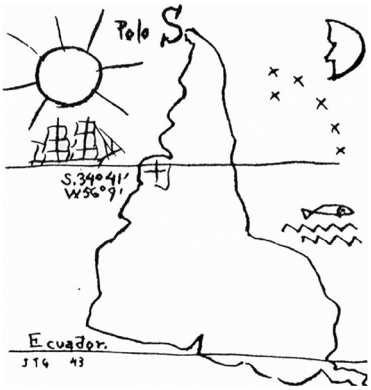
América invertida, Joaquín Torres García, 1943



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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Agencia Nacional de Promoción de Ciencia y Tecnología

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 (oligodendroglía)

[3]Institute of Cellular Neurosciences, Medical Faculty, University of Bonn, Germany
[4]Laboratorio de Fisiología, Faculty of Biological Sciences, Universidad Católica de Chile, Chile

Tanycytes 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 tanycytes 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 tanycytes. **Methods:** We evaluated proliferation in vitro using 5-Bromo-2′-Deoxyuridine (BrdU) incorporation and exposing primary cultures of tanycytes 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 tanycyte cell division. In vivo, continuous infusion of Gap27 prevented the FGF2-induced proliferation only in the β2 tanycyte population. Genetically deletion of Cx43 also impaired hypothalamic cell division. **Discussion:** Our results demonstrate the importance of Cx43 in tanycyte proliferative potential.

The Müller Glial Cells: Are Specialized Glia for Light Detection in the Retina?

Natalia A. Marchese[1], M. N. Rios[1], and M. E. Guido[1]
[1]CIQUIBIC-CONICET Departamefnto de Química Biológica "Ranwel Caputto" Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina

The retina of vertebrates captures ambient light for image and non-image forming (NIF) functions through two sets of specialized photoreceptors: cones and rods responsible for day/night vision, and intrinsically photosensitive retinal ganglion and horizontal cells expressing melanopsin (Opn4). Interestingly, Müller glial cells (MCs), which are derived from neuronal progenitors and represent the most abundant retinal glial cell type, have been shown to express different blue- and UV-light sensitive opsins (Opn3 and Opn5), and the photoisomerase retinal G protein-coupled receptor (RGR). Here we resume our most recent results showing intrinsic cellular responses in primary cultures of avian MCs elicited by blue light stimulation (peak at 480 nm). By means of intracellular calcium level detection with fluorescent probes and real-time microscopy, we identified a subpopulation of MCs (40% of recorded cells) showing direct photic responses, observed as a > 20% increase in intracellular calcium levels and maintained for several minutes after the stimulus. The calcium response was shown to be dependent on opsin activation and specific to blue light stimulation. Light-evoked responses in MC cultures were suppressed by up to 50% after depleting endoplasmic reticulum calcium stores with thapsigargin; no significant inhibition was achieved by modifying extracellular calcium availability with the calcium chelator EGTA. Further testing was performed with the inhibitors for IP3 receptors (2-APB), protein kinase C (U73122), and G protein-coupled receptors (Suramin). Under these conditions, the subpopulation of MCs responding to light with calcium increase was diminished. This would indicate that blue light stimulation in MCs promotes increases in calcium intracellular levels by G-protein signaling towards calcium mobilization from internal stores, pointing at the possibility of a higher level of complexity for light detection in the retina involving photic activation of MCs in light-regulated circuits and pathways.

Müller Glial Cells Alterations in a Retinal Degeneration Mouse Model

Harmonie Vallese-Maurizi[1], Georgina Pamela Coló[1], Luis Politi[1], and Lorena German[1]
[1]Laboratorio de Neurovirología, INIBIBB-UNS-CONICET CCT Bahía Blanca, Argentina

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 pretreatment 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

Julián Del Core[1], Ignacio Jure[1], Alejandro F De Nicola[1,2], and Florencia Labombarda[1,2]

[1]Laboratorio de Bioquímica Neuroendócrina, Instituto de Biología y Medicina Experimental, CONICET, Argentina

[2]Departamento de Bioquímica Humana, Facultad de Medicina, UBA, Argentina

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

Coram Guevara[1], Sebastian Vejar[1], Francisca Villavicencio[2], Margrette Olsen[2], Javier Reyes[3], Cristian Amador[3], Rodrigo Quintanilla[2] and Fernando C. Ortiz[1]

[1]Mechanisms of Myelin Formation and Repair Laboratory, Instituto de Ciencias Biomédicas, Facultad de Ciencias de Salud, Universidad Autónoma de Chile, Santiago, Chile

[2]Laboratory of Neurodegenerative Diseases, Instituto de Ciencias Biomédicas, Facultad de Ciencias de Salud, Universidad Autónoma de Chile, Santiago, Chile

[3]Laboratory of Renal Physiopathology, Institute of Biomedical Sciences, Universidad Autónoma de Chile, Santiago, Chile

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