FIRST MEETING
GLIA CLUB SOUTHERN CONE

The good, the bad, the nice, and the ugly
of glial cells

Hybrid format
University of Buenos Aires
School of Pharmacy and Biochemistry
Buenos Aires, Argentina
October 19–21, 2022

Organizing Committee

Carla Caruso, Depto de Biol. Celular, Histología, Embriología y Genética, e Instituto de Investigaciones Biomédicas (INBIOMED) Facultad de Medicina, Universidad de Buenos Aires CONICET, Buenos Aires, Argentina
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Wednesday, October 19th

I. Oligodendrocytes. Current insights into the mechanisms that draw the myelin landscape
Hosts: Juana Pasquini, Jorge Correale, Florencia Labombarda & Francisco Rivera

14:30–15:30 Conference
Chair: Juana M. Pasquini

Charles ffrench-Constant, PhD, Pro-Vice-Chancellor for Medicine and Health Sciences, University of East Anglia, Norwich Research Park, Norwich, UK.

“From smart wiring to MS: the cell biology of myelination in health and disease.”

15:45–17:45 Symposium
Chair: Jorge Correale

Alerie Guzman de la Fuente, PhD, Instituto Investigación Sanitaria y Biomédica de Alicante (ISABIAL), Alicante, and Instituto de Neurociencias de Alicante CSIC-UMH, San Juan de Alicante, Spain, and Wellcome-Wolfson Institute for Experimental Medicine, Queen’s University Belfast, UK.

“Crosstalk between T cells and oligodendrocyte progenitor cells.”

Francisco Rivera, PhD, Laboratory of Stem Cells and Neuroregeneration, Institute of Anatomy, Histology and Pathology, Faculty of Medicine, Universidad Austral de Chile, Valdivia, Chile, and Molecular and Integrative Biosciences Research Program, Faculty of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland.

“Beyond Haemostasis—Role of Circulating Platelets in Remyelination.”

Florencia Labombarda, PhD, Laboratorio de Bioquímica Neuroendócrina, IBYME-CONICET, Argentina.

“Progesterone and spinal cord injury: The challenge of remyelination.”

Ernesto Bongarzone, PhD, Department of Anatomy & Cell Biology, College of Medicine, the University of Illinois at Chicago, USA.

“Waning efficacy in a long-term AAV-mediated gene therapy study in the murine model of Krabbe disease.”

18:00–19:00 Young investigator talks
Chair: Florencia Labombarda and Francisco Rivera

Vanesa S. Mattera, Departamento de Química Biológica and IQUIFIB, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, CONICET, Argentina.

“Remyelinating effect on OPCs driven by transferrin-loaded extracellular vesicles.”

Bryan Hinrichsen, Laboratory of Stem Cells and Neuroregeneration, Institute of Anatomy, Histology and Pathology, Faculty of Medicine, Universidad Austral de Chile, Valdivia, Chile.

“Macrophages Shape Pericytes Response to Demyelination and their Ability to Facilitate the Generation of Oligodendrocytes.”

Mauricio Galiano, PhD, Departamento de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina.

“Role of arginyl-transferase in glial cells during CNS myelination.”

19:00–20:30 Poster session (oligodendrogliá)
Thursday, October 20th

II. Microglia cells: friend or foe?
Hosts: Laura Pasquini and Fernando Pitossi

9:00–10:00 Conference
Chair: Fernando Pitossi

Soyon Hong, PhD. Dementia Research Institute, Institute of Neurology, University College London, London, UK.
“Microglia-Synapse Interactions: The triggers and the consequences.”

10:15–12:15 Symposium
Chair: Laura Pasquini and Fernando Pitossi

Guillermo Giambartolomei, PhD. Instituto de Inmunología, Genética y Metabolismo (INIGEM), Universidad de Buenos Aires-CONICET, Argentina.
“Innate immune activation of glial cells: lessons to be learnt from an intracellular bacterium.”

Hugo Peluffo, PhD. Neuroinflammation and Gene Therapy Laboratory, Institut Pasteur de Montevideo, Montevideo 11400, Uruguay and Departamento de Histología y Embriología, Facultad de Medicina, Universidad de la República, Montevideo 11800, Uruguay.
“CD300f immune receptor regulates microglial phenotype, psychiatric conditions and aging.”

Elaine Del Bel, PhD. Department of Basic and Oral Biology, School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil.
“Levodopa-induced dyskinesia in Parkinson’s disease increases reactive astrocytes and microglial cells.”

Analia Reines, PhD. Instituto de Biología Celular y Neurociencia “Prof. E. De Robertis” (IBCN), CONICET—Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Farmacología, Universidad de Buenos Aires, Buenos Aires, Argentina.
“Microglial patterns in autism spectrum disorder: Influence of neuronal and astroglial microenvironment.”

12:30–13:30 Young investigator talks
Chair: Carla Caruso

María Julia Pérez. Department of Biological Chemistry, Institute of Chemistry and Biological Physicochemistry (IQUIFIB), School of Pharmacy and Biochemistry, the University of Buenos Aires and National Research Council (CONICET), Buenos Aires, Argentina.
“Transferrin increases microglia phagocytic capacity and induces a neuroprotective phenotype and neuronal differentiation, thus fostering remyelination.”

Berenice Silva. Laboratorio de Terapias Regenerativas y Protectoras del Sistema Nervioso Fundación Instituto Leloir IIBBA, Argentina.
“Environmental enrichment induces microglia polarization and neurotrophin production in a focal chronic cortical model of multiple sclerosis.”

Javier M. Peralta Ramos, PhD. Senior Postdoctoral Research Fellow, Department of Brain Sciences, Weizmann Institute of Science, Rehovot, Israel.
“Alzheimer’s disease modification is mediated by bone marrow-derived macrophages in mouse models of both amyloidosis & tauopathy.”
III. Schwann cells and their involvement in peripheral nerve regeneration and neuropathic pain
Hosts: Patricia Setton and Felipe Court

14:30–15:30 Conference

Chair: Patricia Setton

Felipe Court, PhD, Center for Integrative Biology, Universidad Mayor, Chile and Buck Institute for Research on Aging, Novato, USA.

“Schwann cell exosomes as possible therapeutic intervention to enhance axonal regeneration after peripheral nerve injury.”

15:45–17:45 Symposium

Chair: Felipe Court

Brett Morrison, MD, PhD, Associate Professor of Neurology, Division of Neuromuscular Medicine, Director of Inpatient Neurology Consult Service, Johns Hopkins School of Medicine, Baltimore, Maryland, USA.

“Role of Schwann cell monocarboxylate transporters in development and regeneration.”

Pablo Lopez, PhD, Departamento de Química Biológica Ranwell Caputto-CIQUIBIC, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina.

“Identification of novel molecular mechanisms associated with impaired nerve repair triggered by anti-ganglioside antibodies.”

Patricia Setton, PhD, Instituto de Química y Fisicoquímica Biológica “Alejandro Paladini” UBA-CONICET, Facultad de Farmacia y Bioquímica, Argentina.

“Adult multipotent cells modulate the immune response and the development of neuropathic pain in a model of sciatic nerve injury.”

Margarita Calvo, MD, PhD, Physiology Department, Pontificia Universidad Católica de Chile, Santiago, Chile.

“De novo expression of Kv1.6 as a regulatory mechanism in neuropathic pain.”

Daniela Binaghi, MD, Médica especialista en diagnóstico por imágenes, Médica consultora en patología músculo-esquelética, Jefa de la sección de Nervio Periférico y Plexos, Investigaciones Médicas, Buenos Aires, Argentina.

“Schwann cells seen from a radiological perspective: current use of MR-Neurography.”

18:00–19:00 Young investigator talks

Chair: Pablo López

Paula Soto, PhD, Instituto de Química y Fisicoquímica Biológica “Alejandro Paladini” UBA-CONICET Facultad de Farmacia y Bioquímica. Instituto de Física La Plata, UNLP, CONICET, Facultad de Ciencias Exactas, Argentina.

“Adipose-derived mesenchymal stem cells magnetic targeting to promote sciatic nerve regeneration after traumatic injury.”

Andres Fuentes, PhD, Center for Integrative Biology, Universidad Mayor, Chile.

“Modulation of the Schwann cell phenotype as a strategy for enhancing peripheral nerve repair.”

19:00–20:30 Poster session (microglia & Schwann glia)
Friday, October 21st

IV. Astrocytes; The rising stars of the CNS
Hosts: Javier Ramos & Patricia Cassina

9:00–11:00 Symposium
Chair: Patricia Cassina

Luis Barbeito, PhD, Institut Pasteur Montevideo, Uruguay.
“The crosstalk of astrocytes with mast cells and microglia in ALS.”

Brigitte van Zundert, PhD, Institute of Biomedical Sciences (ICB), Faculty of Medicine & Faculty of Life Sciences, Universidad Andres Bello, Santiago, Chile; CARE Biomedical Research Center, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile, and Department of Neurology, University of Massachusetts Chan Medical School, Worcester, MA, USA.
“PolyP unmasked in ALS/FTD: a deceivingly simple inorganic polymer being around for billions of years.”

Mario E. Guido, PhD, CIQUIBIC-CONICET, Facultad de Ciencias Químicas & Departamento de Química Biológica “Ranwel Caputto,” Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina.
“The implication of the circadian system in the regulation of glioma cell metabolism and growth.”

Millet Violeta, MD, PhD, Department of Ophthalmology, Neuroophthalmology Section at Del Callao Surgical Institute, Buenos Aires, Argentina; Department of Ophthalmology of Bernardino Rivadavia Hospital, Buenos Aires, Argentina, and Department of Ophthalmology, Medical School, University of Buenos Aires, Argentina.
“Neuromyelitis optica spectrum disorders: The astrocytes role, linking basic research, clinical characteristics and therapeutic perspectives.”

11:15–12:15 Conference
Chairs: Patricia Cassina and A. Javier Ramos

Shane Liddelow, PhD, Neuroscience Institute and Departments of Neuroscience, & Physiology, and Ophthalmology, New York University Grossman School of Medicine, New York, NY, USA.
“Reactive astrocytes in inflammation and neurodegenerative disease.”

12:30–13:30 Young investigator talks
Chair: A. Javier Ramos

Ernesto Miquel, MSc, Programa de Desarrollo de las Ciencias Básicas (PEDECIBA)-Biología, sub-área Biología Celular y Molecular, Facultad de Ciencias, Universidad de la República, Uruguay.
“Pyruvate dehydrogenase kinase 2 knockdown restores the ability of ALS-linked SOD1G93A rat astrocytes to support motor neuron survival by increasing mitochondrial respiration.”

Alejandro Villarreal, PhD, Laboratorio de Neuropatología Molecular, Instituto de Biología Celular y Neurociencia “Prof. E. De Robertis” UBA-CONICET, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.
“Changes in histone modifications in hypooosmolar-stressed astrocytes: new insights in tissue response to brain edema after injury.”

Francisca Cornejo Castillo, PhD, Center for Integrative Biology, Universidad Mayor, Chile.
“Role of the ASD-associated gene PTPRD in the development of cortical astrocytes and its implications in neurodevelopmental disorders.”
Glauce Crivelaro do Nascimento, PhD, Department of Oral and Basic Biology, Faculty of Odontology of Ribeirao Preto, University of Sao Paulo, Brazil.

“Dynamic involvement of striatal NG2-glia in L-DOPA induced dyskinesia in parkinsonian rats: effects of doxycycline.”

13:45 Round table
Promoting regional collaborations and sharing resources for neuroscience

Chair: Lorena Rela

Diego Ferrara, Cdor, Responsable de Contrataciones y Comercio Exterior, CONICET, Argentina.

Maite A. Castro Gallastegui, PhD, SEREMI (Secretaría Regional Ministerial), Ministerio de Ciencia, Tecnología, Conocimiento e Innovación, Macrozona Sur (Araucanía, Los Ríos y Los Lagos).
V. Specialized glia: The value of diversity
Host: Lorena Rela

**15:00–16:00 Symposium**
Chair: Ruth Rosenstein

Luis Enrique Politi, PhD, Department of Biology, Biochemistry and Pharmacy, Universidad Nacional del Sur, and Neurobiology Department, Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB) Conicet, Bahía Blanca, Buenos Aires, Argentina.

“Does the impaired crosstalk of Müller glial stem cells and neurons interfere with retinal regeneration?”

Maria Cecilia Sánchez, PhD, Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina, and Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Córdoba, Argentina.

“Müller glia commanding retinal survival and death.”

Mario Perelló, PhD, Grupo de Neurofisiología, Instituto Multidisciplinario de Biología Celular (IMBICE, CIC-CONICET-UNLP), Buenos Aires, Argentina.

“Hypothalamic tanycytes as key players for the central actions of ghrelin.”

**16:15–17:15 Conference**
Chair: Lorena Rela

Bo Chen, PhD, Department of Ophthalmology, Department of Neuroscience, Department of Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

“Müller glia and retinal regeneration.”

**17:15–18:00 Young investigator talks**
Chair: María Cecilia Sánchez

Antonia Recabal, PhD, Departamento de Biología Celular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.

“Role of gap junctions and hemichannels in tanycyte self-renewal.”

Natalia Marchese, PhD, CIQUIBIC-CONICET Departamento de Química Bólogica “Ranwel Caputto” Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina.

“The Müller glial cells: are specialized glia for light detection in the retina?”

Lic. Harmonie Vallese Maurizi, Laboratorio de Neurovirología, INIBIBB-UNS-CONICET, CCT, Bahía Blanca, Argentina.

“Müller glial cells alterations in a retinal degeneration mouse model.”

**18:00–19:00 Closing conference**
Chair: Ruth Rosenstein

Jorge Correale, MD, Departamento de Neurología, FLENI, Instituto de Química y Biofísica Biológicas (IQUBIB), CONICET-UBA, Buenos Aires, Argentina.

“The multifaceted roles of glial cells in inflammatory demyelination: the challenge of achieving homeostasis.”

**19:00–20:30 Poster session (astroglia & specialized glia)**
General organization: Carla Caruso, Laura Pasquini, and Lorena Rela
Abstracts

I. Conferences

Chair: Juana M. Pasquini

From Smart Wiring to Ms: The Cell Biology of Myelination in Health and Disease
Charles ffrench-Constant
1The University of East Anglia, Faculty of Medicine and Health Sciences, Norwich Research Park, Norwich, UK

The axons of the central nervous system (CNS) are often thought of like wiring in a computer—enabling fast conduction but passive, and not part of the brain’s ability to change in response to experience ie to learn. However recent advances in the biology of myelin show that myelin sheaths change in number and size as the brain experiences the outside world, potentially generating what I term “smart wiring.” The signals that regulate these changes are poorly understood, and I will illustrate how endothelins—released by endothelial cells during neural activity—increase sheath numbers. The alteration of sheath number in response to experience is, in part, enabled by the presence of precursor cells throughout the adult brain that can generate new myelinating oligodendrocytes throughout life. This ability to form new oligodendrocytes is also important in driving another example of CNS plasticity—the regenerative response following myelin loss in Multiple Sclerosis. In the second part of my talk, I will describe the use of single nuclear RNA sequencing to dissect this regenerative response in different patients, and show how the results challenge current thinking as to how to promote regeneration and limit progression in this common disabling disease.

Chair: Laura Pasquini and Fernando Pitossi

Microglia-Synapse Interactions: The Triggers and the Consequences
Soyon Hong
1UK Dementia Research Institute University College London, Cruciform Building Gower St, London, UK

Microglia and other resident macrophages in the central nervous system exhibit heterogeneous phenotypes dependent on spatio-temporal context, allowing them to fulfill various functions in health and disease. Microglia, as the primary tissue-resident macrophages of brain parenchyma, listen to changes in local synaptic activity and help sculpt the brain as well as contribute to proper synaptic function throughout life. Interestingly, in models of various neurologic diseases, microglia-synapse interactions have been shown to go awry, contributing to region-specific synapse loss and dysfunction. In particular, in Alzheimer’s disease, many genetic risk factors converge on phagocytic pathways of microglia, raising a critical need for mechanistic insight into microglial phagocytosis in a disease-relevant context. However, what reactivates microglia to engulf synapses is unclear. Here I will discuss our lab’s recent findings on potential triggers and molecular mechanisms that regulate microglia-synapse interaction in adult and diseased brains. Specifically, I will focus on our recent unpublished data that suggest that microglia is heavily influenced by neighboring border-associated macrophages along the vasculature, that is, perivascular macrophages, and how this cross-talk is mediated by SPP1/Osteopontin, to modulate microglial phagocytosis of synapses.

Chair: Patricia Setton

Schwann Cell Exosomes as Possible Therapeutic Intervention to Enhance Axonal Regeneration After Peripheral Nerve Injury
Felipe Court
1Center for Integrative Biology, Universidad Mayor, Chile and Buck Institute for Research on Aging, Novato, USA

Regeneration of injured peripheral nerve axons is critically dependent on the reprogramming of differentiated Schwann cells (SCs) into a repair SC phenotype, specialized for promoting regeneration and tissue homeostasis by secreting neuronal pro-regenerative molecules and extracellular vesicles, formation of a cellular scaffold and mounting an innate immune response. Nevertheless, these events are not efficiently activated during chronic SC denervation and nerve
injuries in aged organisms, which has been associated with a defective SC reprograming into repair SC. In this presentation, I will show you an alternative explanation for the poor regenerative outcome in these conditions, associated with a novel SC phenotype conversion which has a detrimental effect on regeneration. Importantly, these inhibitory SC can be targeted, strongly enhancing axonal regeneration and providing novel therapeutic strategies for nerve repair.

Chairs: Patricia Cassina and A. Javier Ramos

Reactive Astrocytes in Inflammation and Neurodegenerative Disease

Shane A. Liddelow¹,²

¹Department of Neuroscience and Physiology at NYU Grossman School of Medicine, New York, NY, USA
²Department of Ophthalmology at NYU Grossman School of Medicine, Parekh Center for Interdisciplinary Neurology, New York, NY, USA

The study of astrocyte reactivity requires careful identification of heterogeneity via transcriptomic profiling, followed by identification using cell-based systems to model their functional alterations compared to physiologically "normal" astrocytes. Further validation by confirmation in rodent models of disease and in human cells and postmortem tissue provides corroboration of biologically important reactive astrocyte sub-states.

We recently completed a large well-powered scRNAseq analysis of astrocyte reactivity profiles following acute systemic inflammation, highlighting several transcriptomically defined sub-states. Further, using integration with other published datasets we find specific disease-associated sub-states in rodent models of Alzheimer's disease (AD), demyelination, and an acute stab wound. Following, we produced in vitro models to further study the functional alterations of these sub-states of reactive astrocytes and used spatial transcriptomics to localize sub-states of reactive astrocytes to specific brain regions. To improve understanding of transcriptomic heterogeneity in glia during AD, we used snRNA-seq to characterize astrocytes and oligodendrocytes from APOE ε2/3 human AD and age- and genotype-matched non-symptomatic brains. In parallel studies, we also take advantage of recent human and mouse spatial transcriptomics resources to localize heterogeneous astrocyte subtypes to specific regions in the healthy and inflamed brain. Finally, we integrated our data with published AD snRNA-seq datasets, highlighting the power of combining datasets to resolve previously unidentified astrocyte subpopulations. Combined these studies highlight the importance of functional validation of transcriptomically-defined reactive astrocyte sub-states, and identify specific subtypes of astrocytes and multiple sub-states of reactive astrocytes in strategic spatial locations in both rodent and the human brains.

Chair: Lorena Rela

Müller Glia and Retinal Regeneration

Bo Chen¹

¹Department of Ophthalmology, Department of Neuroscience, Department of Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

We examine intrinsic signaling pathways and transcription control in Müller glia (MG), the primary glial cell type in the mammalian retina. The goal is to reprogram them in vivo to generate MG-derived retinal stem cells that are capable of differentiating from new photoreceptors. In cold-blooded vertebrates such as zebrafish, MGs are a source of adult retinal progenitor/stem cells that readily re-enter the cell cycle and replenish lost neurons, including photoreceptors. In mammals, however, MG in the adult retina do not spontaneously re-enter the cell cycle and therefore they lack regenerative capability. One exception is that mammalian MG can restore their progenitor/stem cell status, evidenced by cell cycle re-entry, following a retinal injury such as neurotoxin treatment. This type of injury would be counterproductive for regeneration as it massively kills retinal neurons, precluding recovery of normal retinal function. The injury-induced signals that activate the proliferative response of mammalian MG are unknown. We hypothesized that retinal injury may induce early signaling events, which in turn activates MG proliferation. We further reasoned that by directly activating these signals, MG could be stimulated to re-enter the cell cycle without the introduction of retinal injury. Since then we have made exciting progress on manipulating the Wnt/Lin28 signaling pathway to reprogram these cells to retinal stem/progenitor cells that can be further induced to generate photoreceptors by gene transfer of a defined set of the transcription factors that are essential for photoreceptor differentiation during retinal development. This line of research has opened a door to treating a spectrum of retinal degenerative diseases through activating the retina's own regenerative capabilities.

Chair: Ruth Rosenstein

The Multifaceted Roles of Glial Cells in Inflammatory Demyelination: The Challenge of Achieving Homeostasis

Jorge Correale¹

¹Departamento de Neurología, FLENi, Buenos Aires, Argentina

Glial subtype diversity is emerging in neurobiology and immune-mediated neurological diseases such as multiple sclerosis (MS). While the glial response was initially interpreted only as reactive, new evidence implicates reactive glial cells
as cells that may mediate and perpetuate the immune response and tissue injury. Glial cell types play diverse and sometimes opposing roles in MS pathobiology. These roles can be detrimental, leading to the propagation of inflammation, tissue destruction, and CNS atrophy. Conversely, glial subtypes can also play beneficial roles in clearing debris and promoting repair. Notably, the same cell type could perform multifaceted roles. These opposing functions could be reconciled by differences in the state of activation, regional differences, or different times after injury. Furthermore, due to the relapsing-remitting nature of MS, it could be possible that glial cells cycle between different subtypes according to the specific inflammatory stage. Therefore, targeting these cell subtypes in an attempt to slow neurodegeneration and promote regeneration will prove challenging. Hence, a task for future studies includes identifying these subtype-specific critical regulators of disease processes and developing interventional strategies to modulate glial subtype-specific pathways along spatial and temporal disease trajectories in MS. This might also help us to understand better and eventually promote anti-inflammatory and pro-regenerative cell subtypes to facilitate repair in MS.

II. Symposia

Oligodendrocytes: Current insights into the mechanisms that draw the myelin landscape

Chair: Jorge Correale

Crosstalk Between T Cells and Oligodendrocyte Progenitor Cells

Alerie Guzman de la Fuente1,2,3, Marie Dittmer3, Elise Heesbeen3,4, Nira de la Vega Gallardo3, Jessica White3, Andrew Young3,4, Katie Mayne3, John Falconer3,6, Christopher E. McMurran6, Carmel McVicar3, Mohammed Innayatullah3, Vijay Tiwari3, Rebecca Ingram3, Rosana Penalva3,7,8, and Denise C. Fitzgerald3

1Institute for Health and Biomedical Sciences of Alicante (ISABIAL), Alicante, Spain
2Institute of Neuroscience of Alicante CSIC-UMH, San Juan de Alicante, Spain
3Wellcome-Wolfson Centre for Experimental Medicine, Queen’s University Belfast, Belfast, UK
4Current address: Division of Pharmacology, Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, the Netherlands
5Patrick G Johnston Centre for Cancer Research, Queen’s University Belfast, Belfast, UK
6CRUK Beatson Institute, Glasgow, UK
7Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK
8Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

In multiple sclerosis (MS) lost myelin can be restored by an efficient regenerative process known as remyelination. Unfortunately, like any other regenerative process, remyelination efficiency declines with aging leading to neuronal loss and accrual of permanent disability. Regulatory T cells (Treg) play a key role in driving oligodendrocyte progenitor cell (OPC) differentiation and remyelination. However, it is yet to be determined how aging, a major risk factor for MS progression, affects the ability of Treg to promote remyelination which limits their potential use as therapy to limit MS progression. Aged Treg has an intrinsically impaired regenerative capacity as they fail to promote OPC differentiation in vitro. This impairment can be rescued by a young microenvironment as aged Treg are able to promote OPC differentiation in a young mouse similarly to young Treg, suggesting that the aging process is reversible. To understand the mechanism behind aged-linked impairment of Treg pro-remyelination capacity, we performed transcriptomics analysis and identified 302 genes downregulated with aging. Amongst these, we identified Itga2 and Mcam, as two key Treg-derived factors that drive OPC differentiation in a contact-dependent manner in vitro. These results indicate that age Treg has an impaired regenerative capacity that contributes to remyelination failure in MS progression. However, this impairment can be reversed by a young microenvironment, suggesting that Treg rejuvenation may be a relevant therapeutic strategy to limit MS progression. Exploring the Treg mechanisms identified here will provide new insights into MS progression and new targets for therapeutic gain.

Waning Efficacy in a Long-Term AAV-Mediated Gene Therapy Study in the Murine Model of Krabbe Disease

Ernesto R. Bongarzone1

1Department of Anatomy & Cell Biology, College of Medicine, University of Illinois at Chicago, Chicago, IL, USA

Neonatal gene therapy of twitcher mice, the mouse model of Krabbe disease, started after birth significantly improves neuropathology, normalizes motor deficits, and extends survival. New observations identified the formation of small focal demyelinating foci in the brain of treated twitcher mice after 6–8 months. Rather than diffuse demyelination, these late-onset lesions are surrounded by normal white matter, and in association with reactive astroglia, exhibiting lysosomal alterations with reduced expression of galactosylceramidase (GALC) and increased psychosine levels. Lesions were associated with the extravasation of plasma fibrinogen and activation
of the fibrinogen-BMP-SMAD-GFAP gliotic response. We found that the dysregulation of therapeutic GALC was likely driven by the exhaustion of adeno-associated viral (AAV) episodes within the lesions, paralleling the presence of proliferating oligodendrocyte progenitors and glia. We believe that this is the first demonstration of diminishing expression in vivo from an AAV gene therapy vector with detrimental effects in the brain of a lysosomal storage disease animal model.

Beyond Haemostasis—Role of Circulating Platelets in Remyelination

Amber R. Philp1,2,3, Josselyne Mansilla1,2,
Amar Sharma3, Chao Zhao3, Khalil S. Rawji3, Ginez A. Gonzalez Martinez3, Penelope Dimas3, Carlos Valenzuela1,2, Carolina Reyes1,2, Bryan Hinrichsen1,2, César Ulloa1,2, Maria Elena Silva1,2,4, Maite A. Castro2,6, Pamela Ehrenfeld2,7, Ludwig Aigner8,9, Ilias Kazanis3,10, Cedric Ghevaert11,12, Robin J.M. Franklin3, and Francisco J. Rivera1,2,13

1Laboratory of Stem Cells and Neuroregeneration, Institute of Anatomy, Histology and Pathology, Faculty of Medicine, Universidad Austral de Chile, Valdivia, Chile
2Center for Interdisciplinary Studies on the Nervous System (CISNe), Universidad Austral de Chile, Valdivia, Chile
3Wellcome-MRC Cambridge Stem Cell Institute & Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK
4Laboratory of Pharmacy, Faculty of Sciences, Universidad Austral de Chile, Valdivia, Chile
5Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile
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7Laboratory of Cellular Pathology, Institute of Anatomy, Histology & Pathology, Faculty of Medicine, Universidad Austral de Chile, Valdivia, Chile
8Institute of Molecular Regenerative Medicine, Paracelsus Medical University, Salzburg, Austria
9Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS), Paracelsus Medical University, Salzburg, Austria
10Laboratory of Developmental Biology, Department of Biology, University of Patras, Patras, Greece
11Cambridge Stem Cell Institute, Jeffrey Cheah Biomedical Centre, Cambridge, UK
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Multiple sclerosis (MS) is an autoimmune neuroinflammatory demyelinating disease of the central nervous system (CNS) that affects about 2.5 million people worldwide. In response to demyelination, oligodendrocyte progenitor cells (OPCs) proliferate, migrate and differentiate into mature oligodendrocytes. Microglia/macrophages and pericytes are important regulators of OPC function during myelin repair. Although remyelination represents a robust regenerative response to myelin damage, it extensively fails in MS, and current therapies lack of repair activities. Thus, revealing unknown molecular and cellular cues that control remyelination represents a major goal for MS research. Platelets, small anucleated cells that are essential for hemostasis, have been detected in chronic demyelinated lesions of MS patients. In experimental autoimmune encephalomyelitis mice, an animal model for MS, circulating platelets gradually rise along with disease progression. Here, using in vivo and in vitro experimental approaches we explored the hypothesis that circulating platelets might contribute to remyelination. Our results showed that circulating platelets mediate remyelination by different means. In vivo loss- and gain-of-function experiments revealed that altered numbers of circulating platelets affect OPC differentiation and CNS remyelination. Furthermore, our findings showed that altered number of circulating platelets also affects the behavior of microglia/macrophages and pericytes during remyelination, both potent regulators of OPC function. We observed that platelets directly modulate OPC survival and differentiation in vitro in a dose-dependent manner. This study extends circulating platelets’ function, beyond hemostasis, providing new insights into their role as modulators of myelin repair.

Progesterone and Spinal Cord Injury: The Challenge of Remyelination

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Progesterone is emerging as a myelinizing factor for central nervous system injuries. Successful remyelination requires the proliferation and differentiation of oligodendrocyte precursor cells (OPCs) into myelinating oligodendrocytes, but this process is incomplete following spinal cord injury (SCI). Our group has demonstrated that progesterone treatment during the acute phase increases the number of OPC preventing their apoptosis and decreases the expression of pro-inflammatory cytokines, such as TNFα, IL1β, and IL6, all involved in oligodendrocyte damage. Progesterone also reduces the number of activated microglial cells and reactive astrocytes generating a pro-myelinating environment after SCI. Regarding OPC differentiation into mature oligodendrocytes, progesterone decreases the mRNA expression of transcriptional inhibitors (Id2, Id4, and hes5) and enhances the expression of transcriptional activators of oligodendrogenesis (Olig2, Nkx2.2, Sox10, and Mash1) during the acute phase. BrdU administration immediately after SCI demonstrated that progesterone treatment increases the number of newly mature oligodendrocytes during the chronic phase, identified as CCI+ BrdU+ cells. Progesterone also enhances both the protein and mRNA of PLP and MBP during
the chronic phase. These results suggest that early progesterone treatment induces OPC differentiation into mature myelinating oligodendrocytes. Concerning progesterone mechanisms of action using PRKO mice, we have demonstrated that both progesterone myelinating and anti-inflammatory effects are mediated by the classical nuclear receptor PR. Furthermore, progesterone administration is related to functional recovery. Using the Basso-Bresnahan-Beattie scale for locomotion and CatWalk gait analysis, we have observed that progesterone treatment significantly improves locomotor outcomes. This evidence suggests that progesterone could be considered a promising therapeutic candidate for spinal cord-injured patients.

**Microglia cells: friend or foe?**

**Chairs: Laura Pasquini and Fernando Pitossi**

**Innate Immune Activation of Glial Cells: Lessons to be Learnt From an Intracellular Bacterium**

Guillermo Giambartolomei

**Abstracts**

Central nervous system invasion by bacteria of the genus *Brucella* results in an inflammatory disorder called neurobrucellosis. *B. abortus* infects microglia, eliciting their activation and production of pro-inflammatory mediators. Evidence of neurological demise occurs to varying degrees in the nervous systems of patients with neurobrucellosis. With the aim of determining the putative mechanisms involved in this phenomenon we established murine primary cultures of neurons and microglia to demonstrate that, due to *B. abortus* infection, microglial primary phagocytosis (phagoptosis) actively induces neuronal death, without inducing neuronal apoptosis. This phenomenon was due to microglia-TLR2 activation by *Brucella* lipopolysaccharides. *B. abortus*-activated microglia secretes nitric oxide (NO) and increase their phagocytic ability. NO-induced the exposure of the eat-me signal on neurons (phosphatidylserine, PS). Blocking PS-binding protein milk fat globule epidermal growth factor-8 (MFG-E8) interaction, or microglial vitronectin receptor-MFG-E8 interaction was sufficient to prevent neuronal loss without inhibiting microglia activation. Furthermore, inhibition of purinergic signaling and desyalilation of neurons also prevents phagoptosis of viable neurons by *B. abortus*-activated microglia. Hence, our results demonstrate a novel form of inflammatory neurodegeneration for a bacterial infection, where inflammation causes exposure to eat-me signal on neurons, leading to their death through primary phagocytosis via different non-redundant signaling pathways. These results describe part of the mechanisms whereby *B. abortus* could induce neuronal death during neurobrucellosis.

**CD300f Immune Receptor Regulates Microglial Phenotype, Psychiatric Conditions, and Aging**

Frances Evans, Daniela Ali, Natalia Rego, Maria Luciana Negro-Demontel, Natalia Lago, Fabio Andres Cawen, Bruno Pannunzio, Paula Sanchez-Molina, Laura Reyes, Andrea Paolino, Celia Quijano, Ana Paula Mulet, Geraldine Schlapp, Maria Noel Meikle, Mariana Bresque, Martina Crisp, Eduardo Savio, Carlos Escande, and Hugo Peluffo

Emerging evidence suggests that immune receptors participate in many psychiatric and aging-related processes. CD300f is a lipid-sensing immune receptor that shares many properties with TREM2. It limits the severity of inflammatory conditions by negatively regulating the innate immune system. We asked whether immune receptors expressed on myeloid cells, and in particular, CD300f, can regulate behavior and systemic aging-related processes, such as metabolism, inflammation, and ultimately aging and a healthy lifespan. We will discuss the sex-dependent involvement of CD300f in psychiatric conditions in mice and humans. Moreover, we will focus on the role of this immune receptor in aged CD300f−/− and WT mice which have been followed closely for 30 months. Strikingly, three different cohorts of male and female CD300f−/− mice showed an important reduction in lifespan. This was observed under both specific pathogen-free conditions and in a closer to real-life housing/immunologic environment. No single evident cause of death in CD300f−/− mice could be determined as expected for aging-driven multi-cause deaths. CD300f deficiency during aging enhanced the progressive liver, pulmonary, and brain inflammation, and drove the cognitive decline in 18–24 months old mice, as observed by alterations in Novel Object Recognition and Barnes Maze learning and memory tests. Brain hypometabolism observed by 18FDG PET scans was observed in aged 18 months old CD300f−/− female mice. These brain function alterations were correlated with increased lipid droplet-containing microglia, increased expression of Disease Associated Microglia (DAM) and disease inflammatory...
macrophages (DIM) gene profiles, downregulation of the brain ATF4 pathway, senescence, and alterations in frailty markers. In accordance, aged CD300−/− mice also displayed motor coordination deficits at 25 months. Aging induced reduced hepatic gluconeogenesis and capacity to maintain glycemia in female CD300−/− mice. Taken together, we present novel data supporting the hypothesis that the study of the biology of immune receptors in the context of aging contributes to the elucidation of novel predictors of both health and lifespan, and may identify therapeutic targets for attenuating aging and abrogating age-related diseases.

**Levodopa-Induced Dyskinesia in Parkinson’s Disease Increases Reactive Astrocytes and Microglial Cells**

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Parkinson’s disease is a chronic neurodegenerative disorder associated with the loss of dopamine neurons in the substantia nigra, for which there is still no effective protective treatment. Dyskinesia, or abnormal involuntary movements, is an unfortunate consequence of long-term therapy with L-3,4-dihydroxyphenylalanine (L-DOPA), a gold standard treatment for Parkinson’s disease. Haplessly, the disease continues to progress with aggravation of symptoms and debilitating long-term side effects. The contribution of our work was the discovery that the brain of L-DOPA-treated dyskinetic animals exhibited an increased striatal expression of neuroinflammatory cellular constituents enrolled in the generation/maintenance of dyskinesia. L-DOPA-treated dyskinetic animals exhibited an increased striatal expression of the glial fibrillary acidic protein in reactive astrocytes, an increased number of CD11b-positive microglial cells with activated morphology, and a rise of cells positive for inducible nitric oxide synthase and cyclooxygenase-2 immunoreactivity. Cannabidiol, nitric oxide synthase inhibitor, and the tetracycline antibiotic derivatives with anti-inflammatory activity, doxycycline, minocycline, and COL-3 (the former a derivative with no antibiotic activity), inhibited the development, reduced established dyskinesia, and did not affect L-DOPA’s action. Besides, reactive oxygen species production and increased metalloproteinase activity are involved in the dyskinesia. The dramatic effect of cannabidiol, nitric oxide synthase inhibitor, and the doxycycline in preventing dyskinesia, the glial response, and the indicators of neuroinflammation components in the dopamine-depleted striatum points once more to involvement of neuroinflammation in L-DOPA-induced dyskinesia. The observations indicate a therapeutic strategy for dyskinesia at least in part via preventing inflammatory components of dyskinesia pathogenesis in Parkinson’s disease.

**Microglial Patterns in Autism Spectrum Disorder: Influence of Neuronal and Astroglial Microenvironment**

Anaíl Reinés 1

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Microglia cells are key regulators of synaptic function during early life by exerting trophic and phagocytic tasks at the synaptic level. Indeed, microglia misfunction may compromise neuronal networks by affecting synaptic pruning and connectivity. Concomitantly, the neuronal milieu has a strong impact on microglial cells. Thus, it is not surprising that both neuronal and microglial alterations have been implicated in the neuropathology of autism spectrum disorder (ASD). Synaptic changes and neuroinflammation are present in different brain areas associated with the core symptoms of ASD. Whether microglial and neuronal changes are intrinsic or promoted by alterations in other cell lineages, are still a matter of controversy. Despite astrocytes displaying diverse functions at synapses as well, little attention has been paid to these glial cells in the ASD field. Even though astrogliosis is a common trait found in postmortem samples of people with ASD, the role of astrocytes in this disorder remains highly unexplored. Moreover, astrocyte communication with microglia is essential for synaptic function and plasticity. Consequently, astrocytes provide a higher degree of complexity when studying synaptopathies such as ASD. During the talk, in vivo, and in vitro evidence will be discussed supporting brain area-dependent microglial patterns in an experimental model of ASD. Besides, nicely performed in vitro strategies will be also presented to study the contribution of neuronal milieu and microglia-astrocyte crosstalk in ASD.

**Schwann cells and their involvement in peripheral nerve regeneration and neuropathic pain**

**Chair: Felipe Court**

**Role of Schwann Cell Monocarboxylate Transporters in Development and Regeneration**

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Monocarboxylate transporters (MCTs) are plasma membrane channels for monocarboxylates, particularly lactate, pyruvate,
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Identification of Novel Molecular Mechanisms Associated With Impaired Nerve Repair Triggered by Anti-Ganglioside Antibodies

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Anti-ganglioside antibodies (anti-Gg Abs) are recognized inhibitory cues for nerve repair in GuillainBarré syndrome contributing to impaired/delayed clinical recovery present in about 30% of patients. The most well characterized effects of this Abs include inhibition of axon regeneration by targeting gangliosides present at the growth cones of regenerating nerves, which trigger cell signaling events leading to negative modulation of the growth cone’s cytoskeleton, impeding further extension. Anti-Gg Abs-mediated inhibition of nerve repair involves activation of the small GTPase RhoA and its downstream associated kinase ROCK, in a fashion that partially recapitulates the molecular mechanisms of other well established inhibitors of axon regeneration. However, still remains uncertain the identity of the transducer/s molecule/s responsible for these signaling events, as well as the identification of new non-neuronal cell targets. In the past years, our laboratory has found some clues about these important questions. First, based on a proteomic study approach, we identified tumor necrosis factor receptor 1A (TNFR1A) as a membrane transducer upstream of RhoA responsible for the inhibitory effect of Abs recognizing GD1a ganglioside (but no GT1b Abs) on in vivo and in vitro models of nerve repair. In addition, we identified that regenerating nerves from animals exposed to anti-Gg Abs display a significant failure in the clearance of myelin debris, which we later linked to a direct negative effect of anti-Gg Abs on macrophage function. Moreover, deficient myelin clearance in regenerating nerves was not observed in mice treated with Y-27632, a pharmacological inhibitor of RhoA/ROCK signaling pathways, or in null-mice for gangliosides or TNFR1A. Overall, our results unmask a novel transducer molecule for the inhibition of nerve repair mediated by anti-Gg Abs, and at the same expand our knowledge about a novel cellular target involved in this event. Finally, these findings open therapeutic opportunities for limiting the clinical neurological sequelae associated with high titers of anti-Gg Abs in GuillainBarré syndrome.

Adult Multipotent Cells Modulate the Immune Response and the Development of Neuropathic Pain in a Model of Sciatic Nerve Injury

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The loss of axon-Schwann cell (SC) contact after a peripheral nerve injury, triggers a pathophysiological process characterized by SC proliferation and myelin breakdown known as Wallerian degeneration (WD), useful to evaluate therapeutical approaches after peripheral nerve lesions. Upon a lesion Repaired Bungner SCs together with resident macrophages of the distal stump and within the first 24 h post lesion exert phagocytic activity contributing to myelin debris removal and secrete proinflammatory ILs which promote the recruitment of hematogenous macrophages which complete myelin debris removal to clean the distal stump to assure proper remyelination. Cell therapy is one of the experimental neuroregenerative approaches under study to be applied in peripheral neuropathies promoted by trauma. Among others, bone marrow cell (BMC) transplants have become a therapeutic alternative to mesenchymal stem cells, as culture is not required and phenotypic transformations can be hence avoided. In a reversible model of WD, promoted by
sciatic nerve crush, our group has demonstrated the spontaneous migration of endogenous and systemically transplanted bone marrow mononuclear cells to the lesion area promoting axonal regeneration and remyelination, improving functional recovery and preventing neuropathic pain. In this context, our group is currently digging into the corresponding underlying mechanisms associated with BMC cell regenerative effects, evaluating BMCs ability to transdifferentiate to SC as well as their role in modulating the inflammatory response associated with WD and/or modulating macrophage phenotype. Morphological and functional studies were analyzed to assess the regenerative impact of BMC. Our results encourage us to propose a systemic BMC transplant as a potential therapeutic approach after peripheral nerve injuries.

De novo Expression of Kv1.6 as a Regulatory Mechanism in Neuropathic Pain

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Neuropathic pain following nerve injury is associated with hyperexcitability in damaged myelinated sensory axons, which normalizes over time. We previously showed in rats that after nerve injury Kv1 channel expression at the juxtaparanode decreases acutely, but when hypersensitivity wanes and pain decreases, the Kv1.6 channels are expressed de novo (Calvo et al., eLife 2016). We now examined the functional effect of altered Kv1 subunit composition on axonal excitability and neuropathic pain. For this, we set up rat sensory neuron cultures and performed an axotomy once the axons have regrown. After axotomy Kv1.2 expression was decreased while Kv1.6 was increased. Calcium imaging showed an increased basal level acutely after axotomy that returned to normal after a fortnight. We registered action currents in these axons using a cell-attached voltage-clamp and observed an increased rate of spontaneous firing in acute axotomy that reversed with time. We silenced Kv1.6 using a specific blocker (CPY-Fe1) and we observed an increase in spontaneous currents at later time points after axotomy when non-treated axons had decreased electrical activity. In the same line, we overexpressed Kv1.6 using a lentivirus and observed decreased spontaneous activity acutely after axotomy compared with sham transfected sensory neurons. To explore the role of the Kv1.6 channel in vivo, we used a rat neurona model of neuropathic pain, in which hypersensitivity develops after nerve damage and reverses in 3 weeks. At this time, we injected the Kv1.6 blocker CPY-Fe1 and observed a similar reduction of mechanical thresholds as seen in longer time points after axotomy. These results indicate that Kv1.6 which is expressed in late but not acute axotomy plays a role in regulating axonal excitability after damage. In conclusion, de novo expression of Kv1.6 represents a protective mechanism to suppress the hyperexcitability of myelinated sensory axons that follows nerve injury.

Schwann Cells Seen From a Radiological Perspective: Current Use of MR-Neurography

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Over the past decade, the diagnostic workup to evaluate peripheral nerves has been reviewed continuously with the introduction of new techniques and novel, cutting-edge imaging algorithms. Multiple imaging modalities are available for evaluating the peripheral nerve system, the choice of which is highly dependent on the clinical circumstances. Magnetic Resonance Neurography (MR-N) has become the imaging modality of choice for identifying and characterizing pathology within the peripheral nervous system. MR-N imaging evaluates nerve anatomy, signal intensity, internal pattern, and course, as well as the surrounding tissues and innervated muscles. Nowadays, an MRI technique called Diffusion Tensor Imaging (DTI) is becoming more visible, it allows the assessment of axonal integrity in neural tissues and enables a 3D reconstruction to evaluate neural tracts (Diffusion Tensor Tractography—DTT); however, DTI-DTT is a time-consuming technique with technical difficulties that need to be overcome, also, data interpretation requires experience and future comparisons with surgical and histological findings. The evaluation of demyelinating neuropathies often exposes distinctive features. Recognition of the radiologic appearances when Schwann cells are affected allows for prospective diagnosis and improves clinical management. The aim of this presentation is to provide a comprehensive overview of the MR-N imaging characteristics in demyelinating neuropathies, which should enable colleagues to recognize lesions, especially those that may require targeted biopsy.

Astrocytes: The rising stars of the CNS

Chair: Patricia Cassina

The Crosstalk of Astrocytes With Mast Cells and Microglia in ALS

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Amyotrophic lateral sclerosis (ALS) pathology is characterized by the degeneration of motor neurons, reactive astrogliosis, microgliosis, and immune cell infiltration along the
motor pathways. Dysfunctional astrocytes contribute to ALS pathogenesis, inducing motor neuron damage and accelerating disease progression. Mast cells can enter the CNS parenchyma in neurodegenerative diseases having the potential to induce inflammation and disruption of the blood-spinal cord-barrier (BSCB), through the release of mediators such as cytokines, chemokines, histamine, and proteases. In addition, MCs activate microglia by multiple mechanisms. The pathogenic significance of these cellular interactions between astrocytes and other glial and immune cells accumulating along the central motor pathways in ALS remains unclear. We have identified that Stem Cell Factor and SDF-1 are upregulated in astrocytes in the lumbar spinal cord of ALS patients and SOD1 G93A mice, which is known to stimulate mast cell accumulation and differentiation through the receptors c-Kit and CXCR4. In addition, MCs closely interact with motor neuron somas and peri-neuronal capillaries and were associated with peri-neuronal leakage of Evans blue dye, suggesting a pathogenic role of mast cells in the blood-spinal cord barrier disruption. Pharmacological inhibition of c-Kit with Masitinib in SOD1 G93A mice reduced MC accumulation. Taken together, our results suggest a pathogenic mechanism triggered by astrocytes expressing SCF that leads to MC infiltration in ALS spinal cord, which can be prevented pharmacologically.

PolyP Unmasked in ALS/FTD: A Deceivingly Simple Inorganic Polymer Being Around for Billions of Years
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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are devastating, incurable diseases. While ALS/FTD is characterized by the degeneration of motoneurons in the spinal cord and frontal lobes, independent investigations have shown that non-neuronal cells—specifically astrocytes—release one or more toxic factors that contribute to motoneuron death by increasing neuronal activity. The nature of this toxic factor(s) that mediates this so-called non-cell autonomous toxicity has been elusive. Here we provide evidence that the offending toxic factor is inorganic polyphosphate (polyP), which is enriched in mouse and human astrocytes with diverse ALS/FTD-linked mutant genes (SOD1, TARDBP, and C9ORF72), and derived astrocyte-conditioned media (ACM). The polyP is a ubiquitous, negatively charged inorganic biopolymer of hundreds of PO4 residues found in every tested cell type in nature and conserved across more than a billion years of evolution. While studies in bacteria and yeast have revealed numerous vital physiological functions for polyP, its role in mammalian cells is poorly understood. We found that exposure of spinal cord neurons to polyP reproduced the toxic effects of ALS/FTD-ACM, causing hyperexcitability and enhanced motoneuron death. Conversely, motoneurons can be rescued from the toxic ALS/FTD-ACM by degrading (with i.e., yeast polyphosphatases) or sequestering (with i.e., nano-sized polycationic compounds) polyP. These findings establish excessive astrocyte-derived polyP as a critical factor in non-cell autonomous motoneuron degeneration and a potential therapeutic target for ALS/FTD. Additional studies further reveal that cerebrospinal fluid (CSF) from ALS patients exhibits increased polyP concentrations, indicating that polyP might serve as a new biomarker for ALS/FTD.

The Implication of the Circadian System in the Regulation of Glioma Cell Metabolism and Growth
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Biological clocks present in cells throughout the body and even in immortalized cell lines temporally regulate physiological and molecular processes. The disruption of daily rhythms as a result of modern life (shift work, jetlag, artificial lighting, etc.) can cause metabolic disorders and an increased cancer risk. The complex process of carcinogenesis involves the deregulation of cell growth and division, including the reprogramming of energy metabolism and the evasion of the immune system; however, little is known about the role of the circadian system in the regulation of tumor progression and treatment. In this regard, the aim of our laboratory was to investigate the circadian timing system function on the glioblastoma (GBM) metabolism and progression as well as on the chemotherapeutical treatment of tumor-bearing animal models. Gliomas are solid tumors of the central nervous system (CNS) originated from different glial cells. The World Health Organization (WHO) classified CNS tumors into four groups (1–4) with increasing malignancy. GBM is classified as a grade 4 glioma, with a poor prognosis and only 14-month survival after diagnosis. These tumors are resistant to conventional therapies even when the Stupp protocol that combines surgery and chemoradiotherapy is applied. Nowadays, few novel therapeutic strategies have been used to improve GBM treatment but with relatively modest results. We recently demonstrated that glioma cells (T98G cells derived from human
GBM or murine A530 cells) kept in culture maintained a functional clock driving rhythms in the expression of clock genes, lipid enzymes, and reactive oxygen species with differential temporal responses to chemotherapy. Also, higher tumor growth was observed when glioma (A530) or melanoma (B16) cells were injected in C57BL/6 mice at night compared with the diurnal inoculation. Moreover, Bortezomib chemotherapy (proteosome inhibitor) was more effective when the drug was administered at night in tumor-bearing mice compared with those applied during the day. Results indicate that an intrinsic cellular clock operates in these tumor cells; this tumor clock is composed of the molecular transcriptional and the metabolic oscillators which can be differentially regulated by the molecular clock gene expression disruption (Bmal1 knockdown) or treatment with SR9009, an agonist for REV-ERB (molecular clock loop component that links circadian rhythms with metabolism). It is known now that this cellular clock exerts precise temporal control over tumor growth and therapeutic efficacy, which would depend on the state and susceptibility of the host. At present, this laboratory is focused on identifying key components of the biological clock and/or metabolic targets subject to metabolic reprogramming in tumor cells and involved in glioma growth, in an attempt to address new and more efficient chronopharmacological strategies.

**Neuromyelitis Optica Spectrum Disorders: The Astrocytes Role, Linking Basic Research, Clinical Characteristics, and Therapeutic Perspectives**

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Neuromyelitis Optica spectrum disorder (NMOSD), previously known as Devic Disease, is a chronic autoimmune inflammatory disease of the central nervous system characterized by secondary immune-mediated demyelination and consequent axonal damage. NMOSD clinically preferentially affects the optic nerves (optic neuritis) and the spinal cord (transverse myelitis). An important advance in understanding neuromyelitis Optica (NMO) pathogenesis was the discovery of the AQP-4 antibody that targets the water channel membrane protein aquaporin-4 (AQP-4). AQP-4 is expressed on the end-feet membranes of astrocytes along the blood–brain barrier of astrocytes, in ependymal cell membranes, and in Muller cells present on the fovea in the retina. Serum Aqp4 antibodies existed in approximately 80–90% of patients with NMOSD and more than half of patients with NMOSD. The binding of AQP-4-IgG to AQP-4 causes complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity, and the consequent astrocyte injury. Animal models allow us to understand the pathogenic mechanisms in the immune cascade and help the development of potential drug therapies. Diverse in vivo, ex vivo, and in vitro experimental systems have been used but an ideal animal model of NMO with spontaneous AQP-4-IGG positive optic neuritis and transverse myelitis has yet to be created. Large research is in progress on the pathogenesis, genetic background, serum biomarkers, and optic coherence tomography segmentation. Novel drugs targeting the complement cascade system, IL-6R, and B cells are being studied. Recently, novel therapeutic strategies focused on the induction of antigen-specific immune tolerance by administrating tolerogenic immune-modifying nanoparticles are being developed. Deep research in immune tolerance-based therapies in NMO is likely to be a major step toward improving the treatment outcomes of the disease.

**Specialized glia: The value of diversity**

**Chair: Ruth Rosenstein**

**Does the Impaired Crosstalk of Müller Glial Stem Cells and Neurons Interfere With Retinal Regeneration?**

**Luis Enrique Politi**

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In many retina degenerative diseases, the progressive death of photoreceptors (PHRs) ultimately leads to blindness. The search for therapeutical strategies is focused on preventing cell death and using stem cells (SCs) to replace lost PHRs. Müller glial cells (MGCs) may fulfill both goals, protecting PHRs by providing trophic factors, including glial-derived trophic factor (GDNF), and potentially replacing lost neurons since they are SC. We initially established that MGC supplied PHRs with docosahexaenoic acid (DHA), a PHR trophic factor; MGC took up DHA, incorporated it into its lipids, and channeled it to PHRs. We then focused on the MGC role as SC. Although MGC regenerative capacity is very low in mammals, their potential to regenerate PHRs during retina degenerations, such as retinitis pigmentosa (RP), is being intensely studied. Our early work showed MGC in mixed cultures with retinal neurons, supplemented with GDNF, increase SC markers, stimulate the cell cycle in PHR progenitors and extend their proliferative potential,
suggesting the crosstalk between neurons and MGC regulates their mitogenic capacity. Interestingly, in MGC secondary cultures incubated in a neuronal differentiation media, many progenitor cells expressed neuronal markers. Using the rd1 mouse, an RP animal model, we then established MGC and their crosstalk with PHRs were affected in this disease. MGC in rd1 mixed neuron-glia cultures decreases their stemness and proliferative capacity, downregulating the expression of SC and cell cycle markers compared with wt retinas. Noteworthy, these capacities are restored when rd1 MGC are co-cultured with wt neurons; conversely, they are diminished in wt MGC co-cultured with rd1 neurons. This suggests that alterations in rd1 PHRs affect the regenerative potential of MGC. As a whole, our work points to a central role of MGC in regulating photoreceptor survival and underscores the relevance of neuron-glia crosstalk in controlling the regenerative potential of MGC.

Müller Glia Commanding Retinal Survival and Death

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Müller glial cells (MGCs), the main glial component of the retina, actively participate in retinal development and contribute to tissue homeostasis through many intracellular mechanisms. As there are no homologous cells in other neuronal tissues, it is certain that retinal health depends on MGCs. These macroglial cells are located at the center of the columnar subunit and have a great ability to interact with neurons, astrocytes, microglia, and endothelial cells in order to modulate different events. Several investigations have focused their attention on the role of MGCs in proliferative retinopathies (PRs), such as Diabetic Retinopathy, Sickle Cell Retinopathy, and Retinopathy of Prematurity, where several insults coexist. As expected, data suggest that MGCs display different responses according to the severity of the stimulus, and therefore trigger diverse events throughout the course of the disease. Here, we highlight the physiological functions of MGCs and their participation in inflammation, gliosis and neurotoxicity, oxidative stress, and neovascularization retinal involved in the development and progression of PR. We invite you to consider the protective/deleterious role of MGCs in the early and late stages of the disease. In light of the results, we open up the discussion: is it possible that the modulation of a single cell type could improve or even re-establish retinal function after an injury?

Hypothalamic Tanyctyes as Key Players for the Central Actions of Ghrelin

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Ghrelin is a peptide hormone mainly secreted from the gastrointestinal tract that acts via the growth hormone secretagogue receptor (GHSR), which is highly expressed in the brain. Strikingly, the accessibility of ghrelin to the brain seems to be limited and restricted to a few brain areas. Several studies in mice have shown that ghrelin is internalized in hypothalamic tanyctyes, a specialized subtype of glial cells that is part of the brain via the blood–cerebrospinal fluid (CSF) barrier. However, the molecular mechanisms mediating the internalization of ghrelin or the putative role of tanyctyes transporting ghrelin across the blood–CSF barrier has remained uncertain. In this talk, I will provide a brief summary of our in vivo and in vitro results indicating that hypothalamic tanyctyes internalize ghrelin via clathrin-dependent, but GHSR-independent, mechanisms that end up transporting ghrelin from the cell body to the cellular end foot. Thus, hypothalamic tanyctyes seem to mainly transport ghrelin in a CSF-to-blood direction and, consequently, help to tightly regulate the central actions of ghrelin.

III. Young investigator talks

Oligodendrocytes

Chairs: Florencia Labombarda and Francisco Rivera

Remyelinating Effect on OPCs Driven by Transferrin-Loaded Extracellular Vesicles

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Extracellular vesicles (EVs) are lipid bilayer-bound vesicles released by cells into the extracellular environment. EVs play a major role in cell-to-cell communication in both physiological and pathological scenarios. Since these particles are powerful mediators of information, they may represent a promising therapeutic strategy. Oligodendrocytes (OGDs) are glial cells responsible for myelination in the CNS and are particularly
affected during demyelinating diseases such as multiple sclerosis. Remyelination is promoted by the differentiation of OGD precursor cells (OPCs) into mature OGDs. Glycoprotein transferrin (Tf) is implicated in iron homeostasis and stimulates the differentiation of OPCs into OGDs both in vivo and in vitro. Furthermore, studies have shown the remyelinating effect of aTf in hypoxia/ischemia demyelination models where intranasal aTf administration provided brain neuroprotection, and reduced white matter damage, neuronal loss, and astrogliosis in different brain regions. These data led us to search for a more controlled technique to deliver aTf to the CNS. In previws work, we isolated and successfully loaded EVs from plasma with Tf (EVTf) through the binding of this protein to its receptor (TfTR1), located on the membrane of EVs. In this work, we intranasally administrated fluorescently labeled EVs (EVCT) to track their homing in the brain. By confocal microscopy, we localized the EVCT signal inside OPCs in the corpus callosum 6 h post-administration in an acute model of demyelination induced by cuprizone. Moreover, intranasal administration of EVTf induced a remyelinating effect in cuprizone-demyelinated animals, assessed through immunohistochemistry and Sudan black staining. Finally, we show that the administration of Tf using EVs as transporters protects the cargo and reduces the amount of Tf required to induce remyelination, as compared to non-EV-loaded Tf. Altogether, our results demonstrate that EVTf intranasally administered access OPC sin Vivo and induces a remyelinating effect in an animal model of demyelination.

Macrochages Shape Pericytes Response to Demyelination and Their Ability to Facilitate the Generation of Oligodendrocytes

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Multiple sclerosis (MS) is an autoimmune-demyelinating disease of the central nervous system (CNS) affecting approximately 2.8 million people worldwide by inflicting a progressive deterioration of sensory, motor, and cognitive abilities. In healthy adults, the loss of myelin can be efficiently restored by a process known as remyelination, during which CNS-resident oligodendroglial progenitor cells (OPCs) become activated, proliferate, migrate, and differentiate into myelin-producing oligodendrocytes (OLs). Notably, post-mortem patients who suffer from MS show a reduction of OPC differentiation, leading to failure in the remyelination process. Therefore, revealing new mechanistic insights contributing to OPC differentiation is essential when aiming the development of regenerative therapies for MS. Early after myelin damage, blood-borne pro-inflammatory macrophages (M1-Mac) cross the blood–brain barrier into the abluminal side of the microvascular, facing activated OPCs. While remyelination progresses, anti-inflammatory macrophages (M2-Mac) facilitate OPC differentiation. Previous studies of our group have shown that the proliferation of PDGFRB-expressing pericytes (PCs) is also a prominent feature in the response to demyelination and that these cells are also capable to secrete Laminin alpha 2-chain (Lama2) that stimulate OPCs differentiation during remyelination. Here, we explored whether macrophages’ inflammatory status modulates PCs’ contribution to myelin repair. We showed that pharmacological ablation of macrophages/microglia reduces PCs’ proliferation upon myelin damage decreasing their numbers during remyelination. In vitro assays revealed that soluble factors derived from M1-Mac support PCs’ survival and proliferation but suppress the ability of PCs to promote OPC differentiation. On the contrary, a conditioned medium derived from M2-Mac maintain the ability of PCs to boost the generation of oligodendrocytes from OPCs. Overall, immune progression from pro-inflammatory to anti-inflammatory conditions plays an essential role in controlling PC function and their contribution to remyelination.

Role of Arginyl-Transferase in Glial Cells During CNS Myelination

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Arginyl-transferase (Ate1) mediates the post-translational addition of arginine from Arg-tRNA to different protein substrates. Many reports have defined significant roles for Ate1 in the mammalian central nervous system (CNS), participating in processes such as neuronal migration and neurite outgrowth, protein stability, and neurodegeneration. Our studies represent the first aimed at elucidating the role of Ate1 in glial development, including oligodendrocyte (OL) differentiation and myelination in the CNS. In primary OL cultures, we found a peak in Ate1 protein expression during the myelination process,
whereas transcriptional downregulation of Ate1 reduces the number of OL processes and branching complexity. To study Ate1 function and axonal myelination in vivo, we conditionally ablated Ate1 in mice from OLs using CNP-promoter (Ate1-KO mice). In the corpus callosum of 14-day-old Ate1-KO mice, we found a temporary delay in OL differentiation, compared to wild-type controls, while the local proliferation of OL precursor cells normalizes mature OL populations in 21-day-old Ate1-KO mice. However, 5-month-old Ate1-KO mice showed reductions in mature OLs and myelin thickness along with subtle alterations in motor behaviors. Our results indicate that Ate1 regulates OL differentiation and axonal myelination, in part by modulating the OL actin cytoskeleton. Our findings reveal an essential role for protein arginylation in the maintenance of CNS myelin.

**Microglia**

**Chair: Carla Caruso**

**Transferrin Increases Microglia Phagocytic Capacity and Induces a Neuroprotective Phenotype and Neuronal Differentiation, Thus Fostering Remyelination**

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Transferrin (Tf) is a glycoprotein best known for its role in iron delivery. Moreover, this molecule has been shown to accelerate the process of myelination and remyelination in the central nervous system (CNS) in vivo. Furthermore, Tf treatment has been shown to commit neural stem cells from neurosphere cultures toward an oligodendroglial lineage, to induce oligodendroglial cells maturation and to reduce neuron death and favor the neuronal differentiation process in vitro. While the mechanisms involved in microglial cells have not been fully elucidated yet. Our group has described the effect of Tf on the different glial cell populations. We demonstrate that, after a CNS demyelinating injury, Tf can be incorporated by all glial cells—that is, microglia, astrocytes, and oligodendrocyte progenitor cells—and that, acting on microglial cells in vitro, Tf increases proliferation rates, phagocytic capacity and modulates the phenotype activation of microglial cells. In sum, the present work contributes to the description of the first impact of extracellular Tf on microglia and provides insights into the favorable microenvironment generated by Tf for the CNS regeneration process. It may be then speculated that Tf could be a candidate to be used in therapeutic strategies in neurodegenerative diseases.

**Environmental Enrichment Induces Microglia Polarization and Neurotrophin Production in a Focal Chronic Cortical Model of Multiple Sclerosis**

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The beneficial effects of environmental enrichment (EE) were demonstrated in a few multiple sclerosis (MS) animal models, with the main focus on white matter lesions. EE evaluates the main enrichment paradigms: social interaction, and cognitive and physical stimulation. Additionally, it is known that the combination of both cognitive and physical activities enhances cognitive performance in MS patients. Our group created a focal chronic cortical MS animal model which presents cognitive impairment, depression, and anxiety-like symptoms, along with neuroinflammation, neurodegeneration, and demyelination that lasts for 50 days (Silva et al., 2018). The aim of this work is to study the influence of EE in focal cortical lesions induced by central and peripheral expression of interleukin 1 beta (IL-1ß). Adult rats were injected both in the cortex and in the periphery with an adenovirus expressing IL-1ß. The animals were distributed in either the enriched environments or standard cages (SCs) for 30 days. We performed behavioral tests, and immunohistochemical and molecular biology analyses of peripheral blood and cortical lesions. Results: EE ameliorates depression, anxiety-like symptoms, and short-term memory impairment. Additionally, EE decreased central and peripheral inflammation and astrogliosis activation along with diminished demyelination in EE animals compared to SC animals. Moreover, central production of pro-inflammatory cytokines IL1ß, IL6, and tumoral necrosis factor α are diminished; meanwhile, the expression of anti-inflammatory molecules arginase 1, transforming growth factor ß and brain-derived neurotrophic factor, are increased. Conclusions: Environmental enrichment ameliorates cortical and peripheral inflammation and improves behavioral and cognitive function. It also induces microglia polarization and neurotrophin production. Therefore, EE may act synergistically with other therapeutic agents to facilitate brain repair and general welfare.

**Alzheimer's Disease Modification is Mediated by Bone Marrow-Derived Macrophages in Mouse Models of Both Amyloidosis & Tauopathy**

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Microglia and monocyte-derived macrophages (MDMs) are key players in coping with Alzheimer’s disease. In amyloid-
Schwann cells

Chairs: Pablo López

Adipose-Derived Mesenchymal Stem Cells Magnetic Targeting to Promote Sciatic Nerve Regeneration After Traumatic Injury

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Traumatic peripheral nerve lesions constitute a major concern in public health with high prevalence worldwide. Despite the regenerative capability of the peripheral nervous system, sometimes poor clinical evolution turns these affections into a disabling disease, which is why the development of new regenerative therapies is of great importance. Wallerian degeneration (WD) is an efficient experimental model which mimics the impact of peripheral nerve lesions, usually used to shed light on possible regeneration strategies. Adipose-derived mesenchymal stem cells (AdMSCs) are multipotent adult stem cells that are being fully investigated for regenerative therapies and appear as a promising tool due to their multiple advantages such as multipotentiality, low immunogenicity, and low invasive isolation. On the other hand, magnetic targeting (MT) is a nanotechnological strategy to mobilize magnetic nanoparticles (MNP) or “magnetized” cells—cells loaded with MNPs—using static magnetic fields. In this context, the aim of the present work was to test in an in vivo WD model whether magnetic targeting can optimize cell recruitment of systemically transplanted AdMSCs loaded with MNP (AdMSC-MNP) in the lesion area through an external magnetic field, and thus improve the regenerative ability of AdMSCs upon sciatic nerve lesion. The WD model was promoted by the compression of the sciatic nerve. AdMSC and AdMSC-MNP arrival at the injured nerve were evaluated through microscopy and magnetometry. Also, cell transplantation effects on regeneration were evaluated both in terms of nerve morphology and function by the measurement of the distal latency and amplitude of the compound muscle action potential (CMAP). Our results show that AdMSC can internalize 2-4 pg MNP/cell and that AdMSC-MNP are magnetically attracted and retained exclusively at the injured nerve, enhancing cell arrival and their beneficial effects. Animals treated with AdMSC-MNP and MT showed a partially conserved nerve structure with many intact myelinated axons. Also, a remarkable restoration in myelin basic protein organization, indicative of remyelination, was observed. This resulted in an improvement in nerve conduction, demonstrating functional recovery. In short, our results prove that magnetic targeting of AdMSC-MNP constitutes a novel and valuable tool to promote nerve regeneration by enhancing AdMSC arrival at the lesion site.

Modulation of the Schwann Cell Phenotype as a Strategy for Enhancing Peripheral Nerve Repair

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Efficient axonal regeneration after an injury to the peripheral nervous system (PNS) remains a central goal for regenerative medicine. It has been shown that the reparative phenotype of Schwann cells (rSCs), the glial component of the peripheral nervous system, has a crucial role in promoting axonal regeneration after nerve damage. Nevertheless, this pro-regenerative function is impaired in clinical conditions, being a major cause of morbidity in patients with peripheral nerve injuries. Two factors have been mainly related to this regenerative impairment in rSCs, the age of the patient and delayed target reinnervation, which takes place in long nerves or after a delayed reconnexion surgery. Nevertheless, the
cellular state responsible for rSCs disfunction after chronic denervation and aging in the PNS has not been addressed. Here we analyze the accumulation of dysfunctional SCs in both aged and chronically denervated animals. Then, by implementing an in vitro model, we address the interaction dynamics between these SC and growing neurons in vitro. Finally, we explore pharmacologic approaches to revert the detrimental effect of chronically denervated and aging SCs in axonal regeneration and nerve function. Taken together, we believe that this research will bolster new developments in the field of nerve regeneration.

Astrocytes

Chair: A. Javier Ramos

Pyruvate Dehydrogenase Kinase 2 Knockdown Restores the Ability of ALS-Linked SOD1G93A Rat Astrocytes to Support Motor Neuron Survival by Increasing Mitochondrial Respiration

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Amyotrophic lateral sclerosis (ALS) is characterized by progressive motor neuron degeneration. Various studies using cellular and animal models of ALS indicate that there is a complex interplay between motor neurons and neighboring non-neuronal cells, such as astrocytes, resulting in non-cell autonomous neurodegeneration. Astrocytes in ALS exhibit a lower ability to support motor neuron survival than non-disease-associated ones, which is strongly correlated with low mitochondrial respiratory activity. Indeed, pharmacological inhibition of pyruvate dehydrogenase kinase (PDK) led to an increase in the mitochondrial oxidative phosphorylation pathway as the primary source of cell energy in SOD1G93A astrocytes and restored the survival of motor neurons. Among the four PDK isoforms, PDK2 is ubiquitously expressed in astrocytes and presents low expression levels in neurons. We hypothesized that selective knockdown of PDK2 in astrocytes might increase mitochondrial activity and, in turn, reduce SOD1G93A-associated toxicity. To assess this, cultured neonatal SOD1G93A rat astrocytes were incubated with specific PDK2 siRNA. This treatment resulted in a reduction of the enzyme expression with a concomitant decrease in the phosphorylation rate of the PDH complex. In addition, PDK2-silenced SOD1G93A astrocytes exhibited restored mitochondrial bioenergetic parameters, adopting a more complex mitochondrial network. This treatment also decreased lipid droplet accumulation in SOD1G93A astrocytes, suggesting a switch in energetic metabolism. Significantly, PDK2 knockdown increased the ability of SOD1G93A astrocytes to support motor neuron survival. These results suggest that PDK2 knockdown is able to revert the metabolic reprogramming of SOD1G93A astrocytes and that it could be a cell-specific therapeutic tool to slow the progression of ALS.

Changes in Histone Modifications in Hypoosmolar-Stressed Astrocytes: New Insights in Tissue Response to Brain Edema After Injury

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Reactive astrogliosis involves transcriptional, phenotypic, and functional changes. Astrocyte functional changes have a high impact on brain injury outcomes; however, the epigenetic mechanisms regulating gene expression, such as histone modifications, remain obscure. We have recently shown that astrocytes exposed to pro-inflammatory signals promote changes in histone acetylation, which resembles a situation of advanced injury progression. However, to date, there is no available description of early epigenetic changes in injury-affected astrocytes. Such early changes might “condition” the acquisition of reactive and stable astrocyte phenotype during injury progression while being exposed to other damage and/or pro-inflammatory signals. We hypothesize that hypo-osmolar stress promoted by early edema, prime astrocyte to become reactive during injury progression. In a model of brain cortical injury by pial disruption in adult male Wistar rats we observed, using immunofluorescence, a higher number of astrocytes with lower levels of H3K9ac at 3.5 h in the injured hemisphere when compared to the non-injured hemisphere. Also, the injury promoted an increase in GFAP and AQP4 immunoreactivity, which radically decreased at higher distances from the injury core, probably indicating astrocyte swelling in response to edema. In vitro, primary cultures of astrocytes respond to hypotonic stress with a significant decrease in histone acetylation (H3K9ac and H3K27ac). The levels of both histone acetylation marks are restored after 24 h recovery in the isotonic medium. Inhibition of histone deacetylases with Trichostatin A prevented the decrease of H3K9ac after hypotonic stress. We are currently analyzing changes in astrocyte phenotype and chromatin configuration after hypoosmolar stress and recovery in the pro-inflammatory medium. However, so far our results strongly suggest that astrocytes exposed to an edema-like microenvironment are able to dramatically change the global levels of histone acetylation. During the recovery in histone acetylation levels, chromatin might be re-decorated but in a “reactive epigenome.”
Role of the ASD-Associated Gene PTPRD in the Development of Cortical Astrocytes and its Implications in Neurodevelopmental Disorders
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Neurodevelopmental disorders such as autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), and restless leg syndrome (RLS) are due to an abnormal brain development commonly associated with cognitive impairment, motor disorders, and/or communication disabilities. Although there is not a single mechanism involved in neurodevelopmental disorders outcomes, evidence shows that impaired brain development is produced by mutations in genes associated with neural precursor cells (NPCs) differentiation. This results in an aberrant neuronal formation and often in cognitive impairments. Even though most studies have focused on studying neurodevelopmental disorders phenotypes induced by neuronal impairments, there is scarce evidence showing how impaired NPCs differentiation alters glial cell formation (gliogenesis) and its consequences on brain function. PTPRD is a protein tyrosine phosphatase genetically associated with several neurodevelopmental disorders, including ASD. We have recently demonstrated that PTPRD regulates NPCs differentiation to neurons and their cortical location. Furthermore, since NPCs produce neurons and glial cells from the same cellular pool, we asked whether PTPRD ablation causes aberrant glial development in mice. To assess this, we developed a PTPRD conditional knockout mouse (PTPRD cKO) which lacks PTPRD expression exclusively in NPCs that generate brain cortex. In PTPRD cKO mice we observed a decreased number of glial precursors and astrocytes, an effect that appears to be mediated by reduced activation of the gliogenic pathway JAK/STAT and hyperactivation of the MEK/ERK signaling pathway in the brain cortex, reducing gliogenesis and increasing neurogenesis respectively. These results suggest that PTPRD regulates NPCs differentiation to astrocytes, and its impaired expression in NPCs induce the aberrant activation of neurogenic signaling and hypoactivation of gliogenic signaling pathways, favoring NPCs' differentiation to neurons instead of astrocytes.

Levo-dihydroxyphenylalanine (L-DOPA) therapy for Parkinson’s disease (PD) improves motor symptoms, but long-term treatment induces side effects such as abnormal involuntary movements or levodopa-induced dyskinesia (LID). A rising number of data has linked LID with neuroinflammatory reactions in the striatum implicating microglia and astrocytes. The nerve/glial antigen-2 cells (NG2-glia) are recognized as a class of glial cell whose behavior in pathological situations in the adult nervous system is comparable to microglia. Nevertheless, the function of NG2-glia in the adult brain is poorly understood. Here, we used immunohistochemistry, confocal microscopy, and western blot to characterize the cellular distribution of NG2-glia in the striatum of unilaterally 6-OHDA-lesioned rats chronically receiving L-DOPA and presenting LID. The effect of doxycycline, an antidysskinetic/anti-inflammatory drug was also determined. In the striata of animals presenting LID, NG2-glia density was reduced with cells presenting characterized activated phenotype. Doxycycline antidysskinetic therapy promotes a robust increase in NG2-glia density and protein levels, reducing the reactive NG2-glia state. Dopamine depletion revealed a minor density increase of NG2-glia cells in the dorsal striata. NG2 expression was not detected in astrocytes or microglia. Double labeling of NG2 and GFAP (for astrocytes) or OX-42 (for microglia) revealed a decreased NG2/GFAP and NG2/OX-42 ratio index in LID, doxycycline took it back to the control level. These data revealed that NG2-glia cells diminished in the inflammatory conditions in LID, and the cells density was inversely related to microglia and astrocyte. The capacity of NG2-glia to regulate their dynamics in response to the disease state indicates a dynamic involvement of NG2-glia with LID.

Specialized glia

Chair: María Cecilia Sánchez

Role of Gap Junctions and Hemichannels in Tanyocyte Self-Renewal
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Dynamic Involvement of Striatal NG2-Glia in L-DOPA Induced Dyskinesia in Parkinsonian Rats: Effects of Doxycycline
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 ASN Neuro
Tanyctyes are hypothalamic radial glia-like cells surrounding the walls of the third ventricle; they sense glucose, modulate the activity of neighboring neurons to control feeding behavior, and proliferate and differentiate into functional neurons. One of the shared characteristics between neural precursor cells and tanyctyes is that they are coupled with each other through gap junctions formed by connexin 43 (Cx43).

Here we examined the role of Cx43 gap junctions and hemichannels on the proliferation of hypothalamic tanyctyes. **Methods:** We evaluated proliferation in vitro using 5-Bromo-2'-Deoxyuridine (BrdU) incorporation and exposing primary cultures of tanyctyes to conditions that seek to inhibit or activate Cx43 hemichannels opening. In vivo, Alzet pumps were used to deliver BrdU together with a Cx43 blocker (Gap27 mimetic peptide) directly and continuously to the third ventricle. Finally, Cx30/Cx43 dKO mice were used to evaluate if the absence of Cx43 alters the hypothalamic general proliferation. We used at least three independent cultures or animals per condition and ANOVA or t-student statistics. **Results:** We demonstrated that in vitro, FGF2-induced proliferation is prevented after Gap27 addition. Moreover, ATP released by Cx43-HCs promoted tanyctye cell division. In vivo, continuous infusion of Gap27 prevented the FGF2-induced proliferation only in the β2 tanyctye population. Genetically deletion of Cx43 also impaired hypothalamic cell division. **Discussion:** Our results demonstrate the importance of Cx43 in tanyctye proliferative potential.

**Müller Glial Cells Alterations in a Retinal Degeneration Mouse Model**

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Müller glial cells (MGCs) are stem cells and promote photoreceptors (PHRs) survival in the retina. However, multiple injuries to the retina trigger “reactive gliosis,” which might lead to neuronal death. We previously demonstrated that MGC in rd1 mouse (a retina degeneration model) have early alterations in morphology and in reactive and stem cell markers; and stem cell markers are partially restored when rd1 MGC are co-cultured with wt neurons. This suggests that impaired neuro-glial crosstalk affects the stem cell potential of rd1 MGC. We now investigated whether alterations in the expression of extracellular matrix (ECM) proteins participate in rd1 impaired crosstalk. Using mixed neuro-glial (NG) cultures obtained from postnatal 2 days rd1 and wt mice retinas, we analyzed by immunocytochemistry, osteonectin and fibronectin (FN) expression and focal adhesions (FA) at 6 days in vitro. Also, rd1 mixed NG cultures were seeded on culture dishes previously treated with ECM-enriched conditioned medium (ECM-CM), to analyze rd1 MGC morphology, FAs, proliferation, and PHR survival (using BrdU and DAPI, respectively). In addition, rd1 mixed
NG cultures were supplemented with conditioned medium obtained from wt mixed NG cultures (NG-CM), and conversely, wt NG cultures were supplemented with conditioned medium from rd1 cultures to analyze MGC morphology. Our results showed a decrease in osteonectin expression, a fibrillary FN expression, and a decreased number and length of FAs. Also, FAs cortical locations were different in rd1 and in wt MGC mixed NG cultures. Noteworthy, ECM-CM pre-treatment restored rd1 MGC cytoplasmic extension and their FAs and promoted rd1 MGC proliferation, and decreased PHR death. Likewise, preliminary results showed that wt NG-CM supplementation in rd1 mixed cultures expanded MGC lamellipodia and increased PHR survival. These results suggest that rd1 MGC present alterations in EMC protein synthesis and/or secretion, and that wt ECM supplementation improves MGC morphology and functionality.

IV. Poster Sessions

Oligodendrocytes

O1: Phytocannabinoids Attenuate Astro and Microgliosis Reaction Following Spinal Cord Injury

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Traumatic spinal cord injury (SCI) is a physically disabling and psychologically devastating condition. Reactive gliosis and microglial activation are involved in both secondary damage and the persistence of chronic neuroinflammation after SCI. Therefore, their regulation represents a therapeutic target. In this regard, tetrahydrocannabinol (THC) and cannabidiol (CBD), the main phytocannabinoids of Cannabis sativa, emerge as anti-inflammatory molecules in some experimental models. In the present study, we used a model of SCI in rats to evaluate the effects of oil extracted from a resin composed of THC: CBD 1:1. Spinal cord injured rats received an oromucosal dose (20 mg/kg/day) during 15 days post-injury (dpi) and they were sacrificed at 60 dpi. Immunohistochemistry studies showed that the number of microglial cells (Iba1 + cells) increased with respect to sham rats (p < .001 ANOVA two ways) in the epicenter and in both the white and grey matter of the rostral and caudal segment from the lesion 60 dpi. However, THC: CBD treatment decreased microglial density compared with injured rats (p < .05 ANOVA two ways) in the white and grey matter of all the studied regions.

Regarding astrocytes (GFAP + cells), their number was upregulated after chronic SCI with respect to sham rats (p < .001 ANOVA two ways) in the epicenter and in both the white and grey matter of the rostral and caudal region from the lesion. Unlike microglial cells, after THC: CBD administration, astrocyte density decreased only in the grey matter of the rostral and caudal region with regard to injured rats (p < .05 ANOVA two ways). These results suggest that THC and CBD offer a promising perspective in reducing chronic neuroinflammation and gliosis, which eventually could lead to functional recovery.

O2: Contribution of Nrf2 Antioxidant Pathway in the Neuroinflammation Present in the Cuprizone Model of Multiple Sclerosis

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Multiple sclerosis (MS) is an irreversible progressive disease characterized by the loss of myelin, the presence of glial cell-mediated neuroinflammation, and an overproduction of reactive oxygen species (ROS). These alterations have been linked to impairments of the Nrf2 signaling pathway, a critical antioxidant factor that prevents mitochondrial failure, oxidative damage, and neuroinflammation in the brain. However, it is not clear how this pathway contributes to the pathogenesis and progression of MS. We studied the participation of the Nrf2 pathway in neuroinflammation, mitochondrial function, and behavior of an animal model of MS obtained by feeding mice with 0.25% cuprizone—a demyelinating agent—during 6 weeks. Afterward, mice were fed with normal food for 6 weeks to allow for remyelination. Glial cell dynamics and Nrf2 expression were studied by immunofluorescence at 3, 6, and 13 weeks in demyelinated lesions. Complementary, Nrf2 was evaluated by RT-PCR and WB. Mitochondrial function was estimated by measuring ATP production. Animals’ memory and physical condition were studied using Rotarod and novel object recognition (NOR) tests. At 3 weeks, animals subjected to cuprizone treatment showed a nuclear Nrf2 location in both microglia and oligodendroglia with an increase in ATP levels. At 6 weeks, animals showed morphological changes consistent with
activated microglia were observed. Interestingly, at this age, a decrease in nuclear Nrf2 location and ATP levels, and fewer oligodendroglia were also observed. Finally, at 13 weeks a recovery in ATP levels and oligodendroglia number were observed. However, a decrease in locomotor and memory activity was observed, suggesting a long-term impairment (i.e., neurodegeneration). These preliminary results indicate a possible role of glial Nrf2-activation in demyelinated lesions contributing to the pathogenesis of MS.

O3: Astrocytes Treated With Deferoxamine Impact on Oligodendrocyte Cell Maturation

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Previous work from our laboratory showed that in a gestational iron deficiency model that iron deficiency induces a lesser maturation of oligodendrocytes (OLG) and affects its proliferation, while astrocytes (AST) remain more immature (Rosato-Siri et al., 2018). AST promotes myelination by releasing soluble factors as well as via cell contact with OLG. An in vitro AST model of iron deficiency was used by cell treatment with Deferoxamine (DFX), an iron chelator of Fe^{3+}. To evaluate AST proliferative status, immunocytochemistry was performed and showed that Ki67-positive cells from DFX AST were not different from the control. Regarding AST maturation status, the q-PCR analysis showed reduced AQP4 expression in DFX AST whereas Western blot analysis for GFAP showed no difference. These results indicate that AST treated with DFX remains more immature. To evaluate the impact of iron deficiency in AST on OLG maturation, oligodendrocyte precursor cells (OPCs) primary cultures were incubated with AST-conditioned medium (ACM) from control or DFX AST. Immunocytochemistry showed an increase in OLG immaturity markers (PDGFr) and a decrease in maturity markers (MBP). Moreover, the expression of different growth factors known to promote differentiation of OLG, such as LIF, CNTF, and IGF-1, were downregulated in DFX AST. To assay mitochondrial morphology, AST was incubated with Mitotracker and images showed that DFX AST displayed an increased number and reduced size of mitochondria compared to control cells. Additionally, the q-PCR analysis showed an altered expression of mitochondrial genes, favoring an increment in the fission process. Overall, these results showed that iron-deficient AST displayed cellular alterations, including disruption in mitochondrial dynamics, that impact on OLG maturation.

O4: Aging Alters Pericytes Response to Demyelination and Their Contribution to the Generation of Oligodendrocytes

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Remyelination is a regenerative process in which oligodendrocyte progenitor cells (OPCs) migrate, proliferate, and mature into oligodendrocytes covering demyelinated axons and recovering their functionality. Despite its high efficiency, it largely fails under pathological contexts, such as multiple sclerosis (MS). Thus, revealing new mechanistic insights that contribute to remyelination results as an attractive goal when aiming for regenerative therapies in MS. We have shown that, in response to demyelination, pericytes (PCs) proliferate and secrete soluble factors (such as Lama2) that promote OPC differentiation as well as oligodendrocyte fate choice in neural stem cells (NSCs). Although well known that aging restricts remyelination, however, there is no evidence on how aging alters PCs function and their contribution to myelin repair. In this study, we aimed to determine whether and how aging alters the proliferative response of PCs to stimuli associated with demyelination (such as platelets and platelets-contained molecules) and whether it may affect their ability to induce the generation of oligodendrocytes from NSCs. For this purpose, we performed a demyelinating lesion in 2- and 18-months old rats and observed that aged PCs displayed a lower proliferative response to demyelination in vivo. Additionally, in vitro studies revealed an irregular marker expression profile when comparing to their juvenile counterpart. Although aged PCs show a reduced proliferation rate in vitro, aging did not alter their proliferative response to platelets-derived molecules. Finally, although aging did not affect the ability of PCs to induce oligodendrocyte fate choice in NSCs, it hampered PC’s capacity to...
Iron deficiency has been shown to affect CNS development and induce hypomyelination. Oligodendrocytes (OLGs) are the primary myelinating cells in the CNS and the mayor iron-containing cell, whereas astrocytes (ASTs) accumulate iron but at lower levels. Astrocytes have been shown to promote myelination by releasing soluble factors as well as via cell contact with OLG. Previous work from our laboratory showed that in a gestational iron deficiency model, not only OLG but also AST maturation was altered (Rosato-Siri et al., 2018). In the present study, we generate an in vitro model of iron deficiency by silencing the Divalent Metal Transporter 1 (DMT1) in AST. DMT1 participates in the intracellular export of iron from endosomal compartments. Primary astrocyte cultures were transfected with siRNA for DMT1 (siDMT1), and several parameters were analyzed. These iron-deficient astrocytes showed no changes in the proliferation status (BrdU and Ki67) and displayed an immature profile as indicated by the reduced expression of AQP4. To analyze the impact of iron deficiency AST on OLG maturation, AST conditioned medium (ACM) was added to oligodendrocyte precursor cells (OPC) cultures. Immunocytochemistry analyses showed an increment in OLG immaturity markers (NG2 and PDGFr) and a decrement in OLG maturity markers, (O4 and MBP) in siDMT1 AST, indicating a reduction in the differentiation process. These correlate with a decrement in the expression of secreted factors, such as LIF, NRG1, and IGF-1, known to promote differentiation in OLG. Iron deficiency has been shown to alter mitochondrial functioning. Immunofluorescence analyses showed that siDMT1 AST displayed an increased number and reduced size of mitochondria compared to control cells. Moreover, q-PCR assays showed that the expression of genes related to mitochondrial fission and fusion was altered, favoring an increment in the fission process. Our results showed that iron-deficient AST displayed cellular alterations, such as disruption in mitochondrial dynamics, that compromise OLG maturation.
O7: Exploring Perivascular Cells During Remyelination—Origin, Identity, and Fate
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Remyelination is a process by which myelin sheaths are restored upon damage. Following demyelination in the central nervous system (CNS), oligodendrocyte progenitor cells (OPCs) activate, proliferate, and migrate to the lesion area where they differentiate into mature remyelinating oligodendrocytes. Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system (CNS) in which remyelination fails. Revealing cellular and molecular cues that contribute to remyelination represents a major goal for the development of regenerative therapies in MS. In the CNS, pericytes (PCs) are mural cells that participate in blood–brain-barrier maintenance and regulate cerebral blood flow. We have previously shown that, during remyelination, PDGFRb+ PCs proliferate and secrete soluble factors (such as Lama2) that promote OPC differentiation. Interestingly, in response to demyelination, a non-perivascular located PDGFRb-expressing pericyte-like cells (PLCs) raised within the lesion. The origin, cell identity, and function of these PLCs remain unknown. Here, using a genetic fate mapping mouse model (PDGFRb-CreERT2-TdTTomato) we have explored the origin, identity, and fate of these PLCs during remyelination. We found in the normal-appearing white matter of PDGFRb-CreERT2-TdTTomato mice that only perivascular cells express TdTTomato. Upon lysolecithin-induced focal demyelination in this model, we observed that most PLCs are TdTTomato+, indicating a perivascular origin. Edu studies in this model revealed that perivascular cells do not proliferate prior to infiltration into the parenchyma. At 14 days post-lesion, more than 95% of TdTTomato-expressing PLCs do not express Desmin (PCs and vascular smooth muscle cells marker). Instead, the majority of PLCs do express the perivascular fibroblast (PVF) marker, Colla1. This study indicates that, in response to demyelination, non-proliferating perivascular cells migrate far from vascularity and infiltrate into the lesion displaying, most likely, a PVFs identity. Further studies are necessary to assess PLCs/PVF heterogeneity and their functional role in myelin repair. Funding: Fondecyt Regular 1201706, ANID, Chile.

O8: Myelin Regeneration and Testosterone Treatment in a Murine Model of Amyotrophic Lateral Sclerosis (ALS)
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The wobbler (WR) mouse, a model of ALS, shows motoneuron degeneration, astrocitosis, and microgliosis in the cervical spinal cord (CSC) associated with muscle atrophy and gait disturbances. Male WRs exhibit low androgen levels in the brain and spinal cord. As testosterone plays major role in axon myelination, we analyzed myelin parameters (density of CC1 + oligodendrocytes, luxol fast blue [LFB] staining, and the myelin/axon thick ratio) in the CSC from male WRs after testosterone treatment. Immunoreactivity for glutamine synthetase (GS), a glial enzyme that prevents glutamate toxicity, was also studied. A silastic tube containing testosterone crystals was implanted in WRs at the symptomatic stage for 2 months. Untreated WRs and controls received empty silastic tubes. In the ventrolateral funiculus, the % of LFB reactive area was lower in WRs and controls received empty silastic tubes. In the ventro-lateral funiculus, the % of LFB reactive area was lower in WR versus controls (p < .001) and higher in WR + testosterone (p < .01 vs. WR). WRs showed a low density of CC1 + oligodendrocytes/area versus controls (p < .05) and WR + testosterone (p < .01). An increase in the thickness of the myelin sheaths proportional to the axon diameter was shown in WR + testosterone (Y = 0.2313X + 0.1756; p < .001) and controls.
In the last few years, gold nanoparticles (GNPs) were shown to stimulate the differentiation of several cell types. As the development of new therapies to promote remyelination is a high priority for multiple sclerosis (MS), we used neurospheres (NS) cultures to study the effects of GNP on the different mechanisms involved in brain repair: Polyethyleneimine-stabilized GNP of 55 nm of hydrodynamic diameter were synthesized and used to treat NS cultures. We found a 30% reduction in the metabolic activity of cultures by MTT assay. Although NS numbers were not affected at any dose, we detected a significant reduction in NS diameter at 10 ppm GNP, which was attributed to the downregulation of proliferation observed by the BrdU incorporation assay. In addition, we found a significant inhibition of cell migration in response to GNP treatment and observed some abnormalities in cell adhesion. Finally, NS cultures undergoing cell differentiation and treated with GNP showed a marked increase in the number of mature oligodendrocytes with respect to controls. All these results indicate that GNPs inhibit NS/NPC proliferation and promote cell differentiation towards the oligodendroglial lineage. These findings support the idea that GNP could be used for the development of new regenerative strategies for the CNS.

O10: Human Neural Rosettes Secrete Extracellular Vesicles Enriched in Neural and Glial Cellular Components
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Extracellular vesicles (EVs) participate in neural and glial development in the central nervous system (CNS). Human neural rosettes (hNRs) are radial structures of neuroepithelial cells that assemble during human induced pluripotent stem cell (hiPSCs) differentiation into neural and glial cells. Neural rosettes are an in vitro model that recapitulates some stages of neural tube morphogenesis and express many of the molecular components expressed in vivo during neural tube formation. Here we showed that hiPSCs and hNRs secrete EVs (hiPSC-EVs and hNR-EVs) enriched in proteins related to EVs, exosomes, and components of the endomembrane system. Moreover, hNR-EVs are also associated with neural and glial cellular components involved in CNS development. Through in silico analysis, we found that at the cellular level these proteins exhibit differential expression profiles at various time points during development and culture conditions. These results indicate that EVs secreted by hNRs exhibit neural and glial cellular components and might provide an innovative approach to studying their biological effects during human neural tube formation.

Microglia

M1: Neuropathology, Glial Activation and Cognitive Dysfunction in a Rat Model for Metabolic Syndrome
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Metabolic syndrome (MS) is a term used for the combination of at least three of the following factors: obesity, hyperlipidemia, hyperglycemia, insulin resistance, and hypertension. The spontaneously hypertensive rat (SHR) is an accepted animal model for the study of human MS that reveals all the features of the syndrome when fed high-fat, high-carbohydrate diets. The intake of high-fat diets in rats has been shown to produce brain neuropathology. In humans, MS has been shown to increase the risk of cognitive impairment, dementia, and Alzheimer’s disease. In order to characterize the neuropathology and cognitive dysfunction of MS, we fed SHR with a high-fat diet (4520 kcal/kg) along with a 20% sucrose solution to drink. We also evaluated SHR and Wistar-Kyoto (WKY) fed with the standard diet. At the end of the experiment, SM rats displayed a significant increase in body weight, BMI, and AC/TC ratio compared to SHR, rats fed a normal diet. We also found an increased in fasting glucose levels and in the OGTT in SM rats. Blood pressure was significantly higher in both SHR and SM rats when compared to WKY. SM rats present high blood triglyceride levels. We also measured the expression of IBA1 + microglia in the prefrontal cortex (PFC) and the hippocampus (HC) by immunohistochemistry and classified microglia according to their morphology. We found that while ramified microglia predominated in normotensive rats, SM rats presented an increased proportion of the hypertrophied phenotype. Furthermore, we evaluated hippocampal-dependent memory using the novel object recognition test (NOR) and found SM rats performed poorly when compared to WKY. In conclusion, SHR rats fed a high-fat and high-sucrose diet developed all the characteristics of MS together with neuropathological alterations and cognitive impairments. These results present this as an interesting model for the study of treatments to alleviate neuropathological and cognitive alterations associated with MS.

M2: P2Y6 Receptor Activation is Necessary to Induce Phagoptosis of Neurons by B. abortus-Activated Microglia
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B. abortus-activated microglia kills neurons through primary phagocytosis or phagoptosis. Phagocytosis is a finely regulated process that involves the interaction of different receptors and their ligands. It has been shown that the purinergic pathway is involved in the modulation of different functions of phagocytes. The objective of this work was to investigate whether this signaling pathway is involved in the phagoptosis of neurons mediated by B. abortus-activated microglia. Primary cultures of neurons and microglia from Balb/c mice were infected. Neuron survival was assessed at 48 h by fluorescence microscopy. Co-cultures were treated with apyrase (an enzyme that degrades di and tri nucleotides), Reactive Blue 2 (RB2) (a P2X/P2Y purinergic receptor inhibitor), BBG (a P2×7 specific inhibitor), and MRS2578 (a P2Y6 specific inhibitor). Treatment of B. abortus-infected co-cultures with apyrase inhibited neuronal death, when compared to untreated cultures (p < .05). Treatment of B. abortus-infected co-cultures with RB2 also prevented neuronal death (p > .05). By using the specific inhibitors of P2×7 and P2Y6, we were able to demonstrate that the P2Y6, but not P2×7 purinergic receptor, is involved in the modulation of phagoptosis (p > .05). In all cases microglia activation was not affected since TNF-α secretion was not significantly different between treatments (p > .05). These results demonstrate that the P2Y6 purinergic receptor and the nucleotides that activate it would be necessary for neuronal death mediated by microglia activated by B. abortus, describing new molecular mechanisms involved in the pathogenesis of neurobrucellosis.

M3: Gal-3 and Neuraminidase Activity are Required to Induce Phagoptosis of Neurons by Brucella abortus-Activated Microglia
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We have previously demonstrated that B. abortus-activated microglia kill neurons through primary phagocytosis or phagoptosis. Phagocytosis is a finely regulated process that involves the interaction of different receptors and their ligands. Galectin-3 (Gal-3) is a protein secreted by activated phagocytes and acts as an opsonin through the MERTK receptor. Gal-3 binds to desialylated proteins present on the cell’s surface through the action of neuraminidases. The objective of this work was to investigate if Gal-3 is involved in the phagoptosis of neurons mediated by B. abortus-activated microglia. Primary cultures of neurons and microglia from wild-type (Balb/c or C57/B6) or Gal-3 deficient mice were infected. Neuron survival was assessed at 48 h by fluorescence microscopy. Co-cultures were treated with Oseltamivir phosphate.
(neuraminidase [Neu] inhibitor), and Tacrolimus (inhibitor of the phosphorylation and subsequent secretion of Gal-3). Neu activity on the cell membrane and TNF-α secretion were measured using commercial kits. B. abortus-infected microglia exhibit Neu activity on their cell membrane, which was significantly inhibited by Oseltamivir (p < .05). Moreover, neuronal phagoptosis induced by B. abortus-activated microglia was inhibited by Oseltamivir (p < .05) indicating that the desialylation of neuronal membrane sugars is involved in this phenomenon. Tacrolimus was also able to inhibit neuronal death induced by infected microglia (p < .05). Microglia from Gal-3-deficient mice infected with B. abortus was unable to induce neuronal death, when compared with microglia from wild-type mice (p < .05). In all cases, microglia activation was not affected, since TNF-α secretion was not significantly different between treatments (p > .05). These results demonstrate that Gal-3 and neuraminidase activity would be necessary for the neuronal death mediated by B. abortus-activated microglia, describing new molecular mechanisms involved in the pathogenesis of neurobrucellosis.

M4: Evaluating Microglia Diversity Throughout Rat Life Span

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During aging, the central nervous system (CNS) undergoes a variety of morphological and functional changes. We are interested in studying glia in their natural environment and how aging affects their numbers and functions. Although glial cells are critical for CNS development and maintenance, there is little published research addressing their distribution in different brain regions across the lifespan. Most studies involve mice, which limits the extrapolation of data to the rat. Our aim was to investigate how the number and morphology of microglial cells change in naïve rats at different ages and whether there is a relationship with behavior. Behavioral tests were performed with female Sprague-Dawley rats at 2, 6, 12, and 24 months of age to examine depression-like behavior, short- and long-term memory, exploratory, and anxiety-like behavior. We also analyzed immunoreactive Iba1 cells. We observed impaired spatial memory and anxiety- and depression-like behavior in 24-month-old rats compared with 6- and 12-month-old rats. As for microglia, there was a significant increase in the number of Iba1+ in the hippocampus and stratum radiatum in 24-month-old rats compared with 6-month-old rats. Morphology data showed a difference between young and old rats. Our results suggest that the behavior of female rats is age-dependent, as is the distribution and morphology of microglia. Further studies are needed to explore the phenotype and functions of microglial cells.

M5: Brucella abortus-Activated Microglia Induce Neuronal Death Through IL-6 Signaling

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Neurobrucellosis is an inflammatory disease caused by Brucella spp. infection of the central nervous system. We have previously demonstrated that soluble mediators released by B. abortus-infected astrocytes induce an inflammatory state in microglia, and this bystander activated-microglia elicits neuronal death through phagocytosis of viable neurons. The aim of this work was to investigate possible mediators involved in this mechanism. For this, we used monoclonal antibodies to neutralize TNF-α and IL-6 cytokines in neurons/microglia co-cultures treated with supernatants from B. abortus-infected astrocytes. Neutralization of IL-6, but not TNF-α, prevented neuronal loss (evaluated by fluorescence microscopy; p < .05) and caused a decrease in the phagocytic activity of microglia (evaluated by phagocytosis assay with negatively charged fluorescent beads; p < .005). Considering that both astrocytes and microglia are capable of secreting IL-6, we further investigate the contribution of each cell type to this phenomenon. Astrocytes from wild-type (WT) and IL-6 KO mice were infected or not with B. abortus for 24 h. After that, cell-free culture supernatants were used to stimulate primary murine co-cultures of WT and IL-6 KO microglia with neurons for 48 h. Treatment of WT co-cultures with supernatants from IL-6 KO-infected astrocytes caused a partial inhibition of neuronal death (p < .05). Similar results were obtained when neurons/IL-6 KO microglia co-cultures were treated with supernatants from WT-infected astrocytes (p < .05). Neuronal loss was totally prevented in co-cultures of neurons/IL-6 KO microglia treated with IL-6 KO infected astrocytes (p < .05). Moreover, B. abortus-activated microglia from IL-6 KO mice was unable to induce neuronal death (p < .05). These results indicate that both paracrine and autocrine IL-6 signaling in microglia can be sufficient to induce phagocytosis of viable neurons in the context of a B. abortus infection, and could highlight the relevance of this cytokine in neuropathological mechanisms caused by Brucella spp.
M6: Influence of the Blood–Brain Barrier on Microglia and Astroglia Activation on Ongoing Confined Cortical Lesions

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Inflammation in the central nervous system (CNS) is associated with blood–brain barrier (BBB) breakdown during the early stages of multiple sclerosis (MS), indicating a facilitated entry of waves of inflammatory cells from the circulation to the CNS and glial activation. In the progressive forms of MS, as the lesion becomes chronic, the inflammation remains trapped within the CNS compartment with low inflammation and microglia activation at the lesions edges. Microglia and astroglia activation help to the chronification of the lesions. The chronic expression of interleukin 1β (IL-1β) in the cortex induces BBB breakdown, demyelination, neurodegeneration, microglial/macrophage activation, and impaired cognitive performance. As long as the BBB recovers, the lesion presents low inflammation. Here, we study the effects of peripheral inflammation on cortical central lesions after the recovery of the BBB, as it occurs in the progressive forms of MS. Materials and Methods: Cortical lesions and peripheral inflammation were induced by the chronic expression of IL-1β using an adenovector. We performed histological, and immunohistochemistry on brain tissue and behavioral analyses. Results: The effects of the chronic expression of IL-1β in the cortex resolved within 56 days. Peripheral pro-inflammatory stimulation re-opened the BBB, allowing the reappearance of the microglia and astroglia activation within the cortical lesions, increased demyelination and neurodegeneration, and an increase of cognitive impairment and anxiety-like symptoms. Conclusions: Peripheral inflammation is critically detrimental to the ongoing neurodegenerative process. Its early treatment should be considered in order to protect the brain from disease exacerbation.

M7: Nuclear Localization of Mast Cell Tryptase Identifies Senescent Microglia in Amyotrophic Lateral Sclerosis

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Cellular senescence is a hallmark of normal aging, but it also occurs prematurely in neurodegenerative processes. We have previously reported the accumulation of senescent microglia in the spinal cord of mice expressing SOD1G93A, a model of Amyotrophic Lateral Sclerosis (ALS). These cells are characterized by the expression of p16 and decreased levels of nuclear Lamin B1, together with alterations of nuclear morphology. However, an in-depth characterization of nuclear pathology in ALS senescent microglia and the mechanisms involved remain largely unknown. Here, we have analyzed the nuclear pathology observed in senescent microglia from both the degenerating spinal cord of SOD1G93A mice and in post-mortem tissue from ALS patients and controls. Our results indicate that nuclear pathology in ALS-associated senescent microglia was associated with increased expression of p16 but not p21 and decreased levels of nuclear Lamin B1 and HMGB1. Surprisingly, a subpopulation of senescent microglia also expressed nuclear mast cell tryptase, a proteolytic enzyme typically produced and released by mast cells. Nuclear tryptase localization correlated with nuclear pathology. Moreover, tryptase-negative cells do not present nuclear alterations. Tryptase-expressing senescent microglia also displayed significant alterations in cellular morphology with notorious loss of nuclear circularity. In conclusion, nuclear localization of tryptase identifies a specific subset of senescent microglia which displays major nuclear pathology, suggesting a pathogenic role of nuclear tryptase by degrading nuclear components resulting in an altered transcriptional profile.

M8: Sex- and Area-Dependent Alterations in Glial Cells in the Valproic Acid rat Model of Autism Spectrum Disorder

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Neuroinflammation has been reported in patients and animal models of autism spectrum disorders (ASDs). Both astrocytes and microglia play critical roles in brain development and immune response. However, it is still unknown their role in the physiopathology of ASD. Previously, we described sex-differences in juvenile repetitive and exploratory behavior in the valproic acid (VPA) rat model of ASD. We aimed to study in vivo and in vitro, microglia, and astroglia of the medial prefrontal cortex (mPFC) and hippocampus in the VPA model. We first performed immunofluorescence assays...
to study microglia (Iba1) and astrocytes (GFAP) in juvenile rats. While both male and female VPA rats showed an increased in the number of unramified Iba1+ cells in the mPFC, only males showed a significant increase in GFAP immunoreactivity. In contrast, in the CA3 subfield of the hippocampus, both sexes shared astrogliosis but only females presented a concomitant microgliosis. We prepared microglia cultures and studied their morphology under basal conditions and after exposure to either a pro-inflammatory (lipopolysaccharide) or a phagocytic (synaptosomes) stimulus. Microglia morphology in vitro under basal conditions mimicked in vivo findings: cortical microglia from both male and female VPA animals showed altered morphology and only hippocampal microglia from females evidenced morphological changes. Regardless of their basal condition, microglia from VPA animals of both areas and sexes were able to respond to the pro-inflammatory stimulus but only female cortical microglia responded to the phagocytic stimulus. Since cortical microglia from male VPA animals was resistant to synaptosomes, we evaluated astrocytes synaptosomes phagocytosis in vitro. We observed that astrocytes from male VPA animals showed a reactive phenotype and increased phagocytic activity. Our findings suggest that glial cells in VPA animals show brain area- and sex-dependent alterations that may result in different pathological mechanisms and could explain the contrasting behavioral outcomes.

M9: Repopulating Microglia Rapidly Return to a Cuprizone-Induced Activated State with Area-Dependent Kinetics

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Prolonged cuprizone (CPZ) administration can model progressive multiple sclerosis in triggering chronic demyelination, neurodegeneration, astrogliosis, and exacerbated microglia (MG) activation. MG can be almost completely eliminated from the brain using colony-stimulating factor 1 receptor inhibitors like BLZ945 (BLZ). Our previous results show that continuous BLZ treatment attenuates demyelination but exacerbates axonal degeneration. The present work characterizes MG as they repopulate and determines whether these new cells ameliorate CPZ-induced pathology. Two-month-old mice were fed CPZ chow for 11 weeks, orally gavaged vehicle or BLZ for 3 weeks from the fifth week of CPZ treatment, and evaluated after 0, 1, 2, and 3 weeks of BLZ withdrawal (T0, T1, T2, and T3). Results showed a reduction in the Iba1-positive area at T0 in the cortex (CX) and at T1 in the corpus callosum (CC), reaching repopulation at T3. CPZ induced a more ramified microglial phenotype, while branches were shorter than control only in the CC. BLZ inverted this phenotype in the CX at T0, but the number of branches equaled CPZ very early from T1 and a longer process length was maintained until T3. In the CC, BLZ did not change the phenotype induced by CPZ. Pro- and anti-inflammatory gene expression tended to change with treatment, brain area, and repopulation time. BLZ attenuated demyelination at T0, concomitant with a reduced number of GFAP+ cells, but showed no differences from CPZ at T3 in the CX. In the CC, no differences were found between CPZ and CPZ + BLZ at any time. Axonal neurodegeneration was unaltered by BLZ at T0 but increased at T3 accompanied by an increased number of GFAP+ cells. These results show that the kinetics of MG depletion/repopulation differs between CX and CC and that new MG quickly reactivates due to the pathological state generated by CPZ.

Schwann cells

S1: Cell Therapy Combined With Magnetic Targeting for Treatment of Peripheral Neuropathies

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Bone marrow mononuclear cell (BMMC) is a heterogeneous fraction containing a small population of multipotent cells, good candidates for cell therapy since they are easily isolated, have no culture required, have a high yield and survival rate after transplantation, and have low immunogenicity. We have demonstrated the migration of systemically transplanted BMMC in an 8-s crush model, where they exerted beneficial
effects in terms of morphological features, functional aspects, and prevention of neuropathic pain. Also, we have demonstrated that magnetically assisted delivery of adipose-derived mesenchymal stem cells loaded with iron oxide magnetic nanoparticles (MNP), is a highly promising strategy to promote cell recruitment and sciatic nerve regeneration after injury. In this context, the aim of this work is to evaluate the effect of systemic transplantation of BMMC or BMMC-MNP; magnetic targeting was achieved by placing an external magnet close to the injured site. Morphological and functional studies were performed to evaluate the effect of both treatments. Even though the lesion is more severe and bibliographically considered as an axotomy, BMMC were able to migrate and exert their beneficial effect on regeneration. More myelinated axons were observed in semithin sections of nerves from animals submitted to magnetic targeting. In this group, MBP and βIII-tubulin recovery were earlier than in non-treated or BMMC-treated rats. As regards the sciatic functional index, the magnetic targeting group showed a faster recovery beginning 14 days after treatment. These results encourage us to propose magnetic targeting of BMMC systemically transplanted as a promising strategy for the treatment of acquired peripheral neuropathies.

S2: Transfected BMMC With PLGA Nanocapsules Loaded With Iron Oxide Magnetic Nanoparticles, a Hybrid Platform for Peripheral Nerve Regeneration

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In the context of peripheral nerve injury, reaching full recovery is still difficult since there is a limitation associated with the short time window available for therapeutic intervention. Our group focuses on the development of new strategies to promote functional and morphological nerve regeneration and to prevent neuropathic pain development, using a reversible model of Wallerian degeneration obtained by crushing the rat sciatic nerve. In this model, we previously showed that magnetic targeting of systemically transplanted adipose-derived mesenchymal stem cells loaded with iron oxide magnetic nanoparticles (MNP) optimizes the beneficial effect obtained with cell transplant alone [1]. The aim of the present work was to develop a hybrid platform consisting of a poly-lactic-co-glycolic acid (PLGA) nanocapsule loaded with MNP and functionalized with polyethyleneimine (PEI), useful for transfecting and magnetically labeling bone marrow mononuclear cells (BMMCs) to be magnetically targeted to the injured sciatic nerve after their systemic transplant. To this end, MNP were synthesized by thermal decomposition, and nanocapsules of PLGA (fluorochrome/MNP) were prepared by the double emulsion evaporation technique. The Dynamic Light Scattering (DLS) analysis at 25 °C of PLGA nanocapsules, showed monodisperse nanometric sizes in the range of 220 and 240 nm and PDI values between 0.110 and 0.130. The generation of PLGA: PEI: pDNA complex was confirmed by an electrophoretic retention assay achieving full retention for a relation 6: 3: 5, and the reversibility of PEI: pDNA interaction was checked with heparin. BMMC transfection was evaluated with epifluorescence microscopy in PLGA (rhodamine) or PLGA (FITC). Finally, the systemic transplant of the hybrid platform immediately after the lesion demonstrated the arrival of transfected cells to the injured sciatic nerve as well as the effectiveness of their magnetic targeting. These results encourage us to evaluate if this hybrid platform may be a tool to assess the involvement of mRNA/pDNA of growth factors such as NGF, IGF-1, BDNF, GDNF, or CNTF to better understand nerve regeneration mechanisms and to establish a potential therapeutic approach.

S3: Molecular Mechanisms Triggered by Antiganglioside Antibodies Associated With Impaired Nerve Repair

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Guillain Barré syndrome (GBS) is an acute monophasic polyneuropathy characterized by the presence of ascending muscular paralysis and areflexia. About 30% of patients show the signs of impaired/delayed clinical recovery, most often
associated with the degree/extension of axonal damage of peripheral nerves caused by anti-ganglioside antibodies (anti-Gg Abs). Passive immunization studies using a mAb anti-Gg Ab (anti-GD1a-GT1 b, clone 1b7) in a murine model of axon regeneration confirmed that these antibodies inhibit nerve regeneration by negative modulation of actin and tubulin cytoskeleton in growth cones via RhoA-dependent and independent pathways. Recently, we identified Tumor necrosis factor receptor 1A (TNFR1A) as a transducer for the inhibitory effect of antibodies recognizing GD1a ganglioside on nerve repair in vivo and in vitro models. In addition, we observed that regenerating nerves from animals exposed to anti-Gg display a significant failure in the clearance of myelin debris, suggesting an effect of anti-Gg Abs on non-neural cells. Mice treated with mAb 1B7 after a nerve lesion show a reduced number of macrophage extravasation/migration in sciatic nerves with respect to control nerves associated with a modulatory effect on the macrophage phenotype towards an early anti-inflammatory phenotype. Deficient myelin clearance in regenerating nerves was not observed in mice administered a pharmacological inhibitor of RhoA/ROCK signaling pathways, or mice null for gangliosides or TNFR1A. Overall, our results unmask a novel cellular target in the inhibitory effect that anti-Gg Abs exert on nerve repair.

Astrocytes

A1: Baculoviral Vectors as a Gene Therapy Tool for Gene Delivery into Astrocytes

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In search for better approaches to treat gliomas, we developed baculoviral vectors (BVs) to deliver therapeutic transgenes into these brain tumors. Although recombinant adenoviral (AdV) vectors are the gold standard in neuro-oncology due to their robust transduction efficiency and optimal safety profile, they are highly immunogenic and virtually the entire general population has pre-existing anti-AdV immunity, which leads to transient transgene expression. These limitations impair therapeutic efficacy and have led us to search for alternative therapeutic vectors. Since BVs are natural pathogens of insects, no pre-existing immunity against BVs has been reported in humans so far, making it a great candidate for gene delivery to the brain. We evaluated the transduction efficiency and toxicity of BVs in normal and neoplastic astrocytes in vitro and in vivo and compared them with those of AdV. Our hypothesis was that BVs constitute a useful tool for the persistent expression of therapeutic transgenes to treat brain disorders. Therefore, we constructed AdV and BV encoding fluorescent proteins under the control of the cytomegalovirus (CMV) promoter. Using microscopy and flow cytometry, we found that both vectors exert robust transduction efficiency in murine and human glioma cell lines and short cultures derived from biopsies, as well as rat and mouse astrocytes. To assess transduction efficiency and neuropathology in vivo, vectors were injected by stereotactic surgery into the brain of mice with or without intracranial gliomas. Transduction efficiency was comparable with both vectors, as it was immune infiltration at the injection site, with no obvious signs of neurotoxicity. Both vectors efficiently transduced tumor cells and non-neoplastic astrocytes. Transgene expression was detected 7 and 21 days after injection in naïve brain, and it was lost when mice were preimmunized systemically with BVs. Our findings indicate that BVs are excellent tools for transgene delivery in glioma cells and normal astrocytes.

A2: Astrocytic Glutamate Uptake as a Key Mechanism Involved in Spatial Memory Formation and Disruption

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We studied the role of glutamate transporter GLT-1, specifically located in astrocytes, in learning and memory processes. We used the spatial object recognition (SOR) task in rats to study the effect of GLT-1 inhibition. In this task, a strong training session induced long-term memory (LTM) formation, and a...
A3: The Circadian Clock of Glioblastoma and the Role of its Microenvironment in Tumor Progression
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Glioblastomas (GBM) account for almost half of malignant central nervous system tumors. GBM microenvironment is fundamental in tumor proliferation, where specific cells, such as the astrocytes, secrete molecules that enhance tumor growth, thus having an important role in tumor prognosis. In addition, the circadian clock can enhance or inhibit proliferation and tumor progression, depending on the type of tumor, as well as modulate the efficacy of chemotherapies at 24 h. Our aim was to study how the circadian clock of GBM influences the bidirectional interaction of the tumor with the astrocytes in the microenvironment, affecting tumor proliferation and prognosis. First, through assessing BMAL1 and PER1 oscillations we found that the GBM LN229 cell line has a circadian clock and that these oscillations are impaired in BMAL1-knockdown cells (LN229 E1). When studying proliferation by indirect tumor-astrocyte co-cultures, we found that there is a rhythmic response in LN229 cell proliferation, which is lost in LN229 E1. In vivo assessment of circadian clock regulation of tumor growth and microenvironment was done in nude mice bearing GBM tumors. We found a median survival of 61 days in WT LN229 xenografts, which decreased to 56 days in mice bearing LN229E1. Our preliminary studies show that tumor size was increased in LN229E1-bearing mice at day 25 after implantation when compared to WT. In addition, when assessing by immunohistochemistry reactive astrocytes (Nestin and GFAP) and prognosis tumor marker CD44, we found a higher expression for GFAP and CD44 in LN229E1 mice at day 25 after implantation, but not at an endpoint. These results suggest that the circadian clock of GBM controls the interaction of tumor cells with astrocytes in the tumor microenvironment and that the lack of a circadian clock in cells may increase tumor growth leading to a poorer outcome in mice. Further studies need to be conducted to determine how the tumor circadian clock is influencing this interaction

A4: Altered Secretion of Astrocyte-Derived Extracellular Vesicles Contribute to the Early Metabolic Failure and Redox Imbalance in Huntington’s Disease
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Huntington’s disease (HD) is a neurodegenerative disorder caused by a glutamine expansion at the first exon of the huntingtin gene. Huntingtin protein (Htt) is ubiquitously expressed, and it is localized in several organelles, including endosomes. HD has been associated with a failure in energy metabolism and oxidative damage. Ascorbic acid is a powerful antioxidant highly concentrated in the brain where it acts as a messenger, modulating neuronal metabolism. During the synaptic activity, ascorbic acid is released from glial intracellular reservoirs, and it is taken up by neurons. Using an electrophysiological approach in YAC128 HD slices, we observe a decreased ascorbic acid flux from astrocytes to neurons, which is responsible for alterations in neuronal metabolic substrate preferences. Ascorbic acid
efflux and recycling were decreased in cultures of astrocytes from YAC128 HD mice. Our findings were confirmed in experiments using GFAP-HD160Q, an HD mice model expressing mutant N-terminal Huntingtin mainly in astrocytes. We demonstrated that ascorbic acid is released from astrocytes through extracellular vesicles (EV). Decreased number of particles and exosomal markers were observed in EV fractions obtained from cultured YAC128 HD astrocytes, as well as, from Huntingtin KO cells. Using electronic microscopy, we observed a decreased number of multivesicular bodies (MVBs) in the striatum of YAC128 HD mice. This supports the idea that MVBs biogenesis is altered in presence of mutant Htt. Therefore, we conclude that a decrease in EV-mediated ascorbic release from astrocytes would be responsible for early metabolic failure in HD. Acknowledgments: Chilean FONDECYT grant 1151206 and 1191620, Chilean ANID grant 21190707. Membership: Doctorate in Sciences, mention in cell and molecular biology, Faculty of Science, Universidad Austral de Chile (UACH), Chile.

A5: DAMP or PAMP Exposure Induces Persistent Astroglial DNA Methylation and Downregulation of Homeostatic Genes in Reactive Astrocytes
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Following injury, reactive astrocytes undergo extensive physiological changes that alter their interaction with other members of the neurovascular unit and the molecular microenvironment. Epigenetic mechanisms, such as DNA methylation, regulate DNA transcriptional activity and thus regulate gene expression. In general terms, DNA methylation is known to repress gene expression in the long term. We here explored the DNA methylation and expression of related genes in astrocytes exposed to the PAMP LPS or treated with the DAMP HMGB1 to induce reactive gliosis and astroglial proinflammatory conversion. Using primary astroglial cultures obtained from Wistar rat pups, we exposed them to LPS (25 ng/ml) or HMGB1 (500 ng/ml) for 18 h, culture was then washed and allowed to recover during different periods of time (0 h; 24 h, 72 h, or 7 days). Generalized astroglial hypermethylation was observed by 5-methyl-cytosine (5-metC) specific immunostaining. These results correlated with a chromatin reorganization observed by high-resolution confocal microscopy; a significant increase in DNMT1a and DNMT3a expression and downregulation of homeostatic genes kcnj10 (Kir 4.1) and glutamine synthetase. As expected, proinflammatory genes (IL-1b, c3, and IL-6) were overexpressed following DAMP or PAMP exposure. By bisulfate conversion followed by specific PCR, we also observed an increase in the methylation of the proximal CpG island of the glutamine synthetase promoter. Taken together, these results suggest that changes in DNMT1a/3a expression are concomitant with DNA hypermethylation in astrocytes exposed to proinflammatory DAMP or PAMP. These changes seem to be stable and may affect the function of astrocytes by specifically repressing the expression of homeostatic genes such as kcnj10 and glutamine synthetase thus impairing the astroglial homeostatic ability to support neuronal function in the long term. Supported by grants UBACYT, PICT 2019-0851; PIP Conicet.

A6: Histone 3 Acetylation at Lysine 9 (H3K9ac) is Decreased in Hypoosmolar-Stressed Astrocytes: Early Epigenetic Changes After Brain Injury and Edema Formation
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Astrocytes respond to brain injury through a mechanism known as reactive astrogliosis involving transcriptional, phenotypic, and functional changes. Astrocyte functional changes have a high impact on brain injury outcomes; however, the epigenetic mechanisms regulating gene expression, such as histone modifications, remain obscure. We have recently shown that astrocytes exposed to pro-inflammatory signals increase the level of histone acetylation. However, to date, there is no available description of early epigenetic changes in injury-affected astrocytes. We hypothesize that hypo-osmolar stress, promoted by early edema, triggers epigenetic changes in astrocytes. In a model of brain cortical injury by pial disruption in adult male Wistar rats (Villarreal et al., 2011), we addressed the levels of H3K9ac in astrocyte nuclei at 1.5 and 3.5 h post-injury. We observed, using immunofluorescence, a significative higher number of astrocytes with lower levels of H3K9ac at 3.5 h when compared to the non-injured hemisphere. Also, the injury promoted an increase in GFAP and AQP4 immunoreactivity, which radically decreased at higher distances from the injury core, probably indicating astrocyte swelling in response to edema. In vitro, we exposed a primary culture of astrocytes to hypotonic (20%, 30%, and 40% hypoosmolarity) culture medium to promote hypo-osmolar stress. We further designed a method for automated quantification by creating a macro in FIJI (ImageJ) that allows rapid and accurate analysis of all astrocyte nuclei within a microscopic field. We observed a statistically significant
A7: Role of Ceramide Synthesis in Glial Activation and Communication After a Lipotoxic Stimulus

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Saturated fatty acids (SFAs) are basic components of western-style diets. High systemic and cerebral levels promote chronic inflammation and metabolic disorders. This scenario could lead to brain dysfunction through multiple pathways and be a risk factor for neurodegenerative diseases. Previously, we have shown that a high-fat diet (HFD) induces central and peripheral inflammation, cognitive impairment, and hippocampal glial activation, in young and adult mice (Vinuesa et al., 2016, 2019). One of the mediators involved in these pathways are ceramides, lipidic molecules that, besides their physiological roles, could induce inflammation. In the present work, we aimed to study (1) the impact of palmitate (PA), one of the most abundant SFA in western-style diets, on glial activation and communication and (2) the role of ceramide synthesis mediating the effects of PA. Incubation of microglia (BV2 mouse cell line) with 0.5 μM PA-induced NFκB p65 nuclear translocation (p < .0001) and increased expression of IL1β (p < .05), suggesting the adoption of a pro-inflammatory or M1 phenotype. Treatment of microglia with Cambinol, an inhibitor of ceramide synthesis, ameliorated microglial activation. Treatment of C6 rat astrocytes cell line with PA failed to induce IL1β expression. However, conditioned media (CM) from PA-exposed microglia did (p < .001), and this effect was absent when microglia were pretreated with Cambinol. Isolation of exosomes from PA-microglia CM exerted the same effect, suggesting a relevant function for these extracellular vesicles. Our results suggest a role for glial ceramide synthesis mediating the induction and propagation of inflammation succeeding lipotoxicity. In the present and future experiments, we aim to determine the role of ceramide synthesis in glial-neuronal communication and the promotion of neurodegeneration.

A8: Sox9 Regulates the Choice Between Glial and Late Neuronal Fates in the Developing Spinal Cord

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Neurons and glial cells are sequentially produced during embryonic development, and this temporal order is conserved in all species and regions of the nervous system. Neurogenic events have been considered to be completed by the time gliogenesis begins. However, the spinal cord cerebrospinal fluid-contacting neurons (CSF-cNs) are an exception to this general principle, being generated at late stages together with astrocytes, oligodendrocytes, and ependymocytes. The precise molecular mechanisms that determine the equilibrium between these distinct cell types are unknown. In this work, we show that the transcription factor Sox9 regulates the glial versus neuronal fate choice balance. We found that Sox9 conditional mutants have expanded production of spinal CSF-cNs. This increase is accompanied by impaired astrocyte differentiation, revealed by a reduced number of Sox2+ Nfas + cells in the mantle zone, and lower GFAP immunoreactivity. Moreover, we show that the respecification towards CSF-cN follows a differentiation program initiated by the proneural protein Ascl1 and continued by the postmitotic transcription factors Gata3/2. Altogether, our results demonstrate that Sox9 promotes glial cell fate determination, as its absence leads to failure in astrocytic commitment thus triggering CSF-cN differentiation.

A9: Neurotoxic Glial Phenotypes as Potential Therapeutic Targets in ALS

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Defective neuron-glia interplay and the emergence of aberrant glial phenotypes seem to have a role in the progressive death of motor neurons and muscle atrophy and paralysis in amyotrophic lateral sclerosis (ALS). To test the hypothesis that those aberrant glial cells could be potential targets to decrease in H3K9ac immunofluorescence after 1 and 3 h which were restored to control values 24 h after recovery in the complete isotonic medium. Trichostatin A treatment prevented H3K9ac decrease after 3 h hypoosmolarity. Our results strongly suggest that astrocytes exposed to an edema-like microenvironment are able to dramatically change the global levels of histone acetylation. During the recovery in histone acetylation levels, chromatin might be re-decorated but in a “reactive epigenome.” Funding: PICT2018, ISN-CAEN2019.
A10: Learning Induces Rapid Morphological Plasticity in Mouse Hippocampal Astrocytes

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Astrocytes are key mediators of diverse forms of synaptic plasticity. In addition to the release of soluble factors, astrocytes become hypertrophic and in closer interaction with synapses when animals are exposed to challenging contexts, such as learning paradigms. Many studies have shown how astrocytic structural remodeling takes place in learning paradigms, however, little is known about the time course of astrocyte remodeling in short-lasting learning contexts. In this study, we asked whether astrocytic morphological alterations take place shortly after a motor learning task. To this aim, two different groups of mice were trained in a rotarod task set to an accelerated (learning group) or constant (active control group) speed. In both cases, the brains of these mice were processed for morphological analysis of astrocytes at two-time points (30 min or 24 h post-training). After immunostaining of brain sections for astrocytic markers (GFAP and S100B), we examined hippocampal astrocyte morphology in all groups. Our data show an increased GFAP staining intensity in the hippocampus of the learning group at 24 h post-training, compared to controls (28 ± 3 vs. 20 ± 3 AU, t-test, p = .0492). When exploring astrocyte process complexity through Sholl analysis, we observed a tendency to greater complexity in the learning group, compared to the active control. Unexpectedly, we also discovered a tendency for an astrocytic soma volume reduction in the 24 h learning group when compared to the 24 h active control group. Taken together, these results indicate that astrocytic remodeling takes place shortly after learning and supports the idea that astrocytic remodeling contributes to neural plasticity processes involved in learning.

Specialized glia

SG1: Hypothalamic Tanyocytes Internalize Ghrelin in Their Soma and Vectorially Transport to the Endfeet

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Hypothalamic tanyocytes are polarized glial cells that line the base of the third ventricle. Their somas contact the cerebrospinal fluid (CSF), whereas their terminal sides (endfeet) contact the capillaries of the blood–brain barrier or the fenestrated capillaries of the median eminence, forming an anatomical interface for the transport of molecules between blood and CSF. Using mice and rats, we recently described that tanyocytes internalize the orexigenic hormone ghrelin in vivo and in vitro. Here, we study the cellular mechanisms mediating ghrelin uptake in hypothalamic tanyocytes and their transport direction. Specifically, we incubated primary cultures of rat tanyocytes with a fluorescent ghrelin variant (Fr-ghrelin) in basal conditions or after pharmacological blockage of either clathrin-mediated internalization using Pitstop or Dyno4a, or intracellular transport using colchicine. We also coincubated tanyocytes with Fr-ghrelin and either native ghrelin or desacyl-ghrelin. We then quantified fluorescence intensity in soma, process, and endfeet of each cell. We found that intracellular fluorescence: (1) is found predominantly in the soma of tanyocytes after a 5-min incubation; (2) increased in somas, processes, and endfeet after 30-min incubation as compared to 5-min incubation; (3) was reduced only in terminals in the presence of colchicine; (4) was decreased in all compartments in the presence of Pitstop and Dyno4a; (5) was reduced in somas, processes, and endfeet after coincubation.
with native ghrelin or desacyl-ghrelin. This evidence shows that tanycytes can specifically internalize ghrelin through clathrin-mediated endocytosis in the somas and presumably transport it from the apical to the terminal side.

SG2: Effect of Minocycline in Olfactory Nerve Regeneration
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When toxic damage is applied to the olfactory nerve, it increases the density and reactivity of myeloid cells in the afferent olfactory pathway. It is unclear whether this phenomenon contributes to the regeneration of the olfactory nerve. In this experiment, we assessed whether minocycline a modified tetracycline that inhibits reactivity and proliferation of myeloid cells had an impact on the rate of recovery of the damaged olfactory epithelium. C57BL/6 mice were treated with the olfatotoxin methimazole (75 mg/kg) to damage the olfactory epithelium, and the following day half the mice started consumption of ad libitum regular water whilst the other half received minocycline (0.25 mg/ml in drinking water), for a period of one or two days between PNDs 4–16, preventing functional and structural alterations induced by MSEW. These results show for the first time that ELS has deleterious consequences on mice’s visual function. These results may be explained by an RGC loss, possibly due to microglia activation. Finally, early treatment with mifepristone prevented MSEW consequences. This indicates that corticoid elevated levels during the MS period may be involved in the mechanisms of ELS consequences in later life.

SG4: Glucose and Oxidative Stress as Modulators of Retinal Müller Cells Pluripotency Capacity. Involvement of Histone Deacetylase SIRT6
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In response to a harmful stimulus, retinal Müller glial cells (MGs) can dedifferentiate acquiring neural stem cells properties, proliferate and migrate to the damaged retinal layer and differentiate into lost neurons. However, it is not yet known how this reprogramming process is regulated in mammals. Since glucose and
oxygen are important regulatory elements that may help direct-
ing stem cell fate, we aimed to study the effect of glucose vari-
ations and oxidative stress in MGs reprogramming capacity and
analyze the participation of the histone deacetylase SIRT6 as an
epigeneic modulator of this process. We found that the com-
bination of high glucose and oxidative stress induced a decrease
in the levels of glutamine synthetase, and an increase in the
migration capacity suggesting that these experimental conditions
could induce some degree of dedifferentiation and favor the
migration ability. High glucose-induced an increase in the levels
of the pluripotent factor SOX9 and a decrease in SIRT6 levels
accompanied by an increase in the acetylation levels of H3K9.
Inhibiting SIRT6 expression by siRNA rendered an increase in
SOX9 levels. We also determined SOX9 levels in retinas from
cows with a conditional deletion of SIRT6 in the CNS and evalu-
ated the gene expression profile and performed Gene
Ontology enrichment analysis of MGs from a murine model of
Diabetes. We found several differentially expressed genes
associated with glucose metabolism, cell migration, develop-
ment, and pluripotency. We found that functional categori-
es affected in cells of diabetic animals were directly related to
SIRT6 function. Transcription factors enrichment analysis
allowed us to predict several factors, including SOX9, that
may be involved in the modulation of the differential expression
program observed in diabetic MGs. Our results underline the
heterogeneity of Müller cells response and the challenge that
the study of metabolic impairment in vivo represents.

SG5: Protective Effect of NO2-OA on the
Oxidative Stress, Gliosis, and
Pro-Angiogenic Response in Müller Glial
Cells

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Inflammation, oxidative and nitrosative stress participate in the
pathogenesis and progression of proliferative retinopathies
(PR), which also involves a deregulated neovascularization,
with a dramatic increase of vascular endothelial growth factor
(VEGF) in the retinal tissue. Vascular changes result in alter-
ations of the retinal blood barrier, allowing the extravasation of
α2-macroglobulin (α2M), which induce gliosis in Müller
Glial cells (MGCs) by the increase of GFAP levels. Nitro-fatty acids (NO2-FA) are important electrophilic signal-
ning mediators with anti-inflammatory and cytoprotective prop-
erties (Keap1/Nrf2 pathway). Our goal was to determine the
effect of nitro-oleic acid (NO2-OA) on oxidative stress, gliosis,
and pro-angiogenic response in the human MGC line
(MIO-M1). Pure synthetic NO2-OA induced HO-1 protein
expression in a time- and concentration-dependent manner
in MIO-M1 cells and this up-regulation was abrogated with
the Nrf2 pharmacological inhibitor, trigonelline. To determine
whether NO2-OA could be beneficial against oxidative stress,
we pre-treated MIO-M1 cells with or without NO2-OA
before PMA and LPS stimulus. Both PMA and LPS significantly
increased ROS in MIO-M1 cells compared with control and
NO2-OA prevented the increase in ROS levels induced by
both stimuli. On the other hand, α2 M-induced gliosis is char-
acterized by a significant increase in GFAP and vimentin pro-
tein expression. In addition, α2M also increased ROS levels
and the pre-treatment with NO2-OA reduced α2M induction
of GFAP and ROS to the control level. Finally, to evaluate
whether NO2-OA could modulate the proangiogenic
response of MIO-M1 cells under hypoxic and proinflammatory
conditions, we determined VEGF transcriptional expression
by qRT-PCR. NO2-OA did not affect VEGF mRNA expression
under hypoxic conditions, but in pro-inflammatory conditions,
NO2-OA significantly reduced VEGF-A mRNA levels in
MIO-M1 cells. Furthermore, NO2-OA inhibited endothelial
cell tubulogenesis. Collectively, these results indicate that
NO2-OA may act as an antioxidant protecting MIO-M1 cells
from oxidative damage, gliosis, and the exacerbated proangi-
genic response.

SG6: Annotate_my_Genomes: An
Easy-to-Use Pipeline to Improve Genome
Annotation and Uncover Neglected Genes
by Hybrid RNA Sequencing. The Case of
Radial Glia in Brain Chicken

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By combining high glucose and oxidative stress, MGCs
increase the levels of glutamine synthetase, and an increase in
the acetylation levels of H3K9. Inhibiting SIRT6 expression
by siRNA rendered an increase in SOX9 levels. We also
determined SOX9 levels in retinas from mice with a conditional
deletion of SIRT6 in the CNS and evaluated the gene
expression profile and performed Gene Ontology enrichment
analysis of MGs from a murine model of Diabetes. We found
several differentially expressed genes associated with glucose
metabolism, cell migration, development, and pluripotency.
We found that functional categories affected in cells of
control animals were directly related to SIRT6 function.
Transcription factors enrichment analysis allowed us to
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the heterogeneity of Müller cells response and the challenge
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SG5: Protective Effect of NO2-OA on the
Oxidative Stress, Gliosis, and
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The central nervous system (CNS) development begins soon in embryonic development and is a highly conserved process among vertebrate species. The origin of the CNS is driven by the ectodermal neurulation and posterior vesiculation stages. The neural tube closure defines a virtual symmetrical midline in its dorsal and ventral part, through which the commissural neuron growth cone axons must decide whether to cross or not, guided mostly by attraction/repulsion signals. Located at the most caudal prosomere 1, the posterior commissure (PC) is the first transversal commissure to form and defines the dorsal boundary between the diencephalic and mesencephalic vesicles. Likewise, the radial glial cells located underneath this commissure shape the first secretory structure of the brain to differentiate, the so-called subcommissural organ (SCO). The extracellular matrix components forming the path to the pioneer axons to reach the PC midline have been characterized, however, few individual genes that specifically affect the SCO proliferation and differentiation, and thus, the PC development, have been discovered. In the present work, we used PacBio and Illumina RNAseq analysis to identify a wide spectrum of SCO genes that are significantly up- and downregulated in E4 compared to E7 chick embryos (HH23-HH30, respectively). Moreover, our data was corroborated through quantitative RT-PCR analysis. The data presented here provide a transcriptional panel to identify genes involved in the key proliferative (HH23) and differentiative (HH30) steps of the SCO and its related structure, the PC. We demonstrated the efficiency of this approach by correctly assembling and annotating all exons from the chicken SCO-spondin gene, including the identification of missing genes in the chicken reference annotations by homology assignments. Importantly, the presented data provide the first transcriptional landscapes of SCO in chicken development and the identification of novel key genes and long noncoding RNAs (lncRNAs) for SCO and brain development. We developed an easy-to-use genome-guided transcriptome annotation pipeline that uses assembled transcripts from hybrid sequencing data as input and distinguishes between coding and long non-coding RNAs by integration of several bioinformatic approaches.

**SG7: Damaging Effects Induced by BMAA on Müller Glial Cells**

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β-Methylamine-L-alanine (BMAA) is a non-proteinogenic aminoacidic cyanotoxin produced by several cyanobacteria. This cyanotoxin has been linked with the development of neurodegenerative diseases, like Amyotrophic Lateral Sclerosis, Alzheimer, and of some retinal pathologies. In the retina, it induces damaging effects on neurons and in Müller glial cells (MGCs), the major glial cell type, which have crucial roles in preserving normal retinal functionality. We have previously demonstrated that BMAA promotes neuronal degeneration with no protective effect observed by MGCs. In this work, we studied the direct effects of BMAA on MGCs in pure retinal glial cultures. For that purpose, we treated these cultures, obtained from newborn rat retinas, after cells were reseeded, with BMAA (0.4, 1, and 10 µM) at day 1 or at days 1 and 4. Cells were analyzed in either, in a short and in a long-term BMAA exposure of 3 and 9 days, respectively. We evaluated cell viability by DAPI staining and Trypan Blue assays; cellular metabolic activity by MTT assay, and cytoskeleton integrity by staining actin filaments with phalloidin. Our preliminary results showed that in both, short-term and long-term exposure of cultures to BMAA, MGCs displayed nuclear alterations without affecting the viability of these cells. Additionally, BMAA (1 and 10 µM) promoted an increase in the cellular metabolic activity in short-term, but not in long-term studies. Moreover, our results showed that BMAA induced actin network disorganization. Interestingly, in long-term exposure of cultures to BMAA, this toxin-induced an abnormal cytoplasmic growth. In conclusion, these results imply that BMAA induces several alterations in MGCs at the subcellular level without affecting their viability. Hence, these damages may help to understand neurodegenerative damages elicited by BMAA, which affect human health. Our knowledge of the molecular mechanisms involved in BMAA-induced cell damage could help to develop new therapeutic strategies.

**SG8: Characterization of Cellular Inflammatory Component in a Mice Choroidal Neovascularization Model**

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Age-related macular degeneration (AMD) in its choroidal neovascularization (CNV) stage, where growing neovessels invade the retina inducing photoreceptor degeneration, is the leading cause of vision loss among adults. Mononuclear phagocytic cells (MPCs), such as resident microglia and monocyte-derived macrophages, collaborate in establish a
chronic inflammatory state, which can lead to the onset of CNV. Our lab has previously demonstrated that multi-ligand low-density lipoprotein receptor-related protein 1 (LRP1), is expressed by MPCs in choroid from mice with CNV. In the present study, we aim to evaluate LRP1 expression and localization in retinal tissue during CNV in an animal model. C57BL/6 adult mice were treated with four spots of argon green laser photocoagulation per eye. At 1, 4, 7, 14, and 21 days after laser, they were sacrificed and retinal tissue was processed by WB to study LRP1 protein expression. It has been established that the model shows the highest levels of cell infiltration at 4 days after laser, so at this time point, we evaluated LRP1 transcript levels by rtPCR, its expression on resident MPCs by FACS assay, and its localization in retinal cryosections by Immunofluorescence. We could observe that LRP1 protein expression increases from day 1 to 21. Particularly at 4 days after laser, we found a higher number of MPCs expressing LRP1. However, receptor transcript levels do not show changes. By IF it is possible to observe the activation of both, Muller and microglial cells, together with changes in LRP1 expression close to the CNV area at the inner nuclear layer. These results lead us to conclude that retinal expression of LRP1 might be involved in the inflammatory process during CNV. What is more, MPCs number could be responsible for this effect. Further studies are needed to know the role of LRP1 on the inflammatory component.