMANNSTROST HALLE
DONAU-UNIVERSITÄT KREMS

In 1998 HJ. Meisel was appointed to the Berufsgenossenschaftliche Clinic in Bergmannstrost Halle/Saale as Director of the Clinic for Neurosurgery. Every year his team of 10 surgeons carries out a minimum of 1800 surgeries in the area of cerebral and spinal diseases. Since September 2008 HJ. Meisel has been appointed as the Director of the Center of Neurosciences of the BG-Clinic Bergmannstrost Halle.

HJ. Meisels primary focus in spine surgery and research is for the last 20 years in degenerative spinal diseases. Starting as inventor and designer of spinal implants for the intervertable space in cervical and lumbar in arthroplasty and fixation he developed 1995 the first biological disc repair transplantation system with autologus chondrocytes. HJ. Meisel served as a PI for the first randomized clinical trial to study this regenerative approach (EuroDisc) after running all preclinical evaluations at Emory Spine Center, Atlanta. Together with his Atlanta group they continued the preclinical and clinical work in disc regeneration with adiposed derived mesenchymal stem cells. As a founding member HJ. Meisel helped to start the ETP Platform Nanomedicine for the European Commission.

From 2003 to 2006 HJ. Meisel served AOSpine Europe as a founding member and to develop a new teaching and course platform as a deputy on the neurosurgical side. (Intervertable Disc Course Davos, 2005; Strasbourg Course 2006; Cervical Course Palma de Mallorca, 2006)

In 2005 HJ. Meisel co-founded the Translation Center for Regenerative Medicine at the University of Leipzig and supported there as an Executive Board member the preclinical affairs mentoring since the beginning 4 major spinal projects with the focus in regenerative disc repair and biomaterials financed by the German Ministry of Research (BMBF). In 2005 HJ. Meisel founded the European Network for Regenerative Medicine Regenerate Europe which integrates 10 countries of the European Union that carry out research in the area of Regenerative Medicine and organized the Biospine Meetings (2002, 2007, 2010) and Regulatory Workshops for ATMPs and animal modeling (2010, 2012, 2014). In 2007 HJ. Meisel was appointed by the Vreije University of Amsterdam to become a Visiting Professor in the Department of Orthopedic Surgery for the coordination of international research projects and the development of the European Master Degree in Regenerative Medicine.

Currently there is running the EU COST project Namabio “From nano to macro biomaterials (design, processing, characterization, modelling) and applications to stem cells regenerative orthopaedic and dental medicine” including 250 members in nanomaterial research and clinical application.

In 2013 he became an Honorary Professor in the Department for Health Sciences and Biomedicine at the Donau-University Krems (Austria).

In 2013 he became an elected member of the AO Spine Knowledge Forum Degenerative and Biologics. In 2014 he was elected as chair of the European Technology Platform Nanomedicine (ETPN) Working Group of Nanotechnologies for Regenerative Medicine. HJ Meisel is active member of the EANS, SSE, ICRS, ISSLS, ISASS, AOSpine and others.

08 I INVITED SPEAKER

REGENERATIVE STRATEGIES IN SPINAL DISEASES

MEISEL HJ[1], HOHAUS C[1], MINKUS Y[1], GANEY T[2]

[1] Department of Neurosurgery, BG Clinic Bergmannstrost, Halle/ Saale, Germany; [2] Atlanta Medical Center, Atlanta, USA

INTRODUCTION: Low back pain is an extremely common symptom, affecting nearly three-quarters of the population sometime in their life. Given that disc herniation is thought to be an extension of progressive disc degeneration that attends the normal aging process, seeking an effective therapy that staves off disc degeneration has been considered a logical attempt to reduce back pain. The most apparent cellular and biochemical changes attributable to degeneration include a decrease in cell density in the disc that is accompanied by a reduction in synthesis of cartilage-specific extracellular matrix components. With this in mind, one therapeutic strategy would be to replace, regenerate, or augment the intervertebral disc cell population, with a goal of correcting matrix insufficiencies and restoring normal segment biomechanics. Biological restoration through the use of autologous disc chondrocyte transplantation offers a potential to achieve functional integration of disc metabolism and mechanics.

METHODS: We designed an animal study using the dog as our model to investigate this hypothesis by transplantation of autologous disc-derived chondrocytes into degenerated intervertebral discs. As a result we demonstrated that disc cells remained viable after transplantation; transplanted disc cells produced an extracellular matrix that contained components similar to normal intervertebral disc tissue; a statistically significant correlation between transplanting cells and retention of disc height could displayed.

RESULTS: Following these results the Euro Disc Randomized Trial was initiated to embrace a representative patient group with persistent symptoms that had not responded to conservative treatment where an indication for surgical treatment was given. In the interim analyses we evaluated that patients who received autologous disc cell transplantation had greater pain reduction at 2 years compared with patients who did not receive cells following their discectomy surgery and discs in patients that received cells demonstrated a significant difference as a group in the fluid content of their treated disc when compared to control.

DISCUSSION: Autologous disc-derived cell transplantation is technically feasible and biologically relevant to repairing disc damage and retarding disc degeneration. Adipose tissue provides an alternative source of regenerative cells with little donor site morbidity. These regenerative cells are able to differentiate into a nucleus pulposus-like phenotype when exposed to environmental factors similar to disc, and offer the inherent advantage of availability without the need for transporting, culturing, and expanding the cells. In an effort to develop a clinical option for cell placement and assess the response of the cells to the post-surgical milieu, adipose-derived cells were collected, concentrated, and transplanted under fluoroscopic guidance directly into a surgically damaged disc using our dog model. This study provides evidence that cells harvested from adipose tissue might offer a reliable source of regenerative potential capable of bio-restitution.

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09 I INVITED SPEAKER

DIOGO CASTRO
IGC, LISBOA PORTUGAL

Diogo S. Castro has received his PhD from the Karolinska Institute (Stockholm, Sweden), and performed post-doctoral studies at the MRC National Institute for Medical Research (London, UK) with François Guillemot. He started his own research group at Instituto Gulbenkian de Ciência (Oeiras, Portugal) in 2011, where he is a Principal Investigator sponsored by the FCT investigator programme. He has since long been interested in understanding how global programs of gene expression are regulated during neural development and disease. His research focuses on the function of transcriptional networks in vertebrate neurogenesis, in particular those of proneural transcription factors of the bHLH family.

09 I INVITED SPEAKER

TRANSCRIPTIONAL CONTROL OF VERTEBRATE NEUROGENESIS BY THE PRONEURAL FACTOR ASCL1/MASH1

DIOGO CASTRO
IGC, Lisboa ,Portugal

During neurogenesis, an intrinsic program that relies on the activity of transcription factors and the epigenetic landscape coordinates the progression of neural progenitors through a succession of distinct cellular states. Such program integrates information from local extracellular signals (e.g. through cell to cell contact) or from long range cues (e.g. secreted factors), resulting in a coherent program of cellular differentiation that requires governing the expression of a large number of genes. In order to understand the regulatory logic of the neurogenic process, our work focuses on proneural transcription factors (e.g. Ascl1/Mash1) as these play the role of master regulators by coordinating the various components of the differentiation program. According to the current model, Ascl1 promotes sequentially the proliferation and differentiation of neural/stem progenitor cells along the neuronal lineage, with the concomitant regulation of distinct transcriptional targets in proliferating and differentiating cells. Here I will discuss the contribution of two types of mechanisms to the temporal progression of the Ascl1 transcription program, relying on: i) mutual interactions between Ascl1 and the chromatin landscape, and ii) cross-regulatory interactions with the Notch signaling pathway.

10 I INVITED SPEAKER

ANTÓNIO SALGADO
ICVS, BRAGA, PORTUGAL

António Salgado PhD is a biologist and received is PhD in Materials Science and Engineering- Tissue Engineering and Hybrid Materials, from the University of Minho in 2005. Currently he is a Principal Investigator at the Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho. His research interests are focused on the development of innovative therapies for CNS repair. His main areas of research are: 1) Development of ECM like hydrogels for the transplantation of Mesenchymal Stem Cells into the injured CNS; 2) Role of the secretome of MSCs in neuroprotection and repair and 3) Modulation of MSCs secretome through bioreactor based approaches. He is currently an author of 57 papers in international peer reviewed journals (h-Index=18). He serves as a Biomaterials Editor for Biomed Research International and is an Associate Editor for Biochimie. In 2008 he received the Gulbenkian Award on Cutting Edge Research in Life Sciences, for his project focused on unveiling the neuroregulatory molecules present in the secretome of mesenchymal stem cells. More recently, in 2013, he received the Prize Melo e Castro for Spinal Cord Injury Research, awarded by the Santa Casa de Misericórida de Lisboa.

10 I INVITED SPEAKER

INTEGRATING CELL AND BIOMATERIAL BASED STRATEGIES IN SPINAL CORD INJURY REGENERATIVE MEDICINE

ANTÓNIO J. SALGADO

[1] Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal; [2] ICVS/3B's, PT Government Associate Laboratory, Braga/Guimarães, Portugal

Spinal cord injury (SCI) frequently results in permanent paraplegia and quadriplegia. Up to now no effective therapies have efficiently tackle this problem. The present work aims to establish novel therapeutic routes that overcome the pitfalls of current methodologies. Its backbone is based on an intimate crosstalk between peptide grafted hydrogel-based biodegradable biomaterials and the secretome of mesenchymal stem cells (MSCs). The objective is to develop an integrative strategy where the 3D hydrogel protect sMSCs within the injured spinal cord, while the latter, through their tropich action, modulate inflammation while stimulating axonal outgrowth and neuronal differentiation. A hydrogel based biomaterial, Gellan Gum, used for cell transplantation into thei injured spinal cord at our lab. In subsequent reports the hydrogel phase was further improved by grafting on its structure a GRGDS peptide, trough click chemistry. This modification proved to be pivotal for fostering the growth and differentiation of neural cells within the hydrogel, as well as to stimulate the proliferation and viability of MSCs, with an advantageous impact on their secretome. *In vivo* experiments in a hemissection rat SCI model revealed that the developed strategy lead to significant gains in motor function of afflicted SCI animals, when compared to non-treated controls, thus validating the strategy developed so far.

ACKNOWLEDGMENTS:

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11 I INVITED SPEAKER

DORA BRITES
RESEARCH INSTITUTE FOR MEDICINES; DEPARTMENT OF BIOCHEMISTRY AND HUMAN BIOLOGY, FACULTY OF PHARMACY, UNIVERSIDADE DE LISBOA, LISBOA, PORTUGAL

Dora Brites was graduated in Pharmacy by the Universidade de Lisboa (FF/ULisboa) in 1976 and received her PhD degree in 1988. Dora Brites is Senior Researcher and Invited Full Professor at FF/ULisboa and Group Leader of Neuron Glia Biology in Health and Disease unit of the Research Institute for Medicines. She was recently awarded with the prize Edgar Cruz e Silva from the GEECD Aging group. She is Professor of Physiopathology of Neuroinflammation and of Histology and Embriology in Master Courses. She is member of the Coordinator Council and Executive Director of Mind-Brain College at ULisboa, Member of the Directive Board of the Doctoral Program in Integrative Neuroscience at the Faculty of Medicine-ULisboa and Member of the Mentorship Advisory Committee of the Doctoral i3DU Programme in Medicines and Pharmaceutical Innovation at FF/ULisboa. Dora Brites has authored and co-authored more than 100 refereed publications and book chapters.

11 I INVITED SPEAKER

ROLE OF MICROGLIA IN CNS INFLAMMATION AND PATHOLOGY

DORA BRITES[1,2]

[1] Research Institute for Medicines; 2Department of Biochemistry and Human Biology; [2] Faculty of Pharmacy, Universidade de Lisboa, Portugal

Microglia comprise approximately 12% of the central nervous system (CNS) cells. Microglia are the predominant immune cells in the CNS with a key role in brain inflammation and inflammatory neurodegeneration. In late embryonic brain development and early postnatal brain maturation, microglia evidence an amoeboid shape morphology, actively engulf synaptic material and play a major role in synaptic pruning, while enforce the programmed elimination of neural cells. In mature brains, microglia are consistently distributed throughout the brain parenchyma, exhibit a characteristic ramified morphology with a small, static cell body, but with dynamic and branched processes continuously surveilling their microenvironment for the presence of a pathological factor. When microglia are activated in response to infection or injury undergo dramatic morphologic alterations that include the retraction and thickening of processes and up-regulate a variety of surface receptors, including the major histocompatibility complex class II. In such condition, the cells secrete a host of soluble proinflammatory and neurotoxic factors, such as the cytokines tumor necrosis factor-α and interleukin-1β, and free radicals, like nitric oxide. This classical activated microglia is denominated as the pro-inflammatory M1 phenotype. After these changes, microglia may progress to a "resolution phase" where the alternatively activated M2 cell is amoeboid, highly phagocytic, and produce anti-inflammatory cytokines, like interleukin-10 and transforming growth factor-β in order to resolve the inflammation and clear up the mess. Subsets of M1 and M2 microglia are likely to exist in a state of dynamic equilibrium after injury and their polarization towards each phenotype is suggested to depend on local signals. Indeed, microglia should not be considered as a homogeneous population, once they do not similarly respond to microenvironmental alterations. Accelerated microgliopathy by chronic inflammation and ageing are now suggested as a driving force in neurodegenerative diseases derived from disturbances or loss of microglia function, which may open new opportunities for the treatment of CNS disorders. This presentation will reveal novel data on the acquired microglia phenotypes upon lipopolysaccharide-induced acute inflammation and on their significance to microglia pathological dynamics in models of Alzheimer´s disease.

12 I INVITED SPEAKER

ANTÓNIO CUADRADO
UNIV. AUTÓNOMA DE MADRID, SPAIN

Antonio Cuadrado, obtained his PhD degree in 1985 and enjoyed several postdoctoral stays in the National Cancer Institute-NIH with the help of Fulbright and Fogarty fellowships. He established his independent laboratory as Professor of Biochemistry in 1997 at the Department of Biochemistry, Faculty of Medicine of Autonomous University of Madrid. His main interest is the study of molecular mechanisms involved in initiation and progression of neurodegenerative diseases. For the past 10 years his main lane of research has been the validation of transcription factor Nrf2, master regulator of cell homeostasis, in protection against oxidative, inflammatory and proteopatic stress in Parkinson's and Alzheimer's disease. He is full professor of Biochemistry and Deputy Director of the Institute of Biomedical Research "Alberto Sols" UAM-CSIC. He has published over 100 peer reviewed articles, several book chapters and reviews.

12 I INVITED SPEAKER

TARGETING TRANSCRIPTION FACTOR NRF2 IN PRECLINICAL MODELS OF PARKINSON'S DISEASE

ANTÓNIO CUADRADO
Univ. Autónoma de Madrid, Spain

Rodent models of Parkinson's disease (PD) have failed to provide a disease modifying benefit when translated into humans. This is most likely due to the fact that most of these models do not reproduce the main hallmark of human pathology, which is the synucleinopathy. Our team has been using the stereotaxic delivery to the mouse ventral midbrain of an adenoassociated viral vector expressing human α-synuclein (AAV6-α-SYN). In these mice the dopaminergic nigrostriatal neurons exhibit α-SYN aggregates in cell bodies and fibers reminiscent of Lewy bodies and dystrophic Lewy neurites. To determine the relevance of transcription factor Nrf2, a master regulator of cell homeostasis, as a new target in PD we have been using Nrf2-knockout (*Nrf2*$^{-/-}$) mice and their control littermates (*Nrf2*$^{+/+}$). In these animals, dopaminergic nigrostriatal neurons die in a time window of 0.5-2 months but *Nrf2*$^{-/-}$ mice are more sensitive that *Nrf2*$^{+/+}$ controls. Astroglial and microglial activation is exacerbated in *Nrf2*$^{-/-}$ mice, thus providing evidence of a role of Nrf2 in brain protection against α-SYN toxicity. We used dimethylfumarate (DMF), an activator of the Nrf2 transcriptional response, to determine if Nrf2 targeting might be protective against synucleinopathy. Treatment of microglial BV2 cells with DMF showed a time-dependent increased in mRNA and protein levels of Nrf2-regulated genes like heme oxygenase 1, NAD(P)H quinone oxidoreductase 1 and autophagy related protein p62. Interestingly, DMF treatment decreased the mRNA and protein levels of IL-1β and iNOS induced by α-SYN, indicating that DMF could modulate the inflammatory response produced by α-SYN. In *Nrf2*$^{+/+}$ mice, daily oral gavage of 100 mg/kg DMF provided a very significant protection of nigral dopaminergic neurons against α-SYN toxicity after 3 and 8 weeks following injection with AAV6-α-SYN and at the same time reduced astrogliosis and microgliosis. This protective effect was not observed in the *Nrf2*$^{-/-}$ mice. These experiments provide a significant support to determine if pharmacological targeting of Nrf2 with DMF might be a therapeutic strategy in synucleinopathies.

13 I INVITED SPEAKER

ANA PAULA SILVA
IBILI, COIMBRA, PORTUGAL

Ana Paula Silva obtained her B.Sc. degree in Biology in July 1998 at the Faculty of Sciences and Technology, University of Coimbra, and in January 2003 she obtained her doctorate degree in Cellular Biology at the same University. During her PhD, Ana Paula Silva worked at the Center for Neuroscience and Cell Biology (CNC), Coimbra, and at the Anatomy and Neurobiology Department, Odense University, Denmark, in a project entitled "Neurotoxicity and neuroprotection in the hippocampus: role of neuropeptide Y receptors". During this period she received an Award from Fundação Calouste Gulbenkian and an Honour Mention from Fundação Professor Francisco Pulido Valente. Afterwards, Ana Paula Silva started working as a postdoctoral research fellow at the CNC and at the Neuropharmacology Laboratory, Faculty of Health and Life Sciences, University Pompeu Fabra, Barcelona, Spain. On August 2005, she got a research position at the Institute of Pharmacology and Experimental Therapeutics, Faculty of Medicine, University of Coimbra, and moved to the Institute for Biomedical Imaging and Life Sciences (IBILI), being currently also the coordinator of the Research Support Office at the same Faculty. Her current research is focused on the neurotoxic effects triggered by psychostimulants, with particular attention to blood-brain barrier alterations induced by methamphetamine and methylphenidate.

13 I INVITED SPEAKER

THE IMPACT OF METHAMPHETAMINE ON BLOOD-BRAIN BARRIER FUNCTION: WHAT IS THE ROLE OF INFLAMMATORY MEDIATORS?

V. COELHO-SANTOS[1,2,3], R. A. LEITÃO[1,2,3], F. L. CARDOSO[4], I. PALMELA[4], M. RITO[5], M. BARBOSA [5,6], M. A. BRITO[4,7], C. FONTES-RIBEIRO[1,2,3], A. P. SILVA[1,2,3]

[1]Laboratory of Pharmacology and Experimental Therapeutics, and [2]Institute for Biomedical Imaging and Life Sciences (IBILI), Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [3]CNC.IBILI, University of Coimbra, Portugal; [4]Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal; [5]Neurosurgery Service, Coimbra Hospital and University Centre, Coimbra, Portugal; [6]Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [7]Department of Biochemistry and Human Biology, Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal

Methamphetamine (METH) abuse is a serious public health problem affecting over 35 million users worldwide. The overall negative effects of this drug are well-known[1], including irreversible brain damage that may cause neurological and psychiatric anomalies. However, despite extensive characterization of METH toxicity over the last years, many questions remain unanswered. More recently, it has been suggested that METH-induced neurotoxicity may also result from its ability to compromise the blood-brain barrier (BBB) function[2]. The BBB is a dynamic and complex interface between the blood and the central nervous system (CNS), playing a key role in brain homeostasis and protection[3]. Thus, alterations in BBB function are likely involved in many neurodegenerative diseases, and drug abuse is not an exception. In fact, it was previously shown that METH compromises BBB function, and both matrix metalloproteinase-9[2,4] and nitric oxide[5] are involved in such changes. Importantly, all the components of the BBB play a unique role on its function, and among them, astrocytes modulate endothelial responses under pathological conditions by secreting factors that cause BBB leakage. Despite knowing that METH may induce BBB impairment[2] and neuroinflammation[6], the possible involvement of pro-inflammatory cytokines in METH-induced BBB alterations has never been investigated.

In order to better understand the BBB dysfunction induced by METH, we have recently shown that this psychostimulant rapidly increased the vesicular transport across endothelial cells, followed by an increase of paracellular transport. Moreover, METH triggered the release of tumor necrosis factor-alpha (TNF-α), and the blockade of this cytokine or the inhibition of nuclear factor-kappa B (NF-κB) pathway prevented endothelial dysfunction. Since astrocytes play a crucial role in modulating BBB function, we further demonstrated that conditioned medium obtained from astrocytes previously exposed to METH had a negative impact on barrier properties also *via* TNF-α/NF-κB pathway. Animal studies corroborated the *in vitro* results. Overall, we showed that METH directly interferes with brain endothelium properties or indirectly via astrocytes through the release of TNF-α and subsequent activation of NF-κB pathway culminating in barrier dysfunction. This conclusion may provide an important strategy against BBB dysfunction triggered by METH and consequent brain parenchyma alterations/infections that may occur under such conditions.

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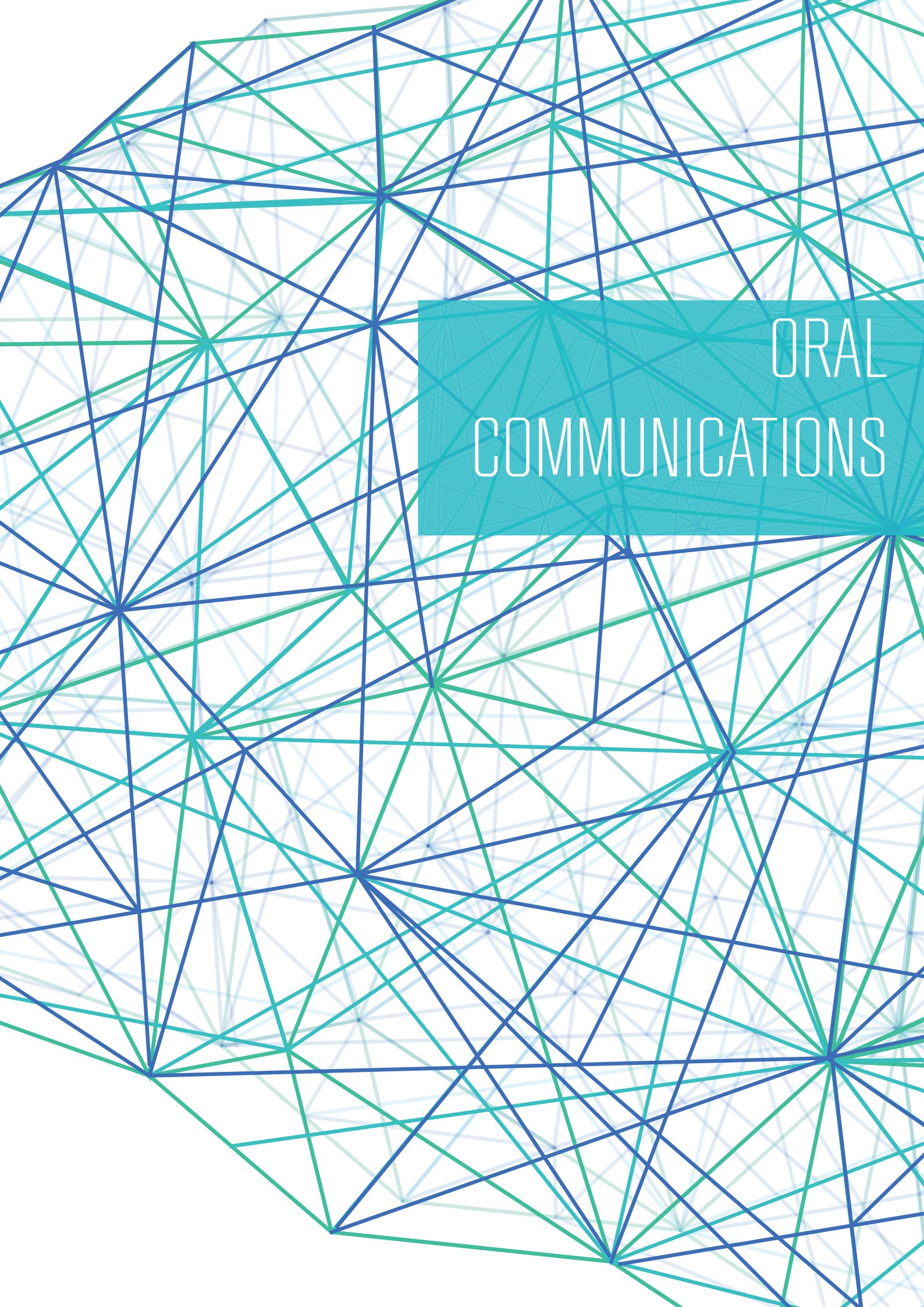

ORAL COMMUNICATIONS

THE TRIAD OF BEHAVIOURAL MODIFICATIONS CHARACTERISTIC OF DEPRESSION IS ABROGATED BY THE SELECTIVE AMYGDALA NEURONAL DOWN-REGULATION OF ADENOSINE A_{2A} RECEPTORS

N. GONÇALVES [1,2], N. J. MACHADO [1], A. P. SIMÕES [1], C. M. SOUZA [1], S. B. FERNANDES [1], I. AMARAL [1], S. G. FERREIRA [1], C. LEMOS [1], A. T. SIMÕES [1], L. P. ALMEIDA [1,3], D. RIAL [1], R. A. CUNHA [1,4]

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Chronic stress dampens mood and memory performances by triggering abnormal amygdala and cortico-hippocampal synaptic plasticity. Since different chronic stressors upregulate adenosine A_{2A} receptors ($A_{2A}R$) in the amygdala, caffeine consumption (a non-selective $A_{2A}R$ antagonist) correlates inversely with the incidence of depression, and the blockade of $A_{2A}R$ prevents aberrant plasticity and impaired memory, we now probed if $A_{2A}R$ deletion only in amygdala neurons is sufficient to control the triad of behavioural alterations characteristic of depression, namely aversive- and reward-based, anxiety and memory impairments caused by repeated restraint stress (RS, 14 days) in male adult rats (Wistar).

One week prior to handling or subjecting to 14 days of RS (4 h daily), all animals were injected bilaterally in the amygdala with lentiviral vectors encoding either a short hairpin for $A_{2A}R$ silencing ($shA_{2A}R$) or a control (shCTR). After RS, rats (n=23-26/group) were behaviourally evaluated in helpless-like (forced swimming, FS), anhedonia-like (splash), anxiety-like (elevated plus-maze, EPM) and memory-related (modified Y-maze) paradigms; then plasma corticosterone (CORT) levels and long-term potentiation (LTP) in amygdala slices were measured.

Restrained rats injected with shCTR displayed, compared to controls, no changes neither in despair-based depression (165.6±5.3 *vs.* 165.7±54 sec of immobility time in FS, n=13-14) nor in locomotion (44.6±2.5 *vs.* 49.5±1.8 m travelled in an open arena, n=14-15), but they presented reward-based deficits (47.6±7.6 *vs.* 24.2±6.6 sec of grooming time in the splash test, n=9-10), increased anxiety (6.7±2.1 *vs.* 15.9±4.8% time exploring open arms in EPM, n=12-14), decreased memory performance (364±2.8 *vs.* 41.0±2.5 % time in the novel Y-maze arm, n=8-12), increased amygdala LTP (241.5±30.6 *vs.* 167.3±20.9%, n=3), and increased CORT levels (925±342 *vs.* 162±43 ng/mL, n=7-8). Lentiviral-mediated down-regulation of $A_{2A}R$ in amygdala neurons reduced by 70% $A_{2A}R$ mRNA expression and exerted an antidepressant activity in FS (145.2±4.9 sec, $p<0.05$, n=13), as significant as the daily administration of the tricyclic antidepressant imipramine [10 mg/kg, i.p.] (128.8±18.3 sec, $p<0.01$, n=6), prevented anxiety in EPM (21.2±4.8%, $p<0.05$, n=11) and memory impairment in Y-maze (novel arm: 37.5±2.1%, n=12) without altering locomotion (48.6±34 m, n=15), and also reduced amygdala LTP (176.8±33.2%, n=3) and reduced the increased CORT levels (518.3±89.8 ng/mL, n=8).

These results consolidate the hypothesis that $A_{2A}R$ may be an attractive and effective target to manage mood-related disorders and identify a key role of amygdala neuronal $A_{2A}R$ in the control of stress-induced modifications associated with depressive-like conditions.

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NEURONAL ADENOSINE A_{2A} RECEPTOR OVEREXPRESSION AFFECTS AMPA AND NMDA CURRENTS IN CA1 HIPPOCAMPAL NEURONS

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Aging and Alzheimer's disease (AD) are associated with cognitive impairments, which are accompanied by structural and functional alterations in the hippocampus. There is compelling evidence of hippocampal upsurge of adenosine A_{2A} receptors ($A_{2A}R$) associated to cognitive deficits. Recently, we have provided evidence that blockade of $A_{2A}R$ in experimental models mimicking aging or AD prevent, or even revert, hippocampus-related impairments (Batalha *et al*, 2013, Mol. Psychiatry; Laurent *et al*, 2015, Mol. Psychiatry). This suggests that dysregulation of hippocampal $A_{2A}R$ function may drive part of the detrimental processes leading to aging and AD. However, the underlying mechanisms are still unknown.

We generated transgenic rats overexpressing the human $A_{2A}R$ under the control of the CAMKII promoter [tg (CAMKII-h$A_{2A}R$)], inducing a specific neuronal overexpression. In order to characterize the impact on neuronal excitability and synaptic transmission, we performed whole-cell patch-clamp recordings in CA1 hippocampal neurons from transgenic *versus* wildtype (WT) animals.

The blockade of $A_{2A}R$ activation in tg(CAMKII-h$A_{2A}R$) animals, by perfusion of the selective antagonist SCH 58261 (50 nM), decreased the mean amplitude of the excitatory post-synaptic currents (EPSCs), suggesting an excitatory tonic effect of overexpressed $A_{2A}R$ on synaptic transmission (n=4-5; $P<0.05$). To evaluate the putative presynaptic effects, we measured Paired Pulse Ratios (PPRs), in the range of 50 - 200ms intervals: a PPR facilitation was observed in neurons from WT animals at all intervals, more evident for the shorter intervals (n=10). The magnitude of PPR was reduced in tg(CAMKII-h$A_{2A}R$) rats when compared to the WT neurons (n=10; $P<0.05$), albeit maintaining the same facilitatory profile, thus suggesting that $A_{2A}R$ overexpression enhances neurotransmitter release probability. These effects were completely rescued by SCH 58261 (50 nM). We assessed the changes in AMPAR and NMDAR contribution, by quantifying the AMPAR/NMDAR which we found to be decreased in tg(CAMKII-h$A_{2A}R$) animals (n=19-21; $P<0.05$). Current-voltage (I-V) relationships in pharmacologically isolated NMDAR or AMPAR EPSCs revealed that AMPAR activation was decreased (n=7; $P<0.05$), while the I-V profile of NMDAR was increased (n=7; $P<0.05$). SCH 58261 partially rescued the mentioned I-V relationships. Such pre- and post-synaptic effects are not due to neuronal excitability, since no differences were observed between WT and tg(CAMKII-h$A_{2A}R$) animals in the following parameters: firing frequency, hyperpolarization-activated currents, resting membrane potential, membrane resistance and single action potential properties (n=11-14).

Altogether these data show that neuronal $A_{2A}R$ overexpression, *per se*, shares some electrophysiological features observed in both AD models and aged animals, strongly suggesting that $A_{2A}R$ overexpression might be one of the key events in the AD- and aging-associated glutamatergic synaptic dysfunction.

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NEUROPROTECTIVE ROLE OF TRANSTHYRETIN THROUGH MEGALIN IN ISCHEMIC BRAIN INJURY

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Transthyretin (TTR) is a protein involved in the transport of thyroxin and retinol, also associated to nerve regeneration in the peripheral nervous system. Recently in our lab, TTR was shown to have a neuroprotective role in focal cerebral ischemia (J. Neurochemistry, (2010), 115, 1434), using a permanent MCAO stroke model. Several studies pointed, in the last years, to the neuroprotective role of TTR in the central nervous system: (i) individuals heterozygous for TTR T119M, a non-pathological mutation of TTR associated with increased levels of the protein in the plasma, show a reduced risk of cerebrovascular disease, and increased life expectancy compared with non-carriers; (ii) TTR can be a good predictor for young strokes, since patients have a worse clinical outcome if they have decreased levels of serum TTR at the time of stroke; (iii) smaller incidence of stroke in women, due to the neuroprotective role of sex steroids (which upregulate TTR in CSF); (iv); in *C.elegans* model, a transthyretin-like protein was determinant in the recognition of apoptotic cells by phagocytes. These effects triggered by TTR might involve transducing receptors, such as megalin, that binds TTR. The goal of this work is to unveil the molecular mechanisms involved in TTR-induced neuroprotection in cerebral ischemia, namely through its interaction with megalin, using *in vitro* ischemia models. We found that cultured hippocampal neurons from TTR KO mice are more sensitive to an glutamate excitotoxic insult than cultured neurons from Wt mice, as neurite proteins MAP2 and Tau are significantly more affected in TTR KO mice neurons. Moreover, if TTR is administered in a therapeutic way, after the excitotoxic stimulus, a clear neuroprotection in the number and lenght of neurites in neurons from TTR KO cultures is observed. This neuroprotective effect of TTR seems to occur mainly in dendrites rather than in axons. More important when TTR is added to Megalin(+/-) TTR KO neuronal cultures after the excitotoxic insult, the neuroprotective effect is lost. Administration of TTR post glutamate stimulation does not prevent the nuclear condensation of apoptotic neurons. However, if TTR is given to cultured hippocampal neurons from TTR KO mice 6h before the glutamate stimulus, as a preventive strategy, a decrease in apoptotic neurons is observed. By opposition in megalin deficient cultures (Megalin(+/-) TTR KO) this soma neuroprotection is lost, indicating that TTR neurite and soma neuroprotection is Megalin dependent. Taken together, our results show that TTR should be explored as a potential therapeutic/neuroprotective protein or even used as an indicator of risk factor in stroke outcome. Further studies to characterize the signaling pathways in *in-vitro/ in-vivo* ischemia models behind this neuroprotection are underway.

CAFFEINE ADMINISTRATION MODULATES NEUROINFLAMMATION AND PREVENTS RAT RETINAL GANGLION CELL LOSS INDUCED BY OCULAR HYPERTENSION

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Neuroinflammation and glial reactivity have been increasingly associated with the onset of glaucoma, the second leading cause of blindness worldwide. This degenerative disease is characterized by retinal ganglion cell (RGC) loss and optic nerve damage. Glaucoma is a multifactorial disease, but elevated intraocular pressure (IOP) is a major risk factor. Strategies designed at reducing microglia-mediated neuroinflammation may offer therapeutic benefits to manage glaucoma.

Caffeine, an antagonist of adenosine receptors, is the most widely consumed psychoactive drug in the world and its consumption has been associated with a reduced risk for development of neurodegenerative diseases. In fact, there is evidence showing that caffeine attenuates neuroinflammatory responses and affords protection upon injury in the central nervous system (CNS).

In this work, we aimed to evaluate whether caffeine administration controls the neuroinflammation and elicits neuroprotection in an animal model of glaucoma.

Ocular hypertension (OHT) was induced in Sprague-Dawley rats by laser photocoagulation of limbar and perivascular veins. Caffeine (1 g/l) was administered in the drinking water, starting 15 days before OHT-induction. Animals were sacrificed 3 or 7 days after the induction of the OHT.

Caffeine decreased IOP of OHT animals, without altering IOP of control animals. Caffeine did not alter retrograde axonal transport in the optic nerve of OHT animals, as assessed by fluorogold tracing. Nevertheless, analysis of Brn3a-immunoreactive cells (RGCs) in retinal whole-mounts showed that caffeine significantly increased RGC survival in OHT animals after 7 days, compared to control animals. In addition, caffeine prevented OHT-induced alterations of inflammatory markers (IL-1β and TNF), markers of microglial reactivity (CD11b and CD200) and phagocytic activity (TREM2), as assessed by qPCR. The mRNA expression of A_1 receptor was not altered in OHT retinas, but mRNA for adenosine A_{2A} receptor ($A_{2A}R$) was significantly increased as compared to control retinas.

These results show that caffeine administration modulates neuroinflammation and prevents RGC loss induced by OHT, possibly by blocking the actions mediated by $A_{2A}R$. This study suggests that caffeine consumption or adenosine receptor antagonists, namely $A_{2A}R$, might be a therapeutic option to manage RGC loss in glaucoma.

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TRANSPLANTED EMBRYONIC NEURONS INTEGRATE INTO ADULT NEOCORTICAL CIRCUITS

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The capacity of the adult mammalian brain to compensate for neuronal loss is very limited. Transplantation studies aiming at cell replacement in animal models of neurological disease or injury have shown host-graft synapse formation and in some cases extension of efferents from the grafted cells to proper anatomical targets through the adult brain (Fricker-Gates *et al.*, 2002; Gaillard *et al.*, 2009). However, it remains largely unexplored whether new afferent synapses are adequate and thus able to convey genuine input information. To tackle this question, we induced cell death of upper layer callosal projection neurons in the primary visual cortex (V1) of adult mice and investigated whether immature neurons transplanted 7-10 days after lesion may integrate properly or aberrantly into the pre-existing circuitry, a critical question for functional reconstruction. We demonstrate here that a great majority of transplanted cells have the appropriate upper layer neuron identity and survive for several months. Using the monosynaptic rabies virus-based tracing approach (Wickersham *et al.*, 2007), previously adapted in our group to target new neurons (Deshpande *et al.*, 2013), we dissected the whole brain host-to-graft circuitry. Local neuronal populations within the visual cortex connect massively with transplanted neurons, but also other sensory and associative areas including the somatosensory cortex, motor cortex, auditory cortex, retrosplenial cortex and the posterior parietal association cortex. Moreover, synaptic input from distant areas including the thalamic lateral geniculate nucleus - the primary relay center for the visual information coming from the optic nerve - and the contralateral V1 was found. A comparative analysis with the endogenous developmentally-generated connectivity suggests that the newly formed circuits strikingly resemble the normal afferents of V1 neurons in the naïve brain. In addition, using two-photon calcium imaging we demonstrate that transplanted neurons become visually responsive and show orientation and/or direction selectivity. Altogether, our results indicate that new neurons may properly integrate and function in an injured neocortex.

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FUNCTIONAL GENOMICS OF HUMAN BRAIN DEVELOPMENT AND EVOLUTION

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Human brain development is a precisely regulated process that unfolds over a protracted period of time. Our understanding of the genetic mechanisms underlying development, and their relevance to both human evolutionary specializations and heightened susceptibility to psychiatric disorders, are impeded by a lack of comprehensive data on the spatio-temporal organization of the brain transcriptome.

To specifically address this problem, we performed a genome-wide transcriptome analysis of hippocampus, amygdala, striatum, thalamus, cerebellum, and 11 neocortical areas, from the left and right sides of post-mortem human brain tissue, encompassing the entire pre- and postnatal development through adulthood. Our results revealed regional and temporal patterns of gene expression and alternative splicing, and identified networks of co-regulated transcripts associated with specific neural circuits and developmental processes. Focusing on the neocortex due to its relevance for perception, behavior, and cognition, we found that inter-areal differences exhibit a temporal hourglass pattern, dividing human neocortical development into three major phases. The first phase, corresponding to prenatal development, is characterized by the highest number of differentially expressed genes among areas and gradient-like expression patterns, including some that are different between human and macaque. The second, preadolescent phase, is characterized by lesser inter-areal expression differences and by an increased synchronization of areal transcriptomes. During the third phase, from adolescence onwards, differential expression among areas reappears driven predominantly by a subset of areas, without obvious gradient-like patterns. Analyses of left-right gene expression revealed population-level global symmetry throughout the fetal and postnatal timespan.

This study provides a comprehensive dataset on the spatiotemporal human brain transcriptome and new insights into the transcriptional foundations of neocortical topographic gene expression.

INCREASED LEVELS OF S100B IN MULTIPLE SCLEROSIS ARE IMPLICATED IN DEMYELINATION AND GLIA REACTIVITY

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Multiple sclerosis (MS) is a primary inflammatory demyelinating autoimmune disorder. Increased levels of S100B were detected in samples of MS patients and correlated with glia reactivity upon demyelination. Under physiological state, S100B low levels are neurotrophic and support oligodendrocyte differentiation/maturation. However, in neuropathological conditions, increased S100B levels may induce glial reactivity and oligodendrocyte demise what may affect myelination/remyelination. Here, we aimed to unravel the role of S100B in the pathogenesis of MS.

S100B levels were determined in CSF and serum of MS patients by ELISA and S100B and its receptor RAGE expression were analyzed in post-mortem samples of MS patients by immunohistochemistry. S100B levels were also determined in cerebellar organotypic slice cultures (COSC) after induction of demyelination with lysophosphatidylcholine (LPC). After antibody neutralization of S100B, the extent of demyelination and glial reactivity were assayed by immunohistochemistry and expression of first line cytokines (TNF-α, IL-1 and IL-6), inflammasome related molecules (IL-18, HMGB1 and NLRP3), and microglia phenotype markers (M1-MHC class II and iNOS, M2-Arg1 and FIZZ1) by qRT-PCR.

At diagnosis, MS patients show elevated S100B levels in the CSF and serum (2.1- and 1.2-fold, $p<0.01$ Mann-Whitney test, respectively). Active MS lesions showed increased expression of S100B in reactive astrocytes and RAGE in activated microglia/macrophages. Treatment of COSC with LPC induced an elevation of S100B (5.0-fold, $p<0.01$) that co-localized mostly with astrocytes. Interestingly anti-S100B prevented LPC-induced demyelination in ~55% ($p<0.01$) and astrocyte reactivity. LPC-induced demyelination markedly increased the expression of TNF-alpha and IL-1beta but decreased that of IL-6 (2.2-, 1.3- and 0.3-fold, $p<0.01$, respectively), while S100B inhibition abrogated TNF-alpha and IL-1beta release and prevented IL-6 reduction ($p<0.01$). LPC also increased inflammasome-related IL-18, HMGB1 and NRLP3 expression levels (10.8-, 14.7- and 11.0-fold, $p<0.01$, respectively) which returned to control values when S100B was neutralized ($p<0.01$). Additionally, after LPC induction microglia became activated with increased expression of both M1 pro-inflammatory markers MHC class II and iNOS (7.3- and 6.5-fold, $p<0.01$, respectively) and M2 anti-inflammatory/damage resolution markers Arg1 and FIZZ1 (4.8- and 2.5-fold, $p<0.01$, respectively). Interestingly, neutralization of S100B not only prevented M1 markers by ~50% but also increased M2 markers expression by more than 30% ($p<0.01$).

Overall, high S100B expression in MS patient samples suggests its use as a new diagnostic biomarker, while the beneficial outcome of its neutralization in our demyelinating model indicates S100B as a potential therapeutic target to reduce damage during MS course.

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ZEB1 POTENTIATES GENE TRANSCRIPTION GENOME-WIDE IN GLIOBLASTOMA MULTIFORME CANCER STEM CELLS VIA A NOVEL LEF1 DEPENDENT MECHANISM

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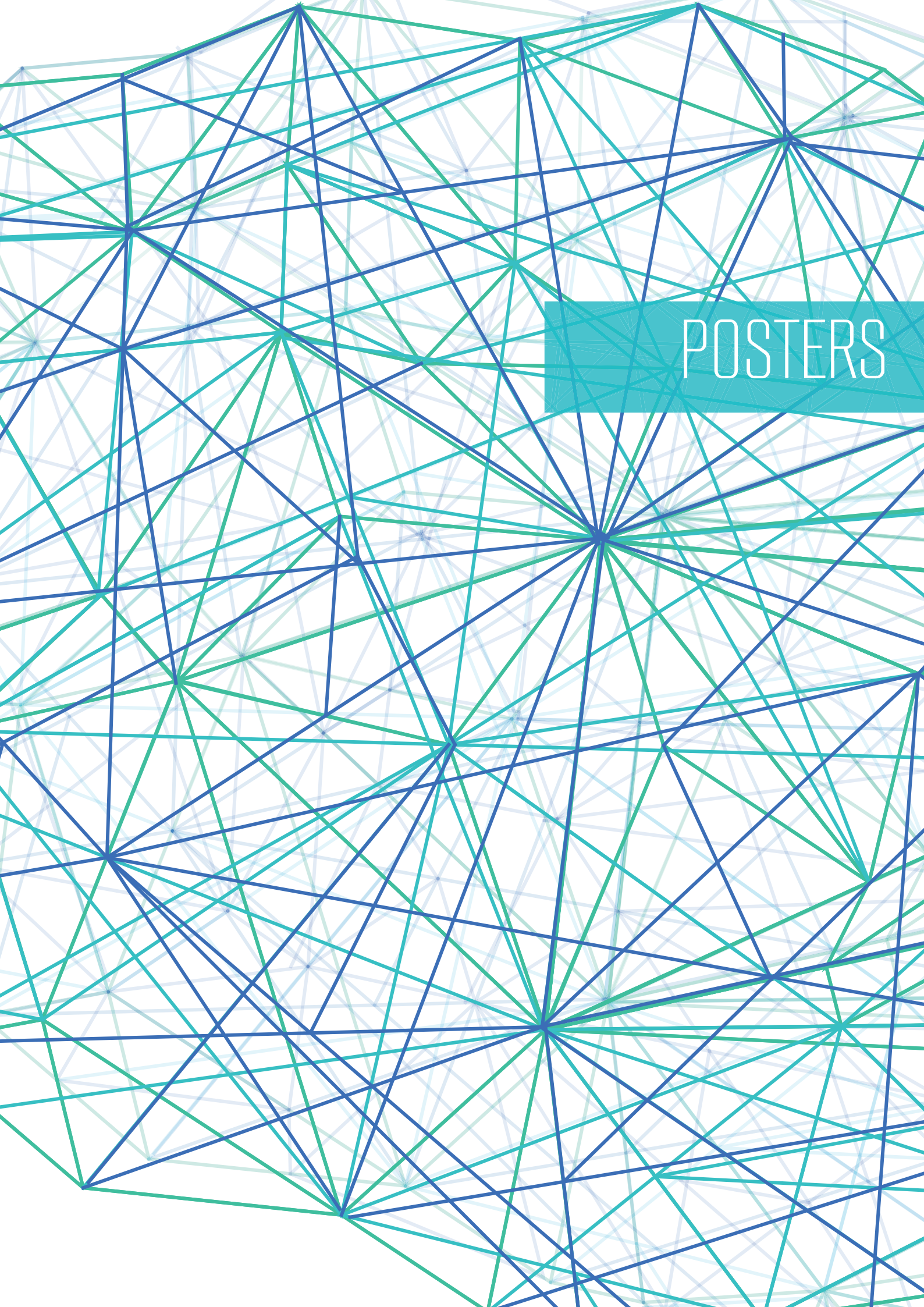
Glioblastoma Multiform (GBM or astrocytoma grade IV according to WHO) is the most prevalent and aggressive type of brain cancer, highly invasive and virtually incurable. GBM harbor stem-like tumor propagating cells (Cancer Stem Cells – CSCs) thought to play a pivotal role in treatment resistance and relapse, making them the subject of intense research. Here we investigated the function of the zinc-finger transcription factor Zeb1 in GBM CSCs, a classical Epithelial to Mesenchymal Transition (EMT) inducer previously implicated in invasion, chemoresistance and tumourigenesis in GBM (Siebzehnrubl *et al*, 2014, EMBO Mol Med). Although a role for Zeb1 in repressing gene transcription by binding to E-box sequences has been well documented in various cellular systems, little is known on the molecular basis for its activities in GBM, and in particular on the identity of its target genes.

With the aim of characterizing the transcriptional program regulated by Zeb1 in GBM CSCs, we combined genome-wide mapping of its binding sites (by ChIP-seq) in NCH421K cell line, with expression profiling upon shRNA mediated knock-down. We identified ~7,000 high-confidence Zeb1 binding events, located mostly at proximal promoter regions. Surprisingly, a *de novo* search for DNA motifs revealed, in addition to the expected E-box, an HMG box sequence highly enriched at a large fraction of peak summits, suggesting indirect recruitment of Zeb1 to DNA via an HMG transcription factor. Peaks associated with the two motifs are differentially associated with changes of gene expression. Those at promoters of repressed genes are enriched with E-boxes, whereas the HMG box motif is found at peaks near genes activated by Zeb1. Moreover, target genes activated or repressed by Zeb1 mediate distinct cellular functions, with Zeb1 repressing genes predicted to regulate neuronal differentiation and an epithelial phenotype, while activating genes associated with cell invasion/migration (e.g. Prex1 or Nrp2). In transcriptional assays, Zeb1 transactivates the enhancers of Prex1 and Nrp2 genes in synergy with the HMG factor LEF1, the main downstream effector of the canonical Wnt signaling pathway in NCH421K cells, and this activity can be further enhanced by activated beta-catenin.

Our results provide a new paradigm whereby Zeb1 can activate and repress gene transcription in the same cellular context, by two distinct modes of recruitment to the cis-regulatory elements of target genes. In addition, they define a putative cross-talk with the canonical Wnt signaling pathway in GBM CSCs, which is currently being investigated.



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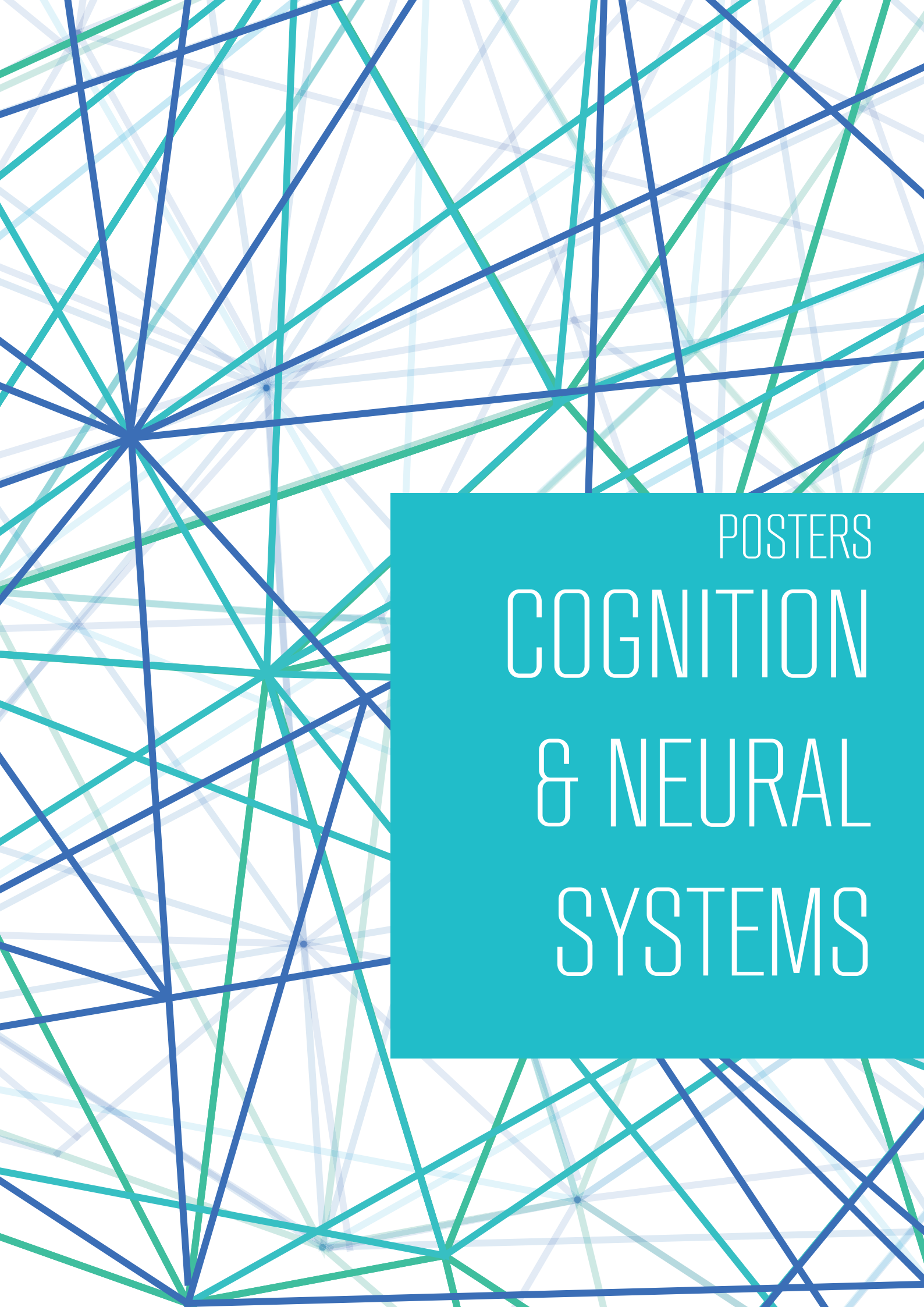

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P2X2/GLUN2B HYBRID RECEPTORS: A NEW CONCEPT OF RECEPTOR

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Fast communication in the nervous system is achieved through ligand-gated ion channels from different families according to their characteristic pharmacological and biophysical properties in function of their structural constraints. Functional interactions between distinct ionotropic receptors have been described, such as those between ATP-gated P2X receptors (P2XR) (Neuron 76(1):51) and nicotinic (nAChR; Nature 406: 405), serotonin (5-HT3; J. Neurosci. 23(4):1246) and GABA (J. Biol. Chem. 279(50):52517) receptors. However, to date no interplay between P2XR and ionotropic glutamate receptors has yet been described, even though ATP is known to be co-released with glutamate from nerve terminals (Pflugers Arch. 452(5):589) and astrocytes (. J. Biol. Chem. 278(2):1354).

We now found that GluN2B subunit of the NMDAR was recovered along with the P2X2R subunits isolated from mouse brain homogenates. Interestingly, no immunoreactivity for the GluN1 was found in the pulldown of P2X2 subunits. This physical interaction could be recapitulated in HEK293 cells expressing both P2X2 and GluN2B (in the absence of GluN1 subunit) and the co-expression of P2X2 rescued the trafficking of GluN2B subunits to the plasma membrane. In whole-cell patch-clamped HEK293 cells expressing P2X2 and GluN2B, NMDA (100 μM) elicited an inward current in the presence of ATP (3 μM), which was not observed in cells solely expressing P2X2 or GluN2B alone. This ATP/NMDA-induced current was blocked by the competitive NMDAR antagonist, APV, and by the generic P2R antagonist, PPADS (Fig. 2c-d), but not by the competitive antagonist of the glycine binding site at GluN1, 5,7-DiChloro Kynurenate (5,7-DiClKyn). The I/V relationship and Mg2+-sensitivity observed for P2X2/GluN2B were similar to those observed for homomeric P2X2Rs, but they do not share P2X2Rs' ability to permeate NMG+ ions. In patch-clamped murine hippocampal neurons, after blocking the classical NMDAR-mediated currents with the potent antagonist for the glycine-binding site at GluN1, 5,7-DiClKyn (25 μM), the simultaneous presence of ATP and NMDA did elicit an inward current, demonstrating the existence of P2X/GluN2 hybrid receptors in native cells.

The identification of a receptor complex encompassing P2X2 and GluN2B only activated upon the simultaneous presence of both ligands, ATP and NMDA/glutamate constitutes the first evidence for the ground-breaking existence of hybrid ionotropic receptors composed by subunits from different and distinct ionotropic receptor families and hence able to sense more than one ligand. These findings establish a new concept of receptor, representing a conceptual breakthrough in receptor signaling systems.

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PROSOCIAL CHOICE IN RATS DEPENDS ON FOOD-SEEKING BEHAVIOUR DISPLAYED BY RECIPIENTS

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Animals often are prosocial, displaying behaviours that result in a benefit to another [1-16] even in the absence of self-benefit [17-21] (but see [22-25]). Several factors have been proposed to modulate these behaviours, namely familiarity [6, 13, 18, 20] or display of seeking-behaviour [16, 21]. Rats have been recently shown to be prosocial under distress [17, 18], however what drives prosociality in these animals remains unclear. To address this issue, we developed a two-choice-task, where prosocial behaviour did not yield a benefit or a cost to the focal rat. We used a double T-maze (one for the focal and the other for the recipient rat) in which only the focal rat controlled access to the food-baited arms of both mazes. In this task, the focal rat could choose between one side of the maze that yielded food only to itself (selfish choice) or the opposite side that yielded food to itself and the recipient rat (prosocial choice). Rats showed a high proportion of prosocial choices. By manipulating reward delivery to the recipient and its ability to display a preference for the baited arm, we found that the display of food-seeking behaviour leading to reward was necessary to drive prosocial choices. In addition, we found that there was more social investigation between rats in selfish trials than prosocial trials, which may have influenced the focals' choices. This study shows that rats provide access to food to others in the absence of added direct self-benefit, while bringing new insights into the factors that drive prosociality thus contributing to future studies on the neural mechanisms underlying these behaviours.

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DECREASED DENSITY OF ADENOSINE A_1 RECEPTORS IN THE AMYGDALA OF SUICIDE COMPLETERS

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Depression is the most common neuropsychiatric pathology, but its neurobiological basis is still poorly understood. There is a strong correlation between the incidence of depression and suicide, since the majority of suicide completers suffer major depressive episodes. Thus, the study of brain tissue from suicide completers provides a unique opportunity to study the neurobiological basis of depressive disorder in humans. Emotion processing in mood disorders is associated with altered amygdala function and deficits of amygdala-frontal connectivity. Furthermore, increasing evidence supports that depression involves cortical synaptic dysfunction, although this has not been confirmed in the amygdala. Adenosine A_1 receptors (A_1R) are important regulators of synaptic efficiency in amygdala synapses, and it is still unknown if they are affected upon depression. We now tested if suicide completers displayed a synaptic imbalance in the amygdala and if this is accompanied by changes of A_1R levels. We compared quality-validated (pH, RIN) postmortem tissue samples from male subjects who committed suicide with age-matched controls. As expected, A_1R were enriched in nerve terminals (compared to total membranes) both in controls (278±133%, n=10) and suicide completers (437±150%, n=10) and were mainly located outside the active zone of nerve terminals, as gauged by subsynaptic fractionation (n=3). Most importantly, A_1R density was 35.2±144% lower in total extracts of suicide completers compared to age-matched controls (n=9). In parallel, there was a tendency for an increased density of a glutamatergic nerve terminal marker (vesicular glutamate transporters type 1) in the amygdala of suicide completers (112.8 ± 32.8%, n=8). These results warren further exploring the role of amygdala A_1R in depression as possible therapeutic targets.

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ECTO-5'-NUCLEOTIDASE (CD73)-GENERATED ADENOSINE ACTIVATES ADENOSINE A_{2A} RECEPTORS TO CONTROL LONG-TERM POTENTIATION IN THE MOUSE PRELIMBIC MEDIAL PREFRONTAL CORTEX

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Adenosine is a neuromodulator controlling synaptic plasticity through facilitatory A_{2A} receptors ($A_{2A}R$) in hippocampal and striatal circuits, in accordance with the ability of $A_{2A}R$ to control memory, motor and reward processes. The selective engagement of $A_{2A}R$ to control adaptive plastic changes is dependent on a particular source of adenosine, i.e. the frequency-dependent release of ATP and its extracellular catabolism through ecto-nucleotidases into adenosine, determined by the activity of ecto-5'-nucleotidase (CD73). $A_{2A}R$ also control behavioral flexibility and we have identified the presence of $A_{2A}R$ in the prefrontal cortex (PFC), although the involvement of $A_{2A}R$ in the control of information processing in the PFC is essentially unknown. Thus, we now aimed at showing the localization of $A_{2A}R$ and CD73 in PFC nerve terminals and testing if CD73-generated adenosine is responsible for the activation of $A_{2A}R$ controlling synaptic plasticity in the prelimbic medial PFC. The triple-labeling of vesicular glutamate transporters type 1 (vGluT1, marker of glutamatergic terminals) $A_{2A}R$ and CD73 in purified nerve terminals from the PFC of C57Bl/6 adult mice showed that 67.8±1.7% of the vGluT1-positive terminals were equipped with $A_{2A}R$, 82.0±1.8% with CD73, while 53.9±1.7% were equipped with both $A_{2A}R$ and CD73 (n=4). Moreover, $A_{2A}R$ and CD73 co-immunoprecipitated in mouse PFC nerve terminal membranes (n=2), suggesting a tight physical interaction. The recording of long-term potentiation (LTP, triggered by 5 trains of 100 Hz, each with 300 pulses, every 3 minutes) of population spikes recorded in layer II/III upon stimulation of layer V of mPFC slices from C57Bl/6 mice, revealed that the selective $A_{2A}R$ antagonist SCH58261 (50 nM) decreased LTP amplitude (36.1±4.8% over basal with SCH58261 *versus* 74.2±5.9% in control slices, n=11, $p<0.05$). Similarly the CD73 inhibitor α,β-methylene ADP (AOPCP, 100 μM) also decreased LTP amplitude (31.6±4.8% over basal with AOPCP *versus* 79.9±12.1% in control slices, n=5, $p<0.05$). Furthermore, the simultaneous blockade of $A_{2A}R$ and inhibition of CD73 was mutually occlusive since the decrease of LTP amplitude observed (27.6±44% over basal with SCH58261+AOPCP *versus* 67.1±7.9% in control slices n=6, $p<0.05$) was not significantly different from that observed when SCH58261 or AOPCP were applied alone ($p>0.05$). Altogether these results show that CD73-mediated formation of extracellular adenosine is responsible for the activation of $A_{2A}R$ that control synaptic plasticity in the PFC.

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THE RNA-BINDING PROTEIN HNRNP K IS REGULATED BY SYNAPTIC ACTIVITY AND BDNF IN THE HIPPOCAMPUS: IMPLICATIONS FOR MRNA METABOLISM IN DENDRITES

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Dendritic protein synthesis plays a critical role in several forms of synaptic plasticity, including in BDNF (brain-derived neurotrophic factor)-mediated long-term synaptic potentiation. Dendritic-localized transcripts are typically transported in a repressed state, as components of large messenger ribonucleoprotein complexes (mRNPs), and then translated upon stimulation in the vicinity of activated synapses. Heterogenous nuclear ribonucleoprotein (hnRNP) K is an RNA-binding protein involved in multiple aspects of mRNA processing such as transcription, splicing and translation. hnRNP K was previously detected in neuronal mRNPs but whether it is involved in the regulation of mRNA metabolism locally at synapses remains to be determined. Here we found that synaptic activity increases hnRNP K protein levels in dendrites in cultured hippocampal neurons by a BDNF-dependent mechanism. Accordingly, exogenous application of this neurotrophin increases the phosphorylation of the ribonucleoprotein at serine 302 and also results in the dendritic accumulation of hnRNP K. Studies with hippocampal synaptoneurosomes showed that several hnRNP K-bound mRNAs, including the transcripts coding for GluA1, GluN1 and BDNF, are released from hnRNP K-containing mRNPs locally at the synapse following BDNF stimulation, most likely to be translated. Our data supports a model in which hnRNP K is a new player involved in the control of dendritic mRNA transport and/or translation, partly mediating the effect of BDNF-TrkB signaling on local protein synthesis. In agreement with this hypothesis, we showed that manipulating hnRNP K levels in hippocampal neurons has an impact on basal rates of translation in dendrites, as assessed by the surface sense of translation (SUnSET) methodology. Additional *in vivo* studies showed that several transcripts are released from hnRNP K complexes during high-frequency stimulation (HFS)-induced long-term potentiation (LTP) in the dentate gyrus in live anesthetized rats. Importantly, the induction of HFS-LTP *in vivo* and the activity-dependent dissociation of hnRNP K-associated transcripts require the extracellular activation of TrkB receptors, presumably by BDNF. Taken together, these results demonstrate that hnRNP K and hnRNP K-associated mRNAs are regulated by synaptic activity and BDNF, both *in vitro* and *in vivo*, and suggest hnRNP K as an important regulator of neuronal function, in particular, in BDNF-induced synaptic plasticity events.

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DOWN-REGULATION OF THE DENSITY OF ADENOSINE A_1 RECEPTORS IN THE HIPPOCAMPUS OF SUICIDE COMPLETERS

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Depression is the psychiatric condition with the highest incidence worldwide, being responsible for the major burden of disease in Europe (Bertolote *et al.*, 2005, World Psychiatry 133:8). Epidemiological studies indicate that regular caffeine consumption (an adenosine receptor antagonist) correlates inversely with the incidence of depression (Lucas *et al.*, 2011, Arch Intern Med 171:1571) and suicide (Kawachi *et al.*, 1996, Arch Intern Med 156:521; Lucas *et al.*, 2014, World J Biol Psychiatry 15:377). This is in remarkable agreement with the proposed involvement of adenosine A_1 and A_{2A} receptors (A_1R and $A_{2A}R$) in the control of mood disorders (van Calker & Biber, 2005, Neurochem Res 30:1205; Cunha *et al.*, 2008, Curr Pharm Des 14:1512). However, neither the neurobiology of depression nor the mechanisms of action of A_1R and $A_{2A}R$ to control mood dysfunction are known. The study of brain tissue from suicide completers offers an opportunity to grasp the neurobiological basis of depression since over 90% of suicide completers have clinical signs of depression (Coryell & Young, 2005, J Clin Psychiatry 66:412; Rihmer, 2007, Curr Op Psychiatry 20:17). We now compared quality-validated (pH, RIN) postmortem tissue samples from male subjects that committed suicide with age-matched controls to test if there was a synaptic imbalance in hippocampus of suicide completers and if this is accompanied by changes of adenosine A_1R levels. We used total extracts and a preparation of purified nerve terminals that we validated as being enriched in different synaptic markers (synaptosomal-associated protein 25 - SNAP-25, synaptophysin, syntaxin and postsynaptic density protein 95 - PSD-95) and de-enriched in a glial marker (glial fibrillary acidic protein - GFAP) when compared with total extracts. Notably, nerve terminals from the hippocampus of suicide completers displayed a decrease of syntaxin density (32.8±10.3%, n=6) and an increase in vesicular glutamate transporter 1 (vGluT1) density (26.8±7.3%, n=6) compared to controls, whereas no change was observed in the immunoreactivity of SNAP-25, synaptophysin and PSD-95. Furthermore, total extracts from the hippocampus of suicide completers displayed a decreased of GFAP (17.5±4.1%, n=6) and of A_1R (23.0±8.8%, n=6) densities, whereas there was no significant changes of the density of A_1R in nerve terminals of suicide completers when compared with age-matched controls. These results further reinforce the relevance of adenosine receptors in the development of new anti-depressant strategies, albeit the functional impact of the altered density of adenosine receptors in the functioning of the hippocampus still remains to be determined.

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AMYGDALAR ADENOSINE A_{2A} RECEPTORS CONTROL SYNAPTIC PLASTICITY AND FEAR MEMORY

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The amygdala encodes emotional memories (Morrison & Salzman, 2010, Curr Opin Neurobiol 20:221) and long-term potentiation (LTP) in this area underlies the learning and expression of fear (Johansen *et al.*, 2011, Cell 147:509). Adenosine A_{2A} receptors ($A_{2A}R$) are known to control synaptic plasticity in hippocampal circuits and to impact on reference memory (Cunha & Agostinho, 2010, J Alzheimers Dis 20:S95). We now studied the role of adenosine receptors in the control of fear memory and of synaptic plasticity in an input pathway to the amygdala. Electrophysiological recordings were performed on horizontal brain slices (400 µm) from mice (male c57bl6, 8 weeks old) by stimulating cortical afferents and recording in the lateral amygdala (LA). The activation of A_1R with CPA (100 nM) decreased (38.9±4.8% of control, n=6, $p<0.001$) and A_1R blockade with DPCPX (100 nM) increased basal synaptic transmission (128.2±9.0% of control, n=6, $p<0.05$) whereas an $A_{2A}R$ antagonist (SCH58261, 50 nM) was devoid of effect. By contrast, SCH58261 decreased LTP amplitude (from 1704±7.1% to 132.7±7.3% of baseline, n=6, $p<0.05$) while both CPA and DPCPX had no effect. Adenosine deaminase (ADA, 2 U/ml; degrades extracellular adenosine) or caffeine (50 µM; non-selective adenosine receptor antagonist) mimicked the effect of DPCPX on basal transmission (124.8±4.2% and 126.8±6.1, n=4, $p<0.05$, respectively) and the effect of SCH58261 on LTP (161.8±8.8% in the control, 120.3±4.3% with ADA and 123.6±10.9% with caffeine, $p<0.05$) further supporting the selective control by A_1R of basal transmission and by $A_{2A}R$ of synaptic plasticity. To evaluate the impact of amygdala $A_{2A}R$ on fear conditioning, mice were injected bilaterally in the amygdala with a lentivirus containing either a sequence to neutralize $A_{2A}R$ ($shA_{2A}R$) or a non-coding sequence (shCTR). Four weeks later, the animals were subjected to fear conditioning by pairing a conditional stimulus (CS, tone) with an unconditional stimulus (US, footshock). Along the 3 trials of the CS-US pairing, the $shA_{2A}R$-treated mice froze less comparing to shCTR-treated mice (17±4.1s shCTR *versus* 4.8±1.4s $shA_{2A}R$, n=5, $p<0.05$). Two and 8 days after fear conditioning, $shA_{2A}R$-treated mice displayed an impaired contextual fear memory compared to the shCTR-treated mice. Accordingly, $shA_{2A}R$-treated mice had a lower LTP amplitude than shCTR-treated mice at cortico-LA synapses (166.8±11.1% in shCTR and 127.1±10.9% in $shA_{2A}R$, n=4, $p<0.05$). Our data reveals a control by $A_{2A}R$ of LTP in the amygdala, which impacts on fear memory supporting the targeting of these receptors in neuropsychiatric disorders.

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REGULATION OF THE ACTIVITY OF THE LOCUS COERULEUS-NORADRENERGIC SYSTEM IN CHRONIC PAIN

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INTRODUCTION: Depression and anxiety disorders may emerge as a consequence of the long-term exposition to severe and chronic painful conditions. This will have repercussions in the psychological state of the patient and will also potentiate the mechanisms underlying the negative effects of chronic pain. However, the mechanistic association of pain to the development of mood disorders is still barely known. The Locus coeruleus (LC) is an important candidate orchestrating the neuronal circuitries behind chronic painful conditions, also having an exceptional role in the regulation of anxiety and depression disorders.

METHODS: Monoarthritis (MA) was induced in male rats by complete Freund´s adjuvant injection as a model of painful arthritis. We evaluated the behavioral attributes of pain, depression and anxiety, as well as LC function by electrophysiology, at distinct time-points of the disease development. Next, we quantified protein expression by western blot and immunohistochemistry.

RESULTS: We observed a late-phase activation of extracellular-signal regulated kinases 1/2 (pERK1/2) accompanied by anxiety and depressive behaviors. These changes were reverted by topical anti-inflammatory therapy in the affected hind paw. We also observed that the electrophysiological activity of LC neurons was altered in the prolonged inflammatory condition. The administration of an ERK1/2 inhibitor (SL327) in the LC of rats submitted to chronic inflammation resulted in reversion of the anxiety-like behavior, reversion of the electrophysiology changes and normalization of the ERK1/2 activation levels in the PFC. Additionally, using a corticotropin-releasing factor (CRF) antagonist, which blocks the activity of the endogenous CRF, we observed reversion of the ERK1/2 levels and anxiety-like behavior in chronic inflammatory conditions but no effects on the nociceptive behavior.

CONCLUSION: The LC plays a role in the increased perception to noxious stimuli and it is related with the emergence of pain-related anxiety. The CRF neurotransmission may act as precursor in these ERK1/2-mediated events.

PERIPHERAL NEUROPHYSIOLOGICAL MEASURES OF EMPATHY IN COUPLES

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Empathic processes include different dimensions such as emotional contagion, cognitive, affective and regulatory abilities. These dimensions have been associated with important neural biomarkers, namely at the level of the autonomic nervous system (ANS) (Coutinho, Oliveira-Silva & Decety, 2014). In this study we analyzed peripheral neurophysiological measures during a dyadic interaction task. Most of the studies about the autonomic correlates of empathy have focused on the sympathetic branch of the ANS, but this study is focused on its parasympathetic branch due to the importance of regulatory skills for empathy. Due to its inhibitory effect on the sympathetic system, the vagal tone has been shown to be important for the physiological and emotional regulation and prosocial behavior. Vagal tone exerts an important influence in the respiratory sinus arrhythmia and consequently in the heart rate variability (HRV). Thus the aim of our study is to analyze the association between HRV and empathy. Specifically this study aims to analyze 1) the relationship between HRV and self-reported empathy and 2) the relationship between HRV and the arousal growth rate during an empathic task when compared to the baseline. Our sample is composed by 30 couples (N=60) married or living together for at least 1 year before the participation in the study, recruited through an online subject pool from the university and the general community. Both spouses performed a videotaped interaction task in the lab. In this task each element is required to talk about positive and negative aspects of their relationship. The other spouse is instructed to pay careful attention and is asked to paraphrase what their partner has just said. This ecological laboratory-based interaction was designed to mimic the couples' daily experiences involving transactional emotional processes, or the reciprocal interaction of emotions, either negative or positive. During the task autonomic measures (interbeat interval, heart rate) from both spouses were recorded using a data acquisition system (BIOPAC SYSTEM MP-150) coupled to two amplifiers for each physiological modality. A 2-minute baseline task was performed by both spouses in order to assess the change scores during the interaction task. After the interaction task self-report measures of dyadic empathy and relationship satisfaction were administered to both spouses. We hypothesize that participants' level of HRV will be positively associated with perceived empathy as assessed by self-report measures. In addition, we hypothesize that HRV will be negatively associated with the arousal growth rate (measured by heart rate) between baseline and empathic task. The results obtained in this study, which are currently under analysis, will be presented. The practical and conceptual implications of this research will also be discussed.

Coutinho, J. F., Oliveira-Silva, P., & Decety, J. (2014). Neurosciences, Empathy, and Healthy Interpersonal Relationships: Recent Findings and Implications for Counseling Psychology. Journal of Counseling Psychology, 61(4), 541–548.

OVER-ACTIVATION OF ADENOSINE A_{2A} RECEPTORS IS SUFFICIENT TO TRIGGER MEMORY IMPAIRMENT

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Adenosine is an ubiquitous modulator in brain synapses acting through inhibitory adenosine A_1 receptors and facilitatory adenosine A_{2A} receptors ($A_{2A}R$) to control basal synaptic transmission and plasticity, respectively, which contributes to the encoding of information salience in neuronal circuits (Cunha, 2008, Neurochem Int 52:65). This impacts on different behaviors raging from locomotion to mood (Chen *et al.*, 2013, Nature Rev Drug Disc 12:265), namely cognition, as heralded by the ability of caffeine, a non-selective adenosine receptor antagonist, to attenuate cognitive dysfunction upon aging or Alzheimer´s disease (AD) in humans, an effect mimicked by the selective blockade of $A_{2A}R$ in animal models (Cunha and Agostinho, 2010, J Alzheimer Dis 20:S95). In order to define if $A_{2A}R$ over-activation might actually be a cause of memory dysfunction, we now tested if $A_{2A}R$ activation was sufficient to trigger memory deficits in naïve mice. Using 3 tests to probe for short-term memory (object recognition, inhibitory avoidance and modified Y-maze), we observed that the activation of $A_{2A}R$ with CGS21680 (0.1 mg/kg intra-peritoneally, i.p.) before the training session was sufficient to trigger memory impairment in naïve mice, an effect prevented by the selective $A_{2A}R$ antagonist SCH58261 (1.0 mg/kg, i.p.). Furthermore, the intra-cerebroventricular administration of CGS21680 (50 nmol) also impaired memory performance in the object recognition task and the bilateral light stimulation of the hippocampus of mice transfected with an AAV expressing opto$A_{2A}R$ (a chimeric rhodopsin-$A_{2A}R$ protein composed of the extracellular and transmembrane domains of rhodopsin, conferring light responsiveness, fused to the intracellular loop of $A_{2A}R$ to confer specific $A_{2A}R$ signaling; Li *et al.*, 2015, Mol Psychiatry doi: 10.1038/mp.201543) during the 5 minutes of testing in a modified Y-maze test, reduced about 2-fold the time spent in the novel arm in comparison with mice transfected with AAV-mCherry (control) only, without locomotor changes. As we observed for age-associated memory deterioration (Costenla *et al.*, 2011, Eur J Neurosci 34:12), memory impairment was associated with an $A_{2A}R$-mediated enhancement of the amplitude of long-term potentiation (LTP) in hippocampal CA1 pyramidal cells: thus, LTP amplitude was larger upon both upon light stimulation (5-min before HFS) of hippocampal slices expressing opto$A_{2A}R$ (197.3±2.9% of baseline, compared to 137.7±2.0% without light stimulation, n=3; $p<0.05$) or in the presence of 30 nM CGS21680 in wild type mice (137.8±2.0% *vs.* 167.9±1.7% without or with CGS21680, n=3; $p<0.05$). Overall, these results show that $A_{2A}R$ over-activation is actually sufficient to trigger spatial reference memory dysfunction, which is associated with an excessive facilitation of hippocampal LTP.

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HIERARCHICAL GLUCOCORTICOID-ENDOCANNABINOID INTERPLAY REGULATES THE METABOLIC STATE-DEPENDENT ACTIVATION OF THE NUCLEUS ACCUMBENS BY INSULIN

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Insulin is thought to produce food-induced reward in the nucleus accumbens. Corticosteroids and endocannabinoids (eCBs) negatively regulate systemic insulin sensitivity and glucose uptake, and are positively coupled with food intake. Besides, corticosteroids can use eCB signaling as an effector system. We now mapped the dependence of the actions of insulin on eCB CB1 and corticosteroid receptors using an *in vitro* ^{3}H-deoxyglucose uptake assay in accumbal slices (400 µm-thick) prepared from 8-10-week-old Wistar rats. In these slices, resting glucose uptake amounted to 684±14 nmol/mg protein (n=141) during the 30 min assay period.

Insulin (3 and 300 nM) produced a rapid >20% increase of glucose uptake. IGF-1 receptor blockade did not prevent the action of insulin, suggestive of the involvement of insulin receptors.

While CB_1R activation by WIN55212 (500 nM) or CB_1R blockade by O-2050 (500 nM) or rimonabant (500 nM) did not affect resting glucose uptake (n≥9, P>0.05 *vs.* DMSO control in all cases), WIN55212 prevented the action of insulin (n=11, P>0.05 *vs.* WIN55212 alone). Dexamethasone (1 and 10 µM) also prevented insulin (300 nM) from stimulating glucose uptake (n≥6, P>0.05 *vs.* dexamethasone alone). This effect of dexamethasone was antagonized with the glucocorticoid receptor antagonist, mifepristone (10 µM, n=11) rather than with the mineralocorticoid receptor antagonist, spironolactone (10 µM), which by itself stimulated glucose uptake by 20% (n=7, P<0.05). Additionally, CB_1R blockade with O-2050 or rimonabant also fully restored the action of insulin in the presence of dexamethasone, and so did tetrahydrolipstatin (10 µM), which is an inhibitor of DAGL, the enzyme responsible for the post-synaptic synthesis of the eCB, 2-arachidonoyl-glycerol (2-AG).

Hence, we tested if the stimulation of endogenous 2-AG levels could prevent the action of insulin. Indeed, the blockade of the post-synaptic 2-AG metabolizing enzyme, αβHD6 with WWL70 (1 µM) prevented insulin from stimulating glucose uptake (n=5, P>0.05 *vs.* WWL70 alone), while the blockade of the presynaptic 2-AG catabolizing enzyme, MAGL with JZL184 (1 µM) failed to do so (n=5, P<0.05 *vs.* JZL184 alone).

Functional CB_1R/InR heterodimers have been previously reported in the pancreas (Kim *et al.*, 2012, Sci Signal 5(216):ra23.), and our above data also suggests that accumbal CB1Rs regulate insulin receptors (InRs). To map if the two receptors dimerize, we attempted to co-IP them. Indeed, we successfully pulled down the CB_1R with the help of anti-InR antibodies in accumbal total protein extract.

Altogether, our data suggest that insulin stimulates accumbal activity, which is negatively regulated by the glucocorticoid-DAGL-2-AG-CB_1R axis. This hierarchical modulation of accumbal insulin action may provide attractive therapeutic targets to manage eating disorders.

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ALTERED DENSITIES OF ADENOSINE A_1 AND A_{2A} RECEPTOR IN THE CAUDATE OF SUICIDE COMPLETERS

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Depression is the psychiatric disability with the highest incidence worldwide. Epidemiological studies revealed that moderate consumption of caffeine (an adenosine receptor antagonist) correlates inversely with the incidence of depression (Lucas *et al.*, 2011, Arch Intern Med 171:1578) and suicide (Kawachi *et al.*, 1996, Arch Intern Med 156:525; Lucas *et al.*, 2014, World J Biol Psychiatry 15:386). However, neither the neurobiology of depression nor the mechanisms of adenosine A_1 and A_{2A} receptor (A_1R and $A_{2A}R$) control of mood dysfunction are known. The investigation of brain tissue from suicide completers provides a window to study the neurobiology of depression since over 90% of suicide completers have clinical signs of depression. We focused on the caudate since this region participates in reward-seeking behaviors that are impaired in depression, in accordance with the decreased volume of the caudate in depressed patients (Krishnan *et al.*, 1992, Arch Gen Psychiatry 49:557; Parashos *et al.,* 1998, Psychiatry Res 84:15). Thus we now compared quality-validated (pH, RIN) postmortem tissue samples from medial caudate (MC) and posterior caudate (PC) of male subjects who committed suicide with age-matched controls, to test if there was a synaptic imbalance and if this was accompanied by a change of adenosine receptors levels. We first optimized the purification of nerve terminals, which was validated by the enrichment of different presynaptic [e.g. synaptophysin or synaptosomal-associated protein 25 (SNAP-25)] and postsynaptic markers [postsynaptic density protein 95 (PSD-95)] and parallel de-enrichment of a glial marker [glial fibrillary acidic protein (GFAP)] when compared with total extracts. We observed a decrease of SNAP-25 density (16.3±6.3%, n=6) in MC and a decrease of PSD-95 density (35.6±104%, n=6) in PC of suicide completers when compared with age-matched controls, whereas there were no alterations of the densities of synaptophysin, syntaxin or vesicular glutamate transporter type 1 (vGluT1). In parallel, we observed an increase of A_1R density (31.8±12.0%, n=6) in total extracts of MC and a decrease of A_1R density (35.0±12.0%, n=5) in PC nerve terminals of suicide completers, together with an increase of $A_{2A}R$ density (29.9±11.2%, n=6) in total extracts of PC of suicide completers. These results suggest the presence of a synaptic imbalance and modifications of adenosine receptors in the caudate of suicide completers, which further supports the interest in targeting adenosine receptors to develop new anti-depressant strategies.

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THE SPATIAL MAP OF PERCEPTION DRIVEN HIGH-FREQUENCY OSCILLATIONS AS REVEALED BY ELECTROCORTICOGRAPHY

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Due to the inherently high spatial resolution, sensitivity and signal-to-noise ratio of electrocorticography (ECoG), this technique has been used to study the dynamics of brain oscillations particularly at high-frequency activity (up to 500Hz) [Jacobs *et al.*, 2012, Kucewicz *et al.*, 2014]. Invasive brain recordings demonstrate that physiological oscillations extend beyond the gamma frequency range (30-150Hz), but their role in human perception and high-level cognitive processing has not been fully elucidated [Kucewicz *et al.*, 2014]. Event-related potentials (ERPs) and frequency oscillations acquired using ECoG are considered to represent neuronal activation elicited by a cognitive task in a complementary way [Crone *et al.* 2006]. We believe that neuronal mechanisms underlying high-gamma activity can best be investigated in humans performing particular cognitive tasks.

In the collaborative setting of clinical functional mapping and to explore the spatio-temporal dynamics of cortical oscillatory activity we acquired task-related Electrocorticography (ECoG) data in collaboration with the Epilepsy unit from the Coimbra University Hospital in two epileptic patients.

These patients underwent extraoperative evaluation with subdural ECoG to better localize seizure foci and pre-surgical functional mapping of different cognitive functions of interest. We investigate high frequency oscillations spanning the high gamma (up to 500Hz) frequency bands using intracranial recordings. We designed two visual perception tasks using ambiguous Mooney stimuli (two tone black and white patches defining possible objects) to help map visual perceptual decision networks. Objects and no objects conditions were presented (103 trials per condition; 250ms duration).

Computed Tomography and Magnetic Resonance anatomical data were acquired and co-registered to precisely localize the positions of the intracranial electrodes. ECoG data were acquired from platinum grid electrodes (n=50 for subject 1, s1; n=60 for subject 2, s2) surgically implanted, with high sampling rate (5kHz). No filters were applied during recording and an average reference was computed for offline analysis. ERPs and Time-Frequency (TF) analysis (5-500Hz) were performed as implemented in EEGLAB.

The ERP results revealed, with excellent spatial and temporal resolution [Lachaux *et al.*, 2012], the task related contribution of local neuronal ensembles in ventral cortex. Moreover, regarding the analysis in the frequency domain we found a clear topographical map for the visual perception tasks, with higher frequencies (>80Hz) mainly present at posterior locations when compared to anterior regions ($P<0.02$). Furthermore, low frequency activity in the beta, alpha and theta bands were more evenly distributed. This spatial map of high frequency activity is reproducible between subjects when an object is perceived and does not old true for the no object perception conditions.

These results are in line and extend our previous EEG and EEG/fMRI work. Notably we could now identify a spatial 'traveling wave' of these oscillations that starts at primary visual regions that propagate to more anterior locations.

In this study it was possible to identify the spatial map of high frequency activity during visual perception tasks with high spatio-temporal resolution. We conclude that there is a spatial map of distinct sub-bands of high frequency oscillations that are perception related.

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ADENOSINE A_{2A} RECEPTOR CRITICALLY CONTROL THE IMPAIRMENT CAUSED BY β-AMYLOID PEPTIDES OF SYNAPTIC PLASTICITY IN MOUSE HIPPOCAMPAL SLICES

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Alzheimer's disease (AD) is characterized by an initial synaptic dysfunction and damage causing progressive cognitive impairment. Amyloid-β peptides (Aβ) have been proposed as culprits of AD and were shown to trigger memory dysfunction and impaired synaptic plasticity, the purported neurophysiological basis of memory. We have previously shown that caffeine, an adenosine receptor antagonist, prevents the synaptotoxicity and memory deficits caused by Aβ, an effect mimicked by the blockade of adenosine A_{2A} receptors ($A_{2A}R$). These $A_{2A}R$ are activated by extracellular ATP-derived adenosine through ecto-5'-nucleotidase (e5N) and are selectively engaged in the control of synaptic plasticity, namely of long-term potentiation (LTP). However, it has still not been defined if $A_{2A}R$ blockade, either directly using $A_{2A}R$ antagonists or indirectly through the modulation of e5N, could rectify the aberrant synaptic plasticity responses triggered by Aβ. By analyzing electrophysiological responses in Schaffer fibers/CA1 pyramid synapses of hippocampal slices, we report that the direct exposure of "naïve" C56BL/6 mice (WT) slices to oligomeric $Aβ_{1-42}$ (50 nM for 40 min) significantly decreased the amplitude of LTP (121.3 ± 3.2 *vs* 152.5 ± 74 of control, n=22). Clearance of extracellular adenosine with adenosine deaminase (2 U/mL) prevented these Aβ-induced LTP deficits. This effect was mimicked by the blockade of $A_{2A}R$, either pharmacologically via the exposure to the $A_{2A}R$ antagonist SCH58261 (50 nM), or genetically by using $A_{2A}R$ knockout mice, whereas the A1R antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, 100 nM) failed to prevent Aβ-induced LTP deficits. Furthermore, the e5N inhibitor α,β-methylene ADP (AOPCP, 100 μM) also abrogated Aβ-induced LTP deficits. We then probed the role of $A_{2A}R$ in hippocampal slices obtained from mice 15 days after an intracerebroventicular (icv) injection with Aβ (2 nmol), when they displayed an impairment of memory performance (modified Y-maze and object displacement test). Synaptosomes from icv-Aβ mice displayed increased levels of $A_{2A}R$ and a diminished K^+-evoked release of ATP and slices from icv-Aβ mice displayed reduced LTP amplitude (33.22 ± 8.99% inhibition, n=5). Notably, the direct incubation of slices from icv-Aβ mice with either SCH58261 (50 nM) or (AOPCP, 100 μM) reverted the deficits of LTP amplitude, returning LTP amplitude to levels similar to the control (vehicle icv-injected) group. The obtained results show that $A_{2A}R$ blockade prevents the impact of Aβ on synaptic plasticity in the hippocampus. Furthermore, our data indicates that the extracellular adenosine generated by the activity of e5N is responsible for the activation of $A_{2A}R$ under pathological conditions and that e5N inhibition might afford a neuroprotection similar to that obtained by directly blocking $A_{2A}R$.

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ANXIETY CAUSED BY PRENATAL IMMUNOMODULATION IS PARALLELED BY AN ATROPHY OF MICROGLIAL PROCESSES IN THE PRE-FRONTAL CORTEX OF FEMALE RATS

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The prenatal exposure to glucocorticoids (GC, effectors of stress responses) such as dexamethasone (DEX), causes deleterious long-term effects, namely chronic anxiety, which is paralleled by changes of synaptic wiring in the pre-frontal cortex (PFC). Microglia cells emerge as putative cellular mediators of GC-induced anxiety since they respond to GC and are actively involved in synapse formation, maturation and elimination during development (Cristovão *et al.*, 2014, Front. Cell. Neurosci. 8:153). Furthermore, both microglia responses (Gyoneva *et al.*, 2014; Neurobiol. Dis. 67:191) and neonatal stress (Batalha *et al.*, 2012, Mol. Psychiatry 18:320) are controlled by adenosine A_{2A} receptors ($A_{2A}R$). This led us to hypothesize that GC trigger $A_{2A}R$-driven microglial alterations impacting on synapse formation that would underlie the behavioral alterations observed at adulthood. To explore this hypothesis, we evaluated the impact of *in utero* exposure to DEX (subcutaneous injection of 1 mg/kg to pregnant Wistar rats on embryonic day 18-19 of pregnancy) on anxiety/depression in adulthood (postnatal day 90, PND 90), microglia morphology and $A_{2A}R$ density in the PFC. The prenatal exposure to DEX induced hyperanxious behavior, although the animals behaved normally in the forced swimming test. We found alterations in microglia morphology (immunohistochemistry with Iba-1, followed by 3D reconstruction of the cells using Neurolucida, Neuroexplorer software), typified by a decrease of the number and length of cellular processes (atrophy), observed from the first post-natal week (number of processes of DEX *versus* saline-treated animals: 4 ± 0.2 *versus* 6 ± 0.6, n=4, $p<0.05$; length of processes of DEX *versus* saline-treated animals: 17 ± 0.8 *versus* 27 ± 3.6 µm, n=4, $p<0.05$) and onwards (at PND 90, number of processes of DEX *versus* saline-treated animals: 25 ± 0,8 *versus* 29 ± 14, n=6, $p<0.05$; length of processes of DEX *versus* saline-treated animals: 111 ± 4.2 *versus* 133 ± 4.7 µm, n=6, $p<0.05$). In addition to microglia atrophy, we observed changes (temporally coincident) in $A_{2A}R$ density in the PFC. The present results indicate that microglia atrophy is a morphological correlate of anxiety observed in adults exposed to high levels of GC during critical periods of brain development. However, further studies are needed to elucidate if there is a causal relation between $A_{2A}R$-driven microglial morphologic alterations and abnormal synapse formation, eventually resulting in the behavioral alterations observed at adulthood.

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BRAIN ACTIVATION IN RESPONSE TO ODORS IN DEAF-BLIND PATIENTS

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Usher syndrome (USH) is a rare hereditary disease and the most common form of human inherited deaf-blindness. Visual and auditory brain cortices are deprived of the normal sensory input and may be recruited to process information from other modalities – cross-modal plasticity[1-2]. Olfactory deficits seems to be also present but are poorly investigated[3-4]. Therefore, this study aims to determine the hypothesis of a putative USH olfactory impairment as assessed using functional magnetic resonance imaging.

Functional and anatomical brain data were acquired in a 3 Tesla scanner. We included nine USH patients (5 females and 4 males; age 32 – 44 years) and six healthy individuals (3 females and 3 males; age 33 – 41 years), all right-handed. A full clinical evaluation including psychophysical tests for olfactory threshold measurement was performed before scanning. Based on olfactory threshold, four different butanol concentrations were presented in a staircase design inside the scanner, using odorless air as baseline. Participants had to press a button whenever they detected the butanol odor. Two functional runs were acquired per participant and analyzed with *BrainVoyager* (multi-study GLM: AR(2) correction, *z*-transformation, FDR $q<0.05$).

Patients presented a trend for higher olfactory threshold (0.164 ± 0.200 %) than controls (0.024 ± 0.017 %) suggesting olfactory deficits, but no significant difference was found ($p=0.093$). Moreover, patients had significantly higher responses in occipital and temporal brain regions during the butanol detection task, mainly for stronger odor concentrations ($F_{(1,10)} \geq 5.347$, $p \leq 0.043$).

These preliminary results suggest increased activation in occipital and temporal cortices during the odor detection task in patients. Interestingly, despite slightly reduced olfactory threshold, USH patients may rely more on olfactory inputs and odor semantic memory to interact with the environment, compensating loss of vision and hearing as previously reported in congenital blindness[1], [2].

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STIMULATION OF DOPAMINE D2-EXPRESSING NEURONS IN THE NUCLEUS ACCUMBENS FACILITATES MOTIVATION

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Dopaminergic neurotransmission has an essential role in complex functions, including motor activity, incentive salience and spatial-temporal organization of goal-directed behaviours. Natural rewards exhibit reinforcing properties by inducing a fast increase in dopamine release in the brain, although the causal biological contribution of this mechanism remains controversial. Mesolimbic signalling comprises dopaminergic projections arising from the ventral tegmental area to the medium spiny neurons (MSNs) of the nucleus accumbens (Nac). The GABAergic MSNs comprise up to 95% of all NAc neurons and are typically segregated into two subtypes – those expressing dopamine D1-type receptors (direct pathway) and those expressing dopamine D2-type receptors (indirect pathway). Whilst activation of D1 MSN excites its projecting target, the substantia nigra pars compacta, and culminates in movement initiation, reinforcement and reward seeking; activation of the D2 MSN projections, mainly to the globus pallidus (GP), antagonizes the direct pathway (1). D1-MSN activation is canonically related to positive rewarding events, inducing persistent reinforcement, whereas D2-MSN signalling is thought to mediate aversion (2,3). Nonetheless, pharmacological and genetic studies raised some questions regarding this functional/behavioural bias of both types of MSNs. Considering the contradictory evidences regarding the impact of selective activation of the NAc D2 neurons in motivation, we decided to use novel optogenetic tools to selectively stimulate accumbal D2 neurons and evaluate its modulatory effects in different motivation-related behaviours. In the present study, we first show that the pattern of activation of neurons within the nucleus accumbens predicts motivational drive. Also, we found a positive correlation between the number of NAc activated dopamine D2 receptor expressing-neurons and behavioural performance in a reward-related task. We subsequently show that optogenetic phasic activation of D2 accumbal neurons is sufficient to enhance motivation. To complement these findings, we show that specific D2-neuronal activation in the NAc normalized motivational deficits in an animal model of anhedonia/motivational deficits. Altogether these results demonstrate that D2-dependent dopaminergic activation in the NAc is sufficient to modulate motivated behaviours.

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PREFRONTAL CORTEX OVER EXCITATION AND CORTICO-SUBCORTICAL STRUCTURAL CHANGES IN AUTISM SPECTRUM DISORDER PATIENTS FACILITATES MOTIVATION

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Prefrontal cortex (PFC) is associated with executive functions, social cognition, and the ability to adjust behavior. These characteristics are known to be impaired in patients with autism spectrum disorder (ASD). It has also been proposed that in ASD patients the PFC presents short-range over-connectivity and long-range underconnectivity, possible caused by increased overall excitation due to an imbalance in the excitation/inhibition ratio (E/I). These connectivity changes impair information flow from/to several cortical and subcortical regions, compromising PFC functions and brain systems to which it connects. Thus, E/I imbalance may also play a role in abnormal neurodevelopment in ASD.

The objective of this work is to investigate a possible imbalance E/I imbalance in the PFC of ASD patients, and whether cortical and subcortical volumetric differences are concomitantly present in this ASD cohort.

ASD group: 25 males; 15±5y.o (range:11-33); positive ADI-R and ADOS; FSIQ:93±14, no epilepsy; typically developing (TD) group: 22 males; 17±6y.o. (range:10-32); FSIQ:122±15; (mean±SD).

Data were acquired in a 3T Siemens scanner. Freesurfer 5.0 was used to obtain regional cortical and subcortical volumes. A 3cm3 single-voxel in the bilateral medial PFC was acquired using MEGA-PRESS for quantification of gamma-aminobutyric acid (GABA) (inhibition) and glutamate (excitation) ratios to N-acetylaspartate + N-Acetyl aspartylglutamic acid (NAA+NAAG). PRESS sequence was acquired in the same location to obtain absolute brain metabolites concentrations: N-acetylaspartate (NAA), Choline (Cho), Inositol (Ins), and Creatine+phosphocreatine (tCr). Ratios and absolute concentrations were quantified with LCModel 6.3 1-D. Groups for neurochemical study were: ASD: 16 males, 15±3y.o (range:12-24); FSIQ:90±14, no epilepsy; TD: 14 males; 11-28 y.o., 18±6y.o (range:11-28); FSIQ:124±16 (mean±SD).

Glu/NAA+NAAG was increased in the ASD group (p=0.01), whereas no changes were observed in GABA/NAA+NAAG, or other main brain measured metabolites.

Volumetric analyses revealed decreased left amygdala and right thalamus in the ASD group (p=0.009 and p=0.025, respectively); the left thalamus showed a tendency for reduced volume (p=0.06).

Furthermore, in the ASD group, cortical volume decreases were detected in the left hemisphere: lateral orbitofrontal cortex (p=0.01), and pars orbitalis (p=0.01); as well as in the right insula (p=0.04). Increases were found in left paracentral gyrus (p=0.03), and right rostral anterior cingulate (p=0.01).

Our results clearly point to an E/I increase in the PFC of ASD patients. Together with the neurochemistry, anatomical results support thalamo-cortical anomalies, as well as altered systems involved in executive function and regulation of social cognition in ASD. PFC over excitation may be related to abnormal brain development and, consequently, to atypical cerebral functioning in ASD patients.

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PAIN FACILITATION FROM THE DORSAL RETICULAR NUCLEUS CONTRIBUTES TO PARADOXICAL OPIOID-INDUCED HYPERALGESIA

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Opiates are a gold standard for the treatment of moderate to severe pain. Chronic use of opioids can induce paradoxical hyperalgesia (opioid-induced hyperalgesia; OIH). OIH is characterized by hypersensitivity to innocuous or noxious stimuli during sustained opiate administration but its molecular mechanisms are not fully understood. The activation of supraspinal pain facilitatory areas is thought to contribute to OIH. Here we studied the involvement of the dorsal reticular nucleus (DRt), an area located in the medulla oblongata, which plays a unique and exclusive pain facilitatory role. We studied the effects of chronic morphine infusion in naïve and neuropathic animals (spared nerve injury- SNI-model) and evaluated the involvement of the DRt in OIH by pharmacological inactivation of the DRt and by knocking down the expression of the μ-opioid receptor (MOR) at the DRt.

Naïve and neuropathic male Wistar rats were anesthetized and implanted subcutaneously with osmotic pumps for morphine (45 $\mu g.\mu l^{-1}.h^{-1}$) or saline infusion. Pain behavior was tested 2, 4 and 7 days later by the von Frey and hot-plate tests in naïve animals and by the von-Frey, pin-prick and acetone tests in SNI animals. Pharmacological inactivation of the DRt was performed in naïve animals, 7 days after subcutaneous morphine or saline infusion, by the injection of lidocaine (0.5 μl; 4% w/v) through a guide cannula stereotaxically implanted at the DRt. Von Frey and hot-plate tests were performed before and 30 min after lidocaine injection. MOR knock down was achieved in naïve and neuropathic animals. For that the animals were stereotaxically injected at the DRt with a lentiviral vector that knocks down MOR and immediately after they were implanted osmotic pumps for morphine or saline infusion. Pain behavior was tested before and 7 days after the surgical procedures. At the completion of the behavioral evaluation the animals were sacrificed by vascular perfusion and the brainstem was removed. MOR expression was evaluated by immunohistochemistry in 40 μm coronal sections encompassing the DRt.

Chronic morphine induced hyperalgesia both in naïve and SNI animals which was fully reversed by lidocaine. MOR expression was significantly higher in the morphine group both in naïve and SNI animals. MOR knock down prevented MOR up-regulation in the morphine group and decreased the expression of MOR in the saline group. MOR knock-down prevented the development of OIH in the morphine group and induced hyperalgesia in the saline group both in naïve and SNI animals.

Our results indicate that chronic morphine exposure induces OIH in naïve and neuropathic animals and, that the DRt is involved in the mediation of OIH, likely through MOR activation whose effects appear to switch from inhibitory to facilitatory upon chronic morphine treatment.

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SPINAL CORD AND DORSAL ROOT GANGLION EXPRESSION OF THE CALCITONIN RECEPTOR AND OF AMYLIN

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Amylin is a peptide secreted by the pancreatic β-cells; however, studies also showed amylin expression in sensory neurons suggesting a nociceptive action for this peptide. The functional amylin receptor consists of the association of a calcitonin receptor (CTR) with a receptor activity modifying protein (RAMP), which confers specificity to amylin. Amylin is expressed by C-fibers projecting to the spinal cord's laminae I-II, so the main targets of endogenous amylin action in nociception should reside there. Furthermore, we observed that the intrathecal delivery of amylin or an amylin-receptor antagonist, modulated tonic pain supporting the existence of relevant spinal cord-mediated mechanisms. Therefore, in order to identify the spinal targets of endogenous amylin, we mapped the expression of CTR in rats' spinal cord by immunohistochemistry. Since there is also indication that amylin-receptors are present in the dorsal root ganglia (DRGs), as there is both CTR and RAMP mRNA, we investigated CTR expression in DRGs.

We found that CTR is expressed at all levels of the spinal cord by neurons located in laminae V and VII, having frequently a spindle or a star shape cell-body. Some neurons were also found in laminae X and in the lateral spinal nucleus (Lsp). Laminae I-II were examined by electron microscopy, which revealed CTR expression on cell membranes and neurotubules in unmyelinated fibers, while there was no expression in neuronal cell bodies or in myelinated fibers. Regarding the DRGs, we found that CTR was expressed only in small-medium neurons (mainly <600 μm^2 cross-sectional area) in about 25-30% of all DRG neurons, and that 32% of the CTR-positive neurons were C-peptidergic, while 17% were C-non-peptidergic and only 6% were A-neurons. Surprisingly, 16% of the CTR-expressing neurons also contained amylin, suggesting that some amylin-receptors may act as auto-receptors, while some amylinergic fibers may act in a paracrine way to activate receptors in neighbor nociceptors. We also further characterized the amylin-expressing neurons and confirmed that the majority are C-peptidergic, as 45% of the CTR-positive neurons co-expressed CGRP. Surprisingly, we found that 32% of all amylinergic neurons were A-fibers, probably Aβ, as they had a medium-large cell body (>600 μm^2), while 11% of the amylin-positive neurons were C-non-peptidergic.

Concluding, we found CTR in C-fibers, so amylin being released by sensory fibers in the superficial laminae of the dorsal horn may bind to auto-receptors or to receptors in neighbor unmyelinated fibers. Furthermore, amylin may bind to CTR in Lsp, which also receives nociceptive input. Amylin expression in Aβ-fibers suggests that a relevant amount of the amylinergic fibers are involved in innocuous mechanical sensation. Additionally, amylin may be involved in the phenotypical switch that occurs in Aβ-fibers in some experimental models of chronic pain, since amylin administration has been shown to modulate chronic pain.

OPTOGENETIC INHIBITION OF MPFC PYRAMIDAL POPULATION IMPROVES WORKING MEMORY PERFORMANCE IN A DELAYED NON-MATCHING TO SAMPLE TASK

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The medial prefrontal cortex (mPFC) is involved in maintaining temporary local neural representations for working-memory and their output to other brain areas. The disruption of these neural interactions can lead to working-memory deficits, particularly at longer information retention intervals. In this study, we evaluated the effects of optogenetic modulation of local mPFC pyramidal cells activity during a delayed non-matching to sample working-memory task performance. Behavioral performance was evaluated using different retention delay-intervals. Extracellular single-unit recordings were made from mPFC pyramidal cells 3 weeks after the injection of an adeno-associated viral vector encoding halorhodopsin (NpHR) under the control of CaMKII promoter (rAAV5/CaMKIIa-eNpHR3.0-EYFP) or a control vector that lacked the NpHR sequence (rAAV5/CaMKIIa-EYFP). Continuous pulses of 5 mW yellow light led-generated were delivered through an implanted optical fiber to block action potential firing of NpHR expressing mPFC pyramidal cells during the first 3 sec of each trial delay-interval. Our results show that the number of error trials increased for longer inter-trial delays and that, in those conditions, inhibitory optogenetic stimulation of mPFC pyramidal cells is associated with an increase of percentage of correct trials. In addition, it was also observed an intensification of populational firing activity signature close to the free-choice lever press during correct trials. Together, these results suggest that the selective optical inhibition of mPFC pyramidal cells at the onset of longer inter-trial delay periods is correlated with an increase of working-memory performance.

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CHRONIC STRESS INDUCES PERSEVERATIVE RESPONDING ON A PARADIGM OF IMPULSIVITY

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Impulsivity, or the tendency to act prematurely without foresight[1], is a multifactorial trait that is associated with various psychiatric diseases where there is a decision-making impairment[2].

Stress is an important modulator of decision-making that has been shown to disrupt decision-making circuits and to induce the adoption of particular patterns of behavior on different decision-making paradigms, namely the adoption of habit-based behaviors3 and a risk-aversive pattern of choices on a rat gambling task[4].

Having these impairments into account, we recently evaluated the performance of chronically stressed animals on a recently developed impulsivity paradigm, the variable delay-to-signal, which provides rapid and simultaneous assessment of response and decision impulsivity in rodents[5].

Our results showed both control and stressed animals exhibited similar levels of impulsivity but the latter group made significantly more perseverative responses relative to control animals. This type of response, as measured in the 5-choice serial reaction time paradigm, has been considered a measure of compulsive behavior[6]. Since compulsivity is seen across a number of psychiatric diseases, most notably addictive disorders and obsessive compulsive disorder, we hypothesized that chronic stress may be an important factor on the development of this pattern of behavior.

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MORPHOFUNCTIONAL HEMISPHERIC ASYMMETRIES ARE ASSOCIATED WITH WORKING MEMORY PERFORMANCE

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Hemispheric asymmetries have been heatedly discussed since Broca's description of language impairments after left hemisphere damage in the XIX century. Macroscopic anatomical distortions such as the Yakovlevian torque and neurochemical biases as in the dopaminergic system, seem to be influenced by numerous factors such as age and sex, and to critically influence cognitive performance. In fact, alterations in the left-to-right balance manifest in some psychiatric conditions as schizophrenia or mild cognitive impairment. A systematic brain-wide description of human brain hemispheric asymmetry as well as its cognitive correlates is yet to be accomplished. Herein, we used magnetic resonance imaging to analyze structural and functional biases in a right-handed aged population (65.2±8.0 years old, 52 male and 53 female) previously classified as good or bad cognitive performers through a battery of cognitive tests. We systematically identified sex- and group-dependent region-specific morphological biases, as well as functional asymmetries during the performance of a working memory task (N-Back). In good cognitive performers, a structural fusiform rightward bias was associated with increased N-Back accuracy, while superior frontal asymmetry correlated with this same outcome irrespectively of group or direction of laterality. Moreover, left superior parietal lobule functional laterality was also correlated with working memory performance. Overall, this work highlighted the presence of ubiquitous structural as well as point functional asymmetries in the human aged brain. Their correlations with N-Back performance may be a step forward towards understanding the neural correlates of working memory.

SIDE SPECIFIC IMPACT OF PERIPHERAL NEUROPATHIC LESIONS IN GOAL-DIRECTED DECISION MAKING: POTENTIAL ROLE OF DOPAMINE

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Humans and animals resort to different decision-making strategies when confronted with different situations. For instance, action repetition drives goal-direct behavior to habit-based behavior, decreasing the attentional demanding. In some conditions (e.g. stress[1]) the capacity to shift back to goal-direct strategies is impaired. No study has yet focus on the impact of chronic pain in this shift.

To understand it food restricted Wistar-Han rats with neuropathic lesions in left (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 reach 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.

SNI-L and SNI-R animals presented an increased sensitivity to light mechanical stimulation when compared with Sham but no differences between them were observed. Concerning decision-making all animals increased lever pressing along sessions, progressing the same way. Also, both Sham and SNI groups decreased the number of lever presses in the devaluated condition in the early test. On the other hand, in the late test, SNI-L, but not SNI-R or Sham, did not devaluate properly, which indicates that left neuropathic lesions shift goal-direct decision-making to habit-based decision-making.

This lateralized impact on decision-making strategies is not explained by differences in stress, as no differences were found between groups both in the corticosterone levels and in dexamethasone suppression test.

Preliminary results from qPCR and HPLC analysis showed lateralized alterations in the dopaminergic system in SNI-L and SNI-R rats, particularly in Orbitofrontal cortex (OFC) and Nucleus Accumbens (NAcc). Further studies are needed to understand if these specific alterations are sufficient to induce a shift from goal-directed to habit based strategies in left lesioned animals and do not impact decision-making in right lesioned ones.

Considering that dopamine depletion in humans (by phenylalanine/tyrosine-restricted diet) shifts decisions from goal directed to habit-based strategies[2] and that peripheral lesions affect mainly the contralateral side, it is possible that SNI-L but not SNI-R decision-making impairments result from a different impact in left and right-hemisphere dopaminergic system.

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THE IMPACT OF THE ADENOSINE NEUROMODULATION SYSTEM IS MORE ROBUST IN THE DORSAL THAN IN THE AND VENTRAL HIPPOCAMPAL CA1 AREA

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Several studies have demonstrated an anatomical and functional segregation along the dorso-ventral axis of the hippocampus: whereas the dorsal hippocampus (DH) is more tightly associated with spatial memory processing, the ventral hippocampus (VH) is more related with emotional responses. Both spatial memory and emotional responses are modulated by adenosine, a brain neuromodulator that inhibits synaptic transmission through A_1 receptors (A_1R) and bolsters synaptic plasticity through $A_{2A}R$. This study aims to characterize possible differences in endogenous adenosine tonus and synaptic plasticity between dorsal and ventral hippocampus to account for the different impact of adenosine on mood and memory. We used dorsal and ventral hippocampal slices from 8-10 weeks old Wistar rats to carry out extracellular electrophysiological recordings in the CA1 stratum radiatum upon low frequency stimulation (0.06 Hz) sub-maximal (50% of maximal responses) of Schaffer afferent fibers. The administration of increasing concentrations of 2-chloroadenosine, a stable adenosine analogue, was more potent to inhibit synaptic transmission in the DH (IC_{50}=0.5 μM, n=10) compared to the VH (IC_{50}=0.9 μM, n=10). When we gauged the endogenous adenosine tonus using 2 U/mL of adenosine deaminase (which converts adenosine into inosine), we observed a larger effect in the DH (20.8 ±3.2% facilitation, n=9) compared to the VH (10.1±1.53% facilitation, n=9; $p<0.05$). Likewise, the selective blockade of A_1R with DPCPX (100 nM) caused a slightly higher facilitation of synaptic response in the DH (124±2.8%, n=8) compared to the VH (9.3±2.3%, n=8), which did not reach statistical significance ($P>0.05$). The comparison of short-term plasticity, revealed that a larger paired pulse facilitation (PPF, at interpulse intervals of 25 and 50 ms) larger in DH than VH (n=5). Likewise, long-term potentiation (LTP) amplitude was significantly larger in DH (161.6±11.8% over baseline, n=4) than in VH (120.0±10.1% over baseline, n=4, $P<0.05$). Blockade of $A_{2A}R$ with SCH58261 (50 nM) only decreased LTP amplitude in DH (161.6±11.8% in the absence and 128.5±14.2% in the presence of SCH58261, n=4), whereas LTP amplitude in VH remained unchanged (120.0±10.1% in the absence and 116.1±2.5% in the presence of SCH58261, n=4). These results indicate an overall more robust modulation by the adenosine modulation system in the dorsal compared to the ventral hippocampus, since the endogenous adenosine tone acting through A_1R to inhibit synaptic transmission and through $A_{2A}R$ to control synaptic plasticity were more evident in the dorsal compared to the ventral hippocampus. This is in global agreement with the more evident effects of the adenosine system to control reference memory performance under different conditions

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DETERMINANTS OF IMPULSIVE BEHAVIOR IN THE VARIABLE DELAY-TO-SIGNAL TASK

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Impulsivity is a natural behavior that involves a complex spectrum of actions and choices that are not deliberated and are difficult to control. Although it is known that impulsivity traits differ across age and gender, when considering impulsive behavior in laboratory these differences are not completely being taken into account. We analyzed the effect of sex, age and strain in the impulsive behavior in the variable delay-to-signal (VDS) paradigm [1].Furthermore, different intrinsic parameters of the applied paradigm were compared between groups such as response latency, perseverant responses and latency to feed. The VDS consists in a series of 1 min trials where the nosepoke triggers the delivery of a sugar pellet, after a variable delay. It starts with an initial block of 3 seconds delay trials (3si) followed by random trials with 6 seconds (6s) and 12 seconds (12s) and again a final block with 3 seconds' delay trials (3sf). Premature responses per minute in the final segment (3sf) provides information regarding delay tolerance and the prematurity in the first second of the initial trial is associated with response impulsivity. Preliminary results demonstrated a major influence of age in impulsivity where young adult animals present an increased intolerance for delay. When considering sex and others intrinsic parameters of the test, no major differences were found. Although our results demonstrate age influence in impulsive choice, it is still essential to explore the influence of age, sex and strain in impulsive action.

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N3DFIX: AN ALGORITHM FOR AUTOMATIC REMOVAL OF SWELLING ARTIFACTS IN NEURONAL 3D RECONSTRUCTIONS

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The functional properties of a neuron are intimately related to its morphology. In order to properly quantify morphological characteristics it is first necessary to translate observational histological data into 3-dimensional (3D) geometric reconstructions of the neuronal structures. This reconstruction process requires several steps including neuronal labeling (e.g. biocytin intracellular injection), histological processing, and data acquisition using specialized software (e.g. MBF Neurolucida [1]). During the process of neuronal reconstruction many types of problems may occur, potentially leading to the incorporation of features into the reconstruction that were not present in the original neurons [2]. The introduction of artifacts such as swelling during the histological preparation is one of the major problems. Such artifacts may alter significantly passive properties of neurons since they change, at least locally, the diameter of a fiber, the total area, axial resistance, capacitance and/or the total membrane conductance.

The algorithm we present here is aimed to automatically detect and fix swelling artifacts. The algorithm is already implemented in two platforms, the NEURON simulation environment [3] and in Py3DN [4] and is currently being ported as a plug-in of Vaa3d [5]. Two preprocessing steps are performed, before the artifacts search and fix algorithm, with the purpose of eliminating two common errors in reconstruction data: a) null, or close to zero, diameter points; and b) overlapping points. The existence of points with very small diameters will lead to models where the corresponding compartment is very thin, blocking almost all the current flow. A parameter setting a lower bound for fiber diameters is used to prevent errors of type a). On the other hand, it is possible that more than one point has the same 3D coordinates. In that case, zero length segments would be created leading to divisions by zero at some future steps of the algorithm. As such, multiple copies of the same point are automatically removed beforehand eliminating errors of type b). After the preprocessing stage the search and fix algorithm takes place.

Swelling artifacts are characterized by abrupt, relatively symmetrical changes in the diameter of a dendrites and/or axons forming a bump. The algorithm is based on a template matching method and searches over all 3D points of the reconstruction for patches of neurite fulfilling four criteria, considered necessary and sufficient to signal a swelling artifact. Whenever a swelling artifact is detected, a pre- and post-region is detected and the fiber diameter is interpolated between both regions. The algorithm was tested and validated using a gold standard dataset of neuronal reconstructions manually corrected by an expert in neuroanatomy.

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GRID CELLS ACTIVITY PROFILES CAN BE OBTAINED IN A NEURONAL CIRCUIT MODEL WHERE SPATIAL INFORMATION IS PROVIDED EXCLUSIVELY BY PLACE CELLS

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Grid cells (GCs) and place cells (PCs) are the main cells involved in spatial information coding in mammals. Since the discovery of PCs the 70's [1] and the discovery of GCs about a decade ago [2], multiple spatial navigation related experiments have been conducted with the aim of characterizing their role in spatial learning. In the framework of computational neuroscience, one of the most challenging problems has been to model the formation of GCs' regular triangular firing pattern. Supported by different experimental evidences, there are three main types of models for grid formation: attractor networks [3], oscillators' interference [4] and self-organizing models [5]. A recent self-organizing model by our team [6] reproduces the hexagonal firing map of a single GC, in a network model architecture which captures the feedforward connections between PCs (in CA_1 layer of hippocampus) and GCs (in medial entorhinal cortex deep layers), and where spatial information is provided solely by PCs. In this present work, we extend our previous model to a local population of GCs, and we are able to show that different grid maps can be generated, providing an extensive coverage of the exploration arena. In this new model, GCs interact among themselves through inhibitory interneurons. When a grid field is generated in a GC, local inhibition acts on neighboring GCs preventing from creating grid nodes. This inhibition lasts until the firing rate of initiating GC decreases below some threshold level. With this mechanism, GCs in the same population are generated with minimum overlap of their grid fields, providing the homogenous coverage of the available environment reported experimentally [2]. Since the scaling and field size is inherited by place cells fields' size, these properties are transferred to GCs within the same recording location. When a group of cells is simulated, the majority produces regular triangular firing nodes with grid scores near 1 while the remaining present less regular tiling. Our results are in accordance with recordings of deeper MEC layers grid cells where only a portion of the principal cells recorded present grid cells typical firing patterns [7]. At this stage of the work, we have not yet incorporated in the model the ability to maintain grid orientation among local GCs. In conclusion, this model is able to reproduce a population of grids cells, with similar properties regarding phase, spacing and field size (orientation is still work in progress) as the ones reported from experimental setups. The model does not rely in the animal's path integration ability, property shared by others in the class of self-organizing models and in contrast with state of the art attractor and oscillators interference models.

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DISTINCT PATTERNS OF SYNAPTIC INTEGRATION IN THE DENDRITES OF TONIC SPINAL LAMINA I NEURONS - EXPERIMENTAL DATA AND COMPUTER SIMULATIONS

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Electrophysiological studies in spinal cord's dorsal horn lamina I show a high prevalence of 'tonic neurons' [1, 2]. This classification, used in many studies of somatosensory information integration in the spinal cord [2, 3], is an oversimplification of the response function of this population of neurons, and can in fact be highly misleading. The reason is that the classification as 'tonic' arises from the experimental observation that these neurons respond with periodic action potentials when subject to the injection of a constant current in the soma – this is, of course, a rather artificial form of stimulation, unlikely to take place in neuronal networks where synaptic activation exhibits complex spatiotemporal properties. This classification is nevertheless used as support for theories regarding information integration in lamina I and as evidence for rate-coding mechanisms [4].

In this present work we question the functional homogeneity of the neurons in this class and show that rather similar 'tonic' neurons can in fact display distinct features in terms of synaptic integration. This multidisciplinary work uses detailed neuronal reconstructions (n=21), including spines locations, to construct realistic computer models with detailed neuronal morphologies [5, 6]. Electrophysiological data for each reconstructed neuron is used to calibrate the dynamics of the associated computer model, allowing the models to fit the firing-frequency response curves recorded experimentally. Each neuron model recreates therefore the tonic properties of the original recorded neuron.

Assuming both passive and active properties in the dendrites, we use the models to show important functional differences within the 'tonic' population. First, spatial distributions of spines (markers of potential synaptic contacts) fall into different spatial organizations suggesting differences in input sources and differences in input integration/processing. The spines locations obtained experimentally are also compared with random synaptic placement in the dendrites. Secondly, calculation of the signal attenuation associated with the location of each spine (as a measure of synaptic efficacy, [7]) show a variety of distributions. Some neurons exhibit pockets with high efficacy spines (not always close to the soma), while other neurons display a relatively uniform spectrum of efficacies. Finally, computer simulations of neuronal activity show that, within the tonic class, distinct neurons can respond differently to similar (same statistical properties) patterns of synaptic activations modelled as Poisson processes. As a whole this work uncovers important functional variability in a prevalent population of neurons in lamina I with a crucial role in the integration, processing and transmission of nociceptive information.

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EFFECTOR MEMORY CD4$^+$ T CELLS ARE ASSOCIATED WITH COGNITIVE PERFORMANCE IN A SENIOR POPULATION

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INTRODUCTION: Longevity is increasing worldwide in part as a result of great improvements of public health and health care. Although with marked inter-individual differences, ageing is associated with a gradual decline in cognitive functions. Thus, the identification of factors that might delay cognition decline, promoting a healthy aging, is an increasingly relevant challenge. In addition to the cognitive alterations, the immune system also progressively changes with age. Recent data on the interplay between cognition and the immune system led to the current vision that the brain rather then being an immune privileged organ, enjoys the privilege of being regulated by the immune system. The immune system has been shown to play modulatory functions in several brain functions, namely cognition.

OBJECTIVE: Immunosenescence and cognitive decline are common markers of the aging process. Taking into consideration the heterogeneity observed in cognitive decline in aging and the now recognized link between lymphocytes and cognition, we herein explored the association between alterations in lymphocytic populations and cognitive performance parameters.

METHODS: In a cohort of cognitively healthy adults (n=114), previously characterized by diverse neurocognitive/psychological performance patterns, detailed peripheral blood immunophenotyping of both the innate and adaptive immune systems was performed by flow cytometry.

RESULTS: Better cognitive performance was associated with lower numbers of effector memory CD4$^+$ T cells and higher numbers of naive CD8$^+$ T cells and B cells. Furthermore, effector memory CD4$^+$ T cells were found to be predictors of general and executive function and memory, even when factors known to influence cognitive performance in older individuals (e.g., age, gender, education and mood) were taken into account.

CONCLUSIONS: This is the first study in humans associating specific phenotypes of the immune system with distinct cognitive performance in healthy aging.

THE IMPACT OF DIFFERENT PERIODS OF NEONATAL MATERNAL SEPARATION ON SOCIAL BEHAVIOR IN AN ADOLESCENT RAT MODEL

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Negative early life experiences, such maternal separation (MS), have been associated with alterations in social behaviors. However, less is known about the impact of short MS on adolescent social behavior. Interactions with peers become particularly important during adolescence and social peer influence is a strong predictor of adolescent risk-taking behaviors, such as drug use. The current study aimed to: 1) Understand the impacts of short daily maternal separation, during two different periods early in life, on the social behavior of adolescent Wistar rats. Since development is an ongoing process, the imprinting consequences of early adversity are highly dependent on the "time window" in which they occur. Here, we approached this issue using two different periods of maternal separation corresponding approximately in humans to a common period of nursery initiation (5 month), or to 3 years of age; 2) Explore the ability of environmental enrichment (EE) to protect from deleterious effects of maternal separation on the adolescent social behavior. The stimulation provided via EE applied early in life impacts both on brain and behavior and may be beneficial for the behavior development; 3) Explore the expression profile of oxytocin (OT) and OT receptor, and correlate it with the effects of MS and EE on adolescent social behavior (given its relevance for both maternal and other social behaviors). In order to achieve these goals we used two periods of MS, between postnatal days PND 2 and 6 or between PND 10 and 14, 2 hours daily, during which half of the litter was in EE and the other half in a standard environment. Effects on male adolescent social behavior, such as social interactions with familiar and unfamiliar peer, were studied at PND40-42. Results showed that daily MS from PND 10 to14 did not affect social interactions with unfamiliar peer, however affected social interactions within familiar peers, reducing social investigation and play behavior. MS from PND 2 to 6 decreased social investigation, play behavior, play solicitation and increased nonsocial activities during interaction with unfamiliar peers. In both MS ages, EE increased play behavior. While analyse of social interactions in familiar peers showed that MS from 2 to 6 days of age reduced social behavior and increased nonsocial activities in the adolescence, rats that experiencing MS from 10-14 days of age displayed increased seeking of social comfort later in life. Taken together, these results provide new evidence that early short periods of maternal absence are able to shape male adolescent social behavior and highlight that distinct social behaviors are differentially affected by maternal separation across ontogeny.

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METABOLIC GLYCOENGINERING IN NEURAL CELLS: CELLULAR UPTAKE OF EXOGENOUSLY SUPPLIED NON-NATURAL SUGARS

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Glycans are involved in a plethora of important physiological processes in both the developing and the adult nervous system: cell migration, neurite outgrowth and fasciculation, synapse formation, and modulation of synaptic efficacy are all related with glycan expression and bioactivity [1]. Targeting elucidation of the glycan role(s) during neurite outgrowth, we synthesised peracetylated azido-functionalized analogues of three natural sugars, namely mannose, glucose and fucose [2]. These unnatural sugars were supplied exogenously to hippocampal neuronal and astrocytes primary cell cultures and the metabolic pathways involved in the glycosylation process were exploited. Specifically, we investigated the influence of the chemical modification on the uptake and biosynthetic pathways of the sugars *in vitro*. Our goal is to study the spatial and temporal distribution of glycans at the cell surface by tracking the modified sugars using click chemistry with labeled cyclooctyne probes.

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BLOCKADE OF ECTO-5'-NUCLEOTIDASE RESPONSIBLE FOR THE EXTRACELLULAR ATP-DERIVED ADENOSINE FORMATION INHIBITS HIPPOCAMPAL SYNAPTIC PLASTICITY AND MEMORY PERFORMANCE

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ATP is stored in synaptic vesicles and it is released together with other neurotransmitters. ATP can directly signal through activation of P2 receptors or it can indirectly activate adenosine A_1 or A_{2A} receptors ($A_{2A}R$) after its extracellular catabolism into adenosine by ecto-nucleotidases, where ecto-5'-nucleotidase (e5'N or CD73) controls the rate of ATP-derived adenosine extracellular formation in brain synapses (Cunha, 2001, Neurochem. Res. 26:979). Since CD73 is selectively responsible for the formation of the ATP-derived adenosine activating $A_{2A}R$ in the striatum (Augusto *et al.*, 2013, J. Neurosci. 33:11890), we now tested the role of CD73 in the control of synaptic plasticity in the hippocampus and in hippocampal-dependent memory performance. Electrophysiological recordings, performed in Schaffer fiber-CA_1 pyramid synapses from hippocampal slices of adult (10 weeks-old) C57/BL6 mice, showed that the pharmacological inhibition of CD73 with α,β-methylene ADP (AOPCP, 100 μM) did not modify basal synaptic transmission, but selectively decreased long-term potentiation (LTP, induced by a 100 Hz train for 1s) by 12.7±2.1% (n=5; $P<0.05$). This effect of AOPCP on hippocampal LTP was abrogated in CD73 knockout (KO) mice, but was still observed upon blockade of $A_{2A}R$ either pharmacologically (with SCH58261, 50 nM) or genetically (in $A_{2A}R$-KO mice). To control for the possible impact of AOPCP in bolstering P2 receptor activation, we tested the impact of AOPCP in the presence of 20 μM PPADS, a non-selective antagonist of P2R. It was observed that the LTP inhibition caused by CD73 blockade remained in the presence of PPADS, which *per se* also decreased LTP amplitude. We then undertook a preliminary behavioral characterization of CD73 KO mice, which revealed that CD73-KO mice displayed a decreased memory performance in the object displacement and modified Y-maze tasks (n=3; $P<0.05$), without locomotor and emotional modifications (open field, elevated plus-maze and accelerated rota-rod). These results indicate that CD73 activity is selectively associated with the control of synaptic plasticity processes in hippocampus, with an impact on hippocampal-dependent memory performance. Although still unresolved, it is anticipated that this effect of CD73 might result from an intertwined mechanism involving the parallel accumulation of extracellular ATP and the reduced formation of ATP-derived adenosine.

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ADENOSINE A_1 RECEPTOR-MEDIATED INHIBITION OF CHOLINERGIC NEUROTRANSMISSION IN THE HUMAN URINARY BLADDER DEPENDS ON TISSUE DISTRIBUTION OF ECTO-NTPDASES

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ATP is a key player in the regulation of human urinary bladder function, which role increases in pathological conditions. However, ATP actions may be restrained by compartmentalization of P2 purinoceptors together with ecto-NTPDases at discrete cell surface domains. This layout may have also profound implications in the activity of P1 receptors by endogenous adenosine formed from ATP catabolism. All four adenosine receptor subtypes (A_1, A_{2A}, A_{2B} and A_3) are expressed in the bladder of experimental animals; these receptors are differently distributed among the urothelium, lamina propria and detrusor layers, but there is a lack of information regarding the purinergic cascade in the human bladder.

We show here that ATP (30 μM) catabolism is faster in the serosal side ($t½$ 26±3 min, n=3) then in the luminal side ($t½$ 35±3 min, n=4) of human urothelium. Higher amounts of ADP compared to AMP were detected following ATP (30 μM) incubation with urothelial strips with the luminal side facing up. This pattern suggests a dominant involvement of NTPDase2, which is a preferential nucleoside triphosphatase hydrolyzing ADP 10 to 15 times less efficiently than ATP with minimal AMP accumulation. AMP (30 μM) was dephosphorylated into adenosine more rapidly in the serosal side (67±13 min, n=5) than in the luminal side (117±32 min, n=3) of the urothelium. Immunoreactivity against NTPDase1 and NTPDase2 spans all layers of the human urothelium, while NTPDase3 stains only the terminally differentiated superficial umbrella cells. Conversely, staining against ecto-5'-nucleotidase/CD73 was more evident in the basal layer of the urothelium. Tissue elements below the urothelium (e.g. nerve fibres), as well as the detrusor smooth muscle layer, stain positively for NTPDase1, NTPDase2 and ecto-5'-nucleotidase.

Neurochemical experiments show that adenosine (100 μM) and its analogues, NECA (1 μM) and R-PIA (0.3 μM, selective A_1 receptor agonist), inhibit [^{3}H]ACh release from stimulated cholinergic nerves of the human detrusor, when these drugs were used in concentrations unable to cause relaxation of acetylcholine (10 μM)-induced myogenic contractions. Blockade of A_1 receptors with DPCPX (100 nM) facilitates [^{3}H]ACh release and prevented NECA (1 μM) and R-PIA (0.3 μM) inhibitory actions.

Data suggest that adenosine formation from the extracellular catabolism of adenine nucleotides via distinct NTPDase subtypes is positioned to favour a dominant inhibitory tonus, via A_1 receptors activation, on cholinergic nerve efferents, which might contribute to control detrusor overactivity in patients.

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ADENOSINE A_{2A} RECEPTORS SELECTIVELY CONTROL LONG-TERM POTENTIATION IN RODENT PRELIMBIC MEDIAL PREFRONTAL CORTEX AND HUMAN CORTICAL AREAS

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Adenosine A_{2A} receptors ($A_{2A}R$) are one of the main targets of chronically consumed caffeine, which is the most widely consumed psychoactive substance in the world. Caffeine and $A_{2A}R$ antagonists control stress-induced emotional and memory alterations that involve information processing in the prelimbic medial prefrontal cortex (PLmPFC). Although $A_{2A}R$ control long-term potentiation in different brain structures, their synaptic localization and role in synaptic transmission and plasticity in the PLmPFC are largely unexplored. Therefore, we recorded population spikes in layer V of slices containing the PLmPFC from male Wistar rats and C57Bl/6 mice upon stimulation of layer II/III, where NMDA-dependent long-term potentiation (LTP) was induced by applying trains of stimuli at 100Hz. $A_{2A}R$ blockade with SCH58261 (50 nM) was devoid of effect in either basal synaptic transmission ($p>0.05$) or paired-pulse ratio ($p>0.05$), but decreased LTP amplitude in either rat (23.5±3.8% over basal with SCH58261 *versus* (vs) 40.3±5.3% in control slices, $n=6$, $p<0.05$) or mouse (28.1±5.1% over basal with SCH58261 vs 79.5±10.9% in control slices, $n=5$, $p<0.05$). $A_{2A}R$ blockade did not affect depotentiation (induced by a 15 min 1Hz train; $p>0.05$) in either species. Having established the selective control of LTP by $A_{2A}R$ in the PLmPFC, we employed whole-cell patch-clamp to dissect their synaptic role in layer V pyramidal neurons. Here, we recorded excitatory postsynaptic potentials (EPSP) from Wistar rat brain slices containing the PLmPFC and human cortical slices (collected from brain surgery of epileptic patients). LTP was induced either by theta burst stimulation (TBS) or by using spike timing-dependent plasticity protocols (STDP). $A_{2A}R$ blockade abolished LTP in both rat and human. In rat layer V pyramidal neurons, with the STDP protocol, the EPSP slope with SCH58261 was 15.0±9.0% ($n=20$, $p>0.05$) over basal vs 48.2±25.0% in control slices ($n=18$, $p<0.05$); with the TBS protocol, the EPSP slope with SCH58261 was 7.0±11.0% over baseline ($n=8$, $p>0.05$) vs 45.0±10.0% in control slices ($n=6$, $P<0.01$). In human layer V pyramidal neurons, with the STDP protocol, the EPSP slope was 5.6±6.1% over basal with SCH58261 ($n=5$, $p>0.05$) vs 36.7±9.9% in control slices ($n=8$, $p<0.05$). An immunocytochemical analysis further showed that glutamatergic nerve terminals of the mPFC of either rat or mouse were endowed with $A_{2A}R$, and Western blot analysis of subsynaptic fractions revealed that $A_{2A}R$ were also present in human cortical tissue, and enriched in the extrasynaptic region. These results provide the first demonstration of the ability of $A_{2A}R$ to selectively control LTP in the mPFC of rodents as well as of humans, and reinforce our hypothesis that $A_{2A}R$ are valid pharmacological targets for the management of PFC-related dysfunctions, such as upon stress, mood disorders or attention deficit and hyperactivity disorders.

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INFRALIMBIC DESCENDING PRONOCICEPTION MEDIATED BY METABOTROPIC GLUTAMATE RECEPTOR-5

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The prefrontal cortex (PFC), an area primarily involved in cognition, participates in the descending modulation of pain. It is also known that metabotropic glutamate receptors (mGluR) partly mediate the output activity of this area. Previous work from our group has highlighted the pronociceptive role of mGluR5 in the infralimbic area (IL) of the medial PFC in healthy rats. In this work, we aim to unveil the descending pathway that modulates mGluR5-mediated pronociception.

We performed single unit electrophysiological recordings in the rostral ventromedial medulla (RVM) and in the dorsal reticular nucleus (DRt) in healthy rats to assess how activating mGluR5 in the IL impacts the cell activity of these two areas following heat noxious stimulation. We observed that after mGluR5 activation, DRt cells, but not RVM ON and OFF cells, presented an altered response to peripheral noxious stimulation.

In addition, transient inhibition of DRt with a local anaesthetic (lidocaine) prevents the decrease of heat-evoked paw withdrawal latency (PWL) resultant of activation of mGluR5 in the IL.

To uncover the spinal pathway(s) through which mGluR5 exerts its facilitatory effect, we measured PWL while simultaneously activating mGluR5 in the IL and blocking several pronociceptive spinal receptors at the L4-L6 level. We observed that the transient receptor potential cation channel subfamily V member 1 (TRPV1) fully reversed the mGluR5's pronociceptive effect in healthy animals.

We conclude that IL's mGluR5-driven pronociception is supraspinally mediated by the DRt, while at the spinal cord level, TRPV1 receptors are involved in this pronociceptive behaviour.

SEGREGATION OF PAIN AND ITS COMORBIDITIES AFTER PERIPHERAL NEUROPATHY

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Neuropathic pain is defined as a pain arising as a direct consequence of a lesion or disease affecting the somatosensory system and it is frequently accompanied by a deterioration of emotional behavior and cognitive function. However, in rat peripheral neuropathies there is temporal dissociation between pain, which appears soon after nerve injury, and anxiety- and depression-like behaviors that manifest later (4-6 weeks after pain onset). Also, left- and right-sided nerve injuries, although having the same allogeneic properties, impact differently on emotional and cognitive behaviors. We tested therefore the hypothesis the nerve injury *per se*, in the absence of pain might play a detrimental role.

A unilateral spared nerve injury (SNI) neuropathy was installed in Sprague-Dawley rats (≅15-20% do not develop mechanical allodynia in this strain), in the left (SNI-L) or in the right (SNI-R) side. One month later animals were tested in a battery of behavioral paradigms – light/dark box (anxiety), forced swimming (learned helplessness), spontaneous burrowing (general wellbeing), fear conditioning and variable delay-to-signal (VDS; impulsivity). The volumes of Cingulate Cortex (Cgl), Prelimbic Cortex (PrL), Infralimbic cortex (IL), Ventral Orbitofrontal Cortex (VO), Lateral Orbitofrontal Cortex (LO), Insular Cortex (IC) and Primary Motor Cortex (M1) were analyzed. Also $Nav_{1.3}$ channel protein levels were measured by Western blot to confirm the effectiveness of the lesion.

Results show that mechanical allodynia is equally reduced in the majority of SNI animals (Low-threshold) but a percentage of 20% of SNI animals (both sides) present similar values to Sham animals (High-threshold). Moreover, low- and high-threshold SNI-L but not SNI-R animals presented an increased anxiety-like profile. On the contrary, both low- and high-threshold SNI-R animals presented increased impulsivity in the VDS task. In the other behavioral paradigms, there are not statistical differences between both SNI groups and Sham animals. The volumes of ipsi- and contralateral side of the Sham and SNI animals were analyzed, and the SNI animals present bilateral changes in volume in both low- and high-threshold. Regarding the Nav1.3 channel, protein levels were measured and an increase expression was observed in either low- and high-threshold SNI animals, demonstrating that the lesion was effective regardless of pain. Our results support the hypothesis that the nerve injury alone, in the absence of pain, is sufficient to affect behavior; further the observed side-specific deficits obey to the same patters previously observed suggesting that the impact on brain function is anatomically restricted.

PREFRONTAL CORTEX PLASTICITY FOLLOWING A PERIPHERAL NERVE INJURY

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The Prefrontal Cortex (PFC) plays an important role in cognitive and emotional functions including attention, emotion, decision-making, goal-directed behavior and working memory. In neuropathic chronic pain conditions, anxiety, depression and cognitive impairments frequently manifest both in humans and experimental models suggesting the involvement of cortical areas associated with higher cognitive functions. Curiously, in the rat, left- and right-sided nerve injuries although having the same allogeneic potential, impact differently on these behaviors suggesting some degree of lateralization in the phenomena.

In this work we studied the impact of chronic neuropathic pain on PFC morphology (Golgi-Cox stain) and volumetry in left- and right-sided lesioned Wistar han rats. The spared nerve injury model (SNI) was installed in left- (SNI-L) or right-side (SNI-R) in the experiments. The model consists in the ligation and distal sectioning of the peroneal and tibial nerves (the sural nerve was spared). Sham operated animals were used as controls. Thresholds to light touch were monitored for 1-month using von Frey filaments after which animals were sacrificed and brains removed for further analysis. Left and right medial PFC (mPFC) – Cingulate Cortex (Cgl), Prelimbic (PrL) and Infralimbic cortex IL - and orbital PFC (OFC) – Ventral and Lateral Orbital Cortex, were then analyzed in terms of neuronal morphology and volumetry.

Results show that mechanical allodynia manifests equally in SNI-L and SNI-R animals. Nevertheless, side-specific morphological alterations were observed in the mPFC particularly in basal dendrites length and spine density. Basal dendrite length in contralateral neurons is increased in the Cgl but is decreased in the PrL in both SNI-L and SNI-R animals, whereas in the IL it is increased in SNI-L but decreased in SNI-R animals. Spine plasticity was also observed in the mPFC particularly in the contralateral IL, SNI-L present an increased spine density while SNI-R presents the opposite trend. Also, SNI animals, independently of lesion side, show a bilateral increase in total volume. Our results provide evidence that neuropathic pain leads to a differential rearrangement in PFC's subregions, which may help defining the cellular basis for cognitive impairments associated with chronic pain.

HETEROGENEITY OF ANTERIOR BNST NEURONAL ACTIVITY

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The bed nucleus of the stria terminalis (BNST) is an anatomically and functionally heterogeneous region that is involved in the regulation of psychiatric disorders such as anxiety and addiction (Stamatakis *et al.*, 2014, Walker and Davis, 2008). Specifically, the anterior division of the BNST (aBNST) receives inputs from limbic areas as the infralimbic cortex (ILCx) and is involved in the modulation of the activity of downstream areas as the paraventricular nucleus of the hypothalamus (PVN) or the ventral tegmental area (VTA) (Choi *et al.*, 2007, Jalabert *et al.*, 2009, Massi *et al.*, 2008). Using an *in vivo* electrophysiology approach in rats, we analyzed the basal activity of aBNST neurons as well as the modulation of their activity by ILCx inputs. We observed distinct basal activities of aBNST neurons, allowing us to categorize them in different groups, according to their firing rate. Additionally, stimulation of ILCx induces an excitatory response from aBNST neurons. Our results contribute to understand the activity of aBNST neurons in basal conditions and highlight the complexity of this region.

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ANTIPSYCHOTIC DRUGS IN DEPRESSION: THE UNEXPLORED ROLE OF NEUROPLASTICITY

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Major depression is one of the most prevalent neuropsychiatric disorders but still without an effective treatment. In fact a high percentage of patients treated with the currently available therapies do not show a full remission and present treatment resistance. Recently, some atypical antipsychotic drugs have received FDA approval for the treatment of antidepressant-resistant forms of major depression. However, the mechanism triggered by these drugs remains widely undisclosed. Growing evidence suggests that neuroplasticity impairments, such as reduced neurogenesis in the hippocampus and changes in gene expression are involved in the pathogenesis and recovery from depression. However, these mechanisms remain unexplored considering the actions of antipsychotics. To address this, an unpredictable chronic mild stress (uCMS) paradigm was implemented during 7 weeks to induce core symptoms of depressive-like behavior in rats[1].

During the last 3 weeks of uCMS, two antipsychotic drugs from different pharmacological classes, clozapine and haloperidol were daily administered. At the end of treatment, the behavioral dimensions commonly affected in depression were assessed and correlated with neuroplastic markers. Learned helplessness was evaluated in the forced swimming test (FST). Cognitive function was assessed by different tasks in the Morris water maze (MWM) test. Anhedonia was assessed using the sucrose preference test. In the brain we measured the levels of neurogenesis in the hippocampus and subventricular zone (SVZ). The mRNA levels of different synaptic remodeling proteins such as neural cell adhesion molecule 1 (NCAM1) and synapsin 1(SYN1) and also brain-derived neurotrophic factor (BDNF) in the hippocampus and prefrontal cortex were also analyzed. The expression of different dopamine receptors in the PFC was also measured.

We found that treatment with clozapine reduced both measures of depressive-like behavior while haloperidol was able to reverse the anhedonic phenotype but aggravated learned helplessness. Moreover, haloperidol-treated animals displayed cognitive impairments in a PFC-dependent task. These observations are in accordance with the differential clinical impact of these drugs in the negative symptoms of schizophrenia. Our findings also suggest that haloperidol and clozapine lead to different neuroplastic adaptive responses. While clozapine promotes neurogenesis, haloperidol leads to a decrease. Regarding the gene expression, antipsychotics increase BDNF but only clozapine increases the levels of SYN1 in the PFC. Furthermore, the expression of dopamine receptors is altered by haloperidol. Thus, the present data suggest a clear and differential impact of different neuroplastic events induced by the use of different classes of antipsychotic in uCMS animals.

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EFFECT OF PSYCHOSIS RISK VARIATION ON THE CIS - REGULATION OF THE CALCIUM CHANNEL, VOLTAGE-DEPENDENT L - TYPE ALPHA 1C SUBUNIT GENE (CACNA1C) IN THE HUMAN BRAIN

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BACKGROUND: Schizophrenia (SZ) and bipolar disorder (BD) are among the most debilitating psychiatric illnesses, leading to significant functional impairments and representing a tremendous public health burden. Many patients respond poorly to medication and relapse frequently, pressing the need for more effective therapies. Staggering advances in the genetic basis of psychosis are currently being made through genome-wide association studies (GWAS) that enable a systematic and unbiased population-based evaluation of individual common DNA variants. GWAS have demonstrated that SZ and BD display a significant genetic overlap, suggesting that some biological pathways confer risk to both disorders. A highly replicated shared risk allele is located in the calcium channel, voltage-dependent L-type alpha 1C subunit (CACNA1C) gene. Single nucleotide polymorphisms (SNPs) located within intron 3 of the CACNA1C gene have been consistently associated with increased risk for both SZ and BD by GWAS. In fact, one SNP within intron 3 of CACNA1C (rs2007044) was ranked number four in order of statistical significance out of all the GWAS hits in the most recent SZ GWAS. Moreover genotype at one particular SNP (rs1006737) has been associated with multiple intermediate phenotypes, related to the functional impairments observed in psychosis. However, the molecular mechanisms that mediate this association have not been clearly defined in the human brain. Given that these SNPs are non-coding it is likely that they will alter the expression or splicing of CACNA1C (cis–effects) in regions implicated in psychosis. This assessment is extremely important given that the protein encoded by this gene possesses key roles in NMDA receptor-independent synaptic plasticity and provides new avenues for therapeutic research.

AIMS: To assess the presence of cis-effects on CACNA1C expression and if these can be accounted for by the risk SNPs for psychosis rs2007044 and rs1006737 in adult DLPFC, caudate, cerebellum and temporal cortex.

Methods: Allele specific expression assays were performed to specifically detect cis – effects on CACNA1C expression and correlate it with rs2007044 and rs1006737 genotype. These assays involved PCR amplification, post–PCR cleanup, SNaPSHOT primer extension genotyping and capillary electrophoresis.

RESULTS: Cis–effects on CACNA1C expression were present in all brain regions. These were significantly associated with risk SNPs genotype in caudate ($p = 0.035$) and cerebellum ($p = 0.001$), but not DLPFC or temporal cortex. The observed effects did not correlate between the brain regions assayed. Within heterozygotes for the risk genotypes there is evidence of differential cis–effects on CACNA1C expression dependent on the brain region, with cerebellum showing most pronounced effects.

CONCLUSIONS: These results pave way for the bridging of GWAS associated common risk variants to psychosis pathophysiology and have implications for psychosis modelling.

ASSESSING COGNITIVE FUNCTION IN OLDER ADULTS VIA VIDEOCONFERENCE

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OBJECTIVE: Neuropsychological testing is mainly used to characterize cognitive functioning. Despite in-person testing being considered the most effective mode of assessment, it can be limiting in reaching those with functional or other limitations. The aim of the study was to determine whether cognitive testing of older individuals using the Telephone Interview for Cognitive Status (TICS), with a delayed recall item (modified, M), via Videoconference (VC, using Skype®), had comparable results with its more traditional application by telephone as well as with the Mini-Mental State Examination (MMSE) administered face-to-face

METHOD: A total of 50 individuals aged 57 to 95 (mean = 71.90, SD = 1.298) were randomly selected from registries of local health centers and day care centers. All participants were tested on cognitive performance using the three experimental conditions: VC and telephone for the TICSM and face-to-face for the MMSE.

RESULTS: Significant associations were obtained between the administration methods with the tVC administration method yielding comparable results to the telephone and face-to-face administrations. The linear regression model analysis indicated that age, gender, education and provenience explained 43.4% of the variance of the TICSM total score via VC, and 35.5% of the variance of the TICSM total score applied by telephone.

CONCLUSIONS: The use of TICSM through VC yields similar outcomes comparatively to those from face-to-face and telephone evaluation. The results suggest for the expediency of further research on the usefulness and suitability of computer-based tools for the assessment of cognitive function to reach diverse populations.

EPIGENETIC CONTROL OF POST-NATAL NEUROPLASTICITY IN THE HEALTHY AND STRESSED BRAIN: EXPLORING METHYLATION AND HYDROXYMETHYLATION LANDSCAPES

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The adult central nervous system (CNS) is endowed with considerable regenerative and neuroplastic potential. Neuroplasticity, in its different forms, is dynamically modulated by intrinsic genetic factors in conjugation with environmentally imposed factors of our everyday life. Nowadays, stress-exposure is a common environmental etiological factor leading to various pathological marks in neurochemical and neuroplastic phenomena, many of them underlying the so-called stress-related disorders. Moreover, several studies have shed light on how epigenetic mechanisms serve as mediators of the pathological effects of stress on brain homeostasis.

Here, we aimed to study the effects of stress on the epigenetic landscapes of the dorsal and ventral hippocampal dentate gyrus (DG). Furthermore, we aimed to explore how chronic exposure to stress impacts on the DG epigenome and its repercussions to fundamental emotional and cognitive modalities. We have exposed young-adult Wistar-Han rats to a pre-validated unpredictable chronic mild stress (uCMS) protocol during 6 weeks and performed a battery of behavioral tests to analyze emotional and cognitive dimensions. After sacrifice, we have analyzed citogenesis and dendritic morphological rearrengements. In addition, we have conducted genome-wide analysis of methylation and hydroxymethylation in these areas.

So far, results demonstrate that stress exposure compromises different neuroplastic processes, affecting different behavioral dimensions. Moreover, hydroxymethylation pathways are likely to be involved in the pathological effects of stress exposure, as they are differentially regulated in control and stress-exposed animals.

EFFECTS OF STRESS IN THE MESOPONTINE CHOLINERGIC CONTROL OF NUCLEUS ACCUMBENS ACTIVITY

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Adverse events occurring during the prenatal period, such as exposure to stress or high levels of glucocorticoids (GCs) may have long-term effects on brain development, increasing the risk of developing neuropsychiatric disorders, such as depression, anxiety or addiction. Such effects are able to reprogram or de-regulate neuronal circuits that are potentially more sensitive to GCs. For example, we have previously shown that prenatal exposure to GCs, specifically the synthetic GC Dexamethasone (DEX), strongly modulates the mesolimbic dopaminergic circuit by altering the expression pattern of the dopamine receptor 2 (Drd2) gene and overall dopamine levels in the nucleus accumbens (NAc) [1], resulting in anxious-like behavior [2], motivational deficits [2] and drug-seeking behavior[1].

Major inputs to the NAc include the canonical ventral tegmental area (VTA), which in turn may be controlled by cholinergic projections arising from the Laterodorsal tegmental area (LDT). Interestingly, a very recent study showed that LDT also directly projects to the NAc, though the type and extension of such connections remains unknown[3]. Moreover, the impact of stress/GCs in this network is poorly explored.

In this work we show that prenatal GC exposure leads to altered expression of enzymes of the cholinergic pathway, namely the choline acetyltransferase (ChAT) and acetylcholinesterase (AChE), in a brain-region specific manner. Specifically, we found altered expression of these enzymes in the LDT and PPTg (but not the nucleus basalis of Meynert), detected very shortly after GC exposure (3 days) until adulthood, suggesting long-term programing of the cholinergic system by GCs.

In addition to the molecular findings, we observed that GC exposure leads to altered electrophysiological transmission from the LDT to the NAc and VTA. These findings suggest that the differences observed in the mesolimbic circuit of GC-exposed animals may be (partially) caused by changes in upstream cholinergic projections, an hypothesis that we are now exploring with the combination of optogenetic stimulation of LDT-NAC projections and behavioral evaluation.

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LESS IS MORE: STRUCTURAL CORRELATES OF COGNITIVE AGING IN THE RAT

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Human aging is associated with memory impairments; however there are some individuals who retain their cognitive abilities until late in life. Understanding these diversities can help us promote "healthy aging". In order to study the underpinnings of this heterogeneity, we decided to use rats as models of cognitive aging. Thus, in this study, we behaviorally characterized a very large cohort of old-age (22-24-month-old) and young (4-6-month-old) Wistar-Han male rats in a battery of swimming-based tests to assess: spatial working and reference memory, and behavioral flexibility. Consistent with previous studies, aged rats as a group showed poorer spatial learning and behavioral flexibility than young subjects, but the degree of cognitive decline was highly variable. Given this heterogeneity, we were able to cluster both aged and young animals by their cognitive performance as: good and bad performers. After this differentiation, we tried to uncover the structural correlates of such behavioral differences, performing stereological and 3-dimensional morphometric dendritic analyses in the hippocampus (HPC) and medial prefrontal cortex (mPFC), two key regions involved in cognitive functioning. Our data revealed that individual differences in the cognitive function can be correlated with structural changes, albeit in different directions in young and aged animals. Interestingly, our data revealed a strong, significant, relation between reference memory performance and hippocampal volume and dendritic tree length that was positive in young animals but consistently negative in aged animals. A similarly inverse relationship between working memory performance and mPFC volume was also found for aged animals. Although counterintuitive, our robust data suggest that, with ageing, increased volume and or dendritic length are associated with worse performance. We think this might be related with enhanced non-productive growth to compensate for effective synaptic communication and are addressing this further. Finally, we also compared patterns of expression of some specific genes, important for learning and memory, comparing them between aged animals that have successfully learned a task and those who failed to learn the different tasks. Our results suggested that different expression patterns of brain-derived neurotrofic factor (*Bdnf*), dopamine receptor type 2 (*Drd2*), and glutamate receptor *Grin2a* and *Grin2b* (also known as *Nr2a* and *Nr2b*), could also explain some of the inter-individual differences in the cognitive ageing process. In conclusion, this study showed that ageing of the brain is a heterogeneous process and revealed some of its morphological and genetic correlates.

These findings may be of potential value to our improved understanding of how aging impacts on the cognitive function.

EXPLORING THE IMPACT OF T CELL SUBSETS ON PHYSIOLOGICAL ASPECTS OF BRAIN FUNCTION

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Until recently, interactions between the immune system and central nervous systems were assumed to be limited to extreme cases of pathological insult. However, recent data have suggested key roles for immune cells in physiological brain functions, including adult neurogenesis, stress responses, and spatial memory (Kipnis J. *et al*, Nat. Rev. Immunol., 2012). For instance, CD4+ T cells have been shown to be involved in learning behavior of mice. While the IFN-γ producing (T helper type 1, Th1) subpopulation has been shown to be detrimental in a model of Alzheimer's disease (Browne T.C. *et al*, J. Immunol., 2013), a pro-cognitive role of the IL-4 producing (Th2) counterpart has been suggested (Derecki N.C. *et al*, J. Exp. Med., 2010). This notwithstanding, it remains unknown whether other T cell subsets may impact on physiological aspects of brain function. In particular, gamma-delta (γδ) T cells and IL-17 producing T cells are two promising candidates, given their role on neuroinflammation in a murine model for multiple sclerosis (Blink S.E *et al*, Curr. Mol. Med., 2009).

To test this, we characterized the behavior performance of C57BL6 mice deficient in γδ T cells or IL-17. Spatial memory was evaluated in the Y Maze test, while the Open Field and Elevated Plus Maze tests were performed to check for exploratory behavior and anxiety, respectively. We observed that in the absence of γδ T cells or IL-17, animals showed no preference for the novel arm of the Y Maze, in contrast to wild-type (WT) controls or IFN-γ -/- mice ($n=5\text{-}8$; $P<0.05$). Mice deficient for γδ T cells or IL-17 also displayed a less anxious behavior than their WT counterparts ($n=7\text{-}10$; $P<0.05$). We are currently indentifying the molecular mechanisms involved in this context. Interestingly, our preliminary results showed a decrease of Brain Derived Neurotropic Factor (BDNF) in the hippocampus of mice deficient for γδ T cells or IL-17.

Altogether, our data suggests that γδ T cells can regulate both cognition and anxiety, possibly through an IL-17-dependent mechanism.

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THE ROLE OF ADENOSINE A_1 IN THE HIPPOCAMPAL SYNAPTIC PLASTICITY MODULATED BY CANNABINOID CB1 RECEPTORS

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Adenosine and endocannabinoids are important modulators of neuronal activity. Cannabinoid CB1 receptor (CB1R) activation inhibits GABA and glutamate release in the hippocampus, these effects being partially reduced by co-activation of adenosine A_1 receptor (A_1R), suggesting an interaction between these modulatory pathways (1,3). We now studied the impact of CB1R on hippocampal (CA1) long-term potentiation (LTP) and the A_1Rs involvement on this modulation.

Extracellular electrophysiological recordings of field-excitatory post-synaptic potentials (fEPSPs) were performed at the CA1 area of hippocampal slices taken from adult (8-18 weeks-old) wild type (WT) or A_1R KO mice (C57Bl/6). LTP was induced by electrical stimulation of the afferents with a *weak*-θ-burst (five 100Hz bursts, 4 stimuli, separated by 200 ms) or a *strong*-θ-burst (ten 100Hz bursts, 4 stimuli, separated by 200 ms). The magnitude of LTP was quantified at 50-60 min after θ-burst stimulation.

Both in WT and A_1R KO mice, LTP magnitude induced by the *weak*-θ-burst in the presence of the CB1R antagonist, AM251 (1 μM), was higher than in its the absence (LTP magnitude in WT: 41±2.3%,n=4 in AM251 vs 30±4.8%,n=4 in its absence, $P<0.05$; in A_1R KO: 39±5.6%;n=6 in AM251 vs 24±6.0%;n=5 in its absence, $P<0.05$). With *strong*-θ-burst stimulation, LTP magnitude was 66±3.8% in WT (n=6) and 78±8% in A_1R KO mice (n=5). Unexpectedly, in A_1R KO mice, AM 251 (1 μM) blocked LTP induced by the *strong*-θ-burst (7.8 ± 24%; n=5, $P<0.05$ vs absence of AM251). In WT mice, the presence of AM251 also attenuated LTP induced by the *strong*-θ-burst, but did not block it (LTP magnitude: 44±3.8%; n=5, $P<0.05$ vs absence of AM251). LTP induced by a *strong*-θ-burst was markedly decreased by AM251 in slices from WT mice with A_1R blocked by the selective antagonist DPCPX (50nM) (DPCPX: 70±74%, n=5; DPCPX+AM251: 31±94%; n=8)

These results suggest that CB1 receptor activation by endogenous cannabinoids is crucial for maintenance of LTP induced by intense neuronal firing under conditions of hampered A_1R signalling.

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INFRALIMBIC NT3 INFUSION RESTORES FEAR EXTINCTION MEMORY IMPAIRMENT IN A MOUSE MODEL OF PANIC DISORDER

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The inability to properly extinguish fear memories is on the foundation of several anxiety disorders. Recent findings report that plasticity in the medial prefrontal cortex (mPFC) is central in the extinction process and capable of modify fear output. Fear extinction, a learning process in which a new memory is formed over previously acquired fear memories, involves different physiological mechanisms including plasticity events. Neurotrophins are a family of growth factors critical in synaptic and behavioral plasticity.

We previously showed that mutant mice with selective deregulation of the neurotrophin tropomyosin kinase, type 3 receptor (*NTRK3*, which encodes TrKC) models panic-like phenotypes (Dierssen *et al.*, 2006) and shows increased fear memories that are resistant to extinction, congruent with an altered activation pattern of the amygdala—hippocampus—mPFC fear circuit (Santos *et al.*, 2013). In the present study we hypothesized that by stimulating plasticity through NT3 infusion in the mPFC-infralimbic region of transgenic (Tg)*NTRK3* animals we would be able to rescue extinction of fear memory deficit.

We found that NT3 expression is increased upon fear extinction in wild type animals implicating, for the first time, this neurotrophin in contextual fear memory extinction. However, this fear-induced regulation in NT3 expression does not occur in Tg*NTRK3* mice, which may indicate a lack of plasticity in this brain region. Indeed, using *ex vivo* mPFC-infralimbic brain slices and electrophysiology recordings we found that Tg*NTRK3* mice have impaired long-term potentiation, which could be completely rescued by local application of NT-3. Moreover, *in vivo* infusion of NT3 into the mPFC of Tg*NTRK3* mice is sufficient to rescue their fear extinction deficit. Moreover, inhibition of ERK phosphorylation, using SL327 antagonist, blocks NT-3 effects on extinction providing a mechanistic ground to extinction-dependent NT3 expression.

Our results show for the first time the involvement of NT3/TrkC and ERK signaling pathway in the modulation of pathological fear and highlight modulation of plasticity in the mPFC, through NT3/TrkC, as a potential therapeutic tool in the treatment of anxiety disorders.

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BONE TO NERVOUS SYSTEM: UNRAVELING THE FEEDBACK LOOP BETWEEN BONE AND CENTRAL AND PERIPHERAL NPY NEURONAL PATHWAYS IN BONE INJURY SETTING

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Neuropeptide Y (NPY) system was demonstrated to be an important regulator of bone mass. Its inhibition induces the increase in bone mass, through central signalling involving the Y2 receptor in the hypothalamus, and peripherally by signalling through the Y1 receptors expressed in the osteoblasts. Recently, we have also showed that central and peripheral NPY neuronal pathways are targeted during the initial steps of bone repair, supporting the involvement of this neuropeptide in the response of nervous system to bone homeostasis challenge. Still, whether/how molecular signals produced locally in bone intervene as messengers of the bone physiologic state to the nervous system, in a system of feedback loop, is not known. In order to clarify this feedback loop, as a first approach, the response of central and peripheral NPY neuronal pathway to soluble factors released after bone injury was investigated through two *ex vivo* experiments: 1) mice dorsal root ganglia (DRG) were treated with plasma collected from mice with injured femur, and 2) hypothalamic organotypic cultures were treated with conditioned medium obtained from an *in vitro* brain blood barrier model after treatment with plasma from mice with femur defect. Treatment with plasma from sham-operated mice was performed as control. The obtained results showed that plasma from mice with femur defect and plasma from sham-operated mice induce different modulation of NPY expression in the hypothalamus, and different modulation of NPY and Y1 receptor expression in the DRG. Overall, our results support that injured bone releases factors able to modulate the NPY-ergic activity in the sensory nervous system, and also the hypothalamic NPY neuronal pathway after cross and/or interact with the BBB.

NEUROGENESIS ASSOCIATED WITH MALE BEHAVIORAL PLASTICITY IN A MARINE FISH, THE PEACOCK BLENNY *SALARIA PAVO*

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Unlike mammals, fish have the remarkable capacity of retaining high levels of adult neurogenesis and are able to significantly recover after neuronal damage. In addition, fish show remarkably high levels of behavioral plasticity, with many species undergoing major behavioral transitions during their lifetime, including changing sex or adopting alternative modes of reproduction. We hypothesized that these extreme behavioral changes in the adult stage rely on a significant capacity for structural rearrangements of neuronal networks that include the integration of new neurons in existing networks. This idea was tested in a species with a high level of behavioral plasticity, the peacock blenny *Salaria pavo*. In this species, young males reproduce by mimicking female behavior and appearance in order to approach nests defended by larger males, deceive the males, enter the nests, and parasitically fertilize part of the eggs inside the nest. The small "sneaker" males transition to the nesting male phenotype, suffering extreme morphological and behavioral changes. To test this singularity, one outdoor pool was set up with 12 nesting males and respective nests, 24 females and 24 sneaker males. The 24 sneaker males were provided with 12 potential nests to induce the transition in approximately half of the sneaker males. In fact, at the end of the experimental period (10 days), some of the sneakers had occupied the available nests and started the morphological and behavioral transition while others had remained sneakers. Fish were injected with the mitotic marker BrdU and sacrificed 2h later. Animals were perfused, the brains extracted, sliced in a cryostat at 15µm and a BrdU immunohistochemistry protocol with DAPI counterstain was applied. Cells were manually counted under a fluorescence microscope. A detailed map of the brain proliferation areas for this species was generated. For the most significant nuclei, differences in the number of proliferating cells between the male morphotypes were analyzed. The results are discussed in light of the hypothesis that periods of extreme behavioral transition are associated with an increase in cell proliferation levels. In conclusion, extensive adult neurogenesis may be a condition for extreme behavioral plasticity and may explain why, throughout vertebrates, adult behavioral plasticity seems to be associated with the capacity for adult neuroplasticity.

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EXPLORING THE ROLE OF PHOSPHORYLATION IN HUNTINGTIN AGGREGATION DYNAMICS

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BACKGROUND: Huntington's disease (HD) is characterized by misfolding and aggregation of mutant huntingtin (HTT), a dynamic process that starts with the association of misfolded HTT monomers into soluble oligomeric structures. Current evidence suggests that dimers and oligomers are the most toxic HTT species while large aggregates may in fact be neuroprotective. To better understand HTT toxicity it is essential to understand the molecular mechanisms of aggregation.

AIMS: Our goal was to elucidate the role of HTT N-terminal phosphorylation in the aggregation and toxicity of mutant HTT.

Methods/Techniques: Bimolecular fluorescence complementation (BiFC) was employed to visualize soluble and insoluble HTT species. We used this model to study the behaviour of HTT phosphomimic and phosphoresistant mutants in living cells. Moreover, we tested several compounds that modulate phosphorylation in *Drosophila* expressing HTT exon 1 (HTT93Q) under the control of the *GAL4/UAS* system. Pan-neuronal expression of HTT93Q by *elavGAL4* gives a variety of disease-relevant phenotypes, including degeneration of photoreceptor neurons. We compared the neurodegeneration of treated or non-treated HD flies using the light microscopy-based pseudopupil assay.

RESULTS/OUTCOME: When phosphomimic mutations were present in HTT103Q BiFC constructs, the generation of inclusion bodies was completely abolished. Phosphomimic and phosphoresistant mutants had varied effects on HTT toxicity, which were not associated with the levels of oligomers or inclusion bodies. A CDC25 phosphatase inhibitor decreased aggregation in cells expressing HTT103Q and increased neurodegeneration in HTT93Q flies. Notably, a specific protein phosphatase 2A (PP2A) inhibitor completely reversed HTT93Q neurotoxicity in *Drosophila*.

CONCLUSIONS: The phosphorylation state of HTT N-terminal region plays a role in aggregation and toxicity of the protein, and this can be pharmacologically modulated by targeting cellular phosphatases.

CELL CYCLE REGULATION OF THE ADULT HIPPOCAMPAL PROGENITOR CELLS IN DEPRESSION AND BY ANTIDEPRESSANTS

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Depression is a complex and multidimensional disorder affecting around 20% of the world population. Alterations in hippocampal dendritic morphology and in adult hippocampal cell proliferation and cell genesis are known to be involved in the pathophysiology of the disorder and in the action of antidepressants (ADs). Indeed, decreased cell genesis and dendritic morphology changes have been detected in the hippocampal neurogenic niche of depressed individuals, whereas ADs treatment was shown to prevent these damages (1). Previous genome-wide studies by our group, using microarray analysis of macrodissected hippocampal dentate gyrus (hDG) in a rat model of depression (uCMS - unpredictable chronic mild stress), have disclosed differential molecular regulation by different classes of ADs. Moreover, these results were correlated with relevant physiological parameters to unveil the integrated network that maintains homeostasis in the hippocampal niche (2). Following this study, the cell cycle mechanisms regulating hippocampal cell proliferation were investigated using both *in vivo* (macrodissected hDG) and *in vitro* (P5 rat hippocampal-derived neurospheres culture) approaches. The hDG of uCMS animals presented a slight increase in the percentage of cells in G1 phase of the cell cycle. Accordingly, these cells had decreased expression levels of cyclin D1 compared to control animals, whereas antidepressant-treated animals reversed these levels to those of controls. To better characterize these responses, an *in vitro* system - the neurospheres culture - was used. This system allowed us to enrich our population in hippocampal-derived progenitor cells. Dexamethasone was applied to the neurospheres cultures for 6 days, to mimic the effects of glucocorticoids elevation occurring in the brains of depressed individuals and animal models of depression. Moreover, neurotransmitters involved in the action of monoaminergic ADs were applied to the dexamethasone-treated cells, in the last 4 days of culturing. A G1 phase arrest was detected in the dexamethasone-treated progenitor cells. A partial reversion of this arrest was observed by the addition of neurotransmitters to the cells. The expression of G1 phase cell cycle regulators, such as the cyclin-dependent kinase inhibitors p21 and p27, was significantly increased in the dexamethasone-treated cells. Interestingly, two other atypical cell cycle regulators, Cdk5 and its activator p35, were increased after dexamethasone exposure. These results suggest a mechanism for the regulation of hDG progenitor cells proliferation upon glucocorticoids increase. Studies are now being conducted to further elucidate the role of these and other cell cycle molecules in the regulation of proliferative phenomena implicated in the pathophysiology of depression and AD treatment.

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MECHANISMS OF SLEEP PATTERNS DISRUPTION IN A FLY MODEL OF PARKINSON'S DISEASE

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Animals use a circadian cycle to shift between activity and sleep. Sleep dysfunction is observed in several neurodegenerative diseases, including Parkinson's disease. However, why 90% of Parkinson's disease patients suffer from sleep patterns defects is still unknown.

We used fruit flies with mutations in the *parkin* gene, which causes Parkinson's disease, to understand the molecular and cellular basis for this defect.

parkin mutant flies mutant present a sleep fragmentation and the disruption of the circadian rhythm. The neurons responsible for circadian cycle are the pigment dispersing factor (PDF) neurons. Our data indicate that the PDF neuropeptide is not properly localized, suggesting defects in its rhythmic release. Parkin plays a critical role in the maintenance of mitochondria homeostasis, and our data indicate that mitochondrial activity in PDF neurons follows the circadian cycle.

This suggests that mitochondrial function, disrupted in a fly model of Parkinson's disease, is linked to the regulation of pdf neurons' rhythmicity and the control of the circadian cycle.

WHAT CAN THE KINETICS OF AMYLOID FIBRIL FORMATION TELL US ABOUT INTERMEDIATE SPECIES AND OFF-PATHWAY AGGREGATION?

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Similarly to other phase transition processes, amyloid fibril formation takes place via a nucleation and growth mechanism until thermodynamic equilibrium is reached. The amyloid pathway may however comprise parallel steps and intermediate species that are no less relevant for the development of amyloidosis than the deposition of fibrils itself. Here we propose a method to identify any measurable effect that secondary processes may produce in protein aggregation curves. Canonical kinetics are expected to show a characteristic hyperbolic to sigmoidal shape and reproducible end-point signals. The information that they provide is limited to the magnitude of nucleation and growth steps and to protein solubility data. Deviations from the characteristic shape and, in some cases, low reproducibility of maximum aggregation rates and end-point signals are indicative of more complex mechanisms and/or pervasive non-fibrillar content. Identified during aggregation of lysozyme under harsh denaturing conditions (pH 1.6, 60 ºC), these kinetic signatures suggest that the obtained amyloid fibrils are only the tip of an iceberg hiding a crowd of oligomeric species. Atomic Force Microscopy (AFM), Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS) and Circular Dichroism (CD) results validated our analysis of thioflavin T (ThT) fluorescence aggregation experiments. Complementary measurements of monomer depletion kinetics show that lysozyme oligomers act as supramolecular crowding agents by inducing volume excluding effects. While contributing to a better understanding of the molecular basis of amyloid diseases, our study may find practical application in future screenings of aggregation inhibitors.

THE ROLE OF ADENOSINE RECEPTORS ON GLUTAMATE TOXICITY IN THE PLANARIA *SCHMIDTEA MEDITERRANEA*

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Adenosine receptors have recently received much attention for the treatment of Parkinson´s disease and other neurodegenerative disorders. Adenosine modulates glutamatergic and dopaminergic neurotransmission, and adenosine receptor antagonists such as caffeine have shown neuroprotective properties in diverse models of neurodegeneration.

Schmidtea mediterranea is a non-parasitic fresh water flatworm that, despite its small size (0.5-1.5 cm), has a complex central nervous system. For example, it contains most neurotransmitters found in mammals, such as glutamate, GABA, serotonin, dopamine and adenosine.

Our main aim was to characterize the interplay between glutamate and adenosine receptors in these planaria. We observed that addition of glutamate (1-10 mM) to the medium showed two dose-dependent toxic effects. Immediately after addition, glutamate produces severe seizures in planaria, which can be easily quantified. Later on, planaria start to disintegrate, eventually dying. The time at which planaria disintegrate is dependent on glutamate concentration, ranging from 8 to 72 hours. A_1 and A_{2A} adenosine receptor agonists [N6-Cyclopentyladenosine and CGS 21680] and antagonists [8-Cyclopentyl-1,3-dimethylxanthine and SCH-58261] modulated glutamate toxicity, also in a dose-dependent manner (1 nM-1 µM). In several occasions, lower concentrations (1-10 nM) showed greater neuroprotective effect than higher concentrations.

Our results indicate that adenosine signaling has potential to prevent glutamate deleterious effects *in vivo*. This observation could have important implications for multiple human pathologies, including Parkinson and Alzheimer diseases, brain trauma, stroke or epilepsy, because all of them show glutamate excitotoxicity, overactivity or both. Our results also support that planaria could be a good *in vivo* model for neuropharmacological and neurotoxicity studies.

METHAMPHETAMINE-INDUCED BLOOD-BRAIN BARRIER DISRUPTION AND BRAIN EDEMA: THE ROLE OF WATER CHANNEL AQUAPORIN-4

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Methamphetamine (METH) is a highly addictive psychostimulant drug of abuse which consumption in Europe has been increased over the last years. Several reports have demonstrated that oxidative stress, mitochondrial dysfunction and neuroinflammation are some of the neurotoxic features of METH. More recently, some studies have suggested that such neurotoxicity can also result from METH´s ability to compromise the blood-brain barrier (BBB) properties. Furthermore, it is also known that METH use may cause cerebral edema leading to severe consequences.

Large water fluxes continuously take place between the different compartments of the brain, as well as between the brain parenchyma and the blood. Disturbances in this well-regulated water homeostasis may lead to brain edema, which will have deleterious effects on brain function. Importantly, the water transport at the BBB is regulated by water channels, the aquaporins (AQPs), with AQP4 being the most important at the Central Nervous System. In fact, AQP4 is express in astrocytic end-feet that surround brain endothelial cells. Furthermore, AQP4 has two isoforms, M1 and M23, and the ratio M23/M1 regulates the brain water homeostasis since M23 stabilizes the channel function, whereas M1 disrupts the AQP4 structure. In fact, brain edema has been observed in several neuropathologies, including under conditions of METH consumption, but nothing is known about the underlying mechanisms.

The present work aims to investigate the effect of METH on AQP4 expression/function and so clarify the role of this water channel on METH-induced BBB permeability and brain edema. Our results show that METH (4× 10 mg/kg, i.p., 2h apart) caused an increase of water content in both mice striatum and hippocampus, together with a clear BBB breakdown and microvessels weakening. However, no effect was observed in pre-frontal cortex. In order to clarify if AQP4 plays a role on the observed BBB alterations, we further demonstrated that the blockade of AQP4 with a specific inhibitor (TGN-020, 200 mg/kg, i.p.) attenuated METH-induced edema and BBB disruption. Moreover, METH interfered with AQP4 expression in the abovementioned brain regions causing an increase in the M1/M23 ratio, which is an indicator of water homeostasis disruption. Also, we demonstrated that brain edema and BBB permeability triggered by METH involve the protein kinase C (PKC) pathway since its inhibition with chelerythrine (4× 5mg/kg, i.p.) prevented both effects.

Overall, our results show that METH interferes with AQP4 expression leading to BBB dysfunction and brain edema *via* PKC signaling pathway. Additionally, we also demonstrated that different brain regions present different susceptibilities to METH.

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METHYLPHENIDATE ACTIVATES SRC PATHWAY LEADING TO CAVEOLAE-MEDIATED TRANSCYTOSIS IN HUMAN BRAIN ENDOTHELIAL CELLS

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Methylphenidate (MPH) is the primary drug of choice in treating attention deficit hyperactivity disorder (ADHD), one of the most common neurobehavioral disorders of childhood. Nonetheless, the central consequences of MPH use are still poorly understood, and its impact on blood-brain barrier (BBB) function has never been addressed before. Thus, the aim of this work was to clarify the effect of MPH on brain endothelial cells, the principal components of the BBB. For that, we used primary cultures and a cell line of human brain microvascular endothelial cells (HBMVECs and hCMEC/D3, respectively). We concluded that MPH increased the macromolecular flux across HBMVECs, without transendothelial electrical resistance (TEER) alterations, suggesting that MPH-induced permeability was not due to changes on the paracellular pathway. In fact, there were no alterations on intercellular complexes, but instead there was a caveolae-dependent vesicular transport of horseradish peroxidase. Accordingly, we also observed a rapid activation of caveolin-1, the major structural component of caveolae vesicles. Additionally, we further concluded that MPH triggered an oxidative stress response by Rac1-dependent NADPH oxidases. To further clarify the intracellular pathway responsible for MPH-induced transcytosis, we proved that MPH activates c-Src kinase in a ROS-dependent manner, which in turn phosphorylated caveolin-1 at Tyr14 and promoted *caveolae* formation. Moreover, the vesicular transport mediated by MPH was completely blocked by the pharmacological inhibition or knockdown of Src pathway, with SKI-1 or c-Src shRNA, respectively.
Overall, our results show that MPH increases brain endothelial cell permeability by promoting vesicular transport. Specifically, we demonstrated that MPH triggers ROS production that will activate c-Src culminating in caveolae-dependent endocytosis.

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SEROTONERGIC SIGNALING SUPPRESSES ATAXIN-3 AGGREGATION AND NEUROTOXICITY IN ANIMAL MODELS OF MACHADO-JOSEPH DISEASE

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Polyglutamine diseases are a class of dominantly inherited neurodegenerative disorders for which there is no effective treatment. Here we provide evidence that activation of serotonergic signaling is beneficial in animal models of Machado-Joseph disease (MJD). We identified citalopram, a selective serotonin re-uptake inhibitor, in a small molecule screen of FDA-approved drugs that rescued neuronal dysfunction and reduced aggregation using a *Caenorhabditis elegans* model of mutant ataxin-3 (ATXN3) induced neurotoxicity (1). Chronic citalopram treatment stalled disease progression and restored survival of mutant ATXN3 animals. Maximum protection required early treatment; furthermore, the extent of drug exposure period was critical, as two days of treatment were insufficient to exert beneficial effects. MOD-5, the *C. elegans* ortholog of the serotonin transporter and cellular target of citalopram, and the serotonin receptors SER-1 and SER-4 were strong genetic modifiers of MJD and necessary for therapeutic efficacy. Moreover, chronic treatment of CMVMJD135 mice (2) with citalopram significantly reduced ATXN3 neuronal inclusions and astrogliosis, rescued diminished body weight and strikingly ameliorated motor symptoms. Suppression of ATXN3 aggregation in *C. elegans* and mice occurred without major changes in mutant ATXN3 protein levels, suggesting that the effect of citalopram in these disease models may affect folding and stability of ATXN3 rather than clearance of the mutant protein. These results suggest that small molecule modulation of serotonergic signaling represents a promising therapeutic target for Machado-Joseph disease, and support a recently emerging role for this signaling in the modulation of proteostasis (3).

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PHARMACOLOGICAL OR GENETIC BLOCKADE OF ADENOSINE A_{2A} RECEPTORS PREVENT COGNITIVE DYSFUNCTION PRECEDING MOTOR IMPAIRMENT IN AN INTRANASAL MPTP MOUSE MODEL OF PARKINSON'S DISEASE

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A growing body of evidence indicates that cognitive (mainly working and procedural memory) deficits precede the classical motor symptoms in Parkinson's disease (PD); these non-motor features of PD respond poorly to dopaminergic medication and represent a major unmet clinical need (e.g. Klepac *et al.*, 2008, Eur J Neurol 15: 128). Convergent epidemiological and pre-clinical data suggest that the blockade of adenosine A_{2A} receptors ($A_{2A}R$) by caffeine or selective antagonists may confer neuroprotection in PD and are now used as anti-Parkinsonian (Chen *et al.*, 2013, Nature Rev Drug Disc 12:265). In parallel both caffeine and $A_{2A}R$ antagonist can attenuate memory impairment in different animal models of brain disease (Cunha & Agostinho, 2010, J Alzheimers Dis 20:S95). Thus, we now tested if the pharmacological or genetic blockade of $A_{2A}R$ could alleviate memory impairment in an animal model of PD based on the intranasal (i.n.) administration of MPTP (Prediger *et al.*, 2011, Curr Pharm Des 17:489).

Male C57BL/6 (6 months-old) were pretreated intraperitoneally with vehicle or SCH58261 (0.3 mg/kg, an $A_{2A}R$ antagonist) 4 days prior plus 14 days after i.n. administration of MPTP (1 mg/nostril). In an independent set of experiments, wild type and $A_{2A}R$ knockout mice were infused i.n. with a single bilateral dose of MPTP (1 mg/nostril). Animals were submitted to a battery of behavioral tasks from 3 to 14 days after MPTP infusion, which included the social recognition, Y-maze, water maze, open field, activity chambers and rota-rod tasks. Upon completing the behavioral experiments, the animals were killed (at day 21, before the onset of motor alteration pathognomonic of PD) to measure the density of tyrosine hydroxylase (TH) and the release of dopamine and glutamate in the prefrontal cortex (PFC), striatum and substantia nigra (SN).

Mice infused with MPTP displayed working and procedural memory deficits without the emergence of alterations in motor performance, which were associated with a significant reduction of TH density in the PFC, striatum and SN. Moreover, the release of both glutamate and dopamine in the PFC, but not in the striatum, was impaired in MPTP-treated mice. The genetic deletion or pharmacological blockade of $A_{2A}R$ prevented these behavioral and neurochemical impairments induced by MPTP administration.

These results prompt considering $A_{2A}R$ as promising therapeutic target to manage cognitive symptoms associated to early phases of PD, possibly acting through the modulation of the frontocortical dopamine and glutamate neurotransmission.

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NADPH OXIDASE 1 REGULATES ALPHA-SYNUCLEINOPATHY IN PARKINSON'S DISEASE

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The interactions between oxidative stress and genetic factors are an important aspect that may help to understand idiopathic Parkinson's disease (PD). Oxidative stress was shown to be involved in dopaminergic neurons loss, as well as in the increased aggregation levels of α-synuclein which was the first genetic cause reported in familiar forms of PD. Mutations in SNCA gene, encoding α-synuclein, give rise to erroneous protein that is prone to form aggregates, a pathologic hallmark of PD. Elevated expression of WT α-synuclein due to multiplications in the SNCA gene has also been identified in early-onset PD leading to the view that increased expression of the protein can cause PD in dose-dependent manner. Although this fact emphasizes the importance of α-synuclein strict regulation, little is known about the regulatory mechanisms involved in α-synuclein expression and aggregation, been clear that both are critically influenced by oxidative stress.

By employing paraquat (PQ)-based *in vitro* and *in vivo* dopaminergic degeneration models, we found that Nox1-derived ROS play a crucial role in α-synuclein pathology as well as in dopaminergic neuronal degeneration.

In human dopaminergic neurons Nox1 and α-synuclein expression increased under PQ exposure. Furthermore, Nox1 knockdown prevented both increased α -synuclein expression and aggregation levels induced by PQ. In rats exposed to PQ we found a clear increase in Nox1 expression levels in the substancia nigra (SN), accompanied by increased α-synuclein expression and aggregation and dopaminergic neuron loss. The selective knockdown of Nox1 in the SN, using adeno-associated virus encoding Nox1 specific shRNA, largely attenuated PQ-mediated dopaminergic neuronal degeneration *in vivo*. Moreover, we observed that Nox1 knockdown *in vivo*, reduced oxidative stress level and dopaminergic neuronal loss, and significantly attenuated the PQ-mediated increase of α-synuclein protein levels as well as α-synuclein aggregates and A11 oligomers in the SN.

These findings shed new light on understanding the regulation of α-synuclein expression as well as aggregation in which Nox1 plays a pivotal role.

EFFECT OF CHRONIC HYPERGLYCEMIA ON NEWLY-GENERATED NEURONS MATURATION AND MEMORY IN A TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE

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Type 2 diabetes mellitus, a pathology associated with increased blood glucose levels (hyperglycemia), has been linked to Alzheimer's disease (AD). Indeed, hyperglycemia, oxidative stress, and dysfunctional insulin signaling are common features of these two age-related diseases. Therefore, elucidate the molecular mechanisms underlying the neurodegenerative process that occurs in AD under hyperglycemia is of utmost relevance. Moreover, little is known about how the perturbation of glucose metabolism affects the formation of new neurons during adult hippocampal neurogenesis, a process that plays an important role in learning and memory. In order to evaluate whether hyperglycemia aggravates the alterations of hippocampal adult neurogenesis in AD and further impair memory, 2 month-old triple transgenic AD (3xTg-AD) mice were treated with an elevated dose of sucrose over 6 months. Non transgenic (NonTg) and 3xTg-AD mice treated with sucrose presented decreased glucose tolerance and increased levels of glycated hemoglobin, cholesterol and leptin, in comparison with the respective non-treated group. Untreated 3xTg-AD mice exhibited increased glucose tolerance, when compared to NonTg, which was associated to increased insulin levels. Interestingly, hyperglycemia had a negative effect on learning and spatial memory of 3xTg-AD mice, leading to the impairment of these cognitive functions. Furthermore, our results show that hyperglycemia potentiated cell proliferation in the subgranular zone of the dentate gyrus of the hippocampus, namely of neuroblasts, which in the case of sucrose-treated 3xTg-AD mice did not contribute to an increase in the total number of neuroblasts or immature neurons. In these mice, we also observed decreased dendrite complexity of immature neurons, when compared to untreated 3xTg-AD mice. These data suggest that hyperglycemia enhances AD pathology, contributing for the impairment in neurogenesis, defective learning and memory loss.

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METHAMPHETAMINE-INDUCED STRUCTURAL DAMAGE ON ENDOTHELIAL CELLS BENEFITS WITH ACETYL-L-CARNITINE TREATMENT

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INTRODUCTION: Methamphetamine (METH) is a potent psychostimulant used worldwide for its addictive properties. There is increasing evidence that METH exerts its toxicity also through disruption of the Blood-Brain-Barrier (BBB) function and integrity. As in other neurological conditions, degradation of tight junctions (TJs) by matrix metalloproteinases (MMPs) was identified as a consistent cause for METH-induced BBB disruption. Based on previous reports where acetyl-L-carnitine (ALC) administration was shown to ameliorate dystrophy and glomerular sclerosis by reducing MMP-9 activity, we hypothesized that ALC could prevent METH-induced MMPs activity contributing to maintain the integrity of the endothelial layer.

METHODS: We used the bEnd.3 endothelial cell line to evaluate the neuroprotective action of ALC over METH-induced damage. METH doses were selected through viability/cytotoxicity assays. ALC 1mM was added to the cultures 30 minutes prior to METH exposure. Twenty-four hours after exposure, cells were evaluated for cytoskeleton alteration and rearrangement and Tight Junctions (TJs) expression and distribution, through immunofluorescence and western blot. Zymography was used to assess MMP-9 activity and ILK knockdown was performed using shRNA-mediated silencing technology. PCR was used to assess mRNA levels alterations.

RESULTS AND DISCUSSION: Our results show that METH led to cytoskeleton disruption and rearrangement concomitant with claudin-5 translocation to the cytoplasm. These events were mediated by MMP-9 activation in association with ILK overexpression, which may contribute to BBB dysfunction. Pretreatment with ALC prevented METH-induced activation of MMP-9, preserving claudin-5 location and the structural arrangement of the actin filaments. The present results support the potential of ALC in preserving the BBB integrity, highlighting ILK as a nouvelle target for ALC therapeutic use.

IPS CELLS DERIVED FROM HUNTINGTON´S DISEASE PATIENTS AND THEIR DIFFERENTIATED COUNTERPARTS EXHIBIT MITOCHONDRIAL DYSFUNCTION AND METABOLIC DISTURBANCES

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Huntington's disease (HD) is an autosomal dominant disease caused by an expansion of CAG repeats in the HD gene encoding for huntingtin. Several pathological mechanisms have been proposed for neurodegeneration, including mitochondrial and metabolic dysfunction and oxidative stress. An attractive model to study disease mechanisms are HD patient-specific induced pluripotent stem cells (HD-iPSC). Indeed, HD-iPSC and HD-NSC can help to reveal the role of mitochondrial and metabolic dysfunction in early stages of HD. In this study we aimed to investigate the pathological mechanisms that have been proposed for neurodegeneration, including mitochondrial dysfunction and oxidative stress in HD patient-specific induced pluripotent stem cells (HD-iPSC) and differentiated HD-NSC. We successfully differentiated HD-iPSC into HD-NSC resorting to SMAD inhibitors. We show that HD-iPSCs mitochondria have more negative membrane potential and increased intracellular basal Ca^{2+} levels, but reduced mitochondrial Ca^{2+} storage capacity. Moreover, increased levels of mitochondrial superoxide anion and hydrogen peroxide production were observed in HD-iPSCs *versus* control iPSC (C-iPSC), and in HD-NSC. Similarly, HD-iPSCs produced higher levels of reactive oxygen species following an acute exposure to hydrogen peroxide. These results are consistent with the increased acetylation and decreased activity of superoxide dismutase 2 (SOD2) in HD cells. Furthermore, HD-iPSCs showed decreased complex I+III activity and mitochondria exhibited altered round shape morphology, however no differences in fission/fusion proteins were found. Additionally, HD-iPSCs and HD-NSC consumed less O_2 and resorted less on oxidative phosphorylation to produce ATP, which is in accordance with the observation that HD cells rely more on glycolysis and have lower ATP/ADP ratio. Moreover, HD-iPSCs showed increased phosphorylation of pyruvate dehydrogenase (PDH) subunit E1α at Ser 232, 293 and 300, reflecting its inactivation; concordantly, increased levels of pyruvate dehydrogenase kinase, isozyme 1 (PDK1) were found. Overall, our study suggests that mitochondrial dysfunction is evident in very early stages of HD cytopathogenesis.

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EFFECTS OF VITAMIN D DEFICIENCY AND/OR HIGH-FAT DIET ON BRAIN MITOCHONDRIAL BIOENERGETICS, INSULIN SIGNALING AND SYNAPTIC INTEGRITY

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The old saying "we are what we eat" reflects the actual facts of modern society. Indeed, epidemiologic evidence reveals that high-calorie diets and low levels of vitamin D increase the risk of developing metabolic disorders (e.g. diabetes) and dementia (e.g. Alzheimer's disease). This study was conducted to uncover the effect of vitamin D deficiency and/or high-fat diet on mitochondrial bioenergetics, insulin signaling and synaptic integrity in the brain cortex of 6-month-old Wistar rats. For this purpose, 2-month-old male Wistar rats were randomly divided into four groups: control (receiving a standard diet); VDD (receiving a vitamin D-free diet); HFD (receiving a high fat diet), and HFD+VDD (receiving a high fat diet free of vitamin D). Rats were maintained on these dietary regimens for a period of 4 months. No significant alterations were observed in the respiratory control ratio (RCR) and ADP/O indexes of brain cortical mitochondria isolated from all groups of rats. However, both VDD and HFD diets induced an increase in insulin receptor, active form of phosphatidylinositol 3-kinase (p^{110} PI3K) and cyclin-dependent kinase 5 (Cdk5) protein levels and a significant decrease in the active form of AMPK (p^{Thr172}-AMPK). Both dietary regimens also caused an increase in the levels of active glycogen synthase kinase-3β (p^{Tyr216}-GSK-3β) and of synaptic markers PSD-95 and SNAP-25. However, vitamin D deficiency did not potentiate the effect of high-fat diet. So far, these results suggest that a compensatory response is triggered in the brain cortex of young rats under vitamin D-free or high-fat dietary regimens in order to protect the brain against a metabolic disarrangement. Because aging is a major risk factor for metabolic disorders, future studies are warranted to determine the role of aging in the above-mentioned mechanisms.

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NEURONAL ADAPTATIONS TO GLYCEMIC VARIABILITY: INVOLVEMENT OF UCP2 IN *IN VITRO* GLUCOSE FLUCTUATIONS-MEDIATED EFFECTS

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Diabetes mellitus is a chronic metabolic disease involving high blood glucose levels which result from dysfunction in insulin secretion and/or action. Accumulating evidence suggests that glucose fluctuations, particularly if accompanied by hypoglycemia, may worsen the damaging effects of diabetes in several organs, including the brain, which is highly vulnerable to glucose and oxidative changes and so, at a higher risk for dysfunction under conditions of metabolic and oxidative stress. Under this theme, neuronal uncoupling proteins (UCP) have proven to have an important role in attenuating mitochondrial reactive oxygen species (ROS) production and in regulating cellular energy transduction. Thus, to assess the effects of glycemic variations in neuronal cells, primary cortical neurons underwent exposure to glucose variations (GV) or to constant high (HG) or low glucose (LG) levels for 10 hours. Furthermore, the role of UCP2 under our experimental conditions was investigated by using genipin, a specific inhibitor of UCP2. Cell viability, ROS production, intracellular antioxidant defenses levels, caspase 3 activation and the protein levels of glycogen synthase 3-beta (GSK-3β), nuclear factor (erythroid-derived 2)-like 2 (NRF2) and uncoupling protein 2 (UCP2) were analyzed. Exposure of cortical neurons to GV promoted a decrease in cell viability, together with an increase in ROS production. GV also induced a decrease in GSK-3β activation and an increase in glutathione levels, as well as of NRF2 and UCP2 protein levels, both proteins described to be involved in the protection against oxidative damage. However, the inhibition of UCP2 by genipin reverted these adaptations, promoting an increase in GSK-3β and in caspase 3 activation and a decrease in NRF2 protein and glutathione levels. In a similar way, the exposure of cortical neurons to LG promoted an increase in NRF2 and UCP2 protein levels, effects that were reverted with genipin, which also led to an increase in ROS production. On the other hand, the constant exposure of cortical neurons to HG promoted a significant increase in ROS production and a slight decrease in NRF2 levels, which was not altered by genipin. Overall, results suggest that primary cortical neurons under GV develop adaptation mechanisms to cope with this metabolic challenge and that UCP2 may have a role in preventing or attenuating glucose fluctuations-mediated neuronal injury.

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ALTERATIONS IN BRAIN MITOCHONDRIA, AUTOPHAGY AND SYNAPTIC INTEGRITY: SHARED FEATURES OF ALZHEIMER'S DISEASE AND TYPE 2 DIABETIC BRAINS

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Maintenance of cellular homeostasis depends on several mechanisms. An important mechanism involved in cellular homeostasis is autophagy, which is involved in the removal of misfolded proteins and damaged organelles. Mitochondria, the primary energy sources of neurons, when damaged should be removed by autophagy. In normal conditions this process is tightly regulated and coupled with mitochondrial biogenesis ensuring the maintenance of a healthy mitochondrial pool. Because mitochondrial impairment was found to be a mechanistic link between Alzheimer's disease (AD) and type 2 diabetes (T2D), this study aimed to explore how autophagy and mitochondrial biogenesis contribute to mitochondrial and, consequently, synaptic anomalies. Isolated brain mitochondria and homogenates from cerebral cortex and hippocampus of 11-month-old male wild type (WT), triple transgenic AD (3xTg-AD) and T2D mice were used to evaluate mitochondrial functional parameters and protein levels of mitochondrial biogenesis, autophagy and synaptic markers, respectively. A significant decrease in mitochondrial respiration, membrane potential and ATP production was observed in brain mitochondria isolated from T2D and 3xTg-AD mice. Also, a significant decrease in the levels of autophagy-related protein 7 (ATG7) and glycosylated lysosomal membrane protein 1 (LAMP1) was observed in the cerebral cortex and hippocampus of T2D and 3xTg-AD mice. Moreover, both brain regions of 3xTg-AD mice presented lower levels of nuclear respiratory factor (NRF) 1 while the levels of NRF2 are lower in both brain regions of T2D and 3xTg-AD mice. Also, a decrease in mitochondrial encoded, nicotinamide adenine dinucleotide dehydrogenase subunit 1 (ND1) was observed in T2D and 3xTg-AD mice although only statistically significant in T2D cortex. Furthermore, a decrease in the levels of postsynaptic density protein 95 (PSD95) in the cerebral cortex of 3xTg-AD mice and in the hippocampus of T2D and 3xTg-AD mice as well as a decrease in the levels of synaptosomal-associated protein 25 (SNAP 25) in the hippocampus of T2D and 3xTg-AD mice were observed suggesting synaptic integrity loss. These results support the idea that alterations in mitochondrial function and biogenesis and autophagy cause synaptic damage in AD and T2D.

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ACTIONS OF THE ANTI-DIABETIC DRUG LIRAGLUTIDE IN ALZHEIMER DISEASE: BRAIN GLUCOSE METABOLISM, NEUROINFLAMMATION AND COGNITION

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The development of cognitive deficits in type 2 diabetes (T2D), and the overlapping pathways between T2D and neurodegenerative diseases opened the door to the study of anti-diabetic therapies in neurodegeneration. Indeed, one of the last drugs approved for the treatment of T2D patients, the long-acting glucagon-like peptide-1 (GLP-1) agonist liraglutide, has already demonstrated some neuroprotective effects in T2D, Alzheimer and Parkinson diseases models. Thus, in our work we hypothesized that the chronic subcutaneous exposure to liraglutide protects against *in vivo* brain metabolic and cognitive dysfunction associated with Alzheimer disease (AD) progression. Hence, we aimed to evaluate the impact of a chronic peripheral liraglutide administration in a mouse model of AD. Therefore, we used blood samples and brain cortical homogenates from mature (11 month-old) female triple transgenic AD (3xTgAD) mice treated with liraglutide (s.c. 0.2mg/kg, once/day, 28 days). Age-matched wild-type female mice were used as controls. We observed that liraglutide was able to normalize blood glucose levels in 3xTgAD mice, decreased glycated hemoglobin (HbA1C) and plasma insulin levels, thus increasing the insulinotropic response. In brain cortical homogenates, we also observed that the regularization of glucose levels in the 3xTgAD females injected with liraglutide was accompanied by increased activities of some enzymes involved in the metabolism of glucose, namely hexokinase, glucose-6-phosphate dehydrogenase and lactate dehydrogenase. Moreover, liraglutide efficacy against glucose dyshomeostasis may underlie the significant reduction in inflammatory (interleukin-1β and C-Reactive Protein) and oxidative stress (thiobarbituric acid reactive substances, 8-hydroxy-2'-deoxyguanosine, nitrites, hydroxide peroxide and carbonyl groups) markers in plasma and brain cortex of 3xTgAD mice. The beneficial effects of liraglutide were also reflected in the amelioration of AD neuropathological hallmarks (as given by the amyloid beta peptide levels and phosphorylated residues of Tau protein) and cognitive function (namely in the spatial memory, analyzed by the Morris watermaze and Y-Maze tests). Our observations strongly suggest that the normalization of glucose homeostasis promoted by chronic peripheral liraglutide therapy may prevent the development of AD pathology, ultimately restoring cognitive function.

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ATP AS A DANGER SIGNAL IN PARKINSON'S DISEASE MODELS - NEUROPROTECTION BY P2Y1 RECEPTOR BLOCKADE

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ATP is the most abundant intracellular molecule responsible for energy transfer; however, it also acts as an intercellular signaling molecule and is a major danger signal in different noxious brain conditions (Rodrigues *et al.*, 2015, Front. Neuropharmacol. in press). Accordingly, antagonists of ATP P2 receptors afford neuroprotection in different brain conditions such as Alzheimer's disease, epilepsy, ischemia or trauma (Rodrigues *et al.*, 2015). Since the possible role of ATP as a danger signal has not been explored in Parkinson's disease (PD), we now tested if ATP might also contribute to neurodegeneration in cellular and animal models of PD. Dopamine-differentiated neuroblastoma SH-SY5Y cells became sensitive to 6-hydroxydopamine (6-OHDA), a toxin used to model PD, as gauged by the decreased reduction potential (MTT reduction), by the appearance of calpain-cleavage products of spectrin and by the release of lactate dehydrogenase (LDH) upon exposure to 30 μM 6-OHDA for 24 hours. This was accompanied by an increase of the extracellular levels of ATP from a basal level of 2.84±0.62 nM to 4.74±0.88 nM at 24 hours of exposure to 30 μM 6-OHDA (n=16). The generic P2 receptor antagonist PPADS (10 μM) prevented 6-OHDA-induced toxicity, an effect mimicked by the presence of apyrase (20 U/mL), an enzyme that hydrolyzes ATP, added before 6-OHDA. Furthermore, the selective P2Y1 receptor antagonist MRS2500 (10 μM) also prevented 6-OHDA-induced toxicity, in accordance with the co-localization of P2Y1 receptor immunoreactivity with tyrosine hydroxylase (a marker of monoaminergic neurons) in the differentiated SH-SY5Y cells. The intra-striatal unilateral injection of 6-OHDA (18 μg/3 μL) in adult rats triggered the pathognomonic PD symptoms of decreased striatal and mesolimbic dopamine and DOPAC levels together with a reduction of tyrosine hydroxylase immunoreactivity in the substantia nigra and striatum after 7 days, accompanied by motor deficits typified by an increased contralateral rotation upon exposure to apomorphine (0.6 mg/kg), an increased ipsilateral limb usage in the cylinder test and a decreased of vertical (not horizontal) exploratory activity in the open field test. The intracerebroventricular perfusion of PPADS (1 nmol/μL with osmotic mini-pumps for two weeks) attenuated the 6-OHDA-induced dopamine loss and motor impairment, an effect mimicked by MRS2500 (2 nmol/μL), whereas both antagonists were devoid of effects in control rats. Overall, these results indicate that ATP acts as a danger signal contributing to neurodegeneration through P2Y1 receptor activation in cellular and animal models of PD, heralding the possibility that P2Y1 receptor blockade might be a novel therapeutic candidate to manage PD.

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PREDOMINANT SUSCEPTIBILITY OF MITOCHONDRIAL MEMBRANE POTENTIAL FROM GLUTAMATERGIC NERVE TERMINALS IN AN *IN VITRO* MODEL OF EARLY ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is defined by a loss of cognitive function associated with an abnormal processing and accumulation of amyloid β-peptide (namely $A\beta_{1-42}$) and by a hypometabolism and hypofunction of brain cortical regions, mainly the hippocampus. With the aim to arrest the evolution of AD, particular attention has been devoted to synaptic dysfunction and loss, which are the precocious modifications accompanying cognitive dysfunction. We have recently reported that glutamatergic synapses are particularly susceptible to damage in an Aβ-based model of AD (Canas *et al.*, 2014, Neuropharmacology 76:51), but the underlying mechanisms are still unsolved. Since mitochondria play a key role in the maintenance of adequate synaptic function, and given that mitochondrial structural and functional abnormalities are well-characterized features of AD, we now tested the hypothesis that mitochondria located in glutamatergic terminals are particularly affected in AD, leading to modifications of calcium balance and energy power supply that underlie the early synaptic degeneration in AD. We carried out live imaging experiments to measure the changes of the mitochondrial membrane potential Δ ($\Delta\psi_m$), using a fluorescent probe, $TMRM^+$, with oligomycin and FCCP used as stimuli, in hippocampal nerve terminals incubated with oligomeric $A\beta_{1-42}$ peptide (500 nM for 2 hours). We report a reduction of 23.0±5.2% (n=6) of $\Delta\psi_m$ after incubation of the nerve terminals with $A\beta_{1-42}$ peptide, without modification of the plasma membrane potential measured using the fluorescent probe PMPI. This reduction was mostly observed in glutamatergic nerve terminals (immunopositive for vesicular glutamate transporters type 1), as confirmed by immunocytochemistry. These results are in agreement with the contention that synaptic mitochondria are an important trigger of "synaptic apoptosis", contributing to synaptic dysfunction and degeneration in AD and further indicate glutamatergic terminals as primary targets of $A\beta_{1-42}$-induced toxicity. This prompts the correction of synaptic mitochondria dysfunction as an increasingly justifiable candidate to therapeutically alleviate early AD.

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DOWNREGULATION OF GABAERGIC SYNAPSES AND NEURONAL DEATH IN CEREBRAL ISCHEMIA

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Cerebral ischemia is a pathological condition caused by insufficient blood supply to the brain, characterized by an early pre- and post-synaptic disruption of GABAergic neurotransmission. GABAergic downmodulation contributes to alter neuronal excitability, causing an imbalance between excitatory/inhibitory neurotransmission and excitotoxic neuronal death. GABAA receptors ($GABA_AR$) are the major players in fast synaptic inhibition in the CNS. These receptors present a dynamic mobility between synaptic and extrasynaptic localization, being the accumulation of $GABA_AR$ at inhibitory synapses regulated by the scaffold protein gephyrin. Furthermore, surface $GABA_AR$ cycle continuously between the plasma membrane and intracellular compartments, and the regulation of the total receptor surface expression plays a key role in the control of the postsynaptic pool size and the strength of synaptic inhibition. We have investigated the molecular mechanisms underlying $GABA_AR$ downregulation in cultured hippocampal neurons subjected to Oxygen Glucose Deprivation (OGD), an *in vitro* model of ischemia. Through biochemical approaches and cell imaging we found that OGD decreases $GABA_AR$/Gephyrin interaction by a calcineurin-dependent mechanism, and induces the internalization of $GABA_AR$ via clathrin-dependent endocytosis. The OGD-induced dephosphorylation and internalization of β3 $GABA_AR$ contributes to neuronal cell death, as demonstrated using a phospho-mutant form of the β3 $GABA_AR$ subunit. The entry of Ca^{2+} following OGD also activates calpains, which were found to cleave gephyrin under *in vitro* and *in vivo* brain ischemia, giving rise to a stable cleavage product. These truncated forms of gephyrin contribute to the disassembly of the postsynaptic gephyrin clusters, with a consequent loss of the neuroprotective activity mediated by synaptic $GABA_AR$.

Under physiological conditions, following internalization, $GABA_ARs$ are rapidly recycled back to the plasma membrane or targeted for lysosomal degradation. The decision regarding the sorting of endocytosed $GABA_ARs$ depends on the interaction of $GABA_AR$ β1-3 subunits with the huntingtin-associated protein 1 (HAP1). We found that OGD also reduces the recycling of $GABA_AR$ back to the plasma membrane and decreases their interaction with the HAP1 protein. Transfection of hippocampal neurons with HAP1 increased the surface expression of $GABA_AR$ and decreased OGD-induced neuronal death. Overall, we propose a new model in which the dissociation of $GABA_AR$/gephyrin, gephyrin cleavage, $GABA_AR$ receptor dephosphorylation and a downregulation of the receptor recycling mechanisms are key steps in GABAergic downmodulation during cerebral ischemia and consequent neuronal cell death.

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ROLE OF TRANSTHYRETIN IN AMYLOID PLAQUES FORMATION USING A CELL MODEL

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Alzheimer's disease (AD) is the most common type of dementia, and it is well known that Abeta fibrillization and extracellular deposition plays a major role in AD's pathology, leading to the formation of amyloid plaques. Thus, preventing the formation of amyloid plaques and, subsequently, some of its symptoms, would be a great progress. For that, it is necessary to have proper AD disease models, either *in vivo* or *in vitro*. Another feature of AD is its complexity, in such way that *in vivo* models (e.g. transgenic mice), despite being closer to reality, require caution when analyzing the results. Thus, the simpler *in vitro* cellular models appear to be best suited to the study of molecular mechanisms. An interesting model for amyloid plaque has been described using THP-1 differentiated cells (1), since it was suggested that microglial cells are of specific importance for the formation, maturation or clearance of amyloid plaques (2). In our lab, we were able to reproduce this model and produce amyloid plaques (confirmed by Congo Red staining). Previously, we have shown that transthyretin (TTR), the major binding partner of Abeta in the CSF, has the ability to inhibit Abeta fibril formation and to disaggregate pre-formed fibrils, *in vitro*. Furthermore, we showed that these effects relate to TTR stability, such that TTR mutations that destabilize the tetramer structure affect negatively its neuroprotective potential, whereas the opposite is observed for stabilizing mutations. In addition, we showed that the TTR/Abeta binding and the TTR-conferred protection in AD can be improved by TTR tetrameric stabilizers (3).

Thus, our general aim is to use the above mentioned cellular model to study the mechanisms underlying TTR protection in AD, namely TTR proteolytic elimination of Abeta amyloid plaques. This is an ongoing project and, so far, we observed that the presence of TTR, whilst not absolute, has significant effect in the number and morphology of amyloid plaques. TTR decreases the amount and size of plaques, both if added prior or after amyloid plaque formation. We have also used a proteolytic inactive mutant of TTR and our preliminary results show an increase in the number of plaques, when comparing with the WT form. Despite being an excellent model for some AD studies, this model uses solely macrophage cells, thus, we are trying to develop a more realistic model using also neuronal cells (SH-SY5Y) to form amyloid plaques.

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EXACERBATED MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE STRESS FOLLOWING SIRT3 OVEREXPRESSION IN HUNTINGTON'S DISEASE MICE STRIATAL CELLS

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Protein acetylation, which is central to transcriptional control as well as other cellular processes, is disrupted in Huntington's disease (HD), a fatal progressive neurodegenerative disorder caused by an expanded polyglutamine stretch in the huntingtin (Htt) protein. Although a direct causative pathway from protein mutation to the selective neostriatal neurodegeneration remains unclear, many lines of evidence suggest that mitochondrial dysfunction and reactive oxygen species (ROS) production play a prominent role in HD pathogenesis. SIRT3 is a member of the sirtuin family of protein deacetylases that is located in mitochondria and serves as a primary regulator of mitochondrial protein acetylation, controlling mitochondrial metabolism, redox status and cell death. Given the versatile nature of SIRT3 metabolic functions, we speculated that its targeting might influence HD neurodegeneration. Here, we showed that STH*dh*$^{Q111/Q111}$ striatal cells, expressing mutant Htt with 111 glutamines, exhibited a significant increase in both SIRT3 protein and mRNA levels, along with increased SIRT3 enzymatic activity, in comparison with STH*dh*$^{Q7/Q7}$ wild-type cells, which is in accordance with increased deacetylation and activity of superoxide dismutase 2, the main mitochondrial antioxidant enzyme regulated by SIRT3. This increase was also verified in lymphoblasts from HD patients. Overexpression (OE) of SIRT3 in striatal cells decreased lysine acetylation and cell viability, which might be triggered by increased levels of ROS, namely mitochondrial superoxide anion and hydrogen peroxide. Additionally, both STH*dh*$^{Q7/Q7}$ and STH*dh*$^{Q111/Q111}$-SIRT3 cells exhibited decreased mitochondrial membrane potential and, in the case of SIRT3-transfected wild-type cells, this was accompanied by increased mitochondrial calcium levels. Overall, data implicate that increased levels of mitochondrial SIRT3 exert negative effects in cells expressing full-length mutant Htt, which may result from higher production of mitochondrial ROS.

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WHY IS AGING THE MAJOR RISK FACTOR FOR BETA-AMYLOID ACCUMULATION?

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Late-onset Alzheimer´s disease is a disease of the elderly, aging being the most critical risk factor. Even in early-onset AD aging is required for the disease to develop; although Aβ production is increased since birth the disease only manifests after 35-45 years. Neurons are highly sensitive to aging because because being mostly post-mitotic they can live a life-span thus accumulate high cellular stress. The underlying cellular and molecular mechanisms of neuronal aging remain elusive.

Primary neuronal cultures of embryonic mouse hippocampus or cortex undergo a stereotyped process of differentiation. When plated these cells differentiate axons and dendrites after a few hours. By two weeks in culture have established synapses. In our hands synapse formation occurs until three weeks in culture (21DIV). After that, at 28DIV we found that synaptic markers no longer increase, instead stabilize or show a small decrease. At 28DIV we did not observe neuronal death or processes degeneration. Instead 28DIV neurons evidence early signs of aging, such as accumulation of late-endosomes/lysosomes and lipofuscin, especially in processes.

In the brain Aβ progressively accumulates with aging, but *in vivo* secreted Aβ cannot be differentiated from Aβ that gets retained intracellularly. In culture, one can measure secreted Aβ in cultured media and cellular Aβ. Indeed, Bart de Strooper and colleagues have described that in wild-type neurons Aβ secretion increases with aging. Aβ intracellular accumulation increases with aging in transgenic early-onset AD models but in normal wild-type conditions it is not clear. We have measured endogenous intracellular Aβ42 (iAβ42) accumulation in neurons using a method developed to analyse the differential accumulation of Aβ42 in the cell body and processes (axons plus dendrites) of wild-type neurons. We have observed that Aβ42 levels were increased by 30% in cell bodies and by 60% in processes of 28DIV neurons compared to 21DIV neurons. This increase might be due to an endogenous increase in APP levels or/and by an increase in APP processing in aging neurons. We found the levels of total APP to be slightly increased by 17%. A more significant increase of 62% was found in the ratio of APP CTFs per total APP in 28DIV neurons compared to 21DIV neurons. More important was the 90% increase in ratio APP β-CTF/APP in 28DIV neurons compared to 21DIV neurons. These results indicate an up-regulation APP processing with aging in particular an increase in BACE-1 activity. BACE-1 brain activity was also found increased with aging. Why BACE1 activity increases with aging is not known. We are currently investigating if alterations in the endocytic and secretory pathways of BACE1 and APP occur in aging neurons.

THE IMPACT OF PHOSPHOLIPASE D GENETIC ABLATION IN THE MOUSE HIPPOCAMPUS

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Over the past years increasing amount of attention has been given to signalling lipids and its modulating enzymes, such as phospholipase D (PLD), that converts phosphatydilcholine to phosphatidic acid (Jenkins and Frohman, 2005). Several studies have been associating PLD1 and PLD2, the two mammalian PLD isozymes, to neurological events, including neurotransmitter release (Humeau *et al.*, 2001), dendritic branching (Zhu *et al.*, 2012; Ammar *et al.*, 2013), cognition and brain development (Burkhardt *et al.*, 2014). Also, the hippocampus has been suggested as one of the brain regions showing the highest PLD activity (Kobayashi *et al.*, 1988) and neurodegenerative conditions such as Alzheimer's disease associated pathways have been shown to be modulated by PLD signalling (Oliveira *et al.*, 2010). Thus, the aim of this project is to understand the potential role of PLD in hippocampal function in adult mice with Pld1 or Pld2 genetic ablation.

Our behavioural characterization, specifically considering motor activity, anxiety and memory, showed that PLD2 knockout mice behaviour is not altered when compared to their wild type littermates. Although most of this was also observed in the animals lacking PLD1, the results indicated an object recognition-dependent short-term memory deficit, and, so our results so far suggest that the ablation of PLD1 may have a greater impact in the hippocampus than PLD2. We are currently evaluating the hippocampal dendritic morphology in our animal models, as well as synaptic protein levels and distribution.

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ROLE OF TRANSTHYRETIN IN Aβ TRANSPORT: FROM THE BRAIN TO THE LIVER

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INTRODUCTION: Transthyretin (TTR) is a protective molecule in Alzheimer's disease (AD) and is decreased in AD patients, contributing to disease development. AD transgenic mice carrying one copy of TTR show increased Aβ brain and plasma levels, when compared to animals with two copies of the gene, leading to the hypothesis that TTR is involved in Aβ brain efflux and/or peripheral clearance (1). It is also known that Aβ can be incorporated in HDL to be further delivered to the liver for degradation (2). Curiously, a fraction of TTR is transported in HDL (3), thus it is possible that TTR participates in Aβ clearance at the liver. This project aims at investigating the influence of TTR in Aβ transport across the blood-brain barrier (BBB) and its uptake by the liver.

METHODS: Human cerebral microvascular endothelial (hCMEC/D3) cells, as a cellular model of the BBB, were incubated with fluorescent Aβ 1-42 (FAM-Aβ) in the presence or absence of TTR, and Aβ internalization levels were assessed fluorometrically in cell lysates. In an *in vivo* assay, FAM-Aβ was injected intracranially in mice with one copy of the TTR gene (TTR+/-) or without TTR gene (TTR-/-). Aβ levels in brain lysates were measured by ELISA. We also evaluated plasma Aβ1-42 and Aβ1-40 levels in AD/TTR female mice at different ages and genotypes for TTR, using ELISA. To assess TTR effects in Aβ uptake by the liver, SAHep cells and primary hepatocytes (from mice with different TTR genetic backgrounds) were incubated with FAM-Aβ and internalization was measured by Flow Cytometry. We also performed immunocytochemistry to observe the colocalization of TTR and FAM-Aβ in SAHep cells using fluorescence microscopy.

RESULTS: In the presence of TTR, Aβ internalization by hCMEC/D3 cells was increased. Importantly, *in vivo* results indicated that brains from TTR+/- mice retained less Aβ than TTR-/- animals, supporting a TTR role in Aβ brain efflux. As for plasma Aβ1-42/Aβ1-40 ratios, these were found elevated in 6 month old AD/TTR+/- mice when compared to AD/TTR+/+, suggesting a role for TTR also in Aβ peripheral elimination. Interestingly, TTR also promoted Aβ internalization in both SAHep cells and primary hepatocytes. Fluorescence microscopy confirmed colocalization of Aβ and TTR in SAHep cells.

CONCLUSION: Based on different approaches used, we propose that TTR promotes Aβ efflux through the BBB, preventing its accumulation in the brain and deposition in extra neuronal plaques. Also, TTR increases Aβ uptake by hepatocytes suggesting its participation in peripheral Aβ clearance at the liver. Given our results, together with the reported decreased TTR levels in AD, restoring TTR levels might constitute a therapeutic approach for AD.

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BLOCKADE OF ADENOSINE A_{2A} RECEPTOR PREVENTS RETINAL GANGLION CELL LOSS THROUGH THE CONTROL OF NEUROINFLAMMATION: POSSIBLE ROLE IN GLAUCOMA

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Neuroinflammation mediated by microglial cells has been associated with the development and progression of retinal disorders. Retinal ischemia contributes to several ocular diseases including diabetic retinopathy and glaucoma, and causes damage to the retina, including loss of retinal ganglion cells (RGCs). Neuroinflammation and microglia reactivity play an important role for the death of retinal cells. Increasing evidence has demonstrated that blocking A_{2A} receptor ($A_{2A}R$) prevents neurodegeneration by modulating the release of noxious factors by activated microglia. The aim of this work was to evaluate whether $A_{2A}R$ blockade prevents microglial reactivity and cell death induced by retinal ischemia-reperfusion (I-R) injury.

Retinal ischemia was induced in one eye by elevating the intraocular pressure (IOP) for 60 min, which was followed by 24 h of reperfusion. The contralateral eye served as the control eye. The $A_{2A}R$ antagonist was administrated by intravitreal injection (SCH 58261; 100 nM, 5 µl) prior to I-R.

Retinal microglial reactivity induced by I-R injury was prevented by administration of SCH 58261. The $A_{2A}R$ antagonist inhibited the increase triggered by I-R injury in the expression and levels of inflammatory cytokines IL-1β and TNF, as well as GFAP expression. The increased number of retinal cell death by apoptosis induced by I-R was significantly decreased in retinas treated with $A_{2A}R$ antagonist. Additionally, blockade of $A_{2A}R$ prevented RGC loss induced by I-R injury. When the actions of TNF and IL-1β were neutralized with antibodies, the loss of RGC induced by I-R was attenuated.

These results show that $A_{2A}R$ antagonist prevents microglia reactivity and affords protection to the retina, and that inflammation contributes to RGC loss induced by I-R. These results indicate that $A_{2A}R$ blockade can be used as potential therapeutic strategy for the treatment of retinal diseases involving neuroinflammation.

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CONDITIONAL FOXP2 DELETIONS DIFFERENTIALLY AFFECT MOTOR-SKILL LEARNING

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Disruptions of the *FOXP2* gene cause a rare speech and language disorder. In the KE family a heterozygous *FOXP2* mutation is dominantly inherited and affected individuals have difficulty producing the sequences of orofacial motor movements necessary for fluent speech. This is considered a core deficit of the disorder, although other expressive and receptive language problems also exist. The FOXP2 transcription factor is expressed in cortico-striatal/ -cerebellar circuits required for sensorimotor integration and motor-skill learning, and imaging studies have identified structural abnormalities in several of these regions in affected KE-family members. FOXP2 is also highly conserved in a number of other vertebrate species, where expression is seen both during development and in adulthood. Mice carrying the KE-family mutation have motor-skill learning deficits and lack striatal long-term depression. They also have abnormally high striatal activity *in vivo* which is aberrantly modulated during the learning of a motor task. Juvenile zebra finches show increased FoxP2 expression during the song learning period in striatal nucleus Area X, and FoxP2 knockdown in this region results in inaccurate and incomplete song imitation. FoxP2 knockdown in Area X of mature birds renders song more variable and abolishes the mediation of song by social context.

We used a conditional *Foxp2* mouse line to selectively delete Foxp2 from the cortex, striatum or cerebellar Purkinje cells. This genetic approach was combined with an operant task where a sequence of 8 lever presses must be completed to obtain a food reinforcer. After 12 days a time constraint was added and the sequence had to be performed at increasingly high speeds. Both cerebellar and striatal Foxp2 mutants showed a reduced rate of lever pressing during training compared to controls. However, analyses of behavioural microstructure revealed that in cerebellar mutants pressing of all speeds is altered, whereas in striatal and cortical mutants rapid pressing is primarily affected. Furthermore, in striatal mutants rapid pressing also becomes more variable.

In a separate experiment we used a tamoxifen-inducible Cre to disrupt Foxp2 globally in adult mice. Around one third of Cre positive animals died, with the first deaths occurring 6 weeks after tamoxifen administration. Surviving animals appeared healthy but showed a reduced press rate on the motor-sequence learning task.

IMPACT OF HIGH-FAT DIET AND VITAMIN D DEFICIENCY IN TYPE 2 DIABETIC BRAINS: A FOCUS ON HIPPOCAMPAL INSULIN SIGNALING

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Type 2 diabetes mellitus (T2DM) is a metabolic disorder that reached epidemic proportions, affecting almost 350 million people around the globe. Most cases of T2DM result from unhealthy eating habits. In fact, consumption of a high-fat diet and high intake of saturated fat are associated with an increased risk of obesity, metabolic syndrome and T2DM. A common characteristic found in T2DM patients is the low levels of serum vitamin D (vitD). VitD is a fat-soluble hormone that shares common signaling pathways with insulin modulating glucose homeostasis and brain function, among other things. Thus, and given the growing evidence that T2DM is a risk factor for the development of neurodegenerative disorders, we hypothesize that high-fat diet and deficiency of vitD potentiate brain insulin signaling alterations in T2DM hippocampus. To test our hypothesis, we studied the insulin signaling pathway in hippocampal homogenates from 6-month-old Wistar (W) and T2DM Goto-Kakisaki (GK) rats subjected to distinct dietary regimens. For this purpose, 2-month-old GK rats were randomly divided in 4 groups and exposed to different diets during 4 months as follows: 1) GK rats fed with standard diet (GK); 2) GK rats fed with a vitamin D-deficient diet (GK w/vitD); 3) GK rats fed with a high-fat diet (GK HFD); 4) and GK rats fed with a vitamin D-deficient HFD diet (GK HFD w/vitD). A slight increase in the protein levels of insulin receptor substrate 1 (IRS1) phosphoinositide 3-kinase (PI3K) p110 as well as in the levels of phosphorylated extracellular signal-regulated kinase (pERK), protein kinase B (pAKT), c-Jun N-terminal kinase (pJNK) and 5' AMP-activated protein kinase (pAMPK) was observed in GK rats. A decrease in glycogen synthase kinase 3 beta (GSK3β) phosphorylated at tyrosine 216 residue (active form) was also observed in GK rats. However, an increase in cyclin-dependent kinase 5 (CDK5) and p25 protein levels was observed in GK rats, resulting in an increase in tau protein phosphorylated at threonine 181 residue. Compared to GK rats, GK HFD rats presented a slight increase in the protein levels of insulin-like growth factor 1 receptor (IGF1R), IRS1, insulin receptor substrate 2 (IRS2), mammalian target of rapamycin (mTOR) and GSK3β phosphorylated at tyrosine 216 residue. A decrease in pAKT and pAMPK protein levels was also observed in GK HFD rats. Furthermore, HFD promoted an increase in tau phosphorylation in both serine 396 and threonine 181 residues. Compared to GK rats, GK w/vitD rats presented a slight decrease in the levels of IRS1, pERK and pAKT. Additionally, a decrease in the levels of IGF1R, IRS1, CDK5 and pIRS2, pERK and phosphorylated tau was observed in GK HFD w/vitD rats when compared with GK HFD rats. Our results suggest that HFD and vitD deficiency affect hippocampal insulin signaling, although compensation mechanisms seem to occur to overcome metabolic disturbances-induced brain damage.

LOSS OF HUMMR PARTICIPATES ON MITOCHONDRIAL TRAFFIC JAMS IN ALZHEIMER'S DISEASE

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Alterations in axonal transport of mitochondria play a critical role in Alzheimer's disease (AD) neuropathology; however, the molecular mechanisms involved remain unexplored. This study was conducted to unveil the role of the hypoxia up-regulated mitochondrial movement regulator (HUMMR - a protein that favors the anterograde movement of mitochondria in a hypoxia-inducible factor 1 (HIF-1α)-dependent process) on defective mitochondrial trafficking during the course of AD pathology. Using human post-mortem brain cortex and hippocampus from AD subjects and differentiated SH-SY5Y cells (resemble mature neurons) exposed to amyloid-β 1-42 ($A\beta_{1-42}$), we evaluated HIF-1α and HUMMR protein levels and mRNA by Western blotting and RT-PCR, respectively, and mitochondrial function and dynamics by fluorimetry and confocal microscopy. A progressive reduction in HIF-1α and HUMMR protein levels and mRNA was observed with increasing AD Braak stage. Interestingly, low levels of the amyloidogenic peptide $A\beta_{1-42}$ (1 μM; 24 hours) increased both HIF-1α and HUMMR protein levels in mature neurons without affecting mitochondrial function and transport and synaptic integrity. Meanwhile, mature neurons treated with high levels of Aβ1-42 (10μM; 24 hours) exhibit a marked reduction in HUMMR protein levels, reduced number of mitochondria presented in the axons with the concomitant accumulation of these organelles in the perinuclear region, neuritic retraction and loss of synaptic integrity as evidenced by diminished SNAP-25 protein levels. These results suggest that during the initial phases of AD pathology, HUMMR sustains the anterograde movement of mitochondria in order to cope with the energetic demands within the synapses, acting as a cell quality control mechanism. However, with the progression of the disease this mechanism fails contributing to an energy crisis and, consequently to synaptic and neuronal loss.

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BRAIN CORTICAL GRAY MATTER VOLUMES ARE CHANGED BY MULTISENSORY DEPRIVATION IN USHER SYNDROME

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Usher syndrome (USH) is a severely debilitating disease being the most common form of inherited deaf-blindness. It is mainly characterized by the occurrence of visual and sensorineural hearing loss. Olfactory and vestibular dysfunction may be also present. Although these patients experience a severe multisensory deprivation, central nervous system structural changes have been scarcely described in the literature. In this work we characterized CNS structural changes using a more comprehensive approach than previously done.

Fourteen USH patients type I and II (8 males, mean age 40±6 years, 1 left-handed and 1 ambidextrous) and 16 age- and gender-matched healthy controls (8 males, mean age 41±11, 1 left-handed and 1 ambidextrous) were scanned in a 3 Tesla Siemens scanner.

All patients had visual peripheral field loss with central vision below 10 degrees.

Using *Freesurfer* 5.3 we obtained global cortical gray matter, white matter and subcortical gray volumes. For further analyses two components of the cortical volume were also extracted: cortical thickness (CT) and surface area (SA), and different subcortical regions were studied.

While no differences were detected for the white matter volume (p=0.23), patients showed an overall reduced cortical gray matter volume (p=0.04). Interestingly, when analyzing CT and SA, increases and decreases were present. Patients had reduced CT in the occipital lobe [bilateral lingual gyrus (p≤0.01), left lateral occipital cortex (p=0.02), right cuneus (p<0.01)]); while increased CT was present in the bilateral superior frontal gyrus (p≤0.03), and in the left supramarginal gyrus (p=0.02).

Cortical SA decreases were detected in the occipital lobe [bilateral cuneus (p≤0.01), left lingual gyrus (p=0.02), right pericalcarine (p=0.02), and right lateral occipital cortex (p<0.01)], while increases were present in the right precentral gyrus (p<0.01), paracentral lobule (p<0.01), pars orbitalis (p=0.04), rostral middle frontal gyrus (p=0.04), and left lateral orbitofrontal cortex (p=0.02).

Additionally, at the subcortical level reduced overall gray matter volume was detected (p=0.03), with reduction of the bilateral globus pallidum volume (p≤0.01).

Our results are in line with previous reports of decreased brain size in USH. Moreover, we were able to determine that this reduction is attributable to reduced overall cortical and subcortical gray matter volumes. However, at the cortical level volume increases and decreases were both present. Areas receiving visual input were withered, while motor, somatosensory, and frontal regions increased their representations suggesting evidence for functional reorganization.

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BIN1 AND CD2AP DIFFERENTIALLY REGULATE THE ENDOCYTIC GENERATION OF AMYLOID-β

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Despite the intensive research on Alzheimer´s disease (AD) an effective treatment is still missing. Amyloid-β (Aβ) production and accumulation plays a central role in the pathogenesis of AD. Aβ is produced in endosomes by sequential cleavage of the amyloid precursor protein (APP) by β- and γ-secretases. The rate-limiting step in Aβ generation is the encounter in endosomes of APP with the aspartyl protease β-site APP-cleaving enzyme-1 (BACE1), the main neuronal β-secretase. Interestingly, APP and BACE1 follow distinct trafficking pathways converging only in early endosomes. Several genes were discovered, by genome wide association studies, to contribute to the risk for late-onset AD. Two strong candidates for increasing AD susceptibility are Bridging Integrator 1 (BIN1) and CD2-associated protein (CD2AP). However, if and how these risk factors contribute to Aβ generation and thus increase the risk of developing AD is not known. BIN1 and CD2AP are endocytic trafficking regulators. Endocytic trafficking has been implicated in Aβ generation and AD. Aβ generation in endosomes is dependent on the endocytic trafficking of APP and BACE1. We hypothesized that BIN1 and CD2AP control Aβ generation by regulation of the endocytic trafficking of APP and BACE1. We demonstrated that both loss and gain of function of BIN1 and CD2AP increase the endogenous production of Aβ in primary neurons and neuronal cells. We have preliminary data suggesting that BIN1 controls Aβ generation in axons whereas CD2AP controls Aβ generation in dendrites. Utilizing pulse chase experiments we investigated whether BIN1 and CD2AP would affect the endocytic trafficking of APP and BACE1 to regulate their encounter in endosomes. We discovered that BIN1 loss of function decreases BACE1 recycling from endosomes to plasma membrane, increasing intracellular BACE1 in early endosomes. On the other hand, we have evidence that CD2AP loss of function decreases APP degradation, increasing intracellular APP in endosomes. Overall our data show that BIN1 and CD2AP differentially control BACE1 and APP sorting; their loss of function leads to an increased encounter of BACE1 and APP in endosomes, facilitating APP processing and Aβ generation.

DISSECTING THE ROLE OF TTR PROTEOLYTIC ACTIVITY IN ALZHEIMER'S DISEASE

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Transthyretin (TTR) is the plasma homotetrameric carrier of thyroxine (T4) and retinol, in the latter case through binding to retinol binding protein (RBP). TTR is mainly synthesized in the liver and choroid plexus of the brain, which constitute the sources of TTR in the plasma and in the cerebrospinal fluid (CSF), respectively. Besides its transport activities, TTR is a metalloprotease having as a substrate Aβ, the main constituent of amyloid plaques in the brains of Alzheimer's disease (AD) patients. TTR proteolysis of Aβ was proposed as a protective mechanism in AD but so far this hypothesis lacks support from *in vivo* data. In this work we further addressed the relevance of TTR proteolysis in AD and demonstrated that TTR proteolytic activity contributes to Aβ clearance. We measured the effect of TTR (either wt or proteolytically inactive (TTR prot-)) in Aβ secretion using Neuro-2A neuroblastoma cells stably expressing human APP carrying the Swedish mutation (N2A-APPswe cells). We observed that while wt TTR led to a reduction in the Aβ levels secreted by N2A-APPswe cells, TTR prot- had no effect. Moreover transmission electron microscopy (TEM) analysis demonstrated that wt TTR, but not TTR prot-, is capable of interfering with Aβ fibrillization by both inhibiting and disrupting fibril formation. In order to assess the effect of TTR in Aβ cytotoxicity *in vitro*, hippocampal neurons will be incubated with Aβ with either wt TTR or TTR prot- and cytotoxicity will be measured by caspase-3 activation. More importantly, we are currently assessing whether TTR proteolysis impacts in the development of AD *in vivo*, by analyzing the effect of intracranial injection of either wt TTR or TTR prot- in a mouse model for AD. If TTR proteolytic activity shows to be relevant for preventing the development of AD *in vivo*, the modulation of TTR proteolysis might constitute a valuable therapeutic strategy.

SIRTUIN 3 OVEREXPRESSION AFFECTS MITOCHONDRIAL DYNAMICS IN STRIATAL CELLS DERIVED FROM HUNTINGTON'S DISEASE KNOCK-IN MICE

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Altered mitochondrial dynamics has been implicated in the pathogenesis of several neurodegenerative disorders, including Huntington's disease (HD)[1]. Sirtuins, NAD+-dependent lysine deacetylases, have emerged as important cellular targets that can interfere with mitochondrial biogenesis, fission/fusion, motility and mitophagy[2]. Among them, sirtuin 3 (SIRT3) is particularly relevant, since it is the main deacetylase located in mitochondria. Here we evaluated the influence of SIRT3 on mitochondrial dynamics using striatal cells derived from HD knock-in mice (STH*dh*$^{Q111/Q111}$) *versus* wild-type cells (STH*dh*$^{Q7/Q7}$). Interestingly, untransfected STH*dh*$^{Q111/Q111}$ cells displayed a significant increase in endogenous SIRT3 protein and mRNA levels in relation to control cells. Untransfected HD cells also exhibited an overall decrease in the levels of mitochondrial biogenesis (PGC-1α) and fusion proteins (Mfn2, Opa1) and an increase in fission-related Fis1. Drp1 (also involved in mitochondrial fission) was preferentially accumulated in the mitochondrial fraction of STHdh$^{Q111/Q111}$ cells, but its protein levels significantly decreased in mutant cells, when comparing with STH*dh*$^{Q7/Q7}$ cells. Overexpression (OE) of SIRT3 apparently reduced the unbalance between fission/fusion by decreasing the protein levels of Fis1 in STH*dh*$^{Q7/Q7}$ and STH*dh*$^{Q111/Q111}$ cells, and Drp1 in STHdh$^{Q7/Q7}$ cells only, with no differences in biogenesis or fusion proteins. Parkin, a marker of mitophagy, was also assessed. Untransfected HD cells exhibited lower Parkin levels. Although no significant differences in Parkin were found after SIRT3 OE in both cells, increased Parkin phosphorylation at activating Ser65 was detected in STH*dh*$^{Q111/Q111}$-SIRT3 cells. In addition, increased LC3-II/I ratio, which evaluates autophagosome formation, was observed in STH*dh*$^{Q111/Q111}$ cells, with an additional significant increase in STH*dh*$^{Q111/Q111}$-SIRT3 cells. Data suggest that enhanced SIRT3 may partially reduce mitochondrial fission and possibly activate mitophagy in HD striatal cells, although no conclusions can still be drawn regarding its termination.

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TACKLING ALZHEIMER'S DISEASE WITH A NEW INHIBITOR OF BACE1: EVALUATION OF PEPTIDE EFFICIENCY *IN VITRO* AND IN AN *IN VIVO* MODEL OF AD

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Alzheimer's disease (AD) is the most common dementia worldwide and at the present an effective therapy is an unmet medical need. It is generally accepted that accumulation of amyloid-β protein (Aβ) in the brain parenchyma represents an early incident on a cascade of events that ends in neurodegeneration and dementia, and thus, Aβ is considered as the etiologic agent of the disease. The formation of Aβ requires the initial cleavage of the amyloid precursor protein (APP) by the β-secretase enzyme (BACE1), followed by the activity of the γ-secretase over the ensuing trasmembrane fragment. So far, only one BACE1 inhibitor previously developed reached the phase II/III clinical trials. Therefore, the goal of this study is the design and development of a new peptide inhibitor for BACE1, a key enzyme for the production of amyloid-β peptide (Aβ), which we expect will overcome some of the limitations of previous BACE1 inhibitors that hindered their clinical use.

The compounds we designed were screened for their ability to inhibit BACE1 by an *in vitro* cell-free assay. The peptides IC50 and the type of enzymatic inhibition were determined. The most promising compounds were then evaluated for their ability to inhibit BACE1 and reduce endogenous Aβ production in a cellular model of AD (Neuro-2a cells overexpressing APPswe, N2A-APPswe). The levels of secreted Aβ40 and Aβ42 as well as the levels of the soluble fragment sAPPβ were assessed by ELISA and Western blot, respectively, after incubation of N2A-APPswe cells with the peptides for 24 hours. In these conditions, we observed that 100 μM of peptide 7 reduced the levels of Aβ40 and Aβ42 by about 70 % and 55 %, respectively, whereas 100 μM of peptide 8 reduced Aβ40 and Aβ42 levels by about 60 %. Accordingly, both peptides induced a reduction in the levels of sAPPβ. Moreover, a 24 h incubation with the new BACE1 inhibitors (peptide 7 and 8) at the concentrations used to decrease Aβ40 and Aβ42 levels, did not change cell viability, as assessed by the MTT assay. Thus, the peptides did not cause cytotoxicity while reducing the endogenous Aβ production by N2A-APPswe cells.

The efficacy of the selected peptides to inhibit BACE1 was also tested in the 3xTg AD mouse model. We observed that both peptides (1.25 mg/kg) reduced plasma Aβ40 by about 25-30% and, at a dose of 5.0 mg/kg, peptide 7 and peptide 8 reduced plasma Aβ42 by about 35-45%, as assessed by sandwich ELISA 24 h after a single peptide administration to 4 months-old mice. Regarding brain soluble Aβ levels, 1.25 mg/kg of the peptides 7 and 8 reduced Aβ40 and Aβ42, by about 30%, 24 h after peptide administration.

Taking into account our preliminary results, we expect to develop a new BACE1 inhibitor that will be able to delay the onset and progression of the disease since it will prevent Aβ production and the subsequent neurotoxic events triggered by Aβ accumulation.

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CYTOSKELETON REMODELING AS A TARGET FOR TTR-INDUCED NEURODEGENERATION

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Familial Amyloid Polyneuropathy (FAP) is a neurodegenerative disease characterized by the extracellular deposition of amyloid fibrils of mutated transthyretin (TTR), particularly in the peripheral nervous system (PNS). As a consequence of TTR deposition, a dying-back axon degeneration occurs ultimately leading to neuronal death. In FAP, considering cytoskeleton damage as a consequence of TTR deposition is pertinent, as alterations in microtubule stability and axonal transport are emerging as common features of dying-back axonopathies. Moreover, WT TTR might also be involved in the regulation of cytoskeleton dynamics, as TTR knockout (KO) mice have impaired axonal transport. In this work, we aim to evaluate whether under physiological conditions WT TTR is involved in the regulation of the neuronal cytoskeleton, and whether TTR aggregates interfere in axonal cytoskeleton dynamics such that axonal degeneration prevails. Supporting cytoskeleton damage as a pathogenic event in FAP, in dorsal root ganglion neurons (DRG) TTR oligomers promote a complete reorganization of both actin and microtubules in the growth cone. Moreover, analysis of microtubule dynamics by live-imaging of DRG neurons transfected with the plus tip protein EB3, demonstrated that the amyloidogenic mutants TTR V30M and TTR L55P decreased microtubule growth speed whereas soluble WT TTR promoted the opposite effect. Moreover, TTR L55P (a mutant with high ability to aggregate) led to growth cone collapse and shrinkage as well formation of axonal swellings, a sign of neurite degeneration. Further supporting an effect of WT TTR in cytoskeleton dynamics, the velocity of the retrograde transport of mitochondria was increased in the presence of WT soluble TTR. Currently, we are analyzing the effect of TTR amyloidogenic mutants on axonal transport. Moreover, we will analyze both microtubule dynamics and axonal transport using a mouse model for FAP. The present results suggest that an effect of WT TTR on microtubules and axonal transport might underlie TTR neuritogenic activity, and that cytoskeleton defects are induced by TTR amyloidogenic mutants leading to neurodegeneration. To confirm our hypothesis we are currently performing a cytoskeleton phospho antibody array in order to determine the intracellular cytoskeleton-related signaling pathways altered by both WT TTR and TTR amyloidogenic mutants.

MICRORNA-155 IS UPREGULATED IN THE SPINAL CORD OF TGSOD1G93A (MSOD1) MICE AT THE PRESYMPTOMATIC STAGE

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Amyotrophic lateral sclerosis (ALS) is the third most common neurodegenerative disease, rapidly progressive and invariably fatal, characterized by the loss of motor neurons (MNs). Most often the disease is sporadic (sALS), while mutations in superoxide dismutase-1 (SOD1) cause a rare form of familial ALS (fALS). Transgenic rodent expressing the G93A mutant human SOD1 (mSOD1) is the most used model with symptoms and pathology similar to ALS patients. Another interesting concept is that activation of microglia and astrocytes are part of the pathological process and neuroinflammation a hallmark of the disease [1]. MicroRNA(miR)-155, miR-124 and miR-146a are considered fine tuners of inflammation [2] and showed to be differently expressed in microglia activated phenotypes [3].

Here we aimed to explore miR-155 as an early biomarker of ALS and to investigate miR-155 associated neuroinflammatory pathways. For that, spinal cord from the mSOD1 mice were collected at pre-symptomatic (4-5 weeks) and symptomatic (14-15 weeks) stages and analyzed by WB and qRT-PCR.

Our results indicated that the three major inflammatory miR-155, miR-124 and miR-146a significantly increase in symptomatic mSOD1 mice, but only miR-155 was upregulated in pre-symptomatic phase, together with the downregulation of its direct target SOCS1 in both pre- and symptomatic stages. Interestingly, the suppression of TGF-β observed at pre-symptomatic stage may derive from miR-155 signalling, while its upregulation at symptomatic stage can in opposite induce miR-155 expression. We also observed miR-155 overexpression in the pre-symptomatic stage, which may cause a decreased clearance function by microglia as it is suggested by the reduced expression of MFG-E8, essential in the phagocytosis of apoptotic cells. These findings preceded the polarization of microglia towards the M1 pro-inflammatory phenotype that we observed only in the symptomatic stage, as indicated by the increased expression of CD11b, Iba1, MHC-II and C/EBPα, together with the decrease in arginase-1 expression. Enhanced expression of the CX3CL1-CX3CR1 axis, HMGB1 alarmin, inflammasome-NLRP3 and IL-1β corroborated the inflammatory milieu at the symptomatic, but not at the pre-symptomatic stage.

Overall, our data strengthen the impact of microRNAs in the ALS-inflammatory profile and suggest that miR-155 is implicated in the pathogenic mechanisms of the disease prior to symptoms onset.

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IDENTIFICATION OF INTERMEDIATE AND OFF-PATHWAY SPECIES DURING AMYLOID FIBRIL FORMATION OF α-SYNUCLEIN

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Increasing evidence suggests pre-fibrilar species as important pathogenic agents in neurodegenerative and prionopathies pathogenesis. The current work aims at the characterization of intermediate and off-pathway species during amyloid fibril formation of α-synuclein, an abundant brain protein whose aggregation is associated to Parkinson Disease (PD). The preliminary conclusions resulting from the analysis of α-synuclein aggregation kinetics are subsequently validated using direct morphological and analytical assays. The first part of this works presents the analysis of *in vitro* progress curves obtained using thioflavin T (ThT) fluorescence and turbidity techniques, while in the second part the results from Gradient Gel Eletrophoresis, Transmition Electron Microscopy (TEM) and Circular Dichroism (CD) techniques are discussed. Besides contributing for a better understanding of the molecular basis of PD, this study envisages the validation of new screening tools targeting fibrillar and non-fibrillar species.

SUPPORT VECTOR MACHINE CLASSIFICATION IN HUNTINGTON'S DISEASE USING EYE-TRACKING PSYCHOPHYSICS DERIVED FEATURES

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BACKGROUND: Oculomotor performance is well known to be one of the first functional alterations in pre-symptomatic stages of Huntington's disease (HD). Therefore, alterations in oculomotor performance have been measured in laboratory settings and studied as possible markers of disease status and progression in early and pre-symptomatic stages of HD, on the basis of traditional analysis methods [1,2,3,5]. Whether oculomotor performance can be used to classify individuals according to HD disease stage has yet to be explored, via application of machine learning methods. Although, these have been recently explored in other neurodegenerative or neuropsychiatric diseases, such as Mild Cognitive Impairment [5] and Parkinson's Disease [6] with accuracies ranging from 87% to 89.6%, respectively.

AIMS: This study aims at applying, in Huntington's disease (HD), support vector machine (SVM) classifiers to oculomotor features pooled from a 4-tasks psychophysical experiment.

METHODS: 22 normal control subjects (CTRL), 14 pre-symptomatic HD (pre-HD) subjects, and 14 early-stage symptomatic HD patients (HD) completed four horizontal saccadic tasks: prosaccade (PS), antisaccade (AS), memory-guided prosaccade (MPS) and memory-guided antisaccade (MAS). Eleven features were extracted from the eye tracking data recorded for each task. Application of SVM for classification was performed for: CTRL *vs.* pre-HD, CTRL vs HD and pre-HD vs HD. The training and testing samples were defined using a leave-one-out approach, and classification performances, for each task, were evaluated.

RESULTS: The best performance was achieved only when one feature was selected within the MAS task. We were able to automatically distinguish CTRL from pre-HD subjects with an accuracy of 7347%, a sensitivity of 74.31% and a specificity of 72.64%; CTRL from HD subjects with an accuracy of 81.84%, a sensitivity of 76.19% and a specificity of 8748%; and pre-HD from HD subjects with an accuracy of 83.54%, a sensitivity of 92.62% and a specificity of 7445%. The Saccade Latency proved to be the best feature to distinguish CTRL from pre-HD subjects, whereas to distinguish CTRL or pre-HD subjects from HD subjects, the percentage of express saccade errors and percentage of direction errors proved to be the best features, respectively.

CONCLUSIONS: The reported results demonstrated that feature selection influence the classifiers' performance, within each oculomotor task. Furthermore, the best performance of SVM algorithm was obtained for different features in each task. These results suggested that the application of machine learning methods to eye movement data in Huntington's disease are a valuable option to distinguish between different groups in Huntington's disease, and may have potential application in follow-up of disease stage.

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IMPAIRED NORADRENERGIC DESCENDING PAIN MODULATION IN A KAOLIN-INDUCED HYDROCEPHALUS RAT MODEL

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Pain transmission at the spinal cord is modulated by descending actions that arise from supraspinal areas which collectively form the endogenous pain control system. Two key areas involved of the endogenous pain control system have a circunventricular location, namely the periaqueductal grey (PAG) and the locus coeruleus (LC). The PAG plays a crucial role in descending pain modulation as it conveys the input from higher brain centers to the spinal cord. As to the LC, it is involved in descending pain inhibition by direct noradrenergic projections to the spinal cord.

In the context of neurological defects, several diseases may affect the structure and function of the brain. Hydrocephalus is a congenital or acquired disease characterized by an enlargement of the ventricles which leads to a distortion of the adjacent tissues. Usually, patients suffering from hydrocephalus present dysfunctions in learning and memory and also have motor deficits.

We used an experimental model of hydrocephalus (the rat injected in the cisterna magna with kaolin) to study descending modulation of pain, focusing on the two circumventricular regions: the PAG and the LC. In order to evaluate the effects of kaolin injection, we measured the degree of ventricular dilatation in sections encompassing the PAG by standard cytoarquitectonic stanings. For the LC, immunodetection of the noradrenaline-synthetizing enzyme tyrosine hydroxylase (TH) was performed, due to the noradrenergic nature of the LC neurons. In general, rats with kaolin-induced hydrocephalus presented a higher dilatation of the 4th ventricle, along with a higher area of the PAG. Increases in the levels of TH in the LC, were detected in hydrocephalic animals. The following pain-related parameters were measured, namely 1) pain behavioural responses in a validated pain inflammatory test (the formalin test) and 2) the nociceptive activation of spinal cord neurons. A decrease in behavioral responses was detected in rats with kaolin-induced hydrocephalus, namely in the second phase of the test (inflammatory phase).

Collectively, the results of the behavioral studies indicate that rats with kaolin-induced hydrocephalus exhibit hypoalgesia. A decrease in Fos expression was detected at the superficial dorsal layers of the spinal cord in rats with kaolin-induced hydrocephalus, further indicating that hydrocephalus decreases nociceptive responses. Since the LC has higher levels of TH in rats with kaolin-induced hydrocephalus, which also appears to increase the noradrenergic innervation in the spinal dorsal horn, it is possible that an increase in the release of noradrenaline at the spinal cord accounts for pain inhibition.

MPTP-INDUCED OXIDATIVE STRESS AND NRF2-DEPENDENT REGULATION OF GSTP GENE PROMOTOR

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Our group has been particularly interested in characterizing the potential neuroprotective role of the Glutathione S-Transferase pi isoform, GSTP, in the context of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced brain lesions. Although the molecular mechanisms underlying dopaminergic neuronal death are still not completely understood, accumulating evidence implicates oxidative stress, namely through the damage caused by reactive oxygen species (ROS), in the pathogenesis of sporadic Parkinson's disease (PD).

Our previous work demonstrated that the sub-acute administration of MPTP to C57BL/6 mice induced GSTP expression in both the midbrain and striata. Moreover, we have shown that the MPTP-induced dopaminergic neuronal degeneration is an earlier event when comparing GSTP null *versus* wild-type mice, suggestive of a protective role for GSTP. One potential defence against the toxicity of ROS is the up-regulation of phase II detoxification enzymes, namely GSTP, through the nuclear factor erythroid 2-related factor 2 (Nrf2) transcription factor.

In this work the main objective is to characterize the transcriptional regulation of Gstp expression by Nrf2 using the sub-acute administration of MPTP to C57BL/6 mice.

We started by evaluating Nrf2 activation status, in our experimental PD model. Western-blot analysis of striatal total protein extracts demonstrated an increase in Nrf2 expression levels in MPTP-treated mice, concomitantly with an increase in the protein levels of the Nrf2 downstream targets GSTP and heme oxygenase 1 (HO-1). Moreover, confirming the activation of Nrf2, an increase in Nrf2 nuclear translocation after MPTP treatment was also observed by Western-blot and immunohistochemistry. Also, by confocal immunofluorescence we detected the co-localization of Nrf2 with GSTP protein in the striatum. Conversely, in GSTP ko mice no changes were detected in the expression levels of Nrf2 protein nor in its downstream target HO-1.

To evaluate if the MPTP-mediated activation of Nrf2 is modulating Gstp transcription, the Gstp mRNA levels were determined by qPCR, in midbrain samples of control and MPTPtreated mice. In parallel, Gstp promoter activity was also assessed in transient cotransfection assays using primary cultures of mouse astrocytes, after MPP+ treatment and/or Nrf2 overexpression. Our results point to the involvement of Nrf2 in the regulation of Gstp transcription. The results obtained so far *in vitro* will be confirmed by Chromatin Immunoprecipitation analysis, *in vivo*.

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EARLY ALTERATIONS IN TYPE I AND II INTERFERON RESPONSES IN THE CHOROID PLEXUS AND IN THE HIPPOCAMPUS OF A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a neurodegenerative disease characterized by a marked decline in cognition and memory function. One of the major pathological hallmarks of AD is the progressive accumulation and deposition of amyloid beta (Aβ) peptides in the brain. Importantly, increased Aβ toxicity and pathology is accompanied by alterations in parallel mechanisms and responses that regulate brain homeostasis and function. Increasing evidence highlights the essential role of neuroinflammatory and immune-related molecules, including those produced at the brain barriers, on brain immune surveillance, decreased Aβ clearance from the brain and increased brain cell dysfunction and pathology in AD. Therefore, understanding the response at the brain barriers may unravel novel pathways of relevance for the pathophysiology of AD. Herein, we focused on the study of the choroid plexus (CP), which constitutes the blood-cerebrospinal fluid barrier, in aging and in AD. Specifically, we used the PDGFB-APPSwInd (J20) transgenic mouse model of AD, which presents early memory decline and progressive Aβ accumulation in the brain, and littermate age-matched wild-type (WT) mice, to characterize the CP transcriptome at 3, 5-6 and 11-12 months of age. The most striking observation was that the CP of J20 mice displayed an overall overexpression of type I interferon (IFN) response genes at all ages. Moreover, J20 mice presented a high expression of type II IFN genes in the CP at 3 months, which became lower than WT at 5-6 and 11-12 months. Importantly, along with a marked memory impairment and increased glial activation, J20 mice also presented a similar overexpression of type I IFN genes in the dorsal hippocampus at 3 months. Altogether, these findings provide new insights on a possible interplay between type I and type II IFN responses in AD and point to IFNs as targets for modulation in cognitive decline.

TUDCA MODULATES NRF2 AND ANTIOXIDANT ENZYME EXPRESSION IN EXPERIMENTAL MODELS OF PARKINSON'S DISEASE

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Several lines of evidence implicate mitochondrial dysfunction along with generation of reactive oxygen species (ROS), as possible mechanisms by which cell death occurs in Parkinson's disease (PD). Importantly, PD patient brains reveal a reduction in complex I activity, increased ROS production and increased lipid, protein, and DNA oxidation. Moreover, complex I inhibitors, such as 1-methyl-4-phenylpyridinium (MPP^+) the toxic metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), induce a cascade of events leading to neuropathological features of the disease in humans and animals. To maintain a proper redox balance, the brain is endowed with an endogenous antioxidant defense mechanism mediated by nuclear factor E2-related factor 2 (Nrf2), which is the master regulator of redox homeostasis. Under oxidative stress, Nrf2 is translocated to the nucleus, where it binds to the antioxidant response element (ARE), present in the promoter of phase II antioxidant enzymes like glutathione peroxidase (Gpx) and heme-oxygenase 1 (HO-1). To achieve protective effects in PD, high amounts of antioxidants are needed, as most exogenous antioxidants do not efficiently cross the blood-brain barrier. This emphasizes the need for alternative strategies, and a promising candidate to limit ROS-mediated damage is the activation of endogenous antioxidant phase II enzymes. Tauroursodeoxycholic acid (TUDCA) is an endogenous molecule; orally bioavailable with no associated secondary effects. This molecule is neuroprotective in mice models of different neuropathological diseases. Importantly, we have showed that TUDCA protects against MPTP-induced neurodegeneration in a mouse model of PD, but the exact mechanisms involved remain unidentified. Thus, this work aims to characterize the effect of TUDCA on Nrf2 pathway and identification of the phase II enzymes and other antioxidant enzymes up-regulated by TUDCA, as well as the effects of TUDCA on cellular redox status in MPP+/MPTP experimental models of PD. Twelve-week-old male C57BL/6 mice were treated with MPTP and/or TUDCA administered before or after the neurotoxin. Our preliminary results show that TUDCA significantly modulates Nrf2 expression, as well as the expression of different downstream phase II and other antioxidant enzymes namely, DJ-1 and superoxide dismutase 2 (SOD2). In addition, in the presence of TUDCA, general ROS production is diminished. Interestingly, TUDCA is also effective when administered after MPTP. Together these results suggest that at least part of the neuroprotective effects of TUDCA is modulated through Nrf2 activation. We hope to demonstrate that Nrf2 is a target for TUDCA to limit ROS-mediated damage in PD, potentially leading to interesting therapeutic perspectives.

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UNRAVELING TUDCA ROLE ON AUTOPHAGY/MITOPHAGY IN MODELS OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is the second most common neurodegenerative disease, characterized by the progressive loss of dopaminergic neurons in the Substantia nigra pars compacta. Accumulating evidence suggests that mitochondrial complex I dysfunction might be the key event in this pathology. Moreover, this evidence is reinforced by the identification of rare PD-associated mutations in genes that affect mitochondrial function such as putative kinase 1 (PINK1) and Parkin. The toxic metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxin, 1-methyl-4-phenylpyridinium (MPP^+), is a mitochondrial complex I inhibitor that gives rise to clinical symptoms similar to those observed in sporadic PD. MPTP causes severe mitochondrial insult that culminates with accumulation of dysfunctional mitochondria. Thus, in addition to the impairment of ROS, one approach toward protection that has recently arisen in PD research is the selective degradation of mitochondria by mitophagy.

The endogenous bile acid tauroursodeoxycholic acid (TUDCA) is an anti-apoptotic molecule shown to interfere with the mitochondrial pathway of cell death. TUDCA is already in use for treatment of chronic cholestatic liver diseases and a recent study shows that is safe and might be effective in amyotrophic lateral sclerosis treatment. Importantly, we have also shown that TUDCA protects against MPTP-induced neurodegeneration in mice, but the mechanisms underlying its neuroprotective action are still unknown.

Here we investigate whether autophagy, and specifically mitophagy are part of the neuroprotective action of TUDCA against MPTP-induced neurodegeneration. Twelve-week-old male C57BL/6 mice were treated with MPTP and/or TUDCA administered before or after the neurotoxin. Mice were sacrificed at different time points and midbrains and striata were dissected. To further characterize the molecular mechanisms involved on TUDCA-induced neuroprotection we used SH-SY5Y cells treated with MPP^+ in the presence or absence of TUDCA. Our preliminary results show that, in the mouse model of PD, TUDCA alters AMPK activation, PINK1 cleavage, Parkin expression levels and LC3 lipidation, indicating that autophagy/mitophagy might be involved. The specific molecular mechanisms involved will be discussed using SH-SY5Y neuroblastoma cells.

Mitochondrial protective agents represent an attractive direction for the development of new therapeutic drugs in PD. We hope to demonstrate and characterize TUDCA-induced mitophagy as a novel and important mechanism underlying the neuroprotective role of this bile acid, contributing to the validation of its application in PD.

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CLASSIFICATION OF HUNTINGTON'S DISEASE STAGE USING SEGMENTED GREY MATTER TISSUE AND DIFFUSION WEIGHTED DERIVED FEATURES

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Identification of early-stage biomarkers in neurodegenerative diseases has been the focus of much research [1], aiming at a valuable assessment of novel neuroprotective therapies. In Huntington's disease (HD), neuroimaging studies have identified neurodegenerative processes up to two decades prior to the onset of clinical symptoms, whereas continuing effort is being made to develop approaches that enable automatic identification of disease stage by machine-learning methods [2–4]. In this study, we applied support vector machines (SVM) to MRI data, encompassing structural and diffusion derived features, such as to improve classification between HD stages. Our dataset was composed of 14 premanifest (Pre-HD) gene carriers, far from estimate disease onset, 12 clinical stage 1 HD (Early-HD) patients, and age- and gender-matched controls (HC). Two high-resolution T1-weighted multi-echo magnetization-prepared rapid gradient echo (MEMRAGE) scans and a diffusion weighted image were collected per participant. We performed binary classifications across groups, using segmented grey matter (GM) and fractional anisotropy (FA), both per imaging modality and with multimodal input. The selected features included whole-brain, subcortical regions of interest (ROIs) chosen on the basis of known pathology, and automated feature selection through the Relief-F algorithm. Our results demonstrate successful classification of Early-HD *vs.* Pre-HD and Early-HD *vs.* HC groups, with accuracy, sensitivity and specificity above 80% ($p<0.01$). As expected, higher weighting values were assigned to striatal regions, once whole-brain or Relief-F were used, with distributed cortical regions and white matter paths contributions, respectively, and in accordance with known neuropathology. Anatomically predefined anatomical ROIs, as well Relief-F features' pre-selection, returned higher accuracy values, in both modalities, whereas a multimodal approach has not demonstrated better performance. Finally, classification of Pre-HD and HC groups was successful once considering FA feature for the caudate ROIs (74% accuracy, $p<0.05$). Hence, classification of disease stage is attained through the usage of supervised methods, and imaging modalities are shown to have different sensitivities if HD gene carriers are estimated to be far from disease onset.

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DISSECTING THE ROLE OF CYTOSKELETON REMODELING IN TRANSTHYRETIN-INDUCED NEURODEGENERATION IN A *DROSOPHILA* MODELAND DIFFUSION WEIGHTED DERIVED FEATURES

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Transthyretin (TTR) is a protein mainly synthesized by the liver and the choroid plexus of the brain, whose mutations are associated with familial amyloid polyneuropathy (FAP). FAP is characterized by the deposition of mutated TTR, in the form amyloid fibrils, particularly in the peripheral nervous system (PNS). As a consequence of TTR deposition there is axonal degeneration that results in neuronal dead with disease progression.

Ongoing studies from our group show that exposure to TTR oligomers induces cytoskeleton reorganization in primary cultures of Dorsal Root Ganglia (DRG) neurons. Aiming at determining whether cytoskeleton remodelling is a target for TTR-induced neurodegeneration *in vivo* we are using a *Drosophila* model for FAP [1]. Fly neurons offer a number of useful features to study the neuronal cytoskeleton machinery, since in this model actin and microtubule binding proteins display high homology with mammalian ones.

The *Drosophila* FAP model is based on the expression of human mutant TTR V30M (the most common pathogenic substitution) in differentiated cells of the fly developing eye (photoreceptor cells (R cells) and accessory cells). Expression of TTR V30M leads to roughening of the eye and degeneration, when compared with flies expressing wild type (wt) TTR. In order to determine whether a defect in axonal cytoskeleton precedes neurodegeneration, we are currently evaluating the cytoskeleton organization of R cells, using Futsch, a microtubule associated protein as a marker, of flies expressing wt TTR or TTR V30M at different developmental stages (larvae, early pupae and adults).

In addition, we are performing a genetic screen to identify enhancers and suppressors of TTR V30M-induced rough eye phenotype to identify the mechanisms associated with neuronal cytoskeleton defects. Flies expressing TTRV30M are being crossed with readily available fly lines for knockdown, through the use of RNAi (UAS-RNAi), candidate genes whose functions are associated with cytoskeleton dynamics. Among these are Rho GTPases, Rho GAFs and GEFs, motor proteins, Cdk5, phosphatases, integrins and protein kinases. Preliminary results suggest a role for Rac proteins in the phenotype associated with TTR V30M expression.

This work will be important to clarify whether cytoskeleton defects underlie TTR induced-neurodegeneration and will pinpoint TTR downstream targets and partners. We will present our latest findings on TTR associated changes cytoskeleton dynamics.

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MAPPING THE DIFFERENTIAL NEURONAL VULNERABILITY TO MUTANT ATAXIN-3 AGGREGATION IN A *C. ELEGANS* MODEL OF MACHADO-JOSEPH DISEASE

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Machado-Joseph Disease (MJD) is a late-onset neurodegenerative disorder, caused by a CAG triplet expansion within the *ATXN3* gene. The mutant ataxin-3 protein (ATXN3) shows a strong tendency to misfold and aggregate leading to the formation of neuronal inclusions in specific brain regions. Knowing that both WT and mutant ATXN3 are ubiquitously expressed throughout the brain and differences in ATXN3 aggregation fail to correlate with ATXN3 tissue/neuron-specific expression levels or heterogeneity of polyQ-length, we posed a challenging question: what causes the neuron-specific pattern of degeneration underlying MJD? To address this question we categorized the 302 *C. elegans* neurons regarding their function and neurochemical content and mapped the neurons showing increased susceptibility to mutant ATXN3 expression in a transgenic *C. elegans* model of MJD pathogenesis. Despite the marked neuronal dysfunction observed, we have found no evidence that ATXN3 aggregation is causing neuronal death in our disease model even at late stages of disease, as assessed by TUNEL and SYTO dye assays and by the detailed analysis of the presence of all individual neurons; hence, we focused on ATXN3 aggregation. Available fluorescent neuronal markers that specifically label distinct neuron subtypes allowed aggregation scoring. *In vivo* confocal analysis allowed classification of neurons as susceptible to mutant ATXN3 expression when ATXN3 aggregates co-localized with the neuronal marker within a single confocal slide. We show that GABAergic neurons localized in the head are severely affected by mutant ATXN3 aggregation while those localized in the ventral nerve cord are mildly affected. Some glutamatergic chemosensory and thermosensory neurons are susceptible to mutant ATXN3 expression, while the cholinergic neurons analyzed so far are only mildly affected. All the neurons that express the biogenic amines serotonin, dopamine and octopamine are severely affected by ATXN3 aggregation, with the exception of dopaminergic neuron PDE, which never presents aggregates.

These results strongly suggest that mutant ATXN3 aggregation in *C. elegans* neurons is not stochastic but neuronal-subtype specific and that the neuronal proteostasis environment in which aggregation-prone proteins are expressed may determine its aggregation state. Our future aim is to investigate the molecular determinants underlying this differential susceptibility.

EFFECT OF MITOCHONDRIAL LIFESPAN-INCREASING MUTATIONS ON MUTANT ATXN3 AGGREGATION IN A *CAENORHABDITIS ELEGANS* MODEL OF MACHADO-JOSEPH DISEASE

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Aging is associated with an increased risk of several neurodegenerative diseases including Machado-Joseph disease (MJD), a disorder caused by an abnormal polyglutamine (polyQ) expansion in ATXN3 protein, culminating with ATXN3 aggregation and neuronal death in specific brain areas.

Since the nematode *C. elegans* has been widely used to study the molecular and organismal regulation of aging, and has allowed the identification of key pathways regulating longevity, we propose to use it as a model to understand how the manipulation of aging-related mechanisms can impact in the progression and onset of different human neurodegenerative diseases characterized by aggregation of pathogenic proteins.

Our previous results in a *C. elegans* transgenic model of MJD, expressing mutant human ATXN3 (AT3q130) in the nervous system, revealed the benefits of slowing down the aging process through the downregulation of insulin/IGF-1 signaling (IIS) pathway and of the activation of HSF-1 signaling, and suggested that not all longevity pathways have an equal effect in the modulation of mutant proteins aggregation and neurotoxicity. Here we studied the impact of mitochondria-related longevity-inducing mutations upon ATXN3 aggregation. ATXN3 aggregates were quantified during aging, when mutant ATXN3 protein was expressed in the background of longevity-associated mutations in the *clk-1* and *isp-1* genes, encoding an enzyme involved in the biosynthesis of the natural antioxidant ubiquinone and a subunit of the mitochondrial respiratory complex III, respectively. A mutation in *isp-1* (qm150) significantly reduced ATXN3 aggregation as the animals aged, whereas distinct mutations within the *clk-1* gene resulted in a milder positive impact. These findings suggest that mitochondrial lifespan-increasing mutations impact positively in the context of MJD, through yet unknown mechanisms. Hypothesizing that the folding/clearance cellular capacity may be key, we will next characterize the proteomic profiles and the status of the proteostasis network in the mutant strains, with the goal of identifying the longevity-associated factors that are most beneficial for ATXN3-mediated neuronal dysfunction and aggregation.

METHAMPHETAMINE-INDUCED CHANGES IN THE MORPHOLOGY OF HIPPOCAMPAL NEURONS IS MEDIATED BY RAC1 ACTIVATION AND RHOA INHIBITION

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Drug-induced modulation of neuronal morphology in the brain's reward circuitry has been associated with the long-lasting behavioral abnormalities that underlie addiction. Exposure to psychostimulants was shown to persistently affect neuronal morphology, increasing the length of dendrites, density of dendritic spines and the number of branched spines, in a region specific way. Both the extent and persistence of the structural changes depend on the dose and period of exposure. Importantly, such changes correlate with the behavioral expression of the drug used. Changes in morphology are mediated by regulation of cytoskeleton-related proteins mainly at the post-translational level, but also by local mRNA targeting and translation. Of note, in the hippocampus, altered neuronal morphology is known to affect long-term-potentiation and learning, exerting a relevant modulatory influence on the reward processing.

Methamphetamine (METH) is a highly neurotoxic psychostimulant that leads to long-term dysfunction in monoaminergic and glutamatergic neurons. Here, we hypothesized that modulation of RhoA and Rac1 may impact METH-induced changes in hippocampal neuronal morphology, providing new targets for therapy development. To test that, a set of experimental approaches was applied to primary hippocampal neuronal cultures obtained from 16 days mice embryos. Total neurite length was obtained by quantifying the dendritic arborization of neurons transfected with enhanced green fluorescent protein (EGFP). The expression of microtubule associated protein 2 (MAP2, a dendritic marker) and of postsynaptic density 95 (PSD95, a postsynaptic marker) was also determined. A dose of 100 µM METH was selected. Following that we verified that under METH 100 µM, neither Rac1 nor RhoA mRNA expression levels were affected by METH. Next, we evaluated the activation state of RhoA and Rac1 using both pull-down assays and specific FRET reporter probes to assess differential topographical activation. Our results evidence that in hippocampal neurons RhoA suffers an initial 10 min increase in activity, followed by a prolonged downregulation, while Rac1 shows a sustained increase in activity, which is more pronounced in neurites. We further confirmed this results by knocking-down Rac1 or over expressing a constitutively active RhoA form, and evaluating METH-induced neurite outgrowth in these mutant neurons.

We show that in hippocampal neurons Rac1 activation and RhoA inhibition are needed for methamphetamine-induced increased complexity to occur. Noteworthy, in nucleus accumbens neurons of mice exposed to cocaine, increased complexity was associated with Rac1 inhibition, showing that these mechanisms are region specific, and that pharmacologic therapies directed RhoGTPases must be carefully evaluated. Nonetheless, as Rac1 activation is the hippocampus is involved in learning processes, Rac1 modulation appears as a relevant element in the acquisition and maintenance of the reward behavior.

EXENDIN-4 THERAPY RESTORES BRAIN GLUCOSE HOMEOSTASIS IN TYPE 2 DIABETIC GOTO-KAKIZAKI RATS: IMPACT ON NEUROINFLAMMATION AND OXIDATIVE STRESS

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Epidemiological studies revealed an increased incidence in both type 2 diabetes (T2D) and Alzheimer disease (AD) worldwide. Moreover, given the higher risk for development of neurodegenerative diseases (namely AD), probably due to a cognitive decay in T2D patients, several main links to these pathologies are being investigated, with highlights to the insulin signaling and the glucose dysmetabolism. Thus, we hypothesized that exendin-4 (Ex-4, which is a potent anti-diabetic with hyperglycemic and insulinotropic effects and action in the central nervous system), modulates insulin signaling pathway and glucose homeostasis in the brains of middle-aged T2D male rats, thereby impacting T2D-brain damage, such as neuroinflammation and oxidative stress. Hence, we aimed to evaluate the impact of a chronic continuously peripheral Ex-4 administration in the Goto-Kakizaki (GK) rat model for T2D. For this purpose, we used blood samples and brain cortical homogenates from middle-aged (8 months-old) GK rats treated with Ex-4 (5 ug/kg/day, 2.5 ul/h, 28 days). Age-matched Wistar rats were used as controls. Ex-4 had a positive effect on peripheral T2D features, by lowering fasting blood glucose levels, glycated hemoglobin (HbA_{1C}) and insulin resistance (HOMA-IR) in GK rats. In addition, we observed an Ex-4 mediated central reduction in the levels of glucose, while glucose metabolism-related enzymes (namely hexokinase, lactate dehydrogenase and glucose-6-phosphate dehydrogenase) also showed enhanced activities that were accompanied by decreased lactate/pyruvate ratio in GK rats. Regarding, the glucagon-like-peptide 1 receptor (GLP-1R)-mediated signaling cascade, which is activated by Ex-4 and converges with the insulin pathway, a general improvement was observed (namely in cAMP, PKA, PI3K and Akt) in brains from the GK rat treated with the drug. Interestingly, Ex-4 action was also able to decrease pro-inflammatory (C-reactive protein and interleukine(IL)-1β) and increase anti-inflammatory markers (IL-4 and IL-10), being accompanied by the attenuation of the oxidative imbalance (as given by thiobarbituric acid reactive substances, hydroperoxide and nitrite levels and carbonyl groups formation) in T2D rats. In conclusion, peripheral Ex-4-mediated improvement in insulin sensitivity and peripheral T2D pathological features also enhanced brain GLP-1R signaling, which may reestablish glucose homeostasis, preventing chronic inflammation and oxidative stress.

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SUBTRACTIVE BRAIN DELIVERY OF DIPHTHERIA TOXIN

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Ablation of molecularly identified cells using tissue specific expression of human diphtheria toxin receptor (DTR) is a powerful genetic tool to access the role of molecularly identified populations of cells in mice [1]. When diphtheria toxin (DT) is administered systemically, it spreads across all organs, brain including. Upon binding to DTR, the A-subunit of DT is internalized, blocking protein synthesis, thus ablating DTR-expressing cells. The penetrance of ablation is determined by the strength and tissue specificity of promoter regions driving expression of DTR. However, several driver lines marking peripheral tissues, such as liver, immune system, or sensory systems, also have considerable expression in the brain. This off target expression limits the utility of DTR for studying peripheral tissues. Hence, a form of DT that wouldn't cross the blood brain barrier (BBB) would be an asset for the study of peripheral populations of cells. Specifically, this tool will enable to ablate populations of autonomic neurons, without compromising those in the brain.

DT was chemically modified so that it doesn't cross the BBB through PEGylation. This method consists in covalent attaching of polyethylene glycol (PEG) chains to proteins or other molecules, so it will increase the apparent size of these molecules, thus reducing the renal filtration and altering biodistribution [2-4]. PEG is a highly investigated polymer for covalent modification of biological macromolecules and surfaces for many applications, such as pharmaceutical and biotechnological. PEGylation of DT was performed using NHS PEG molecules that modified the lysine residues present in DT.

PEG-DT is effective at ablating DTR-expressing cells outside the brain and it doesn't cross the BBB. The ablation of sympathetic neurons leads to a higher speed of weight gain and this allow the study of the role of these neurons in obesity.

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THE ROLE OF IL10 IN DEPRESSION AND ANTIDEPRESSANT THERAPY

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Major depression is among the leading causes of disability worldwide. Despite the several treatments available, ineffectiveness of antidepressants and recurrent depression are commonly encountered. This reflects the poor understanding of depression etiology, which is probably multifactorial, in line with a heterogeneous clinical presentation. Among the different etiological hypothesis, that of a dysfunctional interplay between the nervous and immune systems has received strong support. The findings of increased levels of pro-inflammatory cytokines in patients with depression together with reports symptoms of depression in a substantial proportion of patients treated with the pro-inflammatory cytokine interferon-α (and the fact that these symptoms can be treated with antidepressants or suspension of treatment), strongly suggest that increased levels of pro-inflammatory cytokines are associated with depression. Equally important, although much less discussed, seems to be the role of anti-inflammatory cytokines, namely one of the most relevant, interleukin (IL)10. In fact, studies from our group revealed that female mice lacking IL10 expression, show a depressive-like behavior, a phenotype that is rescued by IL10 administration. In accordance, mice overexpressing IL10 present decreased learned helplessness. Interestingly antidepressant treatment, both in patients with depression and in animal models, increases IL10 production. In fact, the reversion of the inflammatory milieu has been shown to play a role for the efficacy of antidepressant treatment. Indeed, treatment-resistant patients have been shown to present a persistent inflammatory profile after antidepressant treatment. In accordance, the concomitant treatment of those patients with antidepressants and anti-inflammatory drugs improved the efficacy of antidepressant therapy. Thus, the goal of this work is to unravel the mechanisms underlying the role of the anti-inflammatory cytokine IL10 in the etiology of depression and in response to antidepressant therapy. Interestingly, our results suggest that the depressive-like behavior observed in the mice lacking IL10 expression is associated with alterations in the gut inflammatory profile and corticosterone sera levels. Moreover, preliminary data showed that mice that lack the expression of IL10 are resistant to antidepressant therapy (both with fluoxetine and imipramine), suggesting that IL10 expression is crucial for the reversion of the depressive-like phenotype. Further studies using this animal model will help to better understand the causes of treatment-resistant depression and also clarify the role of the immune system in this so complex and devastating disorder.

GENDER INFLUENCES TYPE 2 DIABETIC-RELATED INTRACELLULAR BRAIN SIGNALING AND NEURODEGENERATION MARKERS

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Besides aging, type 2 diabetes (T2D) and female gender have been also described as risk factors for Alzheimer's disease (AD). However, the underlying molecular mechanisms remain unclear. Moreover, given that T2D and AD share several common molecular mechanisms and the increasing interest on a highly specialized personalized, gender-specific medicine, we hypothesized herein that gender differentially affects insulin/IGF-1/estrogen-mediated signaling in T2D brain predisposing to neurodegenerative conditions. Hence, we aimed to analyze how gender may influence insulin/IGF-1/estrogen-related signaling and AD-like hallmarks in T2D rat brains. Under this perspective, we used brain cortical homogenates from middle-aged (8-month-old) male and female Wistar and T2D Goto-Kakizaki (GK) rats to evaluate insulin, IGF-1, cholesterol, sexual steroid hormones and amyloid-beta levels by ELISA, and density of signaling molecules by immunoblotting.

We observed that despite the higher glycemia in both male and female GK rats, no significant changes occurred between genders. Moreover, albeit the increased plasma estradiol levels in Wistar females than in males, its levels were similar between T2D females and males. T2D females showed also impaired brain steroid hormones' metabolism, lower IGF-1 levels and IGF-1 receptor density, whereas their blood and brain insulin levels and insulin receptor densities were increased. Hence, a compensatory mechanism to maintain insulin receptor function and subsequently stimulating Akt activity may occur in GK females, inhibiting BACE activity and ultimately hampering brain amyloid-beta$_{1-42}$ accumulation and lipid and DNA oxidation.

Although brain steroid hormone cascade might be impaired in middle-aged T2D females, these were less susceptible to oxidative stress and AD-like neuropathological markers associated to chronic T2D.

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CHRONIC PERIPHERAL ADMINISTRATION OF EXENDIN-4: DOES IT PROTECT AGAINST BRAIN DYSFUNCTION AND CELL DEATH?

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It has been increasingly demonstrated that type 2 diabetes (T2D) and Alzheimer disease (AD) may be two intimately related pathologies, sharing several common pathological mechanisms that may ultimately lead to brain dysfunction and cell death. Furthermore, it has been suggested that a beneficial treatment against T2D may be also useful against AD, with the insulinotropic drug from the GLP-1 receptor (GLP-1R) agonists' class exendin-4 (Ex-4) being highly promising herein. Therefore, we hypothesized that peripheral Ex-4 attenuates brain cortical dysfunction and neurodegeneration/death associated with chronic T2D. In line with this, we aimed to analyze the role of chronic peripheral administration of Ex-4 in brain cortical injury and neurodegeneration/death from middle-aged T2D rats.

Middle-aged (8-month-old) GK rats were continuously subcutaneously (sc) administered with Ex-4 and their T2D features monitored. Brain cortical homogenates were used to evaluate autophagy (as given by markers of autophagosome nucleation and elongation and other autophagic proteins), necrosis (by RIP1/RIP3 protein expression), apoptosis (by the cleavage of specific colorimetric caspases' substrates, and Bcl2, BAX and cytochrome c protein expression), synaptic integrity and AD-like neuropathological features (Tau p-Ser396 levels, Tau p-Thr181/ total Thr181, APP protein expression and $A\beta_{1-42}/A\beta_{1-40}$).

We observed that sc Ex-4-mediated amelioration of blood HbA_{1C}, insulin resistance and glucose tolerance parameters was accompanied by lower brain cortical P-mTOR expression and higher p62 expression, hence suggesting the induction of autophagy. This was further supported by the increased LC3-II, PI3K III, beclin-1, Atg7, Parkin-1 and glycosylated LAMP-1 expression upon Ex-4 therapy that was followed by a protection against caspase-3-mediated apoptosis upon Ex-4 (further corroborated by the decreased translocation of Bax into mitochondria and the lower released cytochrome c into cytosol). This protection may ultimately account for the decreased formation of Aβ, as indicated by the reduced $A\beta_{1-42}/A\beta_{1-40}$ and by the restoration of synaptic function (as given by the increased PSD-95 and synaptophysin expression) in GK rat brains upon Ex-4 therapy.

In conclusion, by inducing autophagy, sc Ex-4 may protect against T2D-induced brain damage, accumulation of AD pathological features, apoptosis and synaptic dysfunction, thus constituting a promising therapeutic/preventive strategy against T2D-related long-term complications affecting the central nervous system.

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THE IMPACT OF CIRCADIAN CLOCK IN THE NEUROBIOLOGY OF ALZHEIMER'S DISEASE, ACTIVE VS INACTIVE PERIOD IN 3XTG-AD

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Alzheimer's disease (AD) is an age-related disease characterized by a progressive decline in cognition, with a still undefined etiology. The circadian system also undergoes an age-related decline and circadian disturbances have been reported early in AD progression. The triple transgenic (3xTg-AD) mouse model of AD displays abnormalities in circadian rhythmicity prior to AD pathology, but it is still unclear how the circadian clock relates to behavioral, electrophysiological and metabolic alterations linked to AD pathogenesis. The scope of this work was to compare memory performance (Morris water maze), hippocampal long-term potentiation (LTP, a neurophysiological correlate of memory) and mitochondrial function (an index of brain metabolism) in the active (Zt04) and inactive (Zt16) period, in 24 weeks old 3xTg-AD *versus* age-matched non-Tg mice (n=8/group). Acquisition and retention of memory performance was dampened in 3xTg-AD independent of the time of day. A diurnal variation in hippocampus-dependent learning performance was only observed in reverse learning, where the difference of performance between 3xTg-AD and non-Tg mice was more evident at Zt16 than Zt04. This suggests that the pattern of circadian variation of memory performance depends on the type of task and the impact of circadian clock on AD is better outlined in more complex tasks. Analysis of the amplitude of hippocampal LTP, measured extracellularly in Schaffer fibers/CA1 pyramid synapses, showed that LTP amplitude was larger at Zt16 (142.2±2.17%, n=4) than Zt04 (116.9±1.70%, n=4) in slices from non-Tg animals, whereas a circadian variation of LTP amplitude was not observed in the 3xTg-AD (n=4). We then enquired if alterations in mitochondrial function of nerve terminals might accompany the impaired neuroplasticity. Mitochondrial membrane potential ($\Delta\Psi m$, fluorometric probe $TMRM^+$) in cortical synaptosomes was higher in 3xTg-AD than non-Tg mice (1.515±0.07 vs 1.706±0.09, n=4), being the difference larger at Zt16 than Zt04. Susceptibility to oxidative stress was assessed following exposure to hydrogen peroxide (H_2O_2). A greater mitochondrial depolarization in 3xTg-AD vs non-Tg (1.377±0.05 vs 1.609±0.07, n=4) was observed at Zt04 suggesting a greater susceptibility to oxidative stress depending on the Zt. Simultaneously, the measurement of intrasynaptic calcium (Ca_i) levels (fluorometric probe Fura2-AM) revealed an inability of 3xTg-AD mice to maintain Calcium homeostasis. This study demonstrates the impact of the circadian rhythm on key traits of AD pathogenesis and outlines the importance of analyzing diurnal variation for better characterization of AD phenotypes at early symptomatic phases of AD.

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IMPACT OF DIMEHTYLFUMARATE ON COGNITIVE DYSFUNCTION AND ITS CORRELATES IN THE EAE MOUSE MODEL

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Multiple sclerosis (MS) is a demyelinating and neurodegenerative disease of the central nervous system leading to different types of motor, neuropsychiatric and cognitive problems [1]. In MS patients, cognitive deficits correlate poorly with inflammatory activity, being better explained by pathological processes in the grey matter, like demyelination and consequent neuronal damage [2]. MRI data from a phase II trial with dimehtylfumarate (DMF) showed a strong impact of this drug in neurodegeneration, suggesting it could improve cognitive deficits in MS patients [3].

In order to clarify the effects of DMF on cognitive deficits, we immunized C57/Bl6J female mice with MOG35-55 to induce experimental autoimmune encephalomyelitis (EAE), the best-known model of MS, and treated them post-symptomatically with either active drug (EAE treated) or vehicle (EAE non treated) for 18 days, after which their cognitive performance was evaluated in a hippocampal dependent task, the morris water maze (MWM). A group of animals immunized with vehicle was used as controls (non EAE).

Treatment with DMF resulted not only in less EAE associated physical disability but also, in contrast with EAE non-treated, in a cognitive performance at the level of controls (non EAE).

These results suggest that, in the animal model of MS, DMF has a positive impact in cognitive performance, which might be related with its neuroprotective effects.

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NEUROTOXICITY OF WATERBORNE INORGANIC MERCURY IN FISH - MORPHOFUNCTIONAL BRAIN ALTERATIONS AND BEHAVIOURAL SHIFTS IN *DIPLODUS SARGUS*

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The metal mercury (Hg) is extremely neurotoxic to humans and wildlife. In aquatic environments, Hg is present in organic (mainly methylmercury - MeHg) and inorganic (iHg) forms. Both Hg species can be bioaccumulated and biomagnified in fish, inducing toxic effects. In fact, high levels of Hg were recorded in fish brain [1, 2], along with neurodegenerative damage [3, 4], disturbances on sensory processing [5], and behavioural changes [3, 6]. Nevertheless, it remains to be clarified which Hg species (iHg *versus* MeHg) is mainly accumulated in the fish's brain, as well as the contribution of different uptake routes (water *versus* diet).

In this study, we evaluated the swimming performance of the white seabream (*D. sargus*), and identified alterations in brain morphology together with Hg accumulation in brain and eyes. Initially, fishes were exposed to realistic levels of iHg in water (2 µg L^{-1}) during 7 (E7) and 14 days (E14). After that, fish were allowed to recover for 28 days (PE28).

At E7, exposed fish exhibited a significant decrease of the first swimming distance, as well as a lower time for refuge. In parallel, exposed fish also presented lower total cell number in the optic tectum, cerebellum and hippocampus, together with high levels of Hg in brain and eyes. At PE28, previously exposed fish still swam a smaller distance in the first run, exhibited a lower resistance against the water flow (measured as time to immobility), and presented lower total cell number in the optic tectum. Accordingly, Hg levels in eyes and brain did not decrease during the recovery period.

Realistic levels of waterborne iHg can alter fish swimming performance, and trigger total cell number loss in several brain areas responsible for endocrine regulation, sensory and motor-related systems, fine movement and equilibrium/posture without a rapid reversibility. Such impairments could have repercussions in the organism's fitness and survival, leading to increased vulnerability to predation with significant implications for food chain transfer of Hg.

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TAU PROTEIN IS ESSENTIAL FOR STRESS-INDUCED BRAIN PATHOLOGY

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Exposure to chronic stress is frequently accompanied by cognitive and affective disorders in association with neurostructural adaptations. Chronic stress was previously shown to trigger both, depressive- and Alzheimer-like neuropathology, the latter characterized by hyperphosphorylation of Tau protein, neuronal atrophy and memory deficits. We now show that chronic stress fails to elicit pathological behaviors in mice with a deletion of *Tau* (Tau-KO). Further, Tau-KO mice do not exhibit the typical atrophy of hippocampal dendrites and deficits in hippocampal connectivity observed when animals are subjected to chronic stress. These findings implicate Tau as an essential mediator of the adverse effects of stress on brain structure and function.

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THE "STRESSED" AUTOPHAGY: CHRONIC STRESS BLOCKS AUTOPHAGY IN A MODEL OF TAU PATHOLOGY

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Cumulative evidence suggests the importance of Tau-mediated neurodegeneration in a group of neurological diseases named Tauopathies which includes Alzheimer's disease (AD) and frontotemporal dementia with parkinsonism linked to chromosome-17 (FTDP-17). Consistent with suggestions that lifetime stress may be a clinically-relevant precipitant of AD pathology, we previously showed that stress triggers Tau hyperphosphorylation and aggregation but the underlying cellular mechanisms are unknown. In our current work, we monitor the impact of chronic stress in the two main proteostatic mechanisms that regulate degradation of Tau protein by analyzing Ubiquitin-proteasome system (UPS) and autophagic pathways in P301L-Tau Tg mice. We now demonstrate that chronic stress trigger Tau aggregation in insoluble inclusions followed neuronal cell death in prefrontal cortex of P301L-Tau mice while this Tau aggregation was accompanied by impairment in molecular chaperones involved in UPS degradation machinery. Specifically, we analyze several chaperones and co-chaperones involved in Tau proteasome degradation, such as Hsp70, Hsp90 and CHIP detecting a decrease in their protein levels by chronic stress exposure favouring Tau aggregation. In addition, we also monitored the autophagy-lysosome system, where we observed a blockage of autophagy in chronically stressed P301L-Tau mice as revelaed by decrease LC3-II levels and increased levels of p62. Our findings provide novel evidence about how exposure to stressful conditions could impact of the development of AD pathology implicating both UPS and autophagy in the mechanisms through which stress triggers Tau aggregation.

TAU-DEPENDENT REDUCTION OF NEUROGENIC, BUT NOT ASTROGENIC, POOL IN STRESSED HIPPOCAMPUS

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Tau hyperphosphorylation and subsequent malfunction is causally related to neuronal atrophy/dysfunction as well as memory loss that characterize Alzheimer´s disease. Furthermore, our recent studies demonstrate that Tau protein is essential for stress-triggered dendritic atrophy, synaptic loss and cognitive deficits; however, there is no evidence so far, about the involvement of Tau on changes of neurogenesis, the other neuroplastic feature of adult brain, by chronic stress. For clarifying it, we have exposed Tau-knockout (Tau-KO) and WT mice to chronic stress (9 weeks of chronic unpredictable stress) and evaluate different newly-born cell populations in hippocampal neurogenic niche using Ki67, DCX, GFAP and NeuN markers. We found that stress differentiate affected the different populations of newly-born cells between WT and Tau-KO. Specifically, while chronic stress decreased BrdU-labelled and Ki67/BrdU-double labelled cells in DG of WT animals, this stress effects on the above populations was not found in DG of Tau-KO animals. Moreover, neuroblasts (DCX/BrdU-labelled) and newly-born neurons (NeuN/BrdU labelled) were reduced in stressed WT, but not in Tau-KO animals. In contrast, GFAP-labelled cells were decreased in both WT and Tau-KO animals after stress exposure, indicating that Tau is not essential for stress-induced reduction in DG astrocytic pool. These results suggest the importance of Tau protein in stress-driven neuroplastic changes related to neurogenesis, offering novel cellular targets against stress-driven brain damage.

MULTIPLE SCLEROSIS: STUDYING LIPOCALIN 2 AS A NOVEL PLAYER IN THE PATHOPHYSIOLOGY OF THE DISEASE

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Multiple sclerosis (MS) is an immune-mediated demyelinating disease of the central nervous system (CNS), characterized by the presence of demyelination plaques, inflammation and gliosis that consequently lead to axonal damage. The sequence of events that leads to demyelination remains unclear and the pathophysiological mechanisms are diverse.

Recently we found that lipocalin 2 (LCN2), an acute phase protein that is a part of the defense system against bacteria by binding to iron-loaded siderophores, was increased in cerebrospinal fluid (CSF) and serum of MS patients, when compared to control subjects. Similarly, using the experimental autoimmune encephalomyelitis (EAE) mouse model, LCN2 was detected in brain parenchyma astrocytes, in regions typically affected in MS patients. This expression by astrocytes, together with an increased LCN2 level in the CSF, occurs during the active phases of the disease, which could point towards a role for LCN2 secreted by astrocytes in the mediation of inflammatory responses in the EAE model. To further understand the role of LCN2 in MS pathology we are using the EAE mouse model and inducing EAE both in LCN2-null mice and in WT littermate controls. Non-induced EAE animals are being used as controls. LCN2-null mice induced with EAE do not show major alterations in terms of the clinical score when compared with WT littermate controls also induced with EAE. Regarding the activation of astrocytes, we quantified the percentage of area GFAP+ and did a 3D morphology reconstruction of astrocytes, in the white matter of the cerebellum of LCN2-null mice and WT littermates induced with EAE. The EAE animals present an increase in the GFAP+ area, but we saw no differences between genotype. We also evaluated the role of LCN2 in demyelination, in the cerebellum, using the histochemical coloration luxol fast blue, and the LCN2-null mice with EAE show a tendency to present less demyelination than their induced WT littermates. In addition we also studied the thymus, as it is the organ responsible for T cell development and for controlling organ-specific autoimmunity. To do so we performed flow cytometry in the thymus at different time points of the disease: onset [day 16 post-disease induction (PDI)] and chronic phase (day 23 PDI). We found no differences in thymic cell populations of LCN2-null mice in comparison with WT mice in both phases. Interestingly, regarding only the WT animals at the different phases of the disease, we found that at the onset of disease the percentage of double positive cells decreases greatly, and the percentage of single positive cells increases. Of relevance at the chronic phase of disease the populations' percentages are restored to the control levels.

TREHALOSE ALLEVIATES BEHAVIORAL AND NEUROPATHOLOGICAL DEFICITS IN A TRANSGENIC MOUSE MODEL OF MACHADO-JOSEPH DISEASE

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Machado–Joseph disease (MJD), also known as spinocerebellar ataxia type 3, is the most common of the dominantly inherited ataxias worldwide and is characterized by mutant ataxin-3 misfolding, intracellular accumulation of aggregates and neuronal degeneration. No treatment able to modify the disease progression is available. In this project we evaluated whether trehalose, a natural occurring alpha-linked disaccharide with protein stabilizing properties, is able to rescue the behavioral and neuropathological features of a transgenic mouse model of MJD. Transgenic mice were orally treated with 2% Trehalose solution for a period of 30 weeks. Motor behavior was evaluated at different time points during lifetime and neuropathological features were evaluated upon in-life phase termination. We observed that Trehalose treatment significantly improved the locomotive parameters in stationary rotarod and swimming behavioral tests. Moreover, cresyl violet staining of cerebellum revealed that the thickness of the molecular and purkinje cell layers was significantly larger in treated mice as compared to control mice, suggesting a prevention of neurodegeneration in the treated group. In conclusion, this study suggests that Trehalose could be an effective therapeutic agent for MJD disease.

ACKNOWLEDGEMENTS: This study was supported by Bioblast Pharma.

LOSS OF ADENOSINE A_{2A} RECEPTOR-MEDIATED FACILITATION OF CHOLINERGIC MYENTERIC NEUROTRANSMISSION IN THE ILEUM OF TYPE I DIABETIC RATS

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Gastrointestinal (GI) symptoms such as nausea, vomiting, constipation, diarrhea and fecal incontinence, are reported by 76% of diabetic outpatients (Kim *et al.*, 2011, Neurogastroenterol Motil.). Diabetic dysmotility is probably a long-term complication of enteric nervous system glucotoxicity. Knowing that the purinergic control of synaptic transmission in the central nervous system is affected in diabetic individuals, we decided to investigate if purinergic dysfunction could also play a role in type I diabetic enteric neuropathy.

Adult male Wistar rats (~300-400g body weight) were injected once with streptozotocin (STZ, 55mg/kg, IP). Forty eight hours after injection rats became hyperglycemic (404.11±56.01 md/dL, n=9) and blood glucose was kept at high levels until experimental day 14 (497.33 ± 47.17 md/dL, n=9) (*cf.* King, 2012, Br. J. Pharmacol.). Diabetic rats presented a minimal loss of body weight (-3.51±4.35%, n=9) despite obvious polydipsia/polyuria. *Post-mortem* observation of the GI tract of type I diabetic rats revealed a significant ($P<0.05$) increase in caecum size; the caecum weight compared to the total body weight increased from 2.18±0.17% (n=4) to 5.33±0.55% (n=9) in STZ-injected rats compared to control littermates.

In vitro experiments were performed at day 14 on longitudinal muscle-myenteric plexus (LM-MP) of the ileum of control and STZ-injected rats. The extracellular catabolism of ATP (30 μM) and formation of metabolites (ADP, AMP, adenosine, inosine and hypoxanthine) was assessed by HPLC analysis. The half-degradation time of ATP (30 μM) was 7.18±1.14 min (*n*=6) and 4.33±045 min (*n*=3) in control and STZ-injected rats, respectively. Adenosine formation was faster in the LM-MP of diabetic rats reaching a maximum of 12.18 μM at 10 min incubation with ATP (30 μM); the levels of the nucleoside declined thereafter. In control animals, adenosine reached a maximum of 12.81 μM at 30 min, whose levels remained fairly constant towards the end of the protocol. The inhibitory control of [^{3}H]-acetylcholine ([^{3}H]-ACh) release from stimulated myenteric motoneurons (EFS, 5 Hz, 200 pulses, 1 ms) operated by R-PIA (300 nM)-sensitive adenosine A_1 receptors was comparable in control (-36±4%, *n*=4) and STZ-injected rats (-45±8%, *n*=3). Conversely, the adenosine A_{2A} receptor-mediated facilitation of evoked [^{3}H]-ACh release induced by CGS 21680C (3 nM, 53±10%, *n*=4) was abrogated in diabetic animals (-19±7%, *n*=3).

Enzymatic kinetic experiments suggest that although adenosine formation is faster in the LM-MP of the ileum diabetic rats, the nucleoside is rapidly inactivated thus preventing its accumulation in the extracellular milieu. This situation together with the loss of the facilitatory tonus mediated by adenosine A_{2A} receptors located on cholinergic nerve terminals may contribute to constipation, the most common gastrointestinal complaint of diabetic patients.

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CROSS-SECTIONAL ASSESSMENT OF ALTERATION IN HUNTINGTON'S DISEASE: FROM COGNITION AND OCULOMOTOR PERFORMANCE TO BRAIN FUNCTION AND STRUCTURE

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BACKGROUND: Huntington's disease (HD) is an autosomal dominant neurodegenerative disease, characterized by motor alterations, cognitive decline, and psychiatric disturbances. Functional and structural changes in the central nervous system affect primarily the basal ganglia and fronto-striatal circuitry, preceding overt clinical symptoms by up to two decades [1,2]. Current research aims at characterizing earliest neurodegenerative processes and identification of reliable biomarkers that could help in the assessment of novel neuroprotective therapies.

AIM: Characterization in HD gene carriers of: i) cognitive functioning, ii) oculomotor performance with different levels of working memory and fronto-executive load, iii) structural alterations, and iv) changes in brain function during oculomotor tasks.

METHODS: A comprehensive battery of neuropsychological tests was used to assess overall cognitive functioning of participants: 14 EarlyHD patients, 15 preHD gene carriers (on average far from estimated onset), and 22 healthy controls. Oculomotor function was studied through a psychophysics experiments compound of prosaccades (PS), antisaccades (AS), and n(1,2)-back memory-prosaccades (MPS) and memory-antisaccades (MAS), respectively [3-6]. Most participants underwent magnetic resonance imaging, where brain structure and oculomotor function were assessed.

RESULTS: EarlyHD patients exhibited deficits in almost all the neuropsychological measures and showed overall impairments in oculomotor performance, with significantly lower number of successful trials, and higher percentage of direction and express errors. As expected, this group presented obvious morphometric alterations, with pronounced atrophy of basal ganglia structures and cortical thinning throughout an extended area. In contrast, our pool of preHD participants had no overt cognitive decline, and showed similar oculomotor performance to controls with the exception of more automatized responses in the MAS task. Also, stringent morphometric alterations were undetected, with only significant decrease of the left caudate volume. Finally, fMRI results revealed alterations in both groups of gene carriers, with clear differences now unveiled in preHD participants in tasks requiring the involvement of inhibitory processes, encompassing both cortical areas and the caudate.

CONCLUSION: In accordance with previous studies, EarlyHD patients showed alterations in all assessed components. On the other hand, alterations unveiled in our pool of preHD participants may relate to early neurodegenerative processes and neural dysfunction once assessing specific fronto-striatal mechanisms.

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ALPHA-SYNUCLEIN IS TRANSPORTED AND INDUCES NEUROPATHOLOGICAL AND MOTOR BEHAVIOUR ABNORMALITIES UPON OVEREXPRESSION IN THE RAT STRIATUM

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the degeneration of dopaminergic neurons in the *substantia nigra* (SN), striatal loss of dopamine (DA) and the presence of inclusions rich in α-synuclein (α-syn), among other proteins.

Recent studies in cellular models suggest that migration of α-syn from cell to cell may contribute to disease development and progression. Furthermore, development of recombinant viral vectors for *in vivo* transfer of α-syn has given new possibilities to model PD with reproduction of the neuropathological features of the human disease. In this study, we investigated whether α-syn would be transported in the rat brain and could induce neuropathological and motor behaviour abnormalities. For this purpose, we transduced the rat striatum (Str) with adeno-associated viral (AAV) vectors encoding human α-syn and evaluated the localization, transmission between neurons and the neuropathology caused by α-syn transmission to uninfected cells.

Six weeks post-injection, we detected the transgene in the Str and SN, along with alterations in the rotational behaviour of the animals upon amphetamine or apomorphine induction. No differences in the levels of DA in these brain areas, even though an increase was observed in the injected hemisphere of the motor cortex. Furthermore, no changes were found in the levels of TH, DAT, VMAT-2 and DA D2 receptors in the SN and in the Str, suggesting that despite the neuronal dysfunction induced by α-syn expression that led to alterations in rotational behaviour, it did not cause loss of dopaminergic neurons or terminals. A significant loss in striatal DARPP-32 was observed 6 and 12 weeks post-injection with vectors encoding α-syn, suggesting that the depletion of this protein could have contributed to a deregulation of the system and to motor asymmetry. When investigating whether α-syn was transported to other brain areas, we detected the presence of this protein and eGFP in the thalamus and motor cortex, which may also help to explain the changes in the motor circuits. Furthermore, the SN also presented immunoreactivity for α-syn and eGFP. Importantly, in some TH-positive dopaminergic neurons of this region, we detected α-syn labelling only but not eGFP, indicating a specific retrograde transport through nigrostriatal pathways to the SN.

These results suggest that this animal model, although not reproducing some of the main neuropathological features characteristic of PD, may correspond to an initial stage of the disease and may constitute a useful tool to investigate the early mechanisms regarding α-syn spreading that are associated with this neurodegenerative disorder.

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THE MULTIPLE FACES OF HDAC6 IN STRESS-TRIGGERED TAU PATHOLOGY: IMPLICATION FOR ALZHEIMER´S DISEASE

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Several studies reported that histone deacetylases (HDACs) are important epigenetic components of Alzheimer's disease (AD) etiopathogenesis. Indeed, the expression of HDAC6 significantly increases in the hippocampus of AD patients and transgenic animal models of the disease. Our previous studies have shown that chronic stress and stress hormones, glucocorticoids (GC), trigger Tau hyperphosphorylation and aggregation influencing the levels of Hsp90 molecular chaperone, but the exact underlying mechanisms are still unknown. Recently, it was suggested that Tau aggregates might form through the stress granules (SGs) pathway, since SGs colocalize with and stimulate the formation of Tau aggregates. SGs are dense aggregations in the cytoplasm composed of proteins and mRNAs that are suppressed and sequestered when cells are exposed to stress, allowing the fast synthesis of cytoprotective proteins, such as heat shock proteins. The increased expression of HDAC6 in AD might explain the aggregation of Tau, since HDAC6 deacetylates α-tubulin, enabling the formation of SGs and promoting Tau aggregation. Therefore, we hypothesize that HDAC6 may be a master regulator protein in Tau turnover and aggregation, affecting both neuronal cytoarchitecture and function. Using SH-SY5Y cells expressing wild-type and mutated (P301L) human Tau, hereby we provide some novel evidence about the role of stress and GC in Tau aggregation and neurodegeneration and the crosstalk with HDAC6.

HUNTINGTON'S DISEASE YAC128 TRANSGENIC MOUSE MODEL EXHIBITS INCREASED BRAIN LEVELS OF HYDROGEN PEROXIDE

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Huntington's disease (HD) is an inherited dominant autosomal neurodegenerative disorder characterized by neuronal loss in particular brain regions, notably the striatum and the cortex. HD is associated with a dynamic mutation, an expansion in CAG repeats, located in the exon 1 of the *HTT* gene. When the CAG expansion exceeds 35 repeats, progressive motor disability, including chorea and rigidity, cognitive impairment and psychiatric symptoms become apparent. The *HTT* gene codes for a 350 kDa protein with still unclear function, huntingtin. The expanded CAG tract is translated into an elongated polyglutamine (polyQ) domain at the N-terminus of this protein. The elongated polyQ domain confers mutated huntingtin with new cellular toxic properties and an increased propensity for protein aggregation. Mutant huntingtin expression has been associated with excitotoxicity, impaired calcium-handling, transcriptional deregulation, mitochondrial dysfuntion, increased oxidative stress, among other alterations. In several previous studies, oxidative stress and mitochondrial dysfunction have been shown to have a relevant part in HD pathology. Even so, the precise role of these mechanisms in HD is still not entirely understood. To address this issue, we isolated cortical synaptosomes and mitochondria from YAC128 HD transgenic (expressing full-length human mutant huntingtin) and age-matched wild-type mice brains with 9 and 12 months of age. The production of hydrogen peroxide (H_2O_2) was evaluated, using the fluorimetric Amplex Red probe, in cortical synaptosomes and mitochondria pre-exposed to H_2O_2 (1 mM), a strong oxidant or incubated with antimycin A (AA - 2 µM), a selective inhibitor of mitochondrial complex III. HD synaptosomes at 12 months of age exhibited significantly higher levels of H_2O_2 under basal conditions and after a 30-min pre-incubation with H_2O_2. At 9 months of age, a significant increase in H_2O_2 levels of HD synaptosomes was only observed after H_2O_2 pre-incubation. However, isolated cortical brain mitochondria from wild-type and YAC128 with 12 months of age exhibited no differences in H_2O_2 production at basal levels or after AA or H_2O_2 stimuli. This increase in H_2O_2 levels was not associated with differences in oxygen consumption between YAC128 and wild-type synaptosomes or mitochondria. Moreover, wild-type and HD synaptosomes exhibited similar intrasynaptosomal Ca^{2+} levels, even after exposure to H_2O_2 or AA. Also, no differences were detected in mitochondrial membrane potential of HD and wild-type synaptosomes. These data suggest a gradual increase in reactive oxygen species production following disease progression in YAC128 mice that may not be directly associated to an increase mitochondrial production but rather to a possible decrease in antioxidant defenses.

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ENTINOSTAT (MS-275), A HISTONE DEACETYLASE INHIBITOR, REHABILITATES NEUROMUSCULAR TRANSMISSION IN EXPERIMENTAL AUTOIMMUNE *MYASTHENIA GRAVIS*

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Myasthenia gravis (MG) is an autoimmune disease affecting the neuromuscular transmission due to autoantibodies raised against muscle-type nicotinic acetylcholine receptors (nAChR)[1]. Nowadays, therapeutic strategies to control muscle weakness and fatigability in myasthenic patients are mainly devoted to counteract excessive immune responses. Inhibitors of histone deacetylases (HDACs) have recently received attention as important new tools for therapeutic intervention in several types of cancer and autoimmune diseases [2]. This prompted us to evaluate the putative therapeutic impact of HDAC inhibitors in autoimmune *Myasthenia Gravis*.

Experimental autoimmune *Myasthenia Gravis* (EAMG) was generated by immunizing Wistar rats with the R97-116 peptide, a synthetic peptide corresponding to a specific region on the α subunit of the rat nicotinic AChR, made up in a solution containing the Complete Freund's Adjuvant (CFA)[3]. Thirty days after the first inoculation, the animals were boosted with the R97-116 peptide made up in the Inactive Freund's Adjuvant (IFA). Control animals received the CFA emulsion without the peptide. EAMG animals were randomized to receive the selective inhibitor of HDAC1 and HDAC3, entinostat (MS-275, 3.5 mg/kg, i.p.), every 48h from experimental day 31 to 46 (testing period) or its vehicle (DMSO in PBS).

Characterization of cellular immunity imbalance inEAMG animals was determined by increases in serum ADA activity and decreases in FoxP3 expression in $CD4^+CD25^+$T-cell populations (Treg) isolated from lymph nodes (flow cytometry analysis). Compared to control animals, EAMG rats exhibited more intense ($P<0.05$) tetanic failure (fatigue) of diaphragm muscle contractions induced by phrenic nerve stimulation with 50 Hz intermittent bursts. The initial tetanic peak tension rise (123±6%, $n=8$) during the first 30 seconds of stimulation was absent in EAMG rats. This functional pattern is grounded on characteristic morphological changes, including the loss of muscle-type nicotinic receptors and widening of the synaptic cleft [3]. Fifteen-days treatment with entinostat (MS-275) restored the magnitude of the initial facilitatory component to values observed in control animals. Tetanic tension decline reached a minimum of 14±5% ($n=8$) of the initial tetanic peak tension in the diaphragm of EAMG animals, but this value was significantly ($P<0.05$) increased to 34±4% ($n=4$) after treatment with entinostat (MS-275).

Data show here for the first time that inhibition of HDAC1 and/or HDAC3 by entinostat (MS-275) can rehabilitate neuromuscular transmission failure in EAMG animals.

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PHOSPHORYLATION MODULATES CLEARANCE OF ALPHA-SYNUCLEIN INCLUSIONS IN A YEAST MODEL OF PARKINSON'S DISEASE

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Protein aggregation is a common hallmark in neurodegenerative disorders, but is also associated with phenotypic plasticity in a variety of organisms, including yeasts. Alpha-synuclein (aSyn) forms aggregates that are typical of synucleinopathies such as Parkinson's disease (PD), and is phosphorylated at S129, but the significance of phosphorylation in the biology and pathophysiology of the protein is still controversial.

Exploring the power of budding yeast, we found phosphorylation reduces aSyn toxicity and the formation of inclusions and oligomeric species. Moreover, S129 phosphorylation modulates aSyn dynamics in inclusions that are larger and show FRAP heterogeneity when phosphorylation is blocked (S129A aSyn). While no colocalization was observed between the inclusions formed by WT or S129A aSyn with subcellular protein quality control compartments such as the "juxtanuclear quality control" compartment (JUNQ), or the "insoluble protein deposit" (IPOD), neither with the P-bodies, both WT or S129A aSyn colocalize with several trafficking markers from ER to plasma membrane. Endocytic trafficking studies shown a deficiency in the delivery of late endosomes carrying aSyn to the vacuole that is more pronounced in the strain expressing S129A aSyn. Blockade of aSyn phosphorylation also compromises its degradation. Upon blockade of aSyn expression, cells were able to clear the inclusions formed by WT aSyn. However, this process was much slower for the inclusions formed by S129A aSyn. Interestingly, whereas the accumulation of WT aSyn led to a marked induction of autophagy, cells expressing the S129A mutant failed to activate this protein quality control pathway. Interestingly, clearance of aSyn inclusions was reduced in cells where phosphorylation of aSyn at S129 was blocked, correlating with deficient autophagy activation [1].

The finding that phosphorylation alters the ability of cells to clear aSyn inclusions provides novel insight into the role phosphorylation may have in synucleinopathies, and suggests posttranslational modifications might constitute switches cells use to control the aggregation and clearance of key proteins, opening novel avenues for the development of therapeutic strategies for these devastating disorders.

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POSTERS

NEURO-DEVELOPMENT /REGENERATION

01 KAINATE RECEPTORS INDUCE AXON FORMATION AND OUTGROWTH IN MICE HIPPOCAMPAL NEURONS

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KAINATE RECEPTORS INDUCE AXON FORMATION AND OUTGROWTH IN MICE HIPPOCAMPAL NEURONS

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We have recently shown that kainate receptors (KARs) for glutamate bidirectionally modulate DRG neuronal maturation and neurite outgrowth through their dual signal ability (Marques *et al.*, 2013 J Neurosci 33:18298), the canonical ionotropic activity and the non-canonical metabotropic signaling (see Rodrigues and Lerma, 2012 WIREs Memb Transp Signal 1:399). Moreover, we could show that this is achieved through a differential and subcellular-specific regulation of CRMP2 controlling concomitanly Cav2.2 traficking and neurocytoskeleton. This provided the first link between activity and neurocytoskeleton dynamics during neuronal development, raising KAR as the key tether linking synaptic contact formation and neuronal maturation (Marques *et al.*, 2013 J Neurosci 33:18298). As CRMP2 is a key player in the establishment of neuronal polarity and axonal growth (Cell 120(1):137), we now investigated if KARs are also involved in the establishment of neuronal polarity and if their regulation of neurite outgrowth occurs selectively at the axons. For that purpose, low-density mice hippocampal neurons, co-cultured with astrocytes in order to reduce ambient levels of glutamate, were immunolabeled with antibodies against neuron specific β-III-tubulin (neuronal marker), MAP2 (dendritic marker) and/or SMI-32 (axonal marker) in order to visualize the different subcellular compartments at 3 DIV to evaluate axon elongation and branching, and at 6 DIV to examine the formation of secondary axons. Cells were cultured in the presence of different concentrations of kainate, always in the presence of the selective antagonist of AMPA receptors GYKI53655. We observed that low concentrations of KA (0.3 μM-3 μM), but not a higher one (30 μM), increase the axonal length (KA 0.3 μM 20,7 ± 4,43%*; KA 3 μM 25,4 ± 5,32%*; KA 30 μM 5,06 ± 3,67; *p<0.05) and the number of axonal branch tips in comparison to the effect of GYKI53655 alone. Also, KA at 0.3 μM and 3 μM, but not at 30 μM, induced an increase in the percentage of cells presenting multiple axons (control 15,5 ± 3,11%; KA 0.3 μM 43,0±5,75%*; KA 3 μM 54,3 ± 8,82%*; KA 30 μM 12,1 ± 5,23%; GYKI53655 25 μM 20,3 ± 5,41%; *p<0.05). Both effects were prevented by bisindolylmaleimide (0.2 μM), indicating dependency on PKC activation and on the metabotropic signalling of KARs as this KA concentrations did not trigger any KAR-mediated current. Furthermore, we observed that KA at 0.3 μM and 3 μM, but not at 30 μM, decreased the phosphorylation of CRMP2 at T514, reflecting an increase in the inhibitory phosphorylation of GSK3β at Ser9. Both effects were also prevented by bisindolylmaleimide (0.2 μM). Thus, these results demonstrate that the KARs activation induces axon formation and outgrowth through their non-canonical signalling, most likely through the activation of PKC and consequent inhibition of GSK3β activity, leading to de-repression of CRMP2.

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PROTEASOME GOVERNS PRESYNAPTIC DIFFERENTIATION THROUGH MODULATING AN ON-SITE POOL OF POLYUBIQUITINATED CONJUGATES

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Throughout development, the establishment of functional synaptic contacts is pivotal for the correct wiring of neurons and ultimately proper brain function in the adult life. Presynaptic differentiation is a complex albeit fast event that occurs along axons often at sites far distant from the soma. Thus, it is currently believed that the developing axon relies on local mechanisms to support and sustain its prompt response to environmental cues, which then lead to clustering of presynaptic material. The ubiquitin proteasome system (UPS), which is widely known for its role in the degradation of ubiquitin-tagged proteins, has already been shown to act locally at newly-formed synapses. In fact, E3-dependent local downregulation of synaptically localized kinases governs presynaptic differentiation. Surprisingly, in *Aplysia* sensory-motor neuron co-culture, proteasome inhibitors increase the number of synaptic contacts. However, the role of the proteasome in vertebrate synapse formation remains for the most part elusive. Here, we show that proteasome inhibitors have a striking presynaptogenic effect in developing neurons, thus showing that constitutive proteasome activity might function as a developmental brake to the formation of presynaptic sites. Using a microfluidic system to fluidically isolate axons of rat hippocampal neurons, we observed that local proteasome inhibition leads to the formation of orphan presynaptic terminals, as assessed by the clustering of presynaptic markers. We also demonstrated with an FM dye-based live-imaging approach that the number of active terminals being formed is enhanced. Moreover, simultaneous time-lapse of a presynaptic marker and a proteasome degradation reporter revealed that formation of presynaptic clusters on dendrites is accompanied by an on-site decrease in proteasome activity in the differentiating axon. In an attempt for a deeper understanding of the axon-intrinsic mechanism governing proteasome inhibition-induced presynaptic assembly, we underscored a role for polyubiquitination as the trigger for clustering. Indeed, lysine 48-linked polyubiquitinated proteins accumulate at the site of a nascent presynapse. Expression of ubiquitin mutants that prevent polyubiquitination in a linkage-specific fashion allowed us to attribute a novel role for proteasome-related polyubiquitin chains, through lysines 11 and 48, as triggers for presynaptic assembly. Altogether, we conclude that a transient halt in proteasome degradation leads to a subsequent *on-site* accumulation of polyubiquitinated conjugates, which will function as an intra-axonal signal for presynaptic differentiation. We thus propose that ubiquitin signals constitutively recognized as targets for proteasome-degradation can be transiently accumulated to signal a different event within the developing neuron.

INHIBITORY INJURY SIGNALING REPRESSES AXON REGENERATION AFTER DORSAL ROOT INJURY

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Following injury to peripheral axons, besides increased cAMP, the positive injury signals ERK, JNK and STAT-3 are locally activated and retrogradely transported to the cell body, where they induce a pro-regenerative program. Here, to further understand the importance of injury signaling for successful axon regeneration, we used dorsal root ganglia (DRG) neurons that have a central branch without regenerative capacity and a peripheral branch that regrows after lesion. Although injury to the DRG central branch (dorsal root injury-DRI) activated ERK, JNK and STAT-3 and increased cAMP levels, it did not elicit gain of intrinsic growth capacity nor the ability to overcome myelin inhibition, as occurred after peripheral branch injury (sciatic nerve injury- SNI). Besides, gain of growth capacity after SNI was independent of ERK and cAMP. Antibody microarrays of dynein-immunoprecipitated axoplasm from rats with either DRI or SNI revealed a broad differential activation and transport of signals after each injury type and further supported that ERK, JNK, STAT-3 and cAMP signaling pathways are minor contributors to the differential intrinsic axon growth capacity of both injury models. Increased levels of inhibitory injury signals including GSK3β and ROCKII were identified after DRI, not only in axons but also in DRG cell bodies. In summary, our work shows that activation and transport of positive injury signals is not sufficient to promote increased axon growth capacity, and that differential modulation of inhibitory molecules may contribute to limited regenerative response.

ADDUCIN IS NOT ESSENTIAL FOR GENERATING THE PERIODIC PATTERN OF AXONAL ACTIN RINGS BUT IS NECESSARY FOR MAINTAINING AXONAL DIAMETER

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The actin-binding protein adducin was recently shown to be part of actin rings in the neuronal subcortical cytoskeleton (Xu *et al.*, 2013; Zhong *et al.*, 2014). Here we analyzed α-adducin KO mice to uncover adducin's function in neurons. *In vivo*, α-adducin KO mice (Robledo *et al.*, 2008; Robledo *et al.*, 2012) presented progressive axon enlargement that preceded axon degeneration. *In vitro*, lack of adducin impaired axonal transport and cytoskeleton dynamics. Using stimulated emission depletion super-resolution microscopy (Lukinavicius *et al.*, 2014), we observed that in the absence of adducin, the periodicity of actin rings was maintained, whereas their diameter was increased. Our data supports that adducin's actin-spectrin crosslinking activity is not essential for generating the periodic ring pattern, whereas its capping activity is necessary to control actin filament growth within rings. Moreover, we further establish the ubiquitous nature of the periodic neuronal actin rings by observing their presence in dorsal root ganglia and retinal ganglion cells. Finally, our work raises the prospect that changes in neuronal actin rings may trigger degeneration.

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ACTIVATION OF NEUROPEPTIDE Y RECEPTORS MODULATES RETINAL GANGLION CELL PHYSIOLOGY AND EXERTS NEUROPROTECTIVE ACTIONS *IN VITRO*

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Neuropeptide Y (NPY) is expressed in mammalian retina but the location and potential modulatory effects of NPY receptor activation remain largely unknown. Retinal ganglion cell (RGC) death is a hallmark of several retinal degenerative diseases, particularly glaucoma. In purified RGCs, we detected immunoreactivity and mRNA for NPY and NPY receptors. Also, in retinal slices, using [^{35}S] GTPγS binding assay, we found NPY receptor activity in inner retinal layers. Using purified RGCs and *ex vivo* rat retinal preparations, we have measured RGC intracellular free calcium concentration ($[Ca^{2+}]_i$) and RGC spiking activity, respectively. We found that NPY attenuated the increase in the $[Ca^{2+}]_i$ triggered by glutamate mainly via Y_1 receptor activation. Moreover, Y_1 and Y_5 receptor activation increased the initial burst response of OFF-type RGCs although no effect was observed on RGC spontaneous spiking activity. The Y_1 receptor activation was able to directly modulate RGC responses by attenuating the NMDA-induced increase in RGC spiking activity. These results suggest that Y_1 receptor activation, at the level of inner or outer plexiform layers, leads to modulation of RGC receptive field properties. Using *in vitro* cultures of rat retinal explants exposed to NMDA we found that NPY pre-treatment prevented NMDA-induced cell death via activation of Y_1 and Y_5 receptors. However, in an animal model of retinal ischemia-reperfusion injury, pre-treatment with NPY or Y_1/Y_5 agonist was not able to prevent apoptosis or rescue RGCs. In conclusion, we found modulatory effects of NPY application that for the first time were detected at the level of RGCs. However, further studies are needed to evaluate whether NPY neuroprotective actions detected in retinal explants can be translated into animal models of retinal degenerative diseases.

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MESENCHYMAL STEM CELL SECRETOME: A NEW THERAPEUTIC TOOL FOR CENTRAL NERVOUS SYSTEM REPAIR

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The use of Human Mesenchymal Stem Cells (MSCs) has been proposed as a possible therapeutic tool in CNS regenerative medicine. Research in the last decade strongly suggests that MSC-mediated therapeutic benefits is primarily due to their secretion of bioactive molecules (i.e. secretome) [1]. Indeed our lab as previously shown that the secretome of MSCs from different sources increased neurogenesis and cell survival, inhibits apoptosis and has numerous neuroprotective actions in different pathological conditions [2]. More recently, we have found that the use of dynamic culturing conditions, using computer-controlled bioreactors, can further modulate the MSC secretome thereby generating a more potent neurotrophic factor cocktail. Therefore, in this present work we investigated the role that the MSCs secretome collected from dynamic culture conditions, has on (1) the proliferation, survival and differentiation of resident cells in the dentate gyrus (DG) of adult rat hippocampus, and (2) the physiological recovery of a rat model of Parkinson's Disease (PD). For this purpose we injected the secretome of MSCs (no cell transplantation was used) either in the DG of the hippocampus (1) or in the substantia nigra and striatum (2). The use of dynamic culturing conditions modulated and enriched the secretome of MSCs that once injected into the DG of the hippocampus, was able to increase the levels of cell proliferation (Ki-67$^+$ cells) as well as the number of newborn neurons (DCX$^+$ cells) and astrocytes (GFAP$^+$ cells). Additionally, when this MSC secretome was injected into a PD model, it was possible to observe that the secretome potentiated the recovery of dopaminergic neurons, thereby leading to a recovery in the parkinsonian rats' motor performance. With the present work it was possible to observe that the sole use of the secretome may be used for the establishment of novel CNS therapeutic strategies.

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ADIPOSE STEM CELLS AND OLFACTORY ENSHEATHING CELLS: A NOVEL COMBINATORIAL TRANSPLANTATION STRATEGY FOR SPINAL CORD INJURY

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Spinal Cord Injury (SCI) is a highly incapacitating condition for which there is still no treatment. Cellular therapies are seen as a promising tool for SCI repair. From the myriad of cells currently being tested, Mesenchymal like Stem Cells (MSCs) secrete factors that promote neuronal proliferation [1, 2]; in addition, Olfactory Ensheathing Cells (OECs) are characterized by promoting neuronal regeneration and guidance [3]. Interestingly, our group showed that OECs present positive paracrine interactions with different MSC populations, but more evidently with Adipose Stem Cells (ASCs) [4]. In this sense, we studied the effects of combining ASCs and OECs on: 1) an *in vitro* model of axonal regeneration, using dorsal root ganglia explants (DRGs); and 2) an *in vivo* hemisection rat model of SCI. DRGs isolated from neonatal rats (five to seven-days-old) were placed in direct co-cultures with ASCs and/or OECs during 4 days, after which axonal outgrowth was measured. In SCI animals a total of 80.000 cells of a mixture of ASCs and OECs was injected rostral and caudally to the injury site. Rats without treatment were used as controls. The behavioral evaluation included the BBB test, activity box test and average velocity on water. 8 weeks after injury, a histological analysis was performed in order to determine levels of astrogliosis, inflammation, neuronal regeneration and survival of the transplanted cells. *In vitro* results revealed that OECs alone or in co-culture with ASCs promote the highest increase of neurite outgrowth. Moreover, rats treated with ASCs/OECs presented improved locomotor scores on the BBB test. The analysis of the spinal cord tissues revealed a lower inflammatory profile of animals treated with the transplantation of cells. No major alterations were seen in astrogliosis and axonal organization. Transplanted ASCs were detected in the spinal cord 8 weeks post-lesion. Overall, these results support the use of these cells as a therapeutic approach for SCI.

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COMBINING NEUROPROTECTIVE AGENTS: EFFECT OF RILUZOLE AND MAGNESIUM IN A RAT MODEL OF THORACIC SPINAL CORD INJURY

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Damage to the spinal cord can result in irreversible impairments or complete loss of motor, autonomic and sensory functions. Systemically administered riluzole and magnesium chloride have been widely investigated as neuroprotective agents in animal models of spinal cord injury (SCI) and were found to promote both locomotor improvements and tissue sparing after SCI. Therefore, we aimed to investigate the neuroprotective efficacy of individual and combined administration of these drugs. An *in vivo* experiment was set using nineteen female Wistar-Han rats that underwent a thoracic spinal cord contusion (T8) using a weight drop method to induce severe injury. An hour after injury, animals were randomly distributed to receive: 1) riluzole (2.5 mg/kg,) 2) magnesium chloride (24.18 mg/kg) in a PEG formulation, 3) a combined treatment (riluzole and magnesium), or 4) saline. Subsequent treatments were given in 4 intraperitoneal injections (spaced 12 hours apart). The Basso, Beattie, and Bresnahan (BBB) locomotor rating scale, an activity box test, and a swimming test were used to evaluate behavioral recovery. Histological analysis of the spinal cords was performed to measure the extent and volume of the lesion, neuronal survival, inflammation, axonal preservation, seratonergic and glutamatergic fiber sparing, and myelin degeneration. Our results show that only the riluzole treatment significantly improved behavioral recovery, promoted tissue sparing, diminished lesion volume, increased seratonergic fiber sparing and axonal preservation in the caudal portion of the spinal cord (when administered 1 hour post-injury and with 4 subsequent injections, 12 hours apart). The combined treatment, although simultaneously targeting several excitotoxic-related mechanisms, did not further improve behavioral and histological outcome when compared with riluzole given alone.

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MICRORNA-145 REGULATES NEUROGENESIS THROUGH THE SOX2/LIN28/LET-7 SIGNALING PATHWAY

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MicroRNAs (miRNAs or miRs) regulate several biological functions, including cell fate determination and differentiation. In this respect, we and others have recently demonstrated that apoptosis-associated miRNAs have a functional role during neural differentiation through mechanisms independent of cell death. MiR-145 was already shown to repress sex-determining region-box 2 (Sox2), a key transcription factor for self-renewal, to inhibit pluripotency in human embryonic stem cells. Recently, a role for the Sox2/Lin28/let-7 signaling pathway in regulating proliferation and neurogenesis of neural precursors has been reported. In the present study, we aimed to investigate the precise role of pro-apoptotic miR-145 in neural stem cell (NSC) fate decision, and the possible involvement of the Sox2/Lin28/let-7 signaling pathway in the miR-145-regulatory network. For that, NSCs derived from 14.5-dpc mouse fetal forebrain were grown in monolayer and induced to differentiate. Our results showed that miR-145 significantly increased after induction of NSC differentiation, reaching an ~ 10-fold peak at day 3, when compared to undifferentiated cells. Throughout neurogenesis, miR-145 expression remained elevated, while protein levels of Sox2 and Lin28, a well-known suppressor of let-7 biogenesis, decreased. Of note, neuronal differentiation also resulted in let-7b upregulation. The transfection of NSCs with anti-miR-145, in turn, increased both Sox2 and Lin28 while decreased let-7b and neuronal markers, including βIII-tubulin, NeuN, and MAP2. More importantly, Sox2 and Lin28 silencing partially rescued the impairment of neuronal differentiation by anti-miR-145. In conclusion, our results demonstrate a novel role for miR-145 regulation of NSC differentiation, where miR-145 upregulation, and subsequent decrease of Sox2 and Lin28, appear to be crucial for neurogenesis progression.

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NITRIC OXIDE-INDUCED REGULATION OF NEUROGENESIS: IDENTIFICATION OF POST-TRANSLATIONALLY MODIFIED PROTEINS IN NEURAL STEM CELLS

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Nitric oxide (NO) is a well-established regulator of neurogenesis. NO enhances proliferation of neural stem cells (NSC), and is essential for hippocampal injury-induced neurogenesis following an excitotoxic lesion[1]. Although the main effects of NO on NSC proliferation are via activation of the ERK/MAPK and cGMP/sGC/PKG pathways[1,2], S-nitrosylation may have a substantial role in the activation and/or inhibition of several proteins involved in the neurogenic process. Protein S-nitrosylation is a post-translational modification that consists in the formation of a nitrosothiol group (R-SNO) in cysteine residues, which can promote formation of other oxidative modifications in those cysteine residues[3,4]. It is one of the mechanisms underlying non-classical NO cell signaling that regulate many physiological processes, including neuronal plasticity[5-6]. The aim of this work is to identify proteins modified by S-nitrosylation in conditions that promote cell proliferation in NSC derived from the subventricular zone, and that could take part in non-classical NO signaling.

Treatment with S-nitroso-L-cysteine (CysSNO), a physiological permeable nitrosothiol, increased protein cysteine oxidation and S-nitrosylation in NSC, as assessed by a fluorescence switch assay[7]. Separation by two-dimensional electrophoresis and analysis by mass spectrometry resulted in the identification of several proteins that were modified by treatment with CysSNO. From those, p21Ras, PEBP-1, PCNA, 14-3-3 proteins and hnRNP K, were the focus of further validation due to their relevance in the neurogenic context, including their involvement in the ERK/MAPK pathway. By using the biotin switch technique, we show a strong increase in oxidation and, specifically, in S-nitrosylation signal of p21Ras, PEBP-1, PCNA, 14-3-3 and hnRNP K in the presence of CysSNO.

Overall, this work identifies several proteins as a target of S-nitrosylation in NSC and suggests new candidates for NO-induced regulation of neurogenesis.

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ASCL1 COORDINATELY REGULATES GENE EXPRESSION AND THE CHROMATIN LANDSCAPE DURING NEUROGENESIS

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Vertebrate neurogenesis, to a large extent, is regulated by proneural transcription factors of the bHLH family, such as Ascl1/Mash1. Ascl1 is expressed in neural/stem progenitors within the germinal layers of the developing brain and spinal cord regions, where it promotes sequentially their proliferation and differentiation towards a neuronal program. Previous studies have shown that this occurs via the concomitant regulation of distinct transcriptional target genes. However, what determines which subsets of targets are regulated by Ascl1 in proliferating *versus* differentiating cells remains poorly understood. Here we used a cellular model of neurogenesis to investigate how mutual interactions between Ascl1 and the chromatin landscape contribute to differential gene expression in these two cellular contexts. By combining expression profiling with genome-wide mapping of Ascl1 binding sites (ChIP-seq), and DNAse I hypersensitivity sites (DNAse-seq), we found that: i) Ascl1 binding occurs mostly at distal enhancers and is associated with activation of gene transcription; ii) Accessibility of Ascl1 to its target sites remains largely unchanged in proliferating and differentiating progenitors, as judged by the binding profile of overexpressed Ascl1 in both conditions; iii) Ascl1 can bind regions of closed chromatin at a subset of its target genes in proliferating cells, promoting chromatin accessibility and the appearance of new regions of open-chromatin; and iv) New regions of open-chromatin are associated with genes expressed *de novo* during differentiation. Overall, our study suggests that access of Ascl1 to chromatin in proliferating cells is not a major impediment for the activation of differentiation target genes. In addition, it reveals a novel function of Ascl1 in promoting chromatin accessibility during neurogenesis, linking the chromatin landscape at Ascl1 target regions with the temporal progression of its transcriptional program along the neuronal lineage.

Keywords Ascl1 (Mash1) / neurogenesis / ChIP-seq / transcription / chromatin

THE TRANSMEMBRANE PROTEIN KIAA0319 IS A NOVEL REGULATOR OF AXON GROWTH ACTING THROUGH SMAD2 SIGNALING

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KIAA0319, a transmembrane protein associated with dyslexia and neuronal migration, is highly expressed in neurons. Following injury, KIAA0319 expression is downregulated suggesting a regulatory role during axon growth. Supporting this hypothesis, overexpression of KIAA0319 in DRG and hippocampal neurons leads to a specific decrease of axon growth. Using deletion constructs, the inhibitory KIAA0319 activity was mapped to the initial region of its cytoplasmic domain where the putative phosphosite mutant KIAA0319 Y995A was identified as dominant-negative. Neuronal overexpression of KIAA0319 specifically induced increased SMAD2 phosphorylation, which was reverted by overexpression of a KIAA0319 mutant lacking the cytoplasmic domain. Moreover, the presence of a SMAD2 inhibitor reverted KIAA0319 activity, further supporting the central role of the SMAD2 pathway in the KIAA0319-induced repression of axon growth. Given that the myelin-associated neurite outgrowth inhibitor nogo-A induces SMAD2 phosphorylation, we are currently assessing whether KIAA0319-mediated axon growth inhibition is achieved through the nogo-A/ nogo receptor pathway. To assess whether KIAA0319 deletion might generate the opposite effect of KIAA0319 overexpression i.e., enhanced axon growth, we performed downregulation of KIAA0319 by shRNA. Neurons with acutely downregulated KIAA0319 expression had increased neurite outgrowth. To further establish whether KIAA0319 downregulation was capable of promoting axon regeneration *in vivo*, we generated mice with inducible neuronal deletion of KIAA0319. In these mutant animals a modest effect on axon regeneration was observed. We are currently establishing an *in vivo* overexpression model of KIAA0319 to determine whether *in vivo* the overexpression of this transmembrane receptor represses axon growth.

ADENOSINE A_{2A} RECEPTORS CONTROL CORTICAL NEURONS MIGRATION

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Adenosine is an ubiquitous neuromodulator in the central nervous system by mainly activating inhibitory A_1 receptors (A_1R) and facilitatory A_{2A} receptors ($A_{2A}R$). During development, it has been shown that adenosine receptors also fulfills a neuromodulatory action namely in the control of neurite outgrowth, inhibiting through the activation of A_1R (Neuroreport 12, 3057-63) and promoting neurite elongation through $A_{2A}R$ activation (Neurochem. Res. 36, 2259-69). We now found that in addition to a modulation of neurite outgrowth, adenosine receptors are also involved in the establishment of neuronal polarization, in particular in axon specification. We observed in rat hippocampal neurons that the removal of extracellular ATP and ADP by apyrase (20 U/mL) from days *in vitro* (DIV) 1 onwards, leads to the formation of aberrant secondary axons (SMI-31 positive neurites) and a correlated reduction in the number of dendrites (MAP2-positive neurites) in hippocampal neurons at DIV3. This was not due to the lack of P2 receptors activity, since P2 receptors blockade (PPADS 10 μM) did not mimic the effect of apyrase. Instead, the pharmacological activation of $A_{2A}R$ with the selective agonist CGS21680 (30 nM) induced the formation of secondary axons. Furthermore, we could gather evidence that this involved a regulation of CRMP2 protein, a microtubule-associated protein highly expressed in the developing nervous system crucial for axon specification and outgrowth (Cell 120, 137-49). CRMP2 is a cytosolic phosphoprotein negatively regulated by phosphorylation, which disrupts CRMP2-tubulin binding, and hence microtubule assembly, resulting in growth cone collapse leading to the arrest of axonal elongation and/or axon formation (Nat. Cell Biol. 4, 583-91). We found that the pharmacological activation of $A_{2A}R$ with CGS21680 (30 nM) increased the inhibitory phosphorylation of GSK3βS9 and concomitantly decreased the phosphorylation levels of CRMP2 at Thr514 (GSK3β target). These data show that $A_{2A}R$ control axonal specification and hence the establishment of neuronal polarity, most likely through a de-repression of CRMP2 activity. These evidences raise the hypothesis that $A_{2A}R$, in addition to their role on interneurons migration (Sci. Transl. Med. 5: 197ra104), may be also involved in cortical principal neurons migration either by promoting the transition from a multipolar to a bipolar morphology necessary for the radial migration from the intermediate zone, and/or by modulating axonal extension required for cell migration onto the cortical plate.

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INDUCING AXONAL GROWTH IN 3D ECM-LIKE ENVIRONMENTS

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The ability of injured axons to regenerate and re-innervate their targets after an injury is extremely important. However, despite the considerable advances made in microsurgical techniques, complete functional recovery is often non-achieved, varying according to peripheral or central nerve injuries. Therefore, alternative therapies that can successfully repair injured nerves are still essential. The use of biodegradable hydrogels enriched with growth-supporting and guidance cues has been explored for the treatment of nerve injuries, as well as cell transplantation and biomolecular-based approaches [1,2]. In line with this, the aim of this study was to develop a therapeutic construct based on Gellan-Gum (GG) hydrogels enriched with fibronectin-derived peptides (GRGDS), adipose tissue-derived mesenchymal stem cells (ASCs) and Glial cell-line derived neurotrophic factor (GDNF). The goal of this study relayed on assessing the potential of GRGDS-modified GG hydrogels in promoting axonal growth in an *in vitro* model of axonal regeneration based on Dorsal root ganglion (DRG) explants. Furthermore, the effect on neurite outgrowth of ASC encapsulated within the GRGDS-GG hydrogels were also assessed, as well as the addition of the neurotrophic factor GDNF. In order to improve it bioactivity and stability in culture, iron oxide nanoparticles were used as a delivery system for the GDNF (GDNF-NPs) [3]. The impact of hydrogel modification as well as the effect of the nanoparticles on ASCs behavior was also screened.

The results revealed that the GRGDS-GG hydrogel was able to support DRG neurite outgrowth, which was not observed for non-modified gels. The modified gels were also capable of supporting ASC attachment, and the presence of the nanoparticles had no negative impact on ASC behavior. Moreover, hydrogels encapsulated with ASCs showed a tendency to improve the axonal growth. On the other hand, GDNF-NPs alone or combined with ASCs demonstrated to significantly increase neurite outgrowth, suggesting a beneficial role of the proposed strategy for future applications in nerve regeneration.

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PROFILIN-1 IS A KEY REGULATOR OF AXON ACTIN DYNAMICS, GROWTH AND REGENERATION

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Actin is well recognized as a key player in axon growth, but how different actin-binding proteins control its dynamics is still not fully understood. Using the conditioning lesion, a model in which the intrinsic axon growth capacity is promoted, we determined that the actin-binding protein profilin-1 (Pfn1) is increased in regenerating axons. Besides, acute *in vitro* ablation of Pfn1 precluded axon formation in hippocampal neurons and significantly decreased neurite outgrowth in DRG neurons. Collectively, these evidence point to a critical role of Pfn1 during early neuritogenesis and axonal regrowth following injury. To unravel the importance of Pfn1 during axon regeneration, we generated mice with an inducible neuronal deletion of Pfn1 using cre-lox technology. *In vitro*, actin retrograde flow was decreased in neurons lacking Pfn1 and *in vivo*, axons without Pfn1 had decreased regenerating capacity both after conditioning lesion and sciatic nerve injury. Further sustaining the importance of Pfn1 in axon growth, a constitutively active Pfn1 mutant, Pfn1 S137A, strongly increased axon growth *in vitro*. Whether overexpression of constitutively active Pfn1 is capable of increasing axon regeneration *in vivo* is currently being addressed. Our work further revealed that Pfn1 is capable of linking actin and the microtubule cytoskeleton by interfering with intracellular signaling namely with the PI3K/AKT/GSK pathway. In summary, we uncovered Pfn1 as an important determinant in axon formation, growth and regeneration.

PAIRED RELATED HOMEOBOX PROTEIN-LIKE 1 (PRRXL1) IS ELEVATED FOLLOWING SPINAL CORD INJURY IN A MOUSE MODEL

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A common problem in patients with spinal cord injury is the loss of voluntary bladder control. Immediately after injury there is a period of spinal shock where the bladder is arreflexic and where there is urine retention. Over time a C-fiber mediated automatic voiding reflex tends to develop where the bladder muscle (detrussor) involuntarily contracts causing the individual to involuntarily leak urine throughout the day. This condition is referred to as Neurogenic Detrussor Overactivity (NDO) and the incontinence it causes has a negative impact in the lives of the patients who experience it. In order to identify the molecular changes and molecular determinants underlying the reorganization of the neuronal system responsible for the emergence of neurogenic detrussor overactivity (NDO) we have undertaken the evaluation of changes in transcription factors in mice in response to spinal injury.

We lesioned mice by transecting the spinal cord at the lower thoracic level (T9-10) level. Tissue section were collected from these animals from the Dorsal Root Ganglia (DRG) and Dorsal Spinal Cord (DSC) at the S4-5 region and Spinal Cord (SC) at the lesion site (T9-T10) in Sham operated (control) and at 1 and 4 weeks post-lesion animals. We looked for changes in transcriptional regulation by analysing the RT-PCR data using the ddCT method normalizing (by subtraction) the CT of the gene of interest for each condition from the CT of a control gene known to be unmodified in response to this protocol induction (dCT). Changes in expression of several transcription factors involved in development of the peripheral nervous system such as Prrxl1, Lmx1b, Tlx3, Isl1, Pax 3 where evaluated using RT-PCT.

We could find a significant increase in the expression of Prrxl1 gene in the spinal cord, at the site of injury but no other effects. Prrxl1 is a transcription factor important in the development of the peripheral nociceptive system previously shown to be increased in inflammatory but not neuropathic pain. It seems to be involved in maintenance and possible differentiation of glutamatergic cell populations in DRG and DSC from E14 onwards in mice. To further validate these results we have done immunocytochemistry analysis in Intact and 1week post-lesion mice as well as western blot of T9-T10 spinal cord, the site of injury. We found expression levels to be increased at the superficial layers of the spinal cord in lesioned relative to control animals by immunocytochemistry. The functional implication of these results for NDO will be briefly discussed.

TRANSCRIPTIONAL CONTROL OF VERTEBRATE NEUROGENESIS BY PRONEURAL AND NOTCH PATHWAYS

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Proneural transcription factors such as Ascl1 are the main regulators of vertebrate neurogenesis. It has been recently shown that Ascl1 induces sequentially the proliferation and differentiation of neural stem/progenitor cells, with the concomitant regulation of distinct transcriptional targets at distinct stages of the neuronal lineage. To gather insights into the temporal coordination of the Ascl1 program, we have characterized Ascl1 bound regulatory regions in ventral telencephalon, by combining Ascl1 ChIP-seq with expression profiling of Ascl1 null embryos. Strikingly, we found Hes5 gene to be activated in synergy between Ascl1 and Rbpj/ Notch pathways, suggesting a mechanism for the specific activation of Ascl1 targets in RG neural stem cells characterized by high levels of Notch signaling. We are now characterizing the Notch transcriptional targets genome-wide, with the aim of understanding the transcriptional interactions between the Proneural and Notch/Rbpj pathways, and how this may account for the temporal patterning of the Ascl1 transcriptional program.

NEUDESIN IS INVOLVED IN THE REGULATION OF THE ADULT HIPPOCAMPAL NEUROGENESIS - A LINK BETWEEN PROGESTERONE AND ANXIETY?

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Neudesin, also known as neuron derived neurotrophic factor (NENF), is a heme/steroid binding protein. *In vitro* it has been shown to be a potent stimulator of embryonic neuronal precursors proliferation and differentiation, as well as a survival factor for neurons [1]. Surprisingly, *in vivo* NENF's putative neurotrophic action has only been reported to be novel anorexigenic neurotrophic factor [2]. Since NENF presents a cytochrome b5-like heme/steroid binding domain in it's primary structure, it is classified as a member of the membrane associated progesterone receptor family similarly to PGRMC1, a protein that binds progesterone to induce rapid non-genomic effects independent of nuclear receptors. Although NENF's binding to progesterone has not been proven yet, NENF protein structure has 39% of structural homology to PGRMC1 and has been hypothesized to participate in these cascades [3]. In the brain, progesterone and its metabolites are reported to influence, with gender-specificity, cellular plasticity and neurogenesis particularly at the hippocampus [4]. Another important characteristic of progesterone is its anxiolytic effect revealed to act through non-genomic actions [5]. In this study, we used neudesin-null mice and performed a global characterization of neurodevelopment and adult behavior, as well as, assessed the proliferation status of both adult neurogenic niches (hippocampus and sub ventricular zone). We have found that only neudesin-null males have a delayed neurologic development, observed in the development neurologic milestones. In adulthood neudesin-null males show an anxious-like phenotype in novel contextual exposure paradigms, as the elevated plus maze (EPM), light-dark box and the novelty suppress feeding tests. These gender differences were also present in the neurogenic niche of the hippocampus (sub granular zone) but not in the sub ventricular zone. Additionally neudesin-null males show an impaired freezing behavior in the contextual fear conditioning protocol, a task dependent on adult newly born neurons in the dentate gyrus. Neudesin-null mice gender differences together with the possible involvement of neudesin in the non-genomic actions of progesterone made us hypothesized if neudesin might be involved in this phenomenon. Our data might support a mechanistic link between progesterone, neudesin and hippocampal neurogenesis in the modulation of contextual dependent tasks.

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CASZ1 IS EXPRESSED THROUGHOUT DORSAL ROOT GANGLIA AND DORSAL SPINAL CORD DEVELOPMENT

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PrrxI1 is a paired-like homeodomain transcription factor involved in the development/maintenance of both spinal cord (SC) dorsal horn and peripheral nociceptive neurons, dedicated to the processing of pain input(1-3). In previous studies, our lab group has found that PrrxI1 directly regulates Casz1 expression in mouse dorsal SC. Casz1 encodes an evolutionary conserved C2H2 Zn-finger transcription factor. In *Drosophila*, Casz1 homolog, Cas, regulates neural fate(4). More recently, Liu and colleagues(5) have shown that Casz1 is important for cell cycle exit and for differentiation in several human neuroblastoma cell lines. However, Casz1 role in nociceptive system development has not yet been addressed. Here we show that Casz1 is expressed throughout mouse dorsal root ganglia (DRG) and SC development. In the DRG, Casz1 is expressed in all sensory populations during embryonic (E14.5) and early postnatal stages (P7). In dorsal SC, Casz1 expression exhibits a very dynamic pattern. We observed a transient expression in the dorsal most portion of the SC between E10.5-E11.5. At E13.5, Casz1 expression is restarts in glutamatergic neurons located in the superficial laminae until P7. Therefore, further functional analysis will be necessary to unveil the biological importance of Casz1 gene during DRG and dorsal SC development.

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BENEFICIAL EFFECTS OF NEURON-TARGETED BDNF GENE DELIVERY MEDIATED BY NON-VIRAL TMC-BASED VECTORS

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Peripheral neuropathies are very common diseases that still lack an effective treatment option. Envisaging an intervention in peripheral neuropathies it is important to enhance nerve regeneration as well as prevent nerve degeneration. Neurotrophic factors play a crucial role in promoting neuronal trophic support and survival what make them promising disease modulating therapeutic agents to be used in the development of such therapeutic interventions.

Here we propose a non-viral vector based on trimetyl chitosan (TMC) to deliver therapeutic genes to peripheral neurons in order to efficiently promote neuroprotection/regeneration. TMC-plasmid DNA encoding for the brain-derived neurotrophic factor (BDNF) complexes were formed and subsequently targeted to neurons by grafting the non-toxic carboxylic fragment of tetanus toxin, known as neurotropic and able to be retrogradely transported along axons. We investigated whether enhanced expression of BDNF by a peripheral intramuscular administration of these vectors could protect sensorial and spinal motor neurons in a sciatic nerve crush injury animal model.

Our results demonstrate a positive effect of BDNF treatment in the sensorimotor functional recovery as well as a higher expression of neurofilament and Schwann cell markers in the injured nerves of BDNF-treated animals. Besides neuroprotection and neuroregeneration improvement we also observed that BDNF treatment resulted in gastrocnemius muscle protection to denervation that can be responsible for the functional effects.

Altogether, our data show the potential of TMC-based vectors to be used as non-viral gene carriers to deliver therapeutic genes to peripheral neurons and thus provide an effective therapeutic intervention for peripheral neuropathies.

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TUDCA REDUCES AMILOID-BETA PEPTIDE BURDEN IN APP/PS1 MICE BEFORE AND AFTER THE ONSET OF AMYLOID PATHOLOGY

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Alzheimer's Disease (AD) is a neurodegenerative disorder hallmarked by amyloid-β peptide (Aβ) load, leading to memory deficits. Aβ burden may result from impaired production and/or clearance. The lipoprotein receptor-related protein 1 (LRP1) mainly mediates transport of Aβ from brain to periphery. Impaired LRP1 function significantly decreases the Aβ clearance. Tauroursodeoxycholic acid (TUDCA) is a neuroprotective bile acid, which has been shown to ameliorate memory defects in APP/PS1 transgenic mice. However, the therapeutic mechanisms of TUDCA in AD are still partly unknown. Here, we aimed to explore whether TUDCA modulates Aβ load in AD. We fed 2 month-old APP/PS1 mice with a diet containing 04% TUDCA, or no bile acid, for 6 months. In addition, 7 month-old APP/PS1 mice were injected 500 mg/kg bw TUDCA i.p., or vehicle, every 3 days for 3 months. Wild-type littermates were used as controls. Brains were processed for Aβ immunohistochemistry, and total proteins were analyzed by Western blot (WB) and ELISA. The effect of TUDCA in Aβ accumulation was further evaluated *in vitro*, using differentiated N2a cells treated with the bile acid before and after Aβ incubation. We also analyzed the effect of TUDCA in intracellular Aβ levels, after wash-out procedures. Finally, TUDCA modulation of APP processing was assessed in the transgenic APP/PS1 N2a cells.

Our results showed that TUDCA significantly reduced amyloid plaque burden of APP/PS1 mice treated before or after the onset of amyloid pathology. Further, APP/PS1 mice treated with TUDCA presented increased levels of LRP1. *In vitro*, TUDCA treatment of differentiated wild-type N2a cells significantly decreased cell-associated Aβ levels. However, TUDCA did not affect Aβ intracellular levels after washing out Aβ from the culture medium, or APP amyloidogenic processing.

Our data suggest that TUDCA is a potential therapeutic alternative for AD pathology, possibly by modulating Aβ uptake in neuronal and non-neuronal cells.

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NEUROPATHIC PAIN AND COGNITIVE IMPAIRMENT IN A RAT MODEL OF TYPE 1 DIABETES: THERAPEUTIC POTENTIAL OF A NOVEL POPULATION OF MESENCHYMAL STEM CELLS

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One of the several progressive complications associated with diabetes is neuropathy, the most disabling and costly diabetes complication. The efficacy of current treatment strategies for neuropathy is still very limited, with most treatments having undesired side effects. Further, diabetes is a well-recognised risk factor for the development of Alzheimer's Disease (AD) (or AD-like cognitive dysfunction), the most common cause of dementia worldwide and for which there is still no cure. We are currently investigating the therapeutic potential of a novel bone marrow-derived mesenchymal stem cell (MSC) population in the well-established streptozotocin (STZ)-induced Wistar rat model of painful diabetic neuropathy (DN) and cognitive impairment. This novel MSC population results from the development of a new platform technology for MSC based on the Orbsen Therapeutics Ltd. discovery of an exciting novel MSC marker, CD362. Our approach consisted in administering intravenously $CD362^+$ MSCs, or vehicle to STZ-diabetic rats one week after STZ injection. The efficacy of the $CD362^+$ MSCs in preventing the development of behavioural signs of DN, namely altered nociceptive responses to mechanical and thermal stimulation, was evaluated over time. The Randall-Selitto paw-pressure test and the Hargreaves test were used to evaluate mechanical and thermal sensitivities, respectively, and were performed before STZ injection and then every two weeks until the 10^{th} week post-STZ injection. As to the evaluation of the efficacy of the $CD362^+$ MSCs in preventing the development of behavioural signs of cognitive impairment, more precisely spatial learning and memory impairment, animals were tested in a Morris Water Maze at weeks 6 and 7 post-STZ injection. Body weights and blood glucose levels were monitored every two weeks throughout the *in vivo* experiments, and the levels of blood glycated haemoglobin A1C (HbA1C) were quantified at week 10 post-STZ injection. Our results show that treatment of STZ-diabetic rats with $CD362^+$ MSCs significantly improves mechanical hyperalgesia, prevents the development of thermal hypoalgesia, and improves cognitive impairment as compared to non-treated STZ rats. Metabolic parameters typical of this disease model (impaired weight gain, hyperglycaemia, and elevated HbA1C levels) were not affected by intravenous injection of MSCs. Our data strongly suggests that administration of this novel MSC population—$CD362^+$—may be a useful strategy to manage neuropathy and cognitive dysfunction associated with diabetes. It remains to evaluate the mechanisms underlying the effects of these MSCs, a subject that we are currently investigating.

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IN VITRO EVALUATION OF THE ANTICANCER EFFECT OF A NEW HYBRID COMPOUND BASED ON A TRIAZENE-HISTONE DEACETYLASE INHIBITOR COMBINATION

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Gliomas are the most frequent and aggressive primary brain tumors in adults. Temozolomide (TMZ), a triazene alkylating drug, is still the best chemotherapeutic drug available for these type of tumors. However, the treatment with this agent remains only partially successful, demanding for more effective therapies (1). Hybrid compounds may represent a new approach in this challenge. These molecules, result from the combination of two different and independently acting molecules into one covalently linked compound, benefiting from a synergy mechanism with a greater overall pharmacological effect (2). Our research team has synthetized an innovative antineoplastic hybrid compound (HYBCOM) which binds two different molecules – a triazene agent, derived from TMZ, and a histone deacetylase inhibitor drug.

Thus, we tested the chemotherapeutic efficacy of TMZ in comparison to this novel agent. To accomplish this aim, we performed different assays using the mouse glioma GL261 cell line, treated with a single incubation of TMZ (250 μM) or HYBCOM (100 μM), for 72h. In order to compare the anticancer effect of these drugs, we determined cellular viability (MTS test), proliferation (Ki67 immunolabeling) and death (Guava Nexin® Annexin V, flow cytometry). Moreover, we have also assessed autophagy (LC3 II/I and Beclin-1 protein expression, Western Blot), cytoskeleton dynamics (F-actin immunolabeling), cell migration (Boyden chamber assay) as well as the expression of the multidrug resistance-associated protein 1 (Mrp1, Western Blot).

Our results clearly show that HYBCOM possesses a higher chemotherapeutic effect than TMZ alone. In this respect, HYBCOM induced a further loss on cell viability (less 29%, $p<0.01$) and proliferation (less 52%, $p<0.05$). Induction of cell death was similar with both compounds, although detection of autophagy through the LC3 II/I and Beclin-1 protein expression were correspondently 28% ($p<0.05$) and 42% higher with HYBCOM as compared with TMZ. Moreover, HYBCOM significantly turns GL261 cells more susceptible to switch from a polar to a non-polar morphology (more 50% than TMZ, $p<0.01$). This morphological change may be associated with a loss of migratory properties, as demonstrated by our recent results based on the Boyden chamber assay (minus 12% than TMZ). Remarkably interesting is the fact that while the drug resistant phenotype is increased upon TMZ (37%, $p<0.01$), HYBCOM showed the capacity to downgrade Mrp1 expression below control levels (less 42%, $p<0.01$). In conclusion, our data reveal that the synthetic compound HYBCOM has significant and promising chemotherapeutic advantages over TMZ, suggesting that this novel molecule can represent a valuable tool for glioma therapies.

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HYDROGELS FOR NERVE REGENERATION: A COMPARATIVE STUDY

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Damage to the peripheral or central nervous system is mainly characterized by axonal degeneration, demyelination, and interruption of the ascending and descending neuronal pathways[1,2], which hinders a successful recovery of the injured nerves. Therefore, strategies using artificial matrices such as hydrogels has been exploited to support nerve regeneration. Additional benefits include the possibility of cell delivery and proliferation in the lesion site[3]. Moreover, it is possible to modify them with extracellular matrix (ECM)-derived peptides[4] such as collagen, fibronectin and laminin and use different stimulating factors, for instance stem cells[1] or neurotrophic factors to enhance their potential[5]. The use of Mesenchymal stem cells (MSCs) is a promising therapy for this purpose due to the neuroregulatory and neuroprotective role of its secretome[2]. Furthermore, the use of external growth factors, such as Glial cell line-derived neurotrophic factor (GDNF), is known to promote axonal growth and neuronal survival[5]. Herein, we used GDNF conjugated to iron oxide nanoparticles (NPs), to promote a higher stability of the factor and preserve its activity for longer periods of time[5]. In this sense, the first objective of the present work was to study the ability of three substrates - collagen, peptide-modified Gellan-Gum[4], and NVR-Gel[5] – in promoting axonal regeneration using an *in vitro* Dorsal Root Ganglion (DRGs)-based model. Afterwards, we intended to evaluate the beneficial effects of the combination of these substrates with Adipose derived MSCs (ASCs) and GDNF-NPs in axonal growth. Meanwhile, we performed safety studies on the use of NPs with ASCs. Results suggest that the NPs do not have a cytotoxic effect on ASCs. Furthermore, the three hydrogels were able to promote DRGs neurite outgrowth by itself, however this growth was improved by the use of ASCs.

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INFLUENCE OF CELL PASSAGE NUMBER ON THE EFFECT OF ADIPOSE STEM CELLS SECRETOME ON NEURONAL CELLS

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Mesenchymal stem cells (MSCs) have already shown to have a positive effect on central nervous system (CNS) cell populations, regarding their viability, proliferation and densities. Such effect has been mostly attributed to soluble factors, as well as vesicles, present in their secretome. Yet, little is known about the impact that passaging might have in the secretion profile. Aiming at showing how MSCs passage number influences the effect their secretome has in neuronal cell populations, rat post-natal hippocampal neuron primary cultures were incubated with secretomes, collected as conditioned media (CMs), obtained from adipose stem cells (ASCs) in P3, P6, P9 and P12. Simultaneously, to evaluate the influence of passaging in the neurodifferentiating properties of the secretome, human neuroprecursor cell (hNPCs) cultures were incubated with CMs collected the same way. The results show a tendency to increase viability and proliferation of post-natal hippocampal neuronal cultures, reaching the highest value when incubated with CM P9. Using fluorescence microscopy, a decreasing tendency was observed in the number of neurons (MAP-2+ cells). Still, none of this results presented statistical significant differences. Also, no differences were observed when comparing percentages of hNPCs that differentiated after incubation with CMs from different passages. As an attempt to characterize the secretome obtained from different passages, a proteomic analysis was performed using mass spectrometry. The results obtained this way allowed us to identify several proteins, like *Clusterin, Pigment epithelium derived factor (PEDF), DJ1, Interleucin-6 (IL-6), Cystatin and Galectin,* all of which have already been proved to have a neuroprotector/neurodifferentiating role. Our observations lead us to conclude that cellular passaging does not influence significantly ASCs secretome properties concerning their ability to induce survival, proliferation and differentiation in neuronal cells, as does not alter its composition regarding the identified proteins.

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CYP46A1 MODULATES CHOLESTEROL METABOLISM IN NEURONS AND AMELIORATES SOME OF THE PATHOPHYSIOLOGIC FEATURES INDUCED BY U18666A, AN INTRACELLULAR CHOLESTEROL TRANSPORT INHIBITOR

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Accumulation of unesterified cholesterol in endosomal and lysosomal compartments leads to central nervous system abnormalities that share some clinical and histopathological similarities between Alzheimer's disease and Niemann Pick Type C disease (NPC). Interestingly, it has also been shown that Rab GTPases overexpression ameliorates the pathological phenotype through the modulation of cholesterol endosomal trafficking. Since we have shown that overexpression of the neuronal specific cholesterol 24-hydroxylase (CYP46A1) results in a decrease in cholesterol levels, and in an increase in sGTPases activity, we have investigated the protective role of CYP46A1 in a model of pathological intracellular cholesterol accumulation, induced by the amphipathic drug U18666A.

We have determined that cells stably overexpressing CYP46A1 (SH-CYP) do not accumulate free cholesterol when exposed to U18666A, although the basal levels of this sterol are increased when compared to the control cells (SH-EMPTY). Moreover, SH-CYP cells do not present the increase in the production of reactive oxygen species (ROS) and lipid peroxidation induced by U18666A treatment observed in SH-EMPTY cells. Since impairment of intracellular cholesterol traffic induces the activation of the apoptotic cascades, we have determined the effect of U18666A treatment on apoptosis induction. While U18666A treatment induced a significant increase of apoptotic nuclei in the SH-EMPTY cells, it had no effect on SH-CYP cells. Furthermore, in accordance to what was observed for the SH-CYP cells, transient overexpression of CYP46A1 in SH-SY5Y cells and primary cultures of rat neurons abolished the U18666A-mediated increase of ROS levels.

To evaluate if CYP46A1 might promote a protective response through modulation of the expression of oxidative stress-related enzymes, total protein extracts from N15DIV incubated with U18666A and transiently transfected with the Empty or the CYP vector were subjected to SDS-PAGE and blotted for superoxide dismutase-2 (SOD2) and heme oxygenase-1 (HO-1). While U18666A and CYP did not alter the expression of SOD2, U18666A induced a reduction in the expression of HO-1 and, in contrast, CYP induced an increase in the expression of HO-1. Although CYP46A1 did not inhibited the NPC phenotype induced by U18666A, neurons overexpressing CYP46A1 and treated with U18666A showed levels of HO-1 expression similar to control cells. In addition, increased levels of HO-1 were also observed in total protein extracts from cortical tissue of CYP46A1 transgenic mice, when comparing to the wt controls. These results suggest that the increased basal levels of HO-1 in this model may represent a protection against oxidative stress.

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THE ROLE OF LIPOCALIN-2 IN HIPPOCAMPAL NEUROGENESIS ORCHESTRATION

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Stress is defined as a challenge to the homeostatic equilibrium of the organism and when long lasting it severely affects quality of life (1). In fact, stress maladaptation is considered to be an etiological factor in the emergence of mood disorders like depression and anxiety. Stress response elicits a cascade of hormonal and behavioral changes as an attempt to maintain homeostasis and at the level of the central nervous system (CNS), the hippocampus is considered to be a central target and executor of adaptive responses (1). Of interest, the hippocampus is unique in its structural and cellular plasticity in the sense that, even in adulthood, stem cells reside here and continuously give rise to new neurons. This adult neurogenesis is extremely dynamic and modulated by pharmacological, environmental and physiological stimuli and the newborn neurons are considered to highly contribute to the local network. Of interest, adult neurogenesis is well known to be regulated by stress and, conversely, to regulate stress responses (1). Of course there is no simple, linear relationship between stress hormones and adult neurogenesis. As an attempt to disclose putative factors in this crosstalk of stress modulation, in the present work we have assessed the modulation of hippocampal neurogenesis in an animal model with a targeted deletion of lipocalin-2 (LCN2). As part of the lipocalin protein family, LCN2 plays a critical role in the regulation of various physiological processes, such as inflammation and innate immune response (2). Several roles for cell proliferation and death has also been attributed to this protein, including during kidney development (3), in hematopoiesis (4) and even cancer, mainly recognized due to the capacity of LCN2 to traffic iron within cells (3). In addition, LCN2-null mice were described, at a physiological level, to present a sustained activation of the hypothalamic-pituitaryadrenals axis translated into increased levels of corticosterone, accompanied by an anxious and depressive-like phenotypes as well as structural losses in the hippocampus (5). Also it was described to control anxiety and neuronal excitability upon mild stress exposure (6). But specifically how is hippocampal neurogenesis being modulated upon LCN2 deletion and to which extent it contributes to animal's behaviour and stress response is not quite known.

Here we shown that LCN2-null mice exhibit significant cell proliferation deficits, accompanied by impairments in adult neurogenesis progress and in contextual fear conditioning paradigms. Of interest, the neurogenic effects of voluntary wheel running were (partly) sufficient to re-establish neurogenesis in LCN2-null mice, while the effects of stress on adult neurogenesis revealed to be dependent on LCN2. The current observations will certainly contribute to the current interests on the role of hippocampal neurogenesis in the etiology of mood, aiming to identify key regulators of executor of adaptive responses at the hippocampus.

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THE SUBEPENDYMAL ZONE PROLIFERATIVE PATTERNS AND PROGENITOR CELL DISTRIBUTION IN MICE: SPECIES MATTERS!

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The subependymal zone (SEZ), the major neurogenic niche of the adult brain, responds to brain insults and is modified in many neurodegenerative diseases, eliciting expectations for the development of new endogenous regenerative therapies that ultimately envisage human applications. Nevertheless, much remains to be done to fully understand the regulation and the potential of the SEZ cells both in physiological and non-physiological conditions. So far, the most widely used models to study adult neurogenesis in the SEZ are rodents. Additionally, other studies on the SEZ are performed in nonhuman primates such as monkeys and in human postmortem material. Interestingly, when comparing the SEZ niche in different species, differences are observed. To translate studies from animal models to humans for clinical applications, it is necessary to understand the SEZ species-specific variations. In this study, we have determined the proliferative pattern and the progenitor distribution profile along the anterior-posterior and dorsal-ventral axes in mice, similarly to what was previously performed for rat. We found no differences in the proliferation in the anterior, intermediate and posterior SEZ and along the lateral wall. However, we report relevant differences between two close related species, rats and mice, which should be taken into account when extrapolating data on the SEZ from mice to rats and vice-versa.

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CAENORHABDITIS ELEGANS SNX3 MUTATION LEADS TO IMPAIRED DEVELOPMENT AND BEHAVIORAL DEFICITS

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Protein assembly and turn over abnormalities are hallmarks of several neurodegenerative disorders [1]. The Sorting Nexins family of proteins (SNXs) plays pleiotropic functions in protein trafficking and intracellular signal in neuronal and non-neuronal cells, and has been associated with several human diseases that result from abnormal endosomal function, namely, Alzheimer's disease [2]. Despite the reported roles of SNXs in protein homeostasis in neurodegeneration, not much is known about SNXs function in the nervous system. The aim of this project was to use the nematode *Caenorhabditis elegans* that encodes in its genome eight SNXs orthologs, and is a reference model organism to study the function and malfunction of the nervous system, to functionally characterize SNXs. Here we report that SNX3 gene mutation led to an array of developmental defects, namely, reduced brood size, embryonic lethality, delayed hatching, and to a decreased life span. Additionally, SNX3 mutant worms presented distinct behavioral deficits, such as, increased motor uncoordination, impaired chemotaxis and susceptibility to osmotic, thermo and oxidative stresses, which implies perturbed neuronal functions. Altogether, our data supports a prominent role of SNX3 in nervous system development.

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TUDCA IMPROVES MOTOR SYMPTOMS AND REDUCES NEUROINFLAMMATION IN A TRANSGENIC MOUSE MODEL OF MACHADO-JOSEPH DISEASE

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Machado-Joseph disease (MJD), is an autosomal dominant neurodegenerative disorder for which no effective treatment is currently available. We have generated a new transgenic mouse model expressing human ataxin-3 with an expanded CAG tract ubiquitously and at near-endogenous levels. CMVMJD135 mice develop a severe and progressive neurologic phenotype, with intranuclear inclusions in neurons and brain pathology consistent with the human disease. Tauroursodeoxycholic acid (TUDCA) is a bile acid (BA) with neuroprotective action, through its anti-amyloidogenic and chemical chaperone activities and its ability to modulate apoptotic pathways. This BA is orally bioavailable, BBB permeable, and has a very low toxicity profile. In addition, TUDCA has been approved by FDA for chronic use in humans to treat liver disorders. TUDCA has been shown to be beneficial in several models of different neurodegenerative diseases.

The objective of this work was to test the therapeutic efficacy of TUDCA on the motor phenotype and neuropathology of CMVMJD135 mice, and to understand the mechanism of action of this compound in the brain.

Four groups of animals were used: CMVMJD135 and wt littermates under normal or supplemented diet with 0.4% of TUDCA. The treatment regimen was performed from 5 to 34 weeks of age. A battery of motor and non-motor behavior tests were performed every two weeks. At the end stage, the brains were collected for neuropathology analysis. The brainstem was used for western-blot and qRT-PCR analysis.

Our results showed that food supplementation with TUDCA delayed the onset of disease and improved the motor phenotype of CMVMJD135 mice, including balance, motor coordination and gait parameters. Furthermore, TUDCA administration ameliorated neurological reflexes, exploratory movement deficits and partially rescued muscular strength problems. We also observed that TUDCA treatment was able to (i) reduce the steady-state levels of mutant ataxin-3, (ii) reduce the aggregate load of ataxin-3 in the pontine nuclei and (iii) to normalize the levels of TNF-α, Il1β and Il10 in the brain of CMVMJD135 mice. These results demonstrate the therapeutic efficacy of TUDCA in a mammalian model of MJD, and support the involvement of anti-inflammatory properties in its neuroprotective action. We conclude that TUDCA could have important applications for the treatment of neurodegenerative diseases, including MJD.

BRAIN CHOLESTEROL 24-HYDROXYLASE (CYP46A1) MODULATES DENDRITIC MORPHOGENESIS AND SYNAPTIC FUNCTION VIA TRKB-GGTASE I INTERACTION

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The CYP46A1 gene codes for a neuronal specific cytochrome P450 responsible for the conversion of cholesterol to 24S-hydroxycholesterol, the major pathway for brain cholesterol elimination. Cyp46a1 null mice exhibit severe deficiencies in spatial, associative, and motor learning, and hippocampal long-term potentiation, which are probably due to a decrease in the supply of isoprenoids. Conversely, CYP46A1 transgenic mice (C46-HA) have improved cognitive performance accompanied by increased levels of intermediaries of the mevalonate pathway. Accordingly, we have found higher levels of prenylated small guanosine triphosphate-binding proteins (sGTPases) in the brain tissue of CYP46A1 transgenic mice and in primary cultures of rat neurons transfected with this enzyme. Herein, we show that overexpression of CYP46A1 in primary cultures of rat cortical neurons promotes dendritic outgrowth in transfected 4 days *in vitro* neurons (DIV) and, additionally, increases the number of dendritic protrusions and synaptic proteins in 21DIV neurons, in a prenylation dependent manner. We also show an increase in synaptic proteins in the crude synaptosomal fraction (P2) of C46-HA mice brain tissue, which is coherent with our vitro data. Moreover, we observe a significant increase in the phosphorylated levels of Trk B receptor in P2 fraction of C46-HA mice brain when compared to wild type, and an increase in the interaction between p-TrkB and geranylgeranyl transferase I. In conclusion, our results show that CYP46A1 overexpression increases dendritic development and synaptic proteins recruitment that is dependent on protein prenylation, and in the increased interaction of TrkB and GGTase-I as a consequence of membrane cholesterol loss.

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P2Y1 RECEPTOR IS INVOLVED IN THE MIGRATION OF MEDIAL GANGLIONIC EMINENCE-DERIVED INTERNEURONS

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One of the fundamental processes for the establishment of a correctly wired cerebral cortical circuitry is neuronal migration that is crucial to define the final allocation of neurons in the brain and the subsequent formation of synaptic networks. In fact, defects in the migration of cortical GABAergic interneurons have been associated with several neurological and psychiatric conditions (Marin, 2012, 17, 107-120). Thus, unravelling the precise cellular and molecular mechanisms underlying the migration and positioning of interneurons during corticogenesis is of upmost importance. Extracellular purines (ATP and adenosine) operate one of the most primitive receptor signalling systems, which suggests their putative involvement in development. Indeed, purinergic receptors (P1 and P2 receptors) have been identified in early embryonic stages, but their role in brain development remains ill defined.

Our group recently demonstrated that the A_{2A} receptors ($A_{2A}R$) antagonism delays migration and the insertion of GABAergic neurons into the hippocampal circuitry (Silva *et al.*, 2013, 5, 197ra104). Moreover, it has been recently shown that P2 receptors can control axonal outgrowth (del Puerto *et al.*, 2012, 125, 176-188), a crucial event for neuronal migration, suggesting that P2 receptors may also play a role in cortical interneurons migration. Indeed, we now found that P2Y1 receptors (P2Y1R), which promote axonal outgrowth, are involved in medial ganglionic eminence (MGE)-derived interneurons migration. We observed in MGE explants from E13-mice that the selective blockade of P2Y1 receptors (MRS2179 10 μM) reduced the migration of interneurons from the MGE explant. This constitutes the first indication for an involvement of P2Y1R in interneuronal migration, which re-enforces for a central role of purinergic signalling in neuronal migration and in corticogenesis.

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THE ROLE OF THE TRANSCRIPTION FACTOR AP2γ ON THE MODULATION OF ADULT GLUTAMATERGIC NEUROGENESIS AND EMOTIONAL AND COGNITIVE FUNCTION

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Adult neurogenesis represents one form of non-synaptic neural plasticity that is critical for brain homeostasis and for adaptations to the ever-changing environment. Its regulation, by distinct factors, is thus of relevance both to physiological and pathological conditions affecting the CNS. Several studies suggest a crucial role of adult hippocampal neurogenesis in the pathogenesis and treatment of stress-related disorders, such as depression. However, the molecular signature behind these events is still poorly characterized. The understanding of important players in processes such as proliferation, maturation and specialization of neuronal precursors may reveal promising adult neurogenesis modulators with therapeutical potential. Herein, we described AP2γ, a transcription factor known to display a role in neurodevelopmental processes, as a trigger for the generation and maturation of new glutamatergic neurons in the adult brain. Subsequently, we describe the mechanisms by which AP2γ regulates glutamatergic neurogenesis *in vitro* (using transfected cell lines) and *in vivo* (using both AP2γ knock-out and conditional AP2γ mice and over-expressing retroviral vectors). Finally, we demonstrate its critical role in hippocampal and prefrontal cortex activity using structural, electrophysiological and behavioral end-points. Combining the influence of these brain regions, in emotional and cognitive functions, with the behavioral and functional data presented here, it becomes clear the potential relevance of AP2γ in neuronal function in health and in disease. Thus, it became relevant to understand the potential of AP2γ as an effective modulator of the adult glutamatergic neurogenesis process in the pathological context, namely in depression. For that, wild-type (WT) and AP2γ$^{+/-}$ mice were exposed to a chronic mild stress (CMS) protocol, which triggers depression-like features. We accessed the molecular and behavioral impact of AP2γ heterozygous deletion in healthy and depressive-like animals. Results show that, under stress conditions, AP2γ deletion induces an increase of adult neurogenic progenitors consistent with a resilient effect to the CMS-induced deficits in cognitive tasks.

KEYWORDS: AP2γ; neurogenesis; stress; depression; cognition

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CHRONIC VALPROIC ACID TREATMENT LEADS TO LATE AND LIMITED PHENOTYPIC IMPROVEMENT IN A MOUSE MODEL OF MACHADO-JOSEPH DISEASE

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Machado-Joseph disease (MJD), also known as Spinocerebellar Ataxia type 3 (SCA3), is an autosomal dominant neurodegenerative disorder caused by the expansion of a polyglutamine tract (polyQ) in the C-terminus of the ATXN3 gene product, ataxin-3. So far, there is no effective treatment for this disorder. In this study we proposed to target pharmacologically transcriptional dysregulation, one of the proposed pathogenic mechanisms involved in this disease, using valproic acid (VPA), an HDAC inhibitor (HDACi). This compound had been identified as causing a significant reduction in mutant ataxin-3 aggregation and neurological dysfunction in previous studies using a transgenic *C.elegans* model of MJD. Also, several HDACi's such as phenylbutyrate, sodium butyrate or valproic acid (VPA), have been shown to alleviate neurological phenotypes, as motor deficits, increase survival, reverse transcriptional abnormalities by increasing the expression of some neuroprotective genes and attenuate mutant protein toxicity in polyQ disease models. In this study, CMVMJD135 animals were chronically treated with 200mg/kg VPA i.p. injections since 5 weeks until 30 weeks of age. VPA-treated CMVMJD135 animals presented an improvement in foot dragging at the beginning of the disease process that was not maintained over time. VPA treatment also improved the motor performance later in life, as occurred in VPA-treated *C. elegans*, given by the locomotor activity (number of squares travelled in the arena), motor swimming and beam walk tests. Concerning the hanging wire test, tremors and body weight, VPA-treated animals showed only marginal improvement comparing to vehicle-treated transgenic animals. Other motor deficits observed in CMVMJD135 mice, namely motor uncoordination in the rotarod test and limb clasping, were not improved by VPA treatment. At the pathological level, and unlike what happened in *C.elegans*, VPA did not affect ataxin-3 inclusion load in the brainstem of CMVMJD135 animals, at the dosage tested. In summary, our results show that VPA treatment was able to improve some aspects of motor performance in this MJD model, but to a limited extent; even though the results were not clinically very relevant, this does not exclude the use of other dosages of VPA that could exert more pronounced effects, and/or the use of more selective and specific HDACi's. Moreover, the complex activity and a broad range of VPA effects also create the need for further clarification of the effects of this drug on the behavioral phenotype and molecular disease context in the CMVMJD135 mouse model.

ADENOSINE A_{2A} RECEPTORS AS POTENTIAL TARGETS FOR PARKINSON'S DISEASE-RELATED COGNITIVE DEFICITS

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The deposition of alpha-synuclein (aSyn) in cytoplasmic inclusions is the neuropathological hallmark of Parkinson´s disease (PD), and is thought to accumulate throughout the brain prior to cell loss. In addition to the characteristic motor symptoms, cognitive disturbances are also very common in PD and appear to be associated with the described pathological changes. These cognitive deficits, which can predict the development of dementia in later stages, do not respond to dopamine therapies, and represent an unmet need in the treatment of PD.

Recently, adenosine A_{2A} receptors ($A_{2A}R$) emerged as an attractive non-dopaminergic target for the treatment of both motor as well as non-motor symptoms of PD. Nevertheless, the precise molecular mechanisms underlying neuroprotection and the effects of blocking these receptors on synaptic plasticity and cognition remain unknown.

Based on our previous data showing that aSyn oligomers impair long-term potentiation - the molecular paradigm involved in learning and memory[1], we set out to investigate the ability of $A_{2A}R$ blockade to prevent this synaptic damage. We found that hippocampal slices preincubated with the selective $A_{2A}R$ antagonist SCH58260 (110min, 50 nM), did not lose the ability to respond to LTP when preincubated with aSyn oligomers (90 min, 500 nM) (n=5-9, $P<0.01$). Consistently, aSyn oligomers also failed to impair LTP in $A_{2A}R$ KO mice (n=3-4, $P<0.05$). This neuroprotection was achieved through the prevention of NMDA receptors basal overactivation caused by aSyn oligomers.

We then tested a novel selective blocker of adenosine $A_{2A}R$, KW6002, in mice displaying cognitive impairments resembling early PD manifestations, the Thy1-driven human aSyn mice (Thy1-aSyn)[2], in order to establish its potential to rescue memory and cognitive function. Thy1-aSyn mice and their WT littermates were treated for 1 month with KW6002 administered in the drinking water (3 mg/Kg/day) and tested for hippocampal-dependent tasks using the Morris Water maze (MWM) and Y-maze rodent behavior tests. In the MWM, Thy1-aSyn mice presented a slower learning during acquisition and a lack of preference by the target quadrant during probe test. These deficits were reverted by the blockade of $A_{2A}R$ (n=6-11, $P<0.05$). On the Y-maze, Thy1-aSyn animals performed worse than WT, revealing no preference for the novel arm. When treated with the $A_{2A}R$ antagonist, short term working memory was restored (n=6-11, $P<0.05$).

Overall, these results reveal the involvement of $A_{2A}R$ in the aSyn-associated synaptic and memory impairments by showing that long-lasting synaptic and behavioral effects of aSyn can be reverted by targeting adenosine $A_{2A}R$. These findings provide a novel evidence for the use of adenosine $A_{2A}R$ antagonists as potential therapeutic targets in PD-related cognitive deficits.

1. Diógenes MJ *et al* 2012 J Neurosci
2. Chesselet MF *et al* 2012 Neurotherapeutics

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ROLE OF TET3 IN MAINTAINING THE STEM CELL NICHE IN THE ADULT BRAIN

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Hydroxylation of the epigenetic mark 5-methylcytosine (5mC) in the DNA, catalyzed by TET dioxygenases, leads to production of 5-hydroxymethylcytosine (5hmC), an epigenetic mark highly abundant in several brain regions. In particular, it has been shown that high 5hmC content 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 (Santiago *et al.*, 2014).

To study the biological function of Tet3 in forebrain neurons, Tet3 C57BL/6J 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. We treated 6-week-old mice with tamoxifen for 5 days and then, after a 1-week interval, we repeated the administration period. Then, 9 and 23 days after the last administration, the animals were sacrificed and Tet3 ablation was analysed in cortex, hippocampus, dentate gyrus (DG), subependymal zone (SEZ) and striatum. To clarify the impact of Tet3 deficiency, we measured the levels of gene expression of some pluripotency and differentiation markers in the Tet3-depleted brain regions. Our preliminary results indicate that Tet3 is involved in the epigenetic regulation of neural progenitor cells in the adult hippocampus, more specifically in the dentate gyrus. Further analyses are being carried out to assess neural plasticity changes through behavioural tests.

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EPIGENETIC REGULATION OF GENOMIC IMPRINTING DURING NEURAL DIFFERENTIATION

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Genomic imprinting is an essential developmental mechanism that results in monoallelic expression of imprinted genes, dependent on their parental origin. This process plays key roles in brain function and its deregulation is involved in neurodevelopmental disorders. In order for the parental alleles to be distinguished, they are differentially marked through epigenetic modifications. Hydroxymethylation has recently emerged as a novel epigenetic mark that could modulate gene expression state in the brain, where it is highly abundant. In this process, the TET enzymes, of which there are three, oxidize 5-methylcytosine into 5-hydroxymethylcytosine (5hmC) [1].

In this context, the present work aimed to unravel the role of hydroxymethylation and TET enzymes in the control of imprinted genes expression during neuronal differentiation.

For that, we used a stable and inducible knockdown system that relies on site-specific recombination of an shRNA-mir cassete, by Cre recombination, to downregulate Tet genes in neural precursor cells (NPCs) differentiated *in vitro* from embryonic stem (ES) cells. Briefly, ES cells were culture in ES medium containing leukemia inhibitory factor (LIF) on feeders and gelatin and after plated onto non-adherent bacterial dishes for formation of cellular aggregates. Retinoic acid was added to direct neural differentiation and cells were plated on laminin/poly-ornithine coated plates in N2 and N2B27 media. At the NPC stage, doxycycline was added to the medium to induce expression of the shRNA to knockdown Tet1 and Tet3 genes.

After downregulating Tet1 and Tet3, we assessed the expression of specific imprinted genes (*H19, Igf2, Grb10, Dlk1, Mest/Peg1, Peg3, Ube3a, Snrpn*, etc.) in these NPCs by real time-PCR. We observed that knockdown of Tet3 - the member of the TET family which is more expressed in NPCs – increased expression of some of the imprinted genes - *H19, Grb10, Igf2* and *Dlk1*. In conclusion, we postulate that changes in DNA hydroxymethylation and methylation driven by decreased expression of TET enzymes can regulate the expression levels of some imprinted genes during neuronal differentiation.

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FGF22-INDUCED ACTIVATION OF THE PI3K/AKT AND ERK SIGNALING PATHWAYS ARE DIFFERENTIALLY REGULATED IN THE HIPPOCAMPUS

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The information that flows between neurons is crucial for proper brain functioning. Particularly, in the hippocampus the correct assembly of synapses is dependent on the ability of the neuron to undergo structural and functional changes[1,2]. Along this process, a diverse set of molecules will influence when and where synapses are formed, establishing synaptic specificity. The fibroblast growth factor 22 (FGF22) is a presynaptic organizing molecule in the CNS, regulating the formation of glutamatergic synapses[3]. Dysregulation of FGF22 signaling during development has been proposed to increase vulnerability to neuropsychiatric disorders, including epilepsy[4]. However, the signaling pathways activated in response to this neurotrophic factor are not clear. To contribute to the basic understanding of synaptogenesis we investigated the signaling pathways that are activated in response to FGF22 stimulation. We found that FGF22 induces robust activation of the PI3K/Akt and MEK/Erk pathways in hippocampal neurons in culture. Moreover, inhibiting any of these pathways with the corresponding pharmacological inhibitors blocks FGF22-induced synaptogenic effect, and the same was observed in neurons transfected with shRNA against Akt and Erk. PI3K/Akt and Mek/Erk signaling pathways are known for their role in neuronal survival, axonal growth and branching[5,6,7]. Here we demonstrate that these pathways can also regulate synaptogenesis and might have an important role in brain connectivity

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ADENOSINE A_{2A} RECEPTORS INDUCE THE FORMATION OF ABNORMAL SECONDARY AXONS IN RAT HIPPOCAMPAL NEURONS: A NEW TARGET TO ARREST EPILEPTOGENESIS

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Adenosine is an ubiquitous neuromodulator in the adult brain, mainly through the activation of A_1 receptors (A1R) and A_{2A} receptors (A_{2A}R). During development, it was shown that adenosine receptors also play a neuromodulation role namely in the control of neurite outgrowth, inhibiting through A_1R (Neuroreport 12, 3057-63) and promoting it through A_{2A}R (Neurochem. Res. 36, 2259-69). In an on-going study, we found that A_{2A}R are also involved in axon specification/formation: thus, in rat hippocampal neurons, the pharmacological activation of A_{2A}R with the selective agonist CGS21680 (30 nM) induces the formation of abnormal secondary axons. Moreover, we could gather evidence that this involved a de-repression of CRMP2, a microtubule-associated protein crucial for axon specification and outgrowth (Cell 120, 137-49), reflecting a likely inhibition of GSK3β.

One of the structural-functional modifications occurring during epileptogenesis is an abnormal axonal sprouting. An eloquent example of this is the hippocampal mossy fiber (MF) sprouting found in patients and in animal models of temporal lobe epilepsy (TLE) (Neuroscience 14, 375-403). MF sprouting generates an excitatory feedback circuit in granule cells that is thought to contribute to the hyperexcitability underlying the seizure-prone state (Neurochem. Res. 28, 1649-58). However, it is still unknown the mechanism(s) underlying this aberrant re-wiring. Interestingly, CRMP2 has been recently associated to the abnormal axonal sprouting observed in epilepsy (Neuroscience 210, 451-66) and we have previously shown an up-regulation of A_{2A}R in the rat hippocampus upon status epilepticus (Epilepsia 46, 159-65). This prompted us to propose that this newly found A_{2A}R-GSK3β-CRMP2 pathway involved in axon formation during development is reactivated during epileptogenesis, and is responsible or at least contributes to the abnormal axonal sprouting observed in epilepsy.

For that purpose, pilocarpine hydrochloride was administered intraperitoneally (i.p.) at a dose of 380 mg/kg to 6-7 week-old male Sprague Dawley rats to induce SE, characterized by continual stage 3, stage 4 and stage 5 seizures according to the Racine's classification (Electroencephalogr. Clin. Neurophysiol. 32, 269–279). Scopolamine methylbromide (2mg/kg, i.p.) was injected 30 min before pilocarpine to minimize peripheral muscarinic cholinergic effects. Convulsions were terminated with diazepam (10 mg/kg, i.p.) after 2h of SE onset, defined by the occurrence of the first stage 4 seizure. In those animals experiencing SE, hippocampal mossy fiber sprouting was evaluated 42 days later. Using this SE-animal model of temporal lobe epilepsy, we now found that the daily intraperitoneal injection of the selective antagonist of A_{2A} receptors, SCH58261 (0.1 mg/kg, i.p.), from 10 h after SE-induction onwards presented a reduced granule cell layer + molecular layer Timm-positive staining in comparison to vehicle-injected rats (0.1 mg/kgSCH58261 *vs.* vehicle, p=0.009). Altogether, these data demonstrate that A_{2A} receptors contribute to the abnormal hippocampal mossy fiber sprouting, raising A_{2A}Rs antagonists as candidates to arrest epileptogenesis.

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NEUROGENESIS IN THE POSTNATAL BRAIN: THE ROLE OF ADENOSINE A_{2A} RECEPTORS IN THE DENTATE GYRUS

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Constitutive neurogenesis takes place in both adult mammalian subventricular zone and subgranular zone of the dentate gyrus (DG). While BDNF is known to regulate hippocampal neurogenesis, the effects of its modulator A_{2A}R are not known. This study evaluated whether A_{2A}Rs are required for BDNF-induced neurogenesis, namely in cell proliferation and neuronal differentiation, and for the capacity of progenitor cells to divide and self-renew within the DG.

DG stem/progenitor cells were obtained from early postnatal (P1-3) Sprague-Dawley rats. Contrary to BDNF (30ng/mL), A_{2A}R activation with the selective A_{2A}R agonist CGS21680 (30nM) did not change DG cell proliferation, as measured by BrdU staining. However, A_{2A}R blockade with the selective A_{2A}R antagonist, ZM241385 (50nM), prevented BDNF-induced increase of cell proliferation (control: 100%; BDNF: 185±15.7%; ZM+BDNF: 89±13.3%). Moreover, both BDNF and A_{2A}R activation promoted neuronal differentiation (control: 100±0.8%; BDNF: 192±11.9%; CGS21680: 208±18.7%), which were independently prevented by either A_{2A}R blockade (BDNF+ZM: 126±16.3%) or BDNF scavenger (CGS21680+TrkB-Fc: 133±21.9%), the TrkB/Fc chimera (2µg/mL). In addition, A_{2A}R activation increased self-renewal capacity, similarly to BDNF (control: 100%; CGS21680: 162±24.6%; BDNF: 184±30.6%). Finally, a cell-fate study was performed to study cell-type division. We observed that both BDNF and CGS21680 promoted an increase in the number of Sox2+/+ cell-pairs derived from a progenitor cell division (control: 51±14%; BDNF: 61±1.6%; CGS21680: 62±1.6%), with a concomitant decrease in Sox2-/- cell-pairs (control: 41±1.8%; BDNF: 33±1.6%; CGS21680: 33±1.7%).

Data suggest that although A_{2A}Rs have no direct effect on cell proliferation, BDNF effect is dependent on the endogenous co-activation of A_{2A}Rs. On the other hand, A_{2A}R activation promotes neuronal differentiation and its endogenous activation is necessary for the effect of BDNF. In addition, A_{2A}R activation in the DG promotes progenitor cells to divide symmetrically to originate more cells with stem-like properties, Sox2+ cells, which is corroborated by the increase of the self-renewal capacity of progenitor cells. Taken together, A_{2A}R activation is required for postnatal hippocampal neurogenesis.

MICRORNA MODULATION AS A NEW THERAPEUTIC STRATEGY IN ALZHEIMER'S DISEASE

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Aging is considered a major health concern in modern society, making the understanding of senescence a challenge for biomedical research. Brain aging frequently underlies cognitive decline, being a major risk factor for neurodegenerative conditions, such as Alzheimer's disease (AD) [1]. However, the specific mechanisms involved in this relationship between brain aging and neurodegeneration remain unclear [2]. Recent studies have proposed a pivotal role for microRNAs (miRNAs) in ageing, hence in the development of neurodegenerative disorders. These short regulatory RNAs bind to the 3' UTR region of target mRNAs, inhibiting translation or degrading the mRNA, therefore altering expression of their target genes [3].

The identification of "molecular signatures", such as miRNA profiles, may lead to the development of new therapies for Alzheimer's disease (AD). Hence, this study aims to modulate the levels of selected miRNAs predicted to target proteins involved in AD.

Through bioinformatic tools, we have identified miRNAs with high affinity to 3'UTR-APP and 3'UTR-BACE1 and performed a biochemical validation of these binding sites, through luciferase assay, upon co-transfection of HT-22 cells with miRNA mimics. The results demonstrate that the selected miRNAs target APP and BACE1, since there was a decrease in luciferase activity in the presence of these mimics. Additionally, a decrease in APP and BACE1 mRNA levels was observed upon transfection of HT-22 and HEK-293 cells with lentiviral constructions containing the selected miRNAs sequences.

Regarding the results and that miR-31-5p targets both APP and BACE1, we have selected this miRNA to evaluate its therapeutic potential. Therefore, we developed a lentiviral platform able to modulate the levels of the miRNA in the brain of a triple-transgenic animal model of AD, through stereotaxic injection. Our preliminary results show that miR-31-5p must be able to decrease the levels of APP and BACE1 *in vivo*.

Given the high conservation of miRNAs across species, it is likely that new significant insights in ageing process may arise and support new diagnostic and therapeutic avenues to treat ageing-related diseases, such as AD.

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THE ROLE OF STRING/CDC25 PHOSPHATASE IN NEURODEVELOPMENT: AN ENTRY POINT TO DISEASE

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CDC25 phosphatases (PPases) are well-established activators CDKs and their activity is extensively associated to proliferating tissues. Expression and activity of Cdc25 PPases has been reported in normal adult brain tissue, but their potential function and substrates in a non-proliferative context has never been addressed. We are using the *Drosophila* visual system as a model to uncover the function and substrates of CDC25 phosphatases during neuroepithelia development. *Drosophila* Cdc25 homologue is encoded by *string* (*stg/cdc25*). After differentiation onset, Stg expression is up regulated in photoreceptors (PRs) and lamina neurons, two neuronal cell types of the fly visual system. Loss of function (LOF) of *stg/cdc25* in PRs leads to neuronal cell death and degeneration, leading to roughness of the fly adult eye. This suggests that this PPase may play an essential function in post-mitotic neurons. Previous studies have shown that Stg/Cdc25 is a strong suppressor of the rough eye phenotype that characterizes a fly model of tauopathy, which further supports this hypothesis. This tauopathy model is based on the expression of a mutant isoform of the human microtubule associated protein TAU. The mechanisms that underlie Stg-Tau interaction are still unknown. Stg/Cdc25 may act directly as a modulator of Tau phosphorylation levels and activity or indirectly by mediating protein clearance associated mechanisms. We are using a genetic and biochemical approach to address these questions. We will present our latest findings on the role of *stg/cdc25* during late stages of visual system development and as a modulator of Tau neurotoxicity.

FIBRIN HYDROGELS FUNCTIONALIZED WITH α6β1 LIGANDS FOR NEURAL STEM CELL-BASED TRANSPLANTATION THERAPIES

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As in the adult CNS the intrinsic capacity of endogenous neural progenitors to replace lost cells upon injury is insufficient, transplantation of neural stem/progenitor cells (NSPCs) has emerged as a promising approach to compensate for injury-induced lost cells. Herein we aimed at developing a fibrin (Fb)-based hydrogel for transplantation of NSPCs. To enhance Fb specificity towards NSPCs and promote NSPC migration *in vivo*, Fb was functionalized with ligands for selective binding to α6β1 integrin receptors, an integrin highly expressed by NSCs and involved in NSC migration. Six peptides with reported affinity to α6β1 integrin (P3, HYD1, T1, AG10, N4 and A5G81) were investigated.

NSPCs (derived from the ES-46C cell line) were seeded on peptide-adsorbed surfaces, and cell adhesion (in the presence/absence of mAbs against α6β1 integrin subunits), attachment strength, migration and differentiation along the neuronal lineage were quantitatively assessed. Among the tested peptides HYD1, T1, and A5G81 were the most effective in terms of ability to promote NSPC adhesion mediated through α6β1 integrin. Bi-domain peptides with the selected α6β1 ligands at the C-terminus and the factor XIIIa substrate domain at the N-terminus were bound to Fb gels at different concentrations by the action of factor XIIIa. The amount of covalently bound ligands and their stability over time was determined using ^{125}I-labeled peptides. To assess the effect of immobilized ligands on NSPC migration, neurospheres (NSPC aggregates) were seeded on functionalized Fb gels and radial outgrowth (outgrowth area and maximal outgrowth distance) determined after 72 h of cell culture using automatic image analysis. Unmodified Fb and Fb containing laminin (LN) 111/LN 511 were used as controls. The incorporation of soluble LN was not able to promote radial outgrowth in Fb, regardless of the LN isoform used. Functionalization of Fb with A5G81 was not effective in promoting radial outgrowth on Fb, neither. However, immobilization of HYD1 and T1 led to significantly higher (24-fold and 2.2-fold increase) outgrowth area as well as maximal outgrowth distance, for bi-domain peptide concentrations of 20 μM and 40 μM, respectively. Blocking against α6β1 integrin subunits significantly inhibited cell outgrowth from neurospheres on Fb gels functionalized with HYD1/T1, indicating that HYD1/T1 ability to promote NSC migration on Fb gels is partially mediated through α6β1 integrin. Finally, we examined the effect of immobilized HYD1/T1 on neurite extension, quantifying neurite outgrowth from rat E18 dorsal DRGs cultured in functionalized gels. Functionalization of Fb with HYD1/T1 led to significantly higher neurite extension as compared to unmodified Fb, independently of the ligand concentration tested. The *in vivo* biological performance of the functionalized Fb is currently being assessed in a rat model of SCI.

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POSTERS

GLIAL CELL BIOLOGY

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THE OLIGODENDROCYTE "PROCESSOSOME": IDENTIFICATION OF NEW REGULATORS OF DIFFERENTIATION AND MYELINATION

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Oligodendrocytes (OL) are the myelinating cells of the central nervous system (CNS). During CNS development and myelination, OL extend several membrane processes that will contact and wrap axons. The formation and extension of such processes requires controlled reorganization of the cytoskeleton.

We physically separated oligodendrocyte precursor cell (OPC) soma from their processes for transcriptomic analysis (RNA sequencing) in order to identify and characterize mRNAs mainly present in the processes of the OPC. We hypothesize that such molecules will play important roles in OL process extension, wrapping and, later, in myelination of axons.

We observed a substantial enrichment of mRNAs in OPC process related to cytoskeletal dynamics and ribosomes/translation, reinforcing the notion of local regulation of translation of mRNAs involved in oligodendrocyte process extension, as it is already described in neurons. We are currently focused on the study of two undescribed molecules, Dusp19 and Kank2, enriched in OPC processes when compared with soma. These participate in important signalling pathways such as JNK-MAPK and Rho GTPases that modulate the cytoskeleton dynamics and are important for OL differentiation. We observed that the expression of these molecules is modulated both *in vitro* during OL differentiation and *in vivo* during CNS developmental myelination. Knockdown *in vitro* experiments suggest key roles in OL differentiation and myelination.

Altogether, this knowledge will contribute to better understand debilitating demyelinating diseases such as Multiple Sclerosis.

DEVELOPMENT OF NEW CELLULAR MODELS FOR STEP-BY-STEP DISSECTION OF ASTROGLIOGENESIS

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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 IL6/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. These systems are based on bimolecular fluorescence complementation (BiFC) technology. Briefly, two proteins of interest are fused to two non-fluorescent halves of a fluorescent protein. When the proteins of interest interact, they bring together the complementary halves and reconstitute the fluorescence of the reporter protein. 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. These systems are suitable for high-throughput screenings.

The main advantage of our approach stems from the fact that we will be able to situate the genes involved in each rate-limiting step of the astrogliogenic pathway. Furthermore, this approach is more informative, simpler and/or cheaper than current screening strategies based on end-of-pathway readouts (i.e. gene expression profile) or phosphorylation of key proteins (e.g. JAK2, STAT3).

UNVEILING THE ROLE OF ASTROCYTES IN METHAMPHETAMINE-INDUCED MICROGLIA ACTIVATION

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Methamphetamine (Meth) is a highly addictive psychostimulant posing severe health consequences and long-term neurotoxic effects. Microglial cells, the resident immune cells of the central nervous system (CNS), playing a crucial role in inflammatory processes. Upon activation, microglia secrete an array of molecules including pro-inflammatory cytokines, NO and ROS, which can cause damage to the neuronal cells. Of note, microglia activation was reported in Meth users. Recent *in vivo* and *in vitro* studies pointed microglia as a possible major mediator of Meth-induced neurotoxicity. In this study, we investigated the role of Meth in microglia and astrocytes activation, as well as the crosstalk between them. To gain further insight into microglia activation we analyzed the effects of Meth exposure in pro- and anti-inflammatory markers, using primary cortical microglia cultures, immunocytochemistry, quantitative fluorescence microscopy and qRT-PCR. We show that Meth did not trigger a pro-inflammatory or an anti-inflammatory signature in microglia. We also evaluated the Meth effects on primary astrocyte cultures using reactivity markers (f-actin, GFAP and iNOS) and observed that Meth did not trigger astrocyte activation. Moreover, in an attempt to clarify how Meth could affect the crosstalk between microglia and astrocytes, we evaluated the effect of conditioned media from astrocytes (ACM) treated with Meth in microglial cultures. We found that ACM treated with Meth induces a pro-inflammatory signature significantly increasing ROS production, iNOS expression and the phagocytic activity, when compared to cells incubated with conditioned media from naïve astrocytes. In order to isolate possible astrocyte-released factors under Meth exposure, we analyzed the production of pro-inflammatory cytokines (IL-1B; IL-6 and TNF) by qRT-PCR and glutamate release by time-lapse video microscopy coupled with FRET-based high sensitive biosensors. We observed no change in pro-inflammatory cytokine production and a huge increase in glutamate release from astrocytes in the presence of Meth. Unexpectedly, here we describe that Meth *per se* triggers neither cortical microglia nor astrocytes activation. Nonetheless, Meth-induced microglia activation appears to be mediated by soluble factors released from Meth-sensitized astrocytes, suggesting that Meth induces microglial activation in an astrocyte-dependent manner and a possible mediator for this crosstalk is the glutamate.

Keywords: Astrocyte conditioned medium; Astrocyte; Methamphetamine; Microglia; Pro-inflammatory signature.

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VISUALIZING OLIGOMERIZATION OF NORMAL AND MUTANT GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) IN LIVING CELLS

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Astroglia is the most abundant cell type in the central nervous system (CNS), and it plays an essential role in CNS function in normal and pathological conditions. Alexander disease is the first neurodegenerative disorder known to be caused by a defect in astroglia. Mutations in GFAP, an intermediate filament largely specific of astroglia, result in its misfolding and aberrant aggregation. Recent evidence indicates that GFAP toxic oligomeric species are relevant to Alexander disease, but little is still known about how they are formed and how they actually work.

We have recently developed a series of bimolecular fluorescence complementation (BiFC) assays for the visualization of toxic oligomeric species from several neurodegenerative disorders in living cells. We applied this principle to GFAP in order to produce a new cellular model for the study of Alexander disease. We fused normal and mutant GFAP to two non-fluorescent halves of the Venus fluorescent protein. When two GFAP molecules dimerize, they bring the Venus halves back together, reconstituting the functional fluorophore. Fluorescence is therefore proportional to the dimerization/oligomerization of GFAP, and can be easily measured by flow cytometry or microscopy.

We are using this system to identify post-translational modifications, proteins and drugs that influence the dimerization and oligomerization of normal and mutant GFAP. These systems are useful for high-throughput genetic and pharmacological screens.

Our results will provide new insights on the mechanisms underlying normal cytoskeleton organization and aberrant aggregation of misfolded proteins, as well as on the role of astroglia in neuronal survival.

ELEVATED GLUCOSE TRIGGERS INTERLEUKIN-1β PRODUCTION, ACTIVATES MICROGLIA AND DECREASES GLIAL CELL PROLIFERATION IN RETINAL NEURAL CELL CULTURES

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Diabetic retinopathy is considered a microvascular disease, but increasing evidence has shown that retinal neural cells can also be affected. Although hyperglycemia is considered the main trigger of this pathology, diabetes is also regarded as multifactorial disease and therefore it is likely that different factors act in additive or synergistic ways to impair neural homeostasis.

Increasing body of evidence indicates that diabetic retinopathy can also be considered a low-grade chronic inflammatory disease. In the retina, the production of interleukin-1β (IL-1β), a pro-inflammatory cytokine, is increased under diabetes, suggesting that it may play an important role in neural dysfunction.

In this study, we aimed to evaluate if a prolonged exposure to high glucose, *per se*, mimicking chronic hyperglycemic conditions, was capable of changing the expression of IL-1β in retinal cells, and identify which cell types produce IL-1β. Furthermore, the effect of elevated glucose *per se* and IL-1β on retinal cell proliferation was evaluated, giving a particular attention to microglial and macroglial cells.

We used retinal neural cell cultures, which were exposed to high glucose (30 mM) or mannitol (osmotic control; 25 mM plus 5 mM glucose), for 7 days, or otherwise exposed to IL-1β (10 ng/ml) or LPS (1μg/ml) for 24 h.

In primary retinal neural cell cultures, both retinal neurons and glial cells express IL-1β and its receptor, interleukin-1 receptor, type I (IL-1R1). High glucose *per se* upregulated IL-1β mRNA expression and increased IL-1β levels in the culture medium. When retinal neural cells were exposed to 10 ng/ml of IL-1β for 24 h or 30 mM glucose for 7 days, changes in macroglial and microglial cells proliferation were detected. High glucose decreased microglial and macroglial cell proliferation, whereas IL-1β increased their proliferation. Although high glucose *per se* decreased microglial proliferation in retinal neural cultures, it also increased ED1 (cellular marker specific for activated microglia) levels, indicating that high glucose activates microglia.

Concluding, high glucose and IL-1β may play an important role in the pathogenesis of diabetic retinopathy, affecting microglial and macroglial cells. Consequently, the dysfunction of both cell types may ultimately affect neurons and contribute to neural changes observed in diabetic patients.

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IMPACT OF ELEVATED PRESSURE IN THE ADENOSINERGIC SYSTEM AND IN THE RELEASE OF PROINFLAMMATORY CYTOKINES IN MICROGLIA

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Glaucoma is a progressive degenerative disease and the second cause of blindness worldwide, characterized by retinal ganglion cells (RGCs) death and optic nerve atrophy. The degeneration of RGCs is accompanied by a neuroinflammatory response, involving microglia. Elevated intraocular pressure (IOP) is the main risk factor for the development of glaucoma. Adenosine is a neuromodulator in central nervous system involved in inflammatory responses that acts by the activation of four G protein-coupled receptors, A_1, A_{2A}, A_{2B} and A_3. It is unknown whether the increase of elevated hydrostatic pressure (EHP), used to mimic elevated IOP, can affect the adenosinergic signaling system in microglial cells. In this work, we investigated whether EHP affects the protein levels of $A_{2A}R$, concentrative nucleoside transporter 2 (CNT2) and ADA and the pro-inflammatory cytokines TNF and IL-1β.

BV-2 microglia cell cultures were pre-treated with 50 nM SCH 58261, a selective $A_{2A}R$ antagonist, and microglial cells and mice organotypic cultures were exposed to EHP (70 mmHg above atmospheric pressure) for 4h and 24h. Control cells were kept in a standard incubator (normal atmospheric pressure). The protein levels of $A_{2A}R$, CNT2 and ADA were assessed by western blot, and pro-inflammatory cytokines were quantified by enzyme-linked immunosorbent assay (ELISA).

The protein levels of $A_{2A}R$ significantly increased in BV-2 cells after exposure to EHP for 4h and 24h and the protein levels of CNT2 significantly increased only after 24h of EHP exposure. The levels of ADA were not altered by EHP exposure. The levels of TNF increased upon exposure to EHP for 24h, and the blockade of $A_{2A}R$ prevented this increase. The exposure of retinal organotypic cultures to EHP triggered an increase in the levels of TNF and IL-1β.

In conclusion, this study shows that several components of the adenosinergic system are affected by EHP in microglia. In addition, microglial cells release inflammatory cytokines in response to EHP. The blockade of $A_{2A}R$ may modulate microglia activity elicited by EHP.

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GLIAL PLASTICITY AS A KEY MECHANISM UNDERLYING THE PATHOPHYSIOLOGY OF DEPRESSION

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Major depression is a highly prevalent disorder, being the leading cause of years lost due to disability. The pathophysiology of this disease is still poorly understood but growing evidence suggests that impaired neuroplasticity may be a key underlying mechanism for the precipitation of the disorder. The generation of new cells was shown to be particularly relevant for the sustained remission from the cognitive deficits and for the improving actions of antidepressants (ADs), as previously shown by our lab [1]. Recent studies also showed an important role for astrocytes in the pathophysiology of this disorder, which are crucial for neurotransmission and neurovascular coupling, evidenced by astrocytes loss in major depressive disorder (MDD) [2]. However, the importance of pre-existent astrocytes and astrogliogenesis in the precipitation of and recovery from the cognitive deficits in MDD is still largely unknown. Therefore, we proposed to study the role of astrocytes and adult astrogliogenesis, in the precipitation of and recovery from depressive-like cognitive behavior in rats both untreated and treated with ADs -fluoxetine (a selective serotonin reuptake inhibitor,SSRI) or imipramine (a tricyclic agent) - in a longitudinal manner, using the unpredictable chronic mild stress (uCMS) model of depression. Regarding the cognitive dimension, although short-term memory was impaired throughout the course of the disease, treatment with fluoxetine and imipramine was similarly effective in reversing this deficit. Interestingly, when we analyzed the pre-existent astrocytes in the hippocampal dentate gyrus (DG) by immunohistochemistry, at long-term, only fluoxetine-treated animals showed a significant increase in the astrocytic process length ($F_{2,37}=60.66$, $p<0.0001$). Regarding the hippocampal DG newborn astrocytic population, at the long-term perspective, only imipramine was able to elicit a strong pro-gliogenic effect, traduced in an increased percentage of GFAP and BrdU – double positive cells per total BrdU – positive cells ($F_{2,33}=3.662$, $p=0.0366$). Moreover, we are now performing cell fate analysis, using an *in vitro* approach – differentiated neurospheres.

Thereby, we provide some consistent evidences for the causative implication of astrogliogenesis and 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 ADs.

Moreover, our results endorse the view of the adult hippocampal DG astrogliogenesis process as a promising therapeutical target for future therapies in the neuropsychiatric field.

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ANTI-INFLAMMATORY EFFECTS OF SITAGLIPTIN IN RETINAL MICROGLIAL CELLS: INVOLVEMENT OF NPY Y^1 RECEPTOR?

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Diabetic retinopathy is one of the most common complications of diabetes and is the leading cause of vision loss and blindness among working-age population. The breakdown of the blood-retinal barrier (BRB) is a hallmark of this disease and it has been correlated with increased nitric oxide (NO) production in the retina, mainly via inducible nitric oxide synthase (iNOS). Moreover, reactive microglia, which produce high amounts of NO, have been implicated in the pathogenesis of diabetic retinopathy. Neuropeptide Y (NPY) is a neuromodulator in the central nervous system and regulates inflammatory processes. Sitagliptin is an antidiabetic drug that inhibits dipeptidyl-peptidase-IV, an enzyme that processes NPY.

In this study, we aimed to investigate whether sitagliptin is able to inhibit retinal microglia activation and neuroinflammation and whether its effects are mediated through the NPY Y_1 receptor activation. To test this hypothesis, we used primary retinal neural cell cultures and retinal organotypic cultures exposed to lipopolysaccharide (LPS) to trigger an inflammatory response. We evaluated the effects of sitagliptin in microglial reactivity, in the absence or presence of Y_1 receptor antagonist (BIBP3226).

In primary retinal neural cell cultures, sitagliptin was able to inhibit the increase in iNOS immunoreactivity (iNOS-IR) in microglial cells and NO production triggered by LPS. The Y_1 receptor antagonist, BIBP3226, abolished the effect of sitagliptin on NO production. Sitagliptin also appears to attenuate LPS-induced upregulation of IL-1β-IR in microglial cells.

In cultured retinal explants, sitagliptin prevented the alterations in retinal microglia morphology triggered by LPS and inhibited the increase in iNOS-IR in microglial cells. Though sitagliptin did not inhibit the increase in iNOS mRNA expression triggered by LPS, the blockade of Y_1 receptor enhanced the increase in iNOS mRNA expression induced by LPS.

These results indicate that sitagliptin has anti-inflammatory effects by controlling the reactivity of microglial cells. Moreover, the activation of Y_1 receptor might, at least partially, contribute for some of the effects of sitagliptin, but further investigation is required to clarify which effects of sitagliptin on microglia can be mediated by Y_1 receptor activation.

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DYSREGULATED POST-TRANSLATIONAL MODIFICATIONS OF TUBULIN MEDIATE DEMYELINATION-INDEPENDENT AXON PATHOLOGY IN PLASMALOGEN-DEFICIENT MICE

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Nervous tissue is highly enriched in plasmalogens, a class of ether-phospholipids, whose biosynthesis 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, a mouse model for RCDP, we shown that lack of plasmalogens causes myelination defects in the central nervous system. During development, mutant mice initial showed dysmyelination in optic nerves and spinal cord. The pathology in optic nerves developed into a rapid, progressive and severe demyelination, whereas in spinal cord, the dysmyelination period was longer but also developed into a progressive and severe demyelination. Surprisingly, no axonal damage was observed in optic nerves from mutant mice despite the severe demyelination, whereas spinal cord axons showed extensive damage with axonal swellings, axon severing and membrane deformations. The study of the axonal cytoskeleton revealed major dysregulations of its components, including neurofilaments and microtubules. Analysis of tubulin and its post-translational modifications (PTM) in spinal cord lysates from mutant mice revealed abnormally high levels of acetylation, polyglutamylation and detyrosination. The defects in tubulin PTM were not observed in optic nerves lysates from mutant mice at any stage of the pathology. In spinal cords, the dysregulation of tubulin modifications preceded the demyelination stage but was more pronounced at, and during the stages of axonal damage. Combined, our results unraveled the importance of plasmalogens for axons and oligodendrocytes, and highlight that the fine-tuning of tubulin PTM is critical for axon structure, function and integrity.

PERIPHERAL NERVOUS SYSTEM PLASMALOGENS REGULATE SCHWANN CELL DIFFERENTIATION AND MYELINATION

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Schwann cells, the myelinating glia in the peripheral nervous system (PNS) undergo several morphological and biochemical changes that are required for membrane outgrowth and wrapping of individual axons. Myelin is highly enriched in plasmalogens, a class of ether-phospholipids, which may account for 80% of the total glycerophosphoethanolamine pool. The importance of ether-phospholipids is highlighted by the severe clinical presentation of rhizomelic chondrodysplasia puntacta (RCDP), a disorder caused by impairment in the plasmalogens biosynthesis. To investigate the role of these ether-phospholipids in Schwann cell biology and myelination, we examined two mouse models with complete impairments in their biosynthesis, i.e., the Pex7 and Gnpat knockout (KO) mice. The histological characterization of sciatic nerves from KO mice revealed impaired radial sorting and myelination. Impaired myelination was also evident in *in vitro* cultures of embryonic dorsal root ganglia from Gnpat KO mice, and following sciatic nerve crush. Myelin devoid of plasmalogens was less compact and showed abnormal appositions. Myelin compaction in the absence of plasmalogens was partially accomplished through the action of myelin-basic protein (MBP), as a combined defect in plasmalogens and MBP severely affected myelination. These changes in myelin and Schwann cell morphology ultimately lead to an adult-onset severe demyelination with axonal loss. A deficiency in plasmalogens impairs AKT-mediated signaling, causing a dysregulation in GSK3β and modulates the defects observed in Schwann cell differentiation and myelination. In summary, our findings reveal the pivotal role of plasmalogens for myelination and highlight the importance of these phospholipids during neurodegeneration.

HUMAN MICROGLIA PHENOTYPE CHANGES IN THE PRESENCE OF AMYLOID-BETA EXPRESSING NEUROBLASTOMA CELLS

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Pro-inflammatory M1 or anti-inflammatory/damage resolution M2 microglia phenotypes have been described either subsequently or in parallel on Alzheimer's disease (AD) with a determinant role in its progression[1]. Interestingly, we recently found that primary cultures of mouse microglia become less responsive with *in vitro* ageing[2]. Moreover, we also observed impaired mouse microglia reactivity in the presence of Amyloid-beta peptide, which may account for the emergence of AD. Here, we decided to evaluate how human microglial cells are able to react to the presence of Amyloid-beta expressing neuroblastoma cells.

CHME3 human microglia cell line were cultured with SH-SY5Y human neuroblastoma cell line stably expressing wild-type Amyloid precursor protein (APP)695 (APPWT) or Swedish mutant APP695 (APPSwe) for 24, 48 and 72h. Cell culture media and neuronal lysates were analyzed for APP expression by Western Blot. Isolated microglia were analyzed for phenotype-associated microRNAs (miR-155 for M1; miR-124 and miR-146a for M2) and their targets (SOCS1 for miR-155 and CEBP-alpha for miR-124), as well as other phenotype markers (MHC class II and IL-1beta for M1; TGF-beta and Arginase 1 for M2) by qRealTime-PCR.

Cell culture media from CHME3 + SH-SY5Y APPWT showed a time-dependent increase of mature APP 695 levels, while media from CHME3 plus SH-SY5Y APPSwe showed increased values of both mature and immature APP 695. Concerning microglia response, only SH-SY5Y APPSwe promoted a marked increase of microglia miR-155 right after 24h that decreased with time in culture, resulting in an increase of its target SOCS1 along the time of exposure. Interestingly, miR-124 expression was increased in microglia exposed to both SH-SY5Y APPWT or SH-SY5Y APPSwe along time, in parallel with a time-dependent reduction of its target CEBP-alpha. Concerning miR-146 while SH-SY5Y APPWT promoted a gradual increase of miR-146a, SH-SY5Y APPSwe led to a time-dependent decrease of this microRNA. Regarding M1 and M2 markers we observed that expression of M1 phenotype MHC class II and IL-1beta markers decreased along time in microglia exposed to SH-SY5Y APPSwe, whereas expression of M2 phenotype TGF-beta and Arginase 1 increased with time in microglia facing the same SH-SY5Y APPSwe.

Overall, these results suggest a rapid human microglia M1 pro-inflammatory response to increased values of Amyloid-beta peptide, followed by a M2 phenotype-like anti-inflammatory response. Nevertheless, the ability of microglia to promote repair, usually associated to miR-146a expression, appears to be reduced with time when in the presence of increased Amyloid-beta. This data support the notion that microglia show different phenotypes along AD course indicating that to target microglia we must use different therapeutic approaches depending on the stage of disease progression.

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USING A NEW VINYL SULFONE TO INHIBIT MICROGLIA-RELATED INFLAMMATORY SIGNALING PATHWAYS

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Rapid microglial activation and associated inflammatory pathways contribute to immune-defense and tissue repair in the CNS. However, persistent activation of these cells will ultimately result in vast production of pro-inflammatory mediators and other neurotoxic factors, which may induce neuronal damage and contribute to chronic neurodegenerative diseases (1). Therefore, pharmaceuticals with immunomodulatory effects on microglia may be considered as potential strategies to counteract their proinflammatory phenotype and neuroimmune dysregulation in neurodegenerative diseases (2). Recently, the cellular expression of the alarmin high-mobility group box 1 (HMGB1) protein, a mediator of inflammation and a target to confer protection against tissue injury (3), was found enhanced in reactive microglia stimulated with the amyloid-beta (Abeta) peptide (4). Thus, in this study, we aimed to investigate whether a new synthesized vinyl sulfone (VS), with anti-cathepsin S, B and L activity, was able to inhibit the expression of HMGB1 by microglia stimulated with Abeta and, if so, to modulate microglia-mediated neuroinflammation.

The microglia cell line N9 was incubated with 1 microM Abeta peptide alone or in the presence of 20 microM VS, for 24h. Cell viability was assessed by propidium iodide staining and the phagocytic ability of microglia by determining the ingestion of fluorescent latex beads. Microglia reactivity was evaluated by quantifying the activity of matrix metalloproteinases (MMP)-9 and MMP-2, as well as the expression of HMGB1 and its receptor Toll-like receptor-4 (TLR-4). We additionally evaluated the microRNA (miR)-155 (upregulated by HMGB1) and miR-146a (upregulated by IL-1beta), which are associated to inflammation.

Data showed that Abeta increased microglial necrotic death (1.9-fold, $p<0.01$) and decreased the phagocytic capacity (0.7-fold, $p<0.01$). The activity of both MMP-2 and MMP-9 was induced by Abeta (~1.7-fold, $p<0.01$), as well as the expression of HMGB1 (>2.5-fold, $p<0.01$, protein and mRNA), without causing changes in the TLR-4 protein expression. Moreover, Abeta induced the expression of miR-155 (2-fold, $p<0.05$) and marginally of miR-146a (1.2-fold). Interestingly, VS prevented Abeta-induced loss of microglia viability ($p<0.01$) and reduced phagocytic ability. VS also suppressed Abeta immunostimulatory effects on microglia, by retaining the values of MMP-2, MMP-9, HMGB1 (all $p<0.01$), miR-155 ($p<0.05$) and miR-146, close to control levels (without Abeta).

Our study reveals that VS prevents Abeta-induced microglia loss-of-function and associated inflammatory processes, while suggesting its therapeutic potential in neuroinflammatory diseases.

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A_{2A} RECEPTOR BLOCKADE PREVENTS MICROGLIA REACTIVITY ELICITED BY ELEVATED HYDROSTATIC PRESSURE

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Glaucoma is a retinal degenerative disease characterized by damage of the optic nerve and degeneration of retinal ganglion cells (RGCs). One of the major risk factors for the development of glaucoma is the increase in intraocular pressure (IOP). The onset of glaucoma is paralleled by increased microglia reactivity, releasing inflammatory mediators that further contribute to RGC loss. Therefore, drugs that mediate inflammatory responses represent plausible targets for protecting RGCs in glaucoma. Adenosine is a neuromodulator in the central nervous system acting through the activation of four receptors, A_1, A_{2A}, A_{2B}, A_3. In the brain, the blockade of $A_{2A}R$ inhibits microglia reactivity and confers protection in noxious conditions. In this study, we assessed the effects of elevated hydrostatic pressure (EHP) in the reactivity of microglia and investigated the potential protective properties of $A_{2A}R$ blockade. Retinal primary neural cell cultures and microglia cell cultures (BV-2 cell line) were pre-treated with 50 nM SCH 58261, a selective $A_{2A}R$ antagonist, and exposed to EHP (70 mmHg above normal atmospheric pressure), to mimic increased IOP. Control cells were incubated in a standard cell incubator. Microglial cell phagocytosis, proliferation, migration, inflammatory mediators, and cell death were assessed. Exposure of retinal neural cell cultures to EHP increased cell death and the number of microglia with engulfed dead cells. Also, EHP increased microglia reactivity in retinal mixed cultures, increasing cell proliferation and inflammatory response. Exposure of BV-2 cells to EHP increased $A_{2A}R$ expression as well as iNOS mRNA levels, microglia migration and phagocytosis. Pre-treatment with $A_{2A}R$ antagonist prevented cell death, changes in microglia proliferation, phagocytosis and migration induced by EHP. In summary, our results show that EHP triggered microglia reactivity. The blockade of $A_{2A}R$ reduced microglia activation and retinal cell death elicited by EHP, suggesting that $A_{2A}R$ antagonists can be envisaged as a strategy to attenuate microglia reactivity in glaucoma.

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GLIAL ACTIVATION IN THE COLLAGENASE MODEL OF OSTEOARTHRITIS IN RATS

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BACKGROUND: Pain is a major feature of osteoarthritis (OA), but its therapy is still ineffective. Recently, the overexpression of glial fibrillary acidic protein (GFAP), a known marker of satellite glial cell (SGC) and astrocyte activation, has been reported in several animal models of pain. Here, we evaluated if this is also observed in the dorsal root ganglia (DRG) and spinal cord (SC) of rats with OA induced by intra-articular injection of collagenase. Iba-1 expression, a marker of microglia activation, was also quantified in the SC. Additionally, we assessed the effect of the glial inhibitor fluorocitrate on nociceptive behaviour, hoping to contribute to a better understanding of the pain mechanisms in this pathology.

METHODS: OA was induced by two injections of 500U of type II collagenase into the knee joint of rats, separated by 3 days. Movement-induced nociception was evaluated by the Knee-Bend and CatWalk tests during the following 6 weeks. The effect of the epidural administration of fluorocitrate on the nociceptive behaviour was evaluated 6 weeks after the first collagenase injection. L3-L5 DRG from different groups of animals were used for immunodetection and Western Blot analysis of GFAP expression in SGCs; L3-L5 SC was used for immunodetection and Western Blot analysis of GFAP expression in astrocytes and Iba-1 expression in microglia.

RESULTS: Collagenase injected into the knee joint induced an increase in movement-induced nociception evaluated by both the Knee-Bend and Catwalk tests. Fluorocitrate decreased the nociceptive behavior. GFAP expression in the SGC of the DRG of collagenase-injected animals was significantly increased when compared to control animals. An ipsilateral increase in GFAP and Iba-1 expression in the SC of collagenase injected animals was also observed.

Conclusion: OA induced by intra-articular injection of collagenase in the knee of rats leads to the development of nociception associated with movement of the affected joint and to the activation of glial cells in both the DRG and the SC. Inhibition of glial cell activation by fluorocitrate is effective in decreasing these OA-associated nociceptive behaviours. These results suggest that glial cell activation may play a role in the development of chronic pain in this experimental model of OA.

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S100B AFFECTS OLIGODENDROCYTE DIFFERENTIATION AND MATURATION PROCESSES

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Neuroinflammation with marked glia reactivity is a primary hallmark of MS. In this context, we recently showed that S100B is highly expressed in the cerebrospinal fluid, serum and brain samples of Multiple Sclerosis patients, while its inhibition in an *ex vivo* demyelination model was able to prevent demyelination and glia reactivity. S100B is expressed by oligodendrocyte precursor cells (OPC) and it was described to have an essential role in their differentiation; however, high S100B levels released upon brain damage may be toxic for oligodendrocytes (OL). Here, we evaluated the effect of S100B on OL ability to differentiate and migrate.

Primary mixed glial cultures were obtained from rat cortices and OPC isolated after 10 days *in vitro* (DIV). OPC were incubated with 10 or 1000 nM S100B to mimic the physiological or toxic effects of S100B, respectively, for 24 h, just after isolation, or in the first 24 h of differentiation. After 7 DIV cells were evaluated for OL differentiation/maturation and expression of differentiation-related Olig1/Olig2. To study the role of S100B on OPC migration, cells were allowed to migrate to 20 ng/mL FGF for 24h, either in the presence of S100B (chemotaxis) or S100B plus FGF (chemokinesis) using the Boyden chamber.

Exposure to the highest concentration of S100B (1000 nM) during proliferation resulted in a significant reduction of MBP+ cells (0.7-fold, $P<0.05$) with an increase of NG2+ cells (1.6-fold, $P<0.05$), while treatment during initial differentiation induced a less pronounced effect (MBP+, 0.8-fold). To evaluate the effect of S100B on OL morphologic maturation, MBP+ cells were scored in: 1) cells poorly branched, 2) cells with complex branched processes and 3) cells with complex branched processes that partially form membranes. Exposure of OPC to 1000 nM S100B concentration during proliferation reduced maturation by increasing the number of OL in stage 1 (1.6-fold, $P<0.05$) and decreasing them in stages 2 and 3 (0.9-fold and 04-fold, respectively). This effect was enhanced when OPC exposure to S100B occurred during differentiation (1.9-fold for stage 1, $p<0.01$; 0.8-fold for stage 2 and 0.1-fold for stage 3, $p<0.05$). While treatment with 10 nM S100B increased Olig1 (1.7-fold, $p<0.01$, respectively), 1000 nM S100B reduced Olig1 expression (0.6-fold, $p<0.05$) and increased that of Olig2 (1.5-fold, $p<0.05$), corroborating the reduced OPC differentiation upon excessive S100B treatment. Concerning migration, 1000 nM S100B markedly impaired chemotaxis (0.5-fold, $p<0.01$) and chemokinesis ability (04-fold, $p<0.001$).

In sum, these results point to a role of excessive S100B in oligodendrogenesis impairment, being a potential target for new directed therapeutic approaches in inflammatory-related myelin disorders.

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EXPLORING THE ASTROCYTIC NEUROPROTECTIVE FUNCTIONS IN A CHRONIC MILD STRESS MODEL OF DEPRESSION

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Depression is a multidimensional psychiatric disorder that affects millions of people worldwide. Although the pathophysiology of depression is still incompletely understood, dysregulation of monoaminergic systems, neuroplasticity, epigenetics and immunological responses are considered to contribute to the disease. In order to unveil the pathophysiology of this disorder, increasing significance is being given to the study of the abnormal neurotransmission in brain areas affected in depression. Particularly, glutamatergic excitotoxicity has been suggested as one of the possible processes underlying the installation of the disease. Previous studies have suggested that glutamate release is increased in depressed subjects. Astrocytes play an important role in removing synaptic glutamate, mainly through the GLT-1 transporter, which may promote a decreased excitotoxicity. Interestingly, the β-lactamic antibiotic ceftriaxone (CEF) was described to increase glutamate transporter GLT-1 expression thus increasing glutamate uptake. These findings may suggest GLT-1 as a target for disruption in depression and provide clues for the 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 depression features in rats. In this protocol, animals were exposed to different stressors during six weeks. Furthermore, to different antidepressants (fluoxetine and imipramine) already known to impact on behavior and neuronal morphology of uCMS exposed animals were used as comparative controls. Three behavioral dimensions affected in depressive patients will be assessed: mood, anxiety and cognition. Gene and protein expression analysis will allow determining the effect of CEF administration in the levels of GLT-1. These behavioral and molecular 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 further elucidate the potential use of drugs targeting GLT-1 expression in the treatment of depression, paving the way for the development of new therapeutic strategies.

ANALYSIS OF THE IMPACT OF ASTROCYTIC EXOCYTOSIS IN SNARE AGED MICE MODEL

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Astrocytes are in charge of a great variety of complex and fundamental functions in the healthy CNS such as synaptic transmission and information processing by neural circuits. Therefore, any significant dysfunctions of astrocytes would contribute to mechanisms leading to pathological changes in CNS.

Through the release of a great range of transmitters and factors, such as D-serine, ATP and glutamate, astrocytes are able to modulate synaptic and vascular functions. Ca^{2+}-dependent exocytosis could be the main process underlying transmitter release from astrocytes and physiological astrocytic communications. Studies indicate that core proteins of the SNARE complex (synaptobrevin II and syntaxin) are expressed in astrocytes, and underlie Ca^{2+}-dependent glutamate release through a vesicular mechanism. This mechanism may be conditionally blocked in and inducible manner in the dnSNARE mice model.

In our study, we used SNARE model mice to analyze the impact of astrocytic exocytosis in behavior of aged mice. We performed behavior tests to evaluate cognition as well as depressive and anxiety-like behaviors. Furthermore, we did an extended tissue analysis studying the levels of astrocytic markers GFAP and GLT-1 as well as changes in cellular morphology.

THE INFLUENCE OF GLIOTRANSMISSION ON THE COGNITIVE FUNCTION OF THE DNSNARE MICE MODEL

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The modulatory effect of astrocytes in synaptic transmission had already been described supporting that astrocytes are involved in the function of synapses building up the "tripartite synapse", in which a dynamic cross-talk between astrocytes and neurons complements and modulates the communication between pre- and post-synaptic structures. Astrocytes were described to release gliotransmitters (such as glutamate, GABA, ATP or D-Serine) being this process mediated through the vesicular machinery and SNARE complex formation between vesicles and the target membrane. This phenomenon is essential for neurotransmitter release and an alteration on its normal function would be expected to impact on neuronal function and thus on higher cognitive functions. This idea was reinforced with results from Halassa and colleagues pointing out the prevention of cognitive deficits as consequence of sleep loss through the inhibition of gliotransmission. However, the impact of gliotransmission in normal cognitive function such as memory processing is poorly understood.

To dissect the role of gliotransmission on the cognitive function we used a transgenic mouse model with a conditional blockade of gliotransmission, the dnSNARE mice, characterized by having an impaired vesicular release selectively from astrocytes. A battery of behavioural tests was performed covering anxiety and exploratory behaviour - Open-field (OF), Elevated Plus Maze (EPM); anhedonia and learned helplessness – Forced Swim and Tail Suspension Tests (FST and TST); and cognitive function essentially focused on Reference Memory (RM) and Working Memory (WM) formation and behaviour flexibility – Morris Water Maze (MWM) and Y-Maze. High-resolution *in vivo* electrophysiological techniques were combined to search for correlates that can explain the behaviour performances observed taking into account the brain regions that are intimately related to cognitive processing, that is Prefrontal Cortex (PFC) and Hippocampus (Hip). This characterization may open a new window for the understanding/modulation of these cognitive fundamental processes.

THE ROLE OF ASTROCYTIC CALCIUM SIGNALING IN MICE BEHAVIOR

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Astrocytes are emerging as important actors in the regulation of synaptic transmission and plasticity. They possess unique morphologic and phenotypic features that allow them to monitor their neighbourhood and dynamically respond to changes. We hypothesized that impairment of astrocytic function might have implications in neurotransmission, metabolism and brain homeostasis, which may influence neural networks and underlie the pathophysiology of brain disorders. Calcium signalling arises as an important mechanism of astrocyte-function control, yet its molecular pathways and consequences to the network function remain to be elucidated. We propose here the use of IP_3R2KO model of astrocytic calcium dysfunction, to disclose its implications in brain networks by performing a myriad of behavioural tests. Anxiety, depressive-like behaviour and cognitive function were assessed for both IP_3R2KO mice and wild-type littermates. Behaviour tests such as Elevated Plus Maze (EPM) and Open Field (OF) were performed in order to evaluate the general exploratory behaviour of anxious phenotype and learned helplessness was evaluated by the Forced Swim Test (FST). Morris Water Maze (MWM) and Y-Maze tests were applied to assess cognition. Our results suggest that IP_3R2KO mice display no change in anxiety or depressive behaviours, however these animals present severe impairments in behavioural flexibility, a prefrontal cortex (PFC)-dependent task. This behavioural characterization will surely provide us with crucial information on the role of astrocytic calcium signalling in the modulation of neural activity, namely in cognitive processes.

THE APPLICATION OF AN A NOVEL OPEN SOURCE TOOL TO STUDY 3D ASTROCYTIC MORPHOLOGY

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Cell morphology analysis is a key tool to quantify several cellular features: as shape, morphological adaptations and interactions with surroundings cells, allowing the better understanding of its functions and role within the network.

Despite of the emerging importance of astrocytes in brain function, especially in synaptic transmission and plasticity, astrocytic morphology has been poorly studied. Assessing the morphology of astrocytic processes, their close structural association with synapses and their morphologic changes / remodelling will lead to a better understanding of the neuro-glia networks with putative functional implications.

Several remarkable softwares as Neurolucida, FilamentTracer by Imaris, Amira, Neuron 3DMA among others had been used to trace neurons and perform morphometric analysis. All of them have in common an associated license cost.

Here we present an open source software, Simple Neurite Tracer, to trace GFAP-positive astrocytes labelled by immunofluorescence, in image-stacks collected by confocal laser scanning microscope with 1 µm Z steps from brain slices of mice. We have employed this tool to study the morphology of astrocytes of the dnSNARE mice model. This model presents an astrocytic dysfunction that could have an impact in cognitive function of these animals.

We found this tool to be practical, reliable and most importantly to a less degree of user-dependent errors since it traces the processes on a semi-automated manner. Simple Neurite Tracer performs morphometric analysis of dendrites by quantifying their number, ramifications, total length, tortuosity and cell volume. It also provides detailed information about the complexity of the astrocytic morphology by sholl analysis. This software is a valuable tool to assess astrocyte morphology in a simple and economic way.

JMY, AN ACTIN-NUCLEATOR INVOLVED IN OLIGODENDROCYTE PROCESS EXTENSION AND EARLY AXON-GLIA INTERACTION

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Myelin formation in the central nervous system is carried out by oligodendrocytes (OL) that extend processes to contact, wrap and ensheath axons. Proper establishment and maintenance of myelin is essential for nervous system function because it allows for rapid conduction of the nerve impulse and provides metabolic support. Myelination is a highly dynamic process that requires profound changes in cell shape and we and others have shown that actin-related proteins are important regulators of cytoskeleton dynamics in myelinating cells. We found that a novel protein, JMY (also known as junction-mediating and regulatory protein p53 cofactor), is upregulated in the processes of OLs, when compared with its expression in the soma, during the initial phase of process extension. JMY has been described as a dual functional protein that can act as an actin nucleator when in the cytoplasm, through Arp2/3 activation or trough direct actin nucleation by itself, and can also be a p53 cofactor when in the nucleus. With this in mind, we sought to understand if JMY is modulating process extension in OLs and early axon-glia interaction, and if its function regulates axon ensheathment and wrapping. Using primary cultures of rat oligodendrocyte progenitor cells (OPCs), we observed that JMY is present during OL differentiation *in vitro*, with maximal expression in fully differentiated, mature OLs. Knocking down JMY in OLs *in vitro* leads to dramatic morphological defects, including fewer and shorter processes with reduced branching. However, depletion of JMY does not seem to impact the expression of myelin genes, such as MBP (myelin basic protein). This presents as a very interesting functional paradigm to dissect how ubiquitous molecules differentially regulate form and function, especially in a cell where the two are intrinsically linked. We are now using a myelinating co-culture subsystem of dorsal root ganglion (DRGs) and OLs lacking JMY to assess whether JMY is required for normal axon-glia early interaction. We also plan to perform live cell imaging of these co-cultures to get insight on how this dynamic process takes place and the OL morphology impacts the formation of the myelin sheath around an axon.

METHAMPHETAMINE AND NEUROINFLAMMATION: THE CROSSTALK BETWEEN MICROGLIA AND NEURONS

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Exposure to psychostimulants has been classically associated with damage to neuronal terminals. However, it is now accepted that addiction results from the interaction between neuronal and glial cells. 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 individual and collective role(s) of astrocytes and different types of neuronal cells on this inflammatory process. 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. 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 obtained from E16 rat embryos and cultured during 12 days before exposure to well-characterized doses of METH (10 and 100μM). Co-cultures of striatal and mesencephalic neuron were also used. After 3, 6 and 24 hours the conditioned medium was collected and added to primary microglial cells (collected from a mixed glial cell culture obtained from P0-P2 Wistar pups). 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. Although these result are yet not sufficient to exclude a role for neuronal cells in microglia activation, they strengthen the hypothesis that METH-induced neuroinflammation is mediated by astrocyte-released factors, and most likely by glutamate. These results lead us to hypothesize that levelling glutamate, but not dopamine, may prove to be efficient in preventing exacerbated microglial activation and associated neurotoxicity, which could be easily translated into the clinical practice, since there are several available FDA approved medications that target glutamate.

TAUROURSODEOXYCHOLIC ACID ATTENUATES GLIAL ACTIVATION IN MODELS OF ALZHEIMER'S DISEASE AND ACUTE INFLAMMATION

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Tauroursodeoxycholic acid (TUDCA) is an endogenous bile acid with potent neuroprotective and anti-inflammatory properties in several experimental models of Alzheimer's disease (AD). Here, we evaluated the therapeutic effects of TUDCA when administrated after the onset of amyloid pathology, focusing on neuroinflammation.

To assess the effect of TUDCA *in vivo*, we injected TUDCA (500 mg/kg bw i.p., every 3 days) in 7-month old APP/PS1 and wild-type mice, for 3 months. One hemisphere of the brain was processed for immunohistochemistry (IHC) and stained for amyloid-β (Aβ) and markers of astrocytes and microglia. The other hemisphere was processed for RNA and protein extraction, and analyzed by qRT-PCR and Western blot (WB). Further, to evaluate whether TUDCA specifically inhibits microglial activation, we analyzed inflammatory markers in microglial cell lines pre-treated with TUDCA and exposed to lipopolysaccharide (LPS).

TUDCA-treated transgenic mice brains presented reduced amyloidogenic proteolytic fragments of amyloid precursor protein, along with decreased amyloid plaque burden and Aβ levels. Importantly, gliosis was significantly decreased by TUDCA, as evaluated by IHC and WB, with a concomitant decrease in the expression of TNFα, while miRNA-146a and miRNA-155, upregulated in inflammatory conditions, were decreased in the brains of TUDCA-treated transgenic mice. GSK3β hyperactivity, a key event in glial activation, was strongly associated with Aβ accumulation and markedly abrogated by TUDCA. Finally, microglial cell lines exposed to LPS and concomitantly treated with TUDCA presented reduced expression of pro-inflammatory mediators, including TNFα and miRNA-146a.

Overall, our results suggest that TUDCA is a potential therapeutic option not only for the prevention, but also for the treatment of AD after the onset of the disease.

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ETHANOL TRIGGERS FAST GLUTAMATE RELEASE FROM CORTICAL MICROGLIA VIA C-SRC/TNF SIGNALING

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Alcohol consumption leads to neuroinflammation and neurodegeneration. Microglia, the immune resident cells of the central nervous system (CNS), are major players in inflammation-induced neuronal damage. After inflammatory stimuli microglia become activated releasing cytotoxic mediators like glutamate, which might cause neurodegeneration. Here, using Förster Resonance Energy Transfer (FRET)-based live cell imaging together with lentiviral-mediated shRNA protein knockdown, we dissect the signaling events associated with ethanol-induced glutamate release from cortical microglia. We show that ethanol triggers a fast and robust glutamate release from microglia and that this release requires sustained activation of the cytoplasmic tyrosine kinase c-Src. While forced c-Src activation mimics the ethanol effect triggering glutamate release, knocking down c-Src using shRNA abrogates it. In addition, the ethanol/c-Src-mediated glutamate release was prevented in microglia isolated from Tumor Necrosis Factor (TNF) knockout mice, indicating that ethanol triggers glutamate release via c-Src-induced TNF production. These data indicate that inhibiting the c-Src/TNF signaling in microglia could constitute a potential strategy for alleviating ethanol-mediated neuronal damage.

KEYWORDS: Ethanol; Glutamate; Microglia; Src.

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THE SODIUM VITAMIN C CO-TRANSPORTER 2 (SVCT2) DOWNREGULATION IS NECESSARY AND SUFFICIENCY TO INDUCE MICROGLIA PRO-INFLAMMATORY ACTIVATION

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Ascorbate is an antioxidant with many functions in the central nervous system (CNS) such as maturation of glutamatergic neurons and regulation of NMDA receptor. It is transported in a sodium-dependent manner by a plasma membrane transporter belonging to slc23a2 family (sodium vitamin C co-transporter-2; SVCT2). Microglia are a population of resident immune cells in the CNS. When stimulated, these cells trigger neuroinflammatory responses that may lead to neuronal cell death. Here, we demonstrate the SVCT2 expression in microglial cells, explore its importance for microglia response and study how pro-inflammatory stimulation of microglia (ischemia-reperfusion injury and exposure to lipopolysaccharide, LPS) regulates the expression of SVCT2 in the plasma membrane of microglia.

We demonstrate by confocal microscopy, western blotting and PCR analysis that microglial cells express SVCT2. To better understand the role of this protein in microglial physiology, we challenged these cells by LPS intravitreous injections or by performing the ischemia-reperfusion injury in the eye, and evaluated the expression and localization of SVCT2 by high-resolution confocal microscopy. We showed *in vivo* that both LPS injection or ischemia-reperfusion injury in the eye trigger a robust SVCT2 downregulation in retinal microglia. In order to confirm the importance of SVCT2 downregulation in microglia activation, we performed shRNA-mediated SVCT2 Knockdown and observed that a reduction of approximately 50% in protein expression was sufficient to trigger a pro-inflammatory signature in primary retinal microglial cells, with increased ROS production, nuclear translocation of NF-kB, increased iNOS expression and increased pro-inflammatory cytokine production (measured by qRT-PCR and ELISA). Consistent with a critical role for SVCT2 in activating microglia, $SVCT2^{+/-}$ mice showed pronounced microglia reactivity both in the retina and cerebral cortex.

We also analyzed the signaling pathways involved in SVCT2 downregulation. Firstly, we studied the SVCT2 internalization and clearly show the involvement of the tyrosine kinase c-Src. We also analyzed the involvement of Caveolin-1, as downstream c-Src target and observed (by biotinylation, immunoprecipitation and FRET) that c-Src phosphorylates caveolin-1 at tyrosine 14 leading to caveolin-1-mediated SVCT2 internalization. As SVCT2 is a transmembrane protein, we tested whether its degradation was mediated by Lysosomes and observed that the treatment with lysosome degradation blockers totally abolished SVCT2 degradation, concluding that SVCT2 degradation is mediated by lysosomes.

To demonstrate the significance of this pathway in microglia activation we showed that LPS-induced microglia activation was abolished by overexpressing either SVCT2 or caveolin-1 phosphodefective construct (Y14F point mutation). Overall, our data demonstrate an essential role for SVCT2 in controlling the pro-inflammatory polarization of microglia and neuroinflammation.

KEYWORDS: Neuroinflammation; Src kinase; Caveolin-1

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THE ROLE(S) OF RND2, AN ATYPICAL RHO GTPASE, IN NERVOUS SYSTEM MYELINATION

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Precise orchestration of the myelination developmental program involves the proliferation, migration and differentiation of Schwann cells (SCs) and oligodendrocytes (OLs) in the peripheral and central nervous system, respectively. Intrinsic signals, heterologous cell interaction, and extracellular cues tightly regulate these events in a time-controlled manner to ensure correct targeting and ensheathment of axons. Integration of all these signals directs the spatiotemporal control of cytoskeleton remodeling, critical for every step of myelination, and we have shown essential regulatory roles for the classical Rho GTPases Rac1 and Cdc42 in PNS and CNS myelination.

Rho GTPases are master regulators of cytoskeletal dynamics and, for example, during neuronal development, both classical and atypical Rho GTPases take part in integrating intra and extracellular signaling pathways into changes in cell shape dynamics and motility behavior. Unlike their classical counterparts, atypical Rho GTPases do not hydrolyze GTP and their functions and regulatory mechanisms remain largely elusive. These proteins can associate constitutively to membranes, and are thought to be regulated by expression levels, post-translational modifications, and discrete subcellular localizations. Despite their generally high expression in the nervous system and apparent spatiotemporal regulation during development and in disease conditions, information on the possible function(s) of atypical Rho GTPases in myelinating cells is inexistent.

We performed an mRNA level-based screening of five atypical Rho GTPases (Rnd 1, 2, and 3, and RhoU/Chp and RhoV/Wrch-1) during *in vitro* differentiation of primary rat OL progenitors. We found that Rnd3/RhoE and Rnd2 are the most abundant proteins, but while the mRNA levels of Rnd3 decrease steadily during differentiation, Rnd2 is significantly upregulated. The same transcript expression variation was observed in mouse optic nerve and spinal cord (both myelin-rich tissues) collected at different timepoints during developmental myelination. Moreover, immunohistochemical analysis of mouse nervous tissue showed that Rnd2 is highly expressed and co-localizes with MBP (myelin-basic protein) tracts especially during active developmental myelination.

Functional assays in primary cultures of OLs showed that decreasing levels of Rnd2 via lentiviral-mediated RNAi leads to morphological abnormalities in both immature OLs, which display diminished branching of processes, and in mature OLs that appear to not form myelin sheath structures. Interestingly, the expression of mature OL markers is decreased, indicating that Rnd2 may also be important for the differentiation of oligodendrocytes. We are now performing myelinating assays *in vitro* to gain functional insight in the context of axon-glia interaction, and in parallel we are assessing nervous system myelination and remyelination in conditional knockout mouse models of *Rnd2*.

ROLE OF PABPC1 AND YBX1 IN OLIGODENDROCYTE DIFFERENTIATION

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Localization and translational control of mRNAs play a key role on the development and differentiation of different cell types. Such control is mediated by RNA-binding proteins (RBP), which are the main regulators of RNA metabolism. In the central nervous system (CNS), myelin is formed by oligodendrocytes (OL), which extend cytoplasmic processes to myelinate axons. We hypothesize that similarly to axons local translation regulation of mRNA is also important for OL development, axonal targeting and survival. To gain deeper insight into the mechanisms regulating the outgrowth of OL processes, we have performed a comparative analysis of the RNAs present in the soma and processes of OL precursor cells (OPC) by RNA-Seq. Our data revealed an enrichment of mRNAs related to cytoskeletal dynamics and translation in OPC processes. Of particular interest, we found that the mRNA levels of RBPs involved in translation such as poly(A)-binding protein (PABP) cytoplasmic 1 (PABPC1) and Y box binding protein (YBX1), were enriched. Furthermore, we created a bioinformatics tool (PBSFinder), to investigate putative binding sites for RBPs, present within the 3`untranslated region of mRNAs important for OL processes extension such as PABPC1 and YBX1, which we found to be differentially expressed at mRNA and protein level during OL differentiation. Interestingly, shRNA mediated depletion of PABPC1, inhibited OL differentiation and MBP production. Taken together our results suggest that in OPC/OL the translation of several mRNAs is locally controlled and that PABPC1 and YBX1 play a role in this regulatory mechanism.

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