SAN

SOCIEDAD ARGENTINA DE
INVESTIGACIÓN EN NEUROCIENCIAS

XXX ANNUAL MEETING

and SAN-ISN Small Conference and Course

Mar del Plata, Argentina

SEPTEMBER 27th - OCTOBER 1st, 2015

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María Sol Fustiñana
Joaquín Piriz
Lorena Rela

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Dear SAN members, friends and colleagues:

On behalf of the 2015 organizing committee and the SAN Board of Directors, it is my great pleasure to welcome you all to our XXX Annual Meeting, to the SAN-ISN special small conference and to the celebration of the 30th anniversary of the Sociedad Argentina de Investigación en Neurociencias.

SAN was created by a group of pioneers who felt that it was the right time to acquire a differential identity from the classical school of biochemistry that characterized Argentine science. It was born as the Sociedad Argentina de Neuroquímica and one of its foundational missions was to promote the development of neurochemistry in our country. Ranwel Caputto, Department of Biological Chemistry, University of Córdoba, best known forhis work on ganglioside biochemistry in the nervous system, was SAN´s first president. Very quickly Argentine neuroscience grew in size and scope, beyond the borders of neurochemistry. A group of scientists, young investigators and students decided to organize yearly workshops to discuss their work in a friendly atmosphere and in a secluded place that would foster intense scientific discussions. Thus, the Taller Argentino de Neurociencias took shape. It was characterized by an important participation of students who were highly involved in the organization and by its lively discussions. During 2009 and 2010 SAN and Taller organized joint meetings in order to re-unite the entire neuroscience community. SAN renovated its name to Sociedad Argentina de Investigación en Neurociencias, and since 2011 the SAN Annual Meeting has been our yearly reunion.

The Argentinean neuroscience community continues to grow and SAN has more than 600 members. It encompasses all areas of neuroscience, from cellular and molecular to cognitive and computational sciences. The XXX Annual meeting will be a clear reflection of the growth and strength of our society. With 400 participants, a very high quality scientific program and a shift in venue from the hills to the sea, we look forward to a vibrant, lively and exciting meeting.

We want to thank the invited speakers who have traveled long distances to share their recent discoveries and all SAN members and participants for your continuing support to the society. A special thanks to sponsors and exhibitors that have supported this XXX Annual Meeting and celebration of the SAN 30th anniversary. Welcome all and enjoy the meeting.

Ana Belén Elgoyhen
SAN President

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SAN-ISN Course
"State-of-the-art methods in Neuroscience Research"
ROOM TOPACIO

PROGRAM

DAY 1: Sunday September 27th	
18:00-19:00	Registration
19:15-19:30	Welcome words by course organizers
19:30-21:00	**Lecture I:** ***"Mapping neuronal networks with viral tools"*** **María Soledad Espósito**, Friedrich Miescher Institute, Basel, Switzerland
21:00	Dinner

DAY 2: Monday September 28th	
09:00-10:30	**Lecture II:** ***"In vivo 2-photon microscopy for dissection of neuronal circuits"*** **Johannes Letzkus**, Max Planck Institute for Brain Research, Frankfurt, Germany
10:30-11:00	Coffee Break

11:00-12:30	**Lecture III:** ***"Visualizing large-scale neural ensemble dynamics in freely behaving mice"*** **Mark Schnitzer**, Stanford School of Medicine, Howard Hughes Medical Institute, USA
12:30 -13:30	Lunch
14:00-15:30	Session A: Group 1-14 – paper discussion
16:00-17:30	Session B: Group 15-28 – paper discussion
17:30-19:00	**Lecture IV:** ***"Dissecting neuronal circuits using chemical genetic tools"*** **Marcelo de Oliveira Dietrich,** Yale School of Medicine, USA
19:00-19:30	Break
19:30-21:00	**Lecture V:** ***"In vivo optogenetics and electrophysiology"*** **Alexxai Kravitz,** NIDDK, NIDA, USA
21:00-23:00	Dinner and Poster presentation (Room Mediterráneo)

DAY 3: Tuesday September 29th

8:30-10:30	Session A: Group 15-28 – paper discussion
11:00-13:00	Session B: Group 1-14 – paper discussion

XXX REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE INVESTIGACION EN NEUROCIENCIAS

PROGRAM

DAY 1: Tuesday September 29th

09:00-12:00 Registration

14:15 Welcome by Organizers (Room Atlantic)

14:30-15:30 **Special Lecture** (Room Atlantic)
Chair: **Joaquín Piriz**, Instituto de Fisiología y Biofísica Bernardo Houssay, CONICET, Argentina

"Next-generation optical technologies for cracking neural codes"
Mark Schnitzer, Departments of Biological Sciences and of Applied Physics, Stanford School of Medicine, Howard Hughes Medical Institute, USA

15:30-16:00 Coffee break (Room Mediterráneo)

16:00-18:30 **Symposium 1 & Symposium 2**

Symposium 1 GADOR Symposium (Room Atlantic)

"Technological advances to dissect neural circuits controlling behavior"
Chair: **M. Sol Fustiñana** and **M. Soledad Esposito**, Friedrich Miescher Institute, Basel, Switzerland

- ***"Temporal stability of aversive memories is determined by Lateral Habenula activity"***
Joaquin Piriz, Instituto de Fisiología y Biofísica Bernardo Houssay, CONICET, Argentina

- ***"Multitasking of AGRP neurons in metabolism and behavior"***
Marcelo de Oliveira Dietrich, Yale School of Medicine, USA

- ***"Spontaneous neuronal network dynamics reveals circuit's functional adaptations for behavior"***
Sebastián A. Romano, Ecole Normale Supérieure, IBENS, Paris, France

- ***"Disinhibition, a circuit mechanism for associative learning"***
Johannes Letzkus, Max Planck Institute for Brain Research, Frankfurt, Germany

Symposium 2 (Room Topacio)

"Glia at the Round Table: Microglia, Oligodendroglia and Astroglia in a Fruitful Dialogue"
Chair: **Juan María Pasquini**, University of Buenos Aires, School of Pharmacy and Biochemistry, Argentina and **Alberto Javier Ramos**, Instituto de Biología Celular y Neurociencia, Prof. E. De Robertis, UBA-CONICET, Argentina

- "Glia and Nervous System Energy Metabolism"
Bruce Ransom, University of Washington, Seattle, USA

- ***"Analysis of myelination and remyelination in the optic nerve"***
Alain Chedotal, University of Paris, France

- ***"Atypical astrocytes are present in the ischemic lesions and contribute to the expansion of reactive gliosis"***
Alberto Javier Ramos, Instituto de Biología Celular y Neurociencia, Prof. E. De Robertis, UBA-CONICET, Argentina

- ***"Mag as key player of the bi-directional communication between axons and myelin: its impact on postnatal development and disease"***
Pablo Lopez, Instituto de Investigación Médica Mercedes y Martín Ferreyra, CONICET, Argentina

18:45-19:30 **Opening Ceremony and Tribute to Past Presidents** (Room Atlantic)

19:30-20:30 **Opening Lecture** (Room Atlantic)
Chair: **Ana Belén Elgoyhen**, Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, Dr Héctor N. Torres, CONICET, Argentina

"Emergence and mechanisms of cognition"
György Buzsáki, The Neuroscience Institute, New York University School of Medicine, Ney York, USA

21:00 Welcome Reception
Hotel 13 de Julio

DAY 2: Wednesday September 30th

08:30-11:00 **Symposium 3 & Symposium 4**

Symposium 3 (Room Atlantic)

"2015, The International Year of Light: Superresolution microscopies in Neuroscience"

Chair: **Francisco Barrantes**, Instituto Investigaciones Biomédicas, UCA–CONICET, Argentina

- ***"Nanoscopy 2.0. Converging and correlative technologies"***

Alberto Diaspro, Department of Nanophysics at the Istituto Italiano di Tecnologia, Genova, Italy

- "Visualizing the details of neuronal polarity with fluorescence nanoscopy"

Fernando Stefani, Centro de Investigaciones en Bionanociencias, Buenos Aires, Argentina

- ***"Social organization of neurotransmitter receptor nanoclusters"***

Francisco Barrantes, Instituto Investigaciones Biomédicas, UCA–CONICET, Argentina

- ***"Resolft microscopy of living cells in the nervous system"***

Ilaria Testa - Royal Institute of Technology, Stockholm, Sweden

Symposium 4 (Room Topacio)

"*Translational approaches to model and treat SNC diseases*"

Chair: **Elena Avale** and **Marcelo Rubinstein**, Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, Dr Héctor N. Torres, CONICET, Argentina

- ***"Levodopa induced dyskinesias: from classical pharmacology to molecular mechanisms"***
Oscar Gershanik, Instituto de Neurociencias de la Fundación Favaloro, Instituto de Investigaciones Farmacológicas, CONICET, Argentina

- ***"Alzheimer's disease, from a dish to the bed"***
Gorazd Bernard Stokin, St. Anne's University Hospital, Brno, Czech Republic, Division of Neurology, University Medical Centre, Ljubljana, Slovenia

- ***"What Can Movement Circuitry Tell Us About Obesity?"***
Alexxai Kravitz, NIDDK, NIDA, USA

- "Phenotypic rescue in a mouse model of tauopathy using trans-splicing RNA reprogramming"
Elena Avale, Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, Dr Héctor N. Torres, CONICET, Argentina

11:00-11:30 Coffee break

11:30-13:15 **Short Talks by students (1 & 2)**

Short Talks by Students 1 (Room Atlantic)
Chair: **Estela Maris Muñoz**, Instituto de Histología y Embriología "Dr. Mario H. Burgos" and **Pablo Helguera**, Instituto de Investigación Médica Mercedes y Martín Ferreyra, CONICET, Argentina

- "Unraveling the Role of GABAergic-Proopiomelanocortin Neurons in the Hypothalamic Control of Energy Balance"

Milagros Trotta, Instituto de Fisiología y Biofísica Bernardo Houssay, CONICET, Argentina

- "ASCL1 regulates late neurogenic events in the ventral neural tube"

Daniela Di Bella, Fundación Instituto Leloir, Argentina

- "*Evaluating a possible crosstalk between inhibitory and excitatory calcium signals in inner hair cells of the developing inner ear*"

Marcelo Moglie, Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, Dr Héctor N. Torres

- "*Kv1.3 is a candidate target to prevent the hypercholinergic state of parkinsonism*"

Cecilia Tubert, Instituto de Fisiología y Biofísica Bernardo Houssay, CONICET, Argentina

- "*GDNF/GFRα1 complex is a synaptic organizer required for proper hippocampal circuit development*"

Dolores Irala, Instituto de Biología Celular y Neurociencia, Prof. E. De Robertis, UBA-CONICET, Argentina

- "*Delayed coupling to feedback inhibition during a critical period for the integration of adult-born granule cells*"

Silvio Temprana, Fundación Instituto Leloir, Argentina

- "*Involvement of δCaMKII in persistent forms of memory*"

Gisela Zalcman, Instituto de Fisiología, Biología Molecular y Neurociencias, CONICET, Argentina

Short Talks by Students 2 (Room Topacio)
Chair: **Mariano Di Guilmi**, Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, Dr Héctor N. Torres and **Nicolás Unsain**, Instituto de Investigación Médica Mercedes y Martín Ferreyra, CONICET, Argentina

- "*Altered Corticostriatal Connectivity and Exploration-Exploitation Imbalance Emerge as Intermediate Phenotypes for a Neonatal Dopamine Dysfunction*"
Bárbara Braz, Instituto de Fisiología y Biofísica Bernardo Houssay, CONICET, Argentina

- "*Is microglia one of the mediators of IGF-1 effects on aged rats*?"
Eugenia Falomir Lockhart, Instituto de Investigaciones Bioquímicas de la Plata, CONICET, Argentina

- "*Altered maturation through adolescence leads to decreased hippocampal-prefrontal cortex functional connectivity in a mouse model of schizophrenia*"
Rodrigo Alvarez, Instituto de Fisiología y Biofísica Bernardo Houssay, CONICET, Argentina

- "*Differential Reactivation Outcomes on a Single US-Contextual Fear Conditioning: a temporal prediction error account*"
Matías Mugnaini, Laboratorio de Psicología Experimental, Facultad de Psicología, Universidad Nacional de Córdoba

- "*Neurochemical phenotypes rescued in Tau Knock-out mice by human Tau re-expression*"
Ana Damianich, Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, Dr Héctor N. Torres, CONICET, Argentina

"Aversive and appetitive memories are simultaneously formed after a single learning session in the crab Neohelice"
Martín Klappenbach, Instituto de Fisiología, Biología Molecular y Neurociencias, CONICET, Argentina

- "*Hippocampal ERK2 differential activation after memory reconsolidation processes are modulated by α7 nicotinic acetylcholine receptors*"
Maria del Carmen Krawczyk, Laboratorio de Neurofarmacología de los Porcesos de Memoria- Cátedra de Farmacología- Facultad de Farmacia y Bioquímica- UBA, Argentina

13:00-15:30 Lunch Break

15:30-16:30 **Eduardo de Robertis Lecture** (Room Atlantic)
Chair: **Cecilia Bouzat**, Instituto de Investigaciones Bioquímicas Bahía Blanca, CONICET, Argentina

"Opioid receptors and brain function - mouse genetic approaches"
Brigitte L Kieffer, Douglas Research Center, Department of Psychiatry McGill University Montréal, Canada

16:30-19:00 Poster Session & Networking (Room Mediterráneo)
16:30-17:45 ***ODD NUMBER poster presentation***
17:45-19:00 ***EVEN NUMBER poster presentation***

19:00-21:00 Asamblea Anual SAN

DAY 3: Thursday October 1st

08:30-11:00 **Symposium 5 & Symposium 6**

Symposium 5 (Room Atlantic)
"Neurophysiology of conscious states"
Chair: **Mariano Sigman**, Universidad Torcuato Di Tella and **Jacobo Sitt**, ICM Research Center, Pitié Salpêtrière Hospital, Paris, France

- ***"Broadband Cortical Desynchronization Underlies the Human Psychedelic State"***
Robin Carhart-Harris, University College London, England

- ***"Large-scale brain dynamics and connectivity across different states of consciousness"***
Enzo Tagliazucchi, Netherlands Institute for Neuroscience, Netherlands

- ***"Putting control back into free will"***
John Dylan-Haynes, Bernstein Center for Computational Neuroscience, Berlin, Germany

- ***"Imaging neural signatures of consciousness: 'What', 'When', 'Where' and 'How' does it work?"***
Lionel Naccache, ICM Research Center, Paris, France

Symposium 6 ISN Symposium (Room Topacio)
"Regulation of neuronal ion channels by G protein coupled receptors"
Chair: **Jesica Raingo**, Instituto Multidisciplinario de Biología Celular, CONICET, La Plata, Argentina.

-***"Neuronal calcium channels: From one to thousands"***

Diane Lipscombe, Brown University, Providence, USA

- ***"Receptors coupled to Gq modulate ion channels by lipid signaling"***

Bertil Hille, Washington University, Seattle, USA

- ***"Novel clustering of diverse ion channels in neurons mediated by AKAP79/150"***

Mark Shapiro, University of Texas Health Science Center, San Antonio, USA

- ***"New regulation of neuronal calcium channels by G protein coupled receptors constitutive activity"***

Jesica Raingo, Instituto Multidisciplinario de Biología Celular, CONICET, La Plata, Argentina

11:00-11:30 Coffee break

11:30-13:00 **Young Investigator Symposia 1 & 2**

Young Investigator Symposium 1 (Room Atlantic)
Chair: **Diego Gelman**, Instituto de Biología y Medicina Experimental, CONICET, Argentina

- ***"The BDNF prodomain (pBDNF) induces neuronal morphological changes associated with neuropsychiatric and neurodegenerative diseases"***

Agustin Anastasia, Instituto de Investigación Médica Mercedes y Martín Ferreyra, CONICET, Argentina

- ***"Recovery of locomotor activities in Spinal Cord Injury: A novel treatment to produce intra-axonal decrease of peroxide levels and reactivation of cytoskeleton dynamics in damaged axons"***

Ramiro Quintá, Instituto de Química y Fisicoquímica Biológicas, CONICET, Argentina

- ***"A two-pronged approach against Alzheimer's disease neurodegeneration: amyloid-beta synthesis and clearance are both regulated by glial metabotropic glutamate receptor"***
Daniela Durand, Instituto de Investigaciones Biomédicas, CONICET, Argentina

- ***"Progesterone prevents chronic pain after spinal cord injury"***
María Florencia Coronel, Instituto de Biología y Medicina Experimental, CONICET, Argentina

Young Investigator Symposium 2 (Room Topacio)
Chair: **Rafael Pagani**, Instituto de Fisiología y Biofísica Bernardo Houssay, CONICET, Argentina

- ***"Theta-oscillations in visual cortex emerge with experience to convey expected reward time and experienced reward rate"***
Camila Zold, Instituto de Fisiología y Biofísica Bernardo Houssay, CONICET, Argentina

- ***"VTA and LC control protein synthesis required for long-term memory formation during the behavioral tagging process"***
Diego Moncada, Instituto de Biología Celular y Neurociencia, Prof. E. De Robertis, UBA-CONICET, Argentina

- ***"Dilp8 requires the neuronal relaxin receptor Lgr3 to couple growth to developmental timing"***
Andres Garelli, Instituto de Investigaciones Bioquímicas Bahía Blanca, CONICET, Argentina

- ***"Muscarinic regulation of dopamine and glutamate transmission in the nucleus accumbens"***
Martín F. Adrover, National Institute on Alcohol Abuse and Alcoholism, NIH, Bethesda, USA

13:00-15:30 Lunch break

15:30-18:00 Poster Session (Room Mediterráneo)
15:30-16:45 ***EVEN NUMBER poster presentation***
16:45-18:00 ***ODD NUMBER poster presentation***

18:00-19:00 **Ranwell Caputto Lecture** (Room Atlantic)
Chair: **Pedro Bekinschtein,** Instituto de Biología Celular y Neurociencia, Prof. E. De Robertis, UBA-CONICET, Argentina

"30 years of persistence"
Jorge Medina, Instituto de Biología Celular y Neurociencia, Prof. E. De Robertis, CONICET, Argentina

19:00 Award to Best Talk by Students (Sponsored by Laboratorios ELEA and ABCAM)
Closing Remarks

Meeting Adjourns

21:00 Finger food and Party at Tío Curzio!!!!!

RECIPIENTS OF YOUNG ISN NEUROCHEMISTRY AWARDS

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Alessandro Maria soledad, Buenos Aires, Argentina
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Zalcman Gisela, Buenos Aires, Argentina
Zappala Cecilia, Ciudad Autónoma de Buenos Aires, Argentina

ABSTRACTS

COURSE / LECTURES

Room Topacio

DAY 1: Sunday September 27th, 19:30-21:00

Lecture I

"Mapping neuronal networks with viral tools"

María Soledad Espósito

Friedrich Miescher Institute, Basel, Switzerland

The ability of the brain to process multiple sensory information and select the appropriate behavioral outcome requires the existence of precise neuronal networks. In order to study those circuits neuroscientists count now with a battery of viral tools to target specific neuronal subpopulations according to gene expression and projection pattern, and to identify pre- and post-synaptic circuits in a high throughput manner. Furthermore, one exclusive property of viruses compared to other tracing techniques is their ability to carry genes of interest, making possible to unravel not only the structure but also the function of specific neuronal networks.

DAY 2: Monday September 28th, 09:00-10:30

Lecture II

"In vivo 2-photon microscopy for dissection of neuronal circuits"

Johannes Letzkus

Max Planck Institute for Brain Research, Frankfurt, Germany

2-photon microscopy (2-pm) is a powerful experimental approach that over the last two decades has consistently enabled breakthrough discoveries in neuroscience. It is particularly well suited to dissect the function of neuronal circuits in vivo and in relation to the animal's behavior. This lecture will cover the basic principles of 2-pm and how they relate to the conditions found in in vivo experiments. Next, the practical implementation of 2-pm will be covered in depth with a focus on the mouse as a genetically tractable model. We will discuss the unique strengths (as well as the inherent limitations) of 2-pm when combined with other state-of-the-art methods such as viral and transgenic tools, electrophysiology and behavior. Finally, to illustrate the power and versatility of the technique, we will briefly discuss a few recent studies that used 2-pm in experiments that provided major conceptual advancements in the field of circuit neuroscience. The overall aim of the lecture is to provide an accessible and interactive introduction to this important experimental approach.

DAY 2: Monday September 28th, 17:30-19:00

Lecture IV

"Dissecting neuronal circuits using chemical genetic tools"

Marcelo de Oliveira Dietrich
Yale School of Medicine, USA

Santiago Ramon y Cajal doctrines predict that neuron circuits are responsible to control behaviors. Cajal's major breakthroughs came from conceptually novel ideas extrapolated from using newly developed techniques to stain the nervous system. Only recently, techniques to control the electrical activity of specific populations of neurons have been developed, based on genetically encoded proteins. Here, it will be provided an overview of several techniques used to activate and/or inhibit neuronal activity (and or specific neuronal projections) in a variety of model organisms. The pros and cons of each technique will be discussed and experimental data will be presented to exemplify the use of these particular techniques to answer biologically relevant questions in neuroscience.

DAY 2: Monday September 28th, 19:30-21:00

Lecture V

"In vivo optogenetics and electrophysiology"

Alexxai Kravitz
NIDDK, NIDA, USA

Dr. Kravitz will provide an introduction to optogenetics, starting with the technical aspects of various opsins and light-activated molecules, as well as considerations about illumination strategies. He will review several behavioral discoveries aided by these technologies, and finally discuss the integration of optogenetics with in vivo electrophysiology. In closing, he will discuss some conceptual and theoretical concerns when designing optogenetic experiments, including how optogenetic stimulation parameters do, and don't, relate to physiological activity of neural circuits.

MEETING / PLENARY LECTURES

DAY 1: Tuesday September 29th, 14:30-15:30

Special Lecture - Room Atlantic

Chair: **Joaquín Piriz**, Instituto de Fisiología y Biofísica Bernardo Houssay, CONICET, Argentina

"Next-generation optical technologies for cracking neural codes"

Mark Schnitzer

Departments of Biological Sciences and of Applied Physics, Stanford School of Medicine, Howard Hughes Medical Institute, USA

A longstanding challenge in neuroscience is to understand how populations of individual neurons contribute to animal behavior and brain disease. Addressing this challenge has been difficult partly due to lack of appropriate brain imaging technology for visualizing cellular dynamics in awake behaving animals. I will discuss several new optical technologies of this kind. The miniature integrated fluorescence microscope allows one to monitor the dynamics of up to ~1000 individual genetically identified neurons in behaving mice over weeks. I will describe ongoing studies using this technology to understand the neural codes underlying episodic, emotional and reward related memories. Toward elucidating the interactions between brain areas during active behavior, multi-axis optical imaging can record the dynamics of two or more neural ensembles residing in different brain regions. Lastly, genetically encoded voltage indicators are progressing rapidly in their capacities to allow high fidelity detection of neural spikes and accurate estimation of spike timing, and with further improvements might soon be ready for use in behaving animals.

DAY 1: Tuesday September 29th, 19:30-20:30

Opening Lecture - Room Atlantic

Chair: **Ana Belén Elgoyhen**, Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, Dr Héctor N. Torres, CONICET, Argentina

"Emergence and mechanisms of cognition"

György Buzsáki

The Neuroscience Institute, New York University School of Medicine, Ney York, USA

The fundamental goal of the brain is to predict the future. More complex brains evolved multiple hierarchical loops between their outputs and inputs to make prediction more reliable in more complex environments and at longer time scales. With extensive training these prediction mechanisms have become 'internalized'. At the center of this model are self-propagating loops of neuronal coalitions connected by modifiable synapses that can be propelled forward without external cues. The implication of this conjecture is that brain networks are endowed with internal mechanisms that can generate a perpetually changing neuronal activity even in the absence of environmental inputs. I will discuss examples and mechanisms of this framework.

DAY 2: Wednesday September 30th, 15:30-16:30

Eduardo de Robertis Lecture - Room Atlantic

Chair: **Cecilia Bouzat**, Instituto de Investigaciones Bioquímicas Bahía Blanca, CONICET, Argentina

"Opioid receptors and brain function - mouse genetic approaches"

Brigitte L Kieffer

Douglas Research Center, Department of Psychiatry McGill University
Montréal, Canada

Opiates have been used since thousand years for their pain-relieving and rewarding properties. Opiates produce their potent effects by activating opioid receptors in the brain, highjacking the endogenous opioid system. This neuromodulatory system includes three opioid receptors, mu, delta and kappa, normally stimulated by endogenous opioid peptides to control pain and stress responses, as well as emotional and addictive behaviors. All three receptors are coupled to inhibitory G protein and reduce neuronal activity, but their distributions throughout brain circuits differ, and each receptor fulfills distinct functions in the brain. Gene targeting approaches in the mouse have been instrumental to identify the role of each opioid receptor in brain physiology and disease. Using gene knockout, we have demonstrated that mu receptors mediate morphine analgesia and reward, as well as reinforcing properties of non-opioid drugs of abuse and natural rewards, and have further shown that deficient mu receptor signaling leads to autistic-like behaviors. We also demonstrated opposing roles for mu and delta receptors in emotional processing and motor impulsivity, and positioned delta receptors as a potential target for mood disorders. On another front, we have achieved functional imaging of delta receptors in vivo, using an eGFP knock-in strategy. This is the first example of a G protein coupled receptor directly visible in vivo. This unique tool led us to visualize real-time receptor trafficking in live neurons, and demonstrate physiological relevance of receptor internalization for analgesic efficacy and tolerance. A similar approach targeting the mu opioid

receptor reveals brain sites of mu/delta receptor co-expression in vivo. Finally, we recently developed non-invasive MRI-based imaging of live mutant animals, and detected significant consequences of receptor deletion on whole-brain functional connectivity. Our findings have both fundamental and therapeutic implications in pain, mood and addiction research, as well as for GPCR biology and disease.

MEETING / SYMPOSIA

DAY 1: Tuesday September 29th, 16:00-18:30

GADOR Symposium - Room Atlantic

"Technological advances to dissect neural circuits controlling behavior"

Chair: **M. Sol Fustiñana** and **M. Soledad Esposito**, Friedrich Miescher Institute, Basel, Switzerland

"Temporal stability of aversive memories is determined by Lateral Habenula activity"

Joaquin Piriz

Instituto de Fisiología y Biofísica Bernardo Houssay, CONICET, Argentina

The Lateral Habenula (LHb) encodes negative value signals. It has been shown recently that LHb activation is sufficient to induce aversive associative learning; however the key question about whether LHb activation is required for an aversive memory to be formed has not been addressed. We studied the function of the LHb in memory formation using the Inhibitory Avoidance task (IA).We found that LHb inactivation during IA training does not disrupt memory when assessed 24h after, but abolishes it 7 days later, indicating that LHb activity during memory acquisition is not necessary for memory formation, but regulates its temporal stability. We are currently analyzing how LHb relates to brain regions involved in memory storage such as the hippocampus.

"Multitasking of AGRP neurons in metabolism and behavior"

Marcelo de Oliveira Dietrich

Yale School of Medicine, USA

The brain is very complex and little is known about how behaviors are controlled. In the basal part of the brain is located the hypothalamus, an evolutionarily conserved region consisting of heterogeneous, widely projecting neurons. These neurons are classically involved in the control of homeostatic functions, such as food intake, reproduction and sleep. We hypothesize that in addition to control homeostatic needs, hypothalamic neurons are also involved in other complex behaviors and decision-making. In this talk, I will provide evidence for the role of hunger-promoting AGRP neurons in the organization of behaviors in mice, revealing the fascinating function of old brain regions in controlling more complex behaviors. We have been using state-of-the-art techniques to manipulate neuronal function combined with advanced behavior analysis tailored towards understanding innate behaviors. With these experiments, we have identified novel functions for AGRP neurons relevant for the control of many diverse behaviors.

"Spontaneous neuronal network dynamics reveals circuit's functional adaptations for behavior"

Sebastián A. Romano

Ecole Normale Supérieure, IBENS, Paris, France

The brain spontaneously produces activity patterns, even in the absence of sensory stimulation. This ongoing activity was once considered as neuronal noise with no functional value. However, spontaneous activity dynamically engages in network states that mimic patterns of sensory-induced activities, potentially playing a role in brain computations. Nevertheless, the neuronal interactions underlying these spontaneous activity patterns, and their true biological relevance, remain elusive. I will present a recent work1 that sheds light over these issues. Using 2-photon calcium imaging of intact GCaMP-expressing transgenic zebrafish larvae, I monitored the spontaneous activity in the optic tectum. In zebrafish, the tectum is the most complex visual region, containing a retinotopic visual map that is essential for visually guided prey detection and capture. Spontaneous tectal activity was organized in neuronal clusters, representing visual assemblies that specifically grouped functionally similar neurons. Collectively, they reflected the tectal retinotopic map, even in the absence of retinal inputs. These assemblies consisted of all-or-none-like cooperative sub-networks shaped by competitive dynamics, a mechanism suited for their efficient recruitment. Notably, the spontaneous visual assemblies were tuned to the same angular sizes and spatial positions as larva's prey-detection performance in behavioral assays, and their spontaneous activation predicted directional tail movements. These results reveal that structured spontaneous activity represents "preferred" network states tuned to behaviorally relevant features, emerging from the functionally adapted intrinsic non-linear dynamics of neuronal circuits. 1 – Romano et al., Neuron 85, p. 1070-85, 2015.

"Disinhibition, a circuit mechanism for associative learning"

Johannes Letzkus

Max Planck Institute for Brain Research, Frankfurt, Germany

Learning causes a change in how information is processed by neuronal circuits. Whereas synaptic plasticity, an important cellular mechanism, has been studied in great detail, we know much less about how learning is implemented at the level of neuronal circuits and, in particular, how interactions between distinct types of neurons within local networks contribute to the process of learning. Activity in neuronal circuits is tightly regulated by inhibition supplied by distinct types of inhibitory interneurons. We employed a combination of cell-type specific recordings and optogenetic activity manipulations to determine how inhibition in auditory cortex and the amygdala contributes to auditory fear learning. Our results indicate that foot-shocks, which drive learning in this paradigm, lead to inhibition of parvalbumin-positive interneurons contacting the perisomatic domain of principal cells in both brain areas. In auditory cortex, this response is mediated by acetylcholine release leading to time-locked firing of layer 1 interneurons, which in turn inhibit parvalbumin-positive interneurons. Together with optogenetic manipulations of learning and recordings from putative principal neurons, these results suggest that foot-shocks gate plasticity induction through disinhibition in both auditory cortex and the amygdala. In contrast, during the auditory conditioned stimulus, amygdala parvalbumin-positive interneurons are excited and indirectly disinhibit the dendrites of principal neurons via inhibition of somatostatin-positive interneurons, thereby enhancing auditory responses and promoting cue–shock associations. Together with other recent work, our results suggest that disinhibition is a key circuit mechanism for induction of experience-dependent plasticity during associative learning. In addition, our experiments demonstrate that plasticity induction is dynamically regulated by the stimulus-specific activation of distinct disinhibitory microcircuits through precise interactions between different types of local interneurons.

Symposium 2 - Room Topacio

"Glia at the Round Table: Microglia, Oligodendroglia and Astroglia in a Fruitful Dialogue"

Chair: **Juan María Pasquini**, University of Buenos Aires, School of Pharmacy and Biochemistry, Argentina and **Alberto Javier Ramos**, Instituto de Biología Celular y Neurociencia, Prof. E. De Robertis, UBA-CONICET, Argentina

"Glia and Nervous System Energy Metabolism"

Bruce Ransom

University of Washington, Seattle, USA

"Analysis of myelination and remyelination in the optic nerve"

Alain Chedotal

University of Paris, France

The optic nerve (ON) has been used extensively as an experimental model of axon injury in the central nervous system as it contains only one type of axons, which originate from retinal ganglion cells (RGCs). It also contains endothelial cells and all types of CNS glial cells (oligodendrocytes, astrocytes and migroglia). Electron microscopy studies showed that one oligodendrocyte might form 15-20 myelin internodes in the rat ON. Therefore, even a minor axonal injury might have a consequence on a large number of axons and the death of a small number of oligodendrocytes might demyelinate many axons. The goal of this project was to study demyelination and remyelination after injury in the ON. Previous attempts to answer these questions relied on imaging techniques that did not allow to discriminate individual oligodendrocytes. To solve this problem, we have used two transgenic mouse lines that express fluorescent proteins in subsets of ON oligodendrocytes. Mice expressing a tamoxifen inducible form of CRE recombinase under the proteolipid protein promoter (PLP::CReERT2) were crossed to either CAGBOW mice (which express a Brainbow cassette under the ubiquitous β-actin promoter) or Tau:lox-STOP-lox-mGFP (TauGFP) mice. We found that in both lines, spontaneous recombination occurs which results in the

expression of fluorescent proteins in a small subsets of ON oligodendrocytes. The tri-dimensional morphology of single oligodendrocytes could be reconstituted with Imaris software. After tamoxifen injection a large number of ON oligodendrocytes were labelled. Next, we performed ON crush in adult transgenic mice and collected the ON at various time points post injury (3 days-3 months). Injured and non injured ONs were imaged using confocal microscopy. Our preliminary results showed that a large number of oligodendrocytes survived in the distal part of the optic nerve, where Wallerian degeneration occurs but that they rapidly retract myelin internodes. However, many of their processes were still aligned along the ON long axis. Birthdating experiments (Edu, BrDU) showed that the lesion was also accompanied by a significant proliferation of olig2+ cells in the lesioned ON. We are now using this strategy to study remyelination in models of optic nerve regeneration.

"Atypical astrocytes are present in the ischemic lesions and contribute to the expansion of reactive gliosis"

Alberto Javier Ramos

Instituto de Biología Celular y Neurociencia, Prof. E. De Robertis, UBA-CONICET, Argentina

Reactive gliosis is a general but largely complex and graded glial response to brain injury. Its biological role in the induction of neuronal survival or death is still under debate but it is clear that the expression of proinflamatory genes induce a pro-neurodegenerative phenotype in reactive astrocytes. In addition to the complexity of reactive gliosis, astroglial population has a -previously underestimated- high heterogeneity with cells differing in their morphology, gene expression profile and response to injury.

In this work we show that a subset of reactive astrocytes, showing several atypical characteristics, are present in early ischemic lesions and can be directly amplified in vitro. Atypical ischemic astrocytes (AIA) were isolated from early ischemic penumbra and core (3-7 days post-ischemia), a period characterized by brain-blood barrier breakdown and nestin expression. AIA express markers of immature glia, are not originated from myeloid precursors, proliferate in vitro with high cell division rate, show reduced senescence and grow in the presence of macrophages within the limits imposed by the glial scar. AIA conditioned medium induce reactive gliosis on resting glia and facilitate neuronal death of OGD-exposed neurons. When cultured AIA are re-introduced in normal brains, they induce focal reactive gliosis and persist as clustered cells. We propose that AIA is a highly reactive and less differentiated astroglial subtype induced by the injury and likely to be involved in the propagation of reactive gliosis and neuroinflammation.

Supported by grants PICT 2012-1424, CONICET, UBACYT

"Mag as key player of the bi-directional communication between axons and myelin: its impact on postnatal development and disease"

Pablo Lopez

Instituto de Investigación Médica Mercedes y Martín Ferreyra, CONICET, Argentina

The identities of molecules that contribute to the nurturing effects of myelin on neurons have emerged over the past several years. One such molecule is MAG, a minor component of the nervous system preferentially expressed on the periaxonal layer of myelinated axons. MAG regulates axonal caliber, controls the distribution of molecules at nodes of Ranvier and promotes axon stability under physiological conditions. MAG can also mediate signals coming from the axons that strongly impact on oligodendrocytes (OL), highlighting the bidirectional nature of axon-myelin communication. We have recently identified MAG as a new regulatory component on the apoptosis of motoneurons (MNs) during their postnatal development. Thus, the protection exerted by MAG emerges as a critical factor to maintain survival of MNs during the first postnatal week, a period during which these neurons remain sensitive to deprivation of neurotrophins from their end-target organ. Interestingly, the anti-apoptotic signaling triggered by MAG seems to recapitulate the developmental program of MNs by activating RhoA/ROCK signaling pathway downstream of the low affinity neurotrophin receptor p75NTR. On the other hand preliminary results from our lab unmasked a novel mechanism modulating high extracellular glutamate (Glu) concentrations in the central nervous system triggered by activation of MAG at the cell membrane of OL. Antibody-mediated activation of MAG prevented neuronal and OL death in cerebellar organotypic cultures exposed to high Glu. The molecular mechanisms underlying this effect were further studied in primary OL cultures using FRET-based Glu biosensors and pharmacological inhibitors of Glu transport. We identified that MAG's activation leads to an increase in intracellular Glu by OL and further conversion to glutathione, the main cellular antioxidant. This was prevented by pharmacological inhibitors of Glu transporters such as System Xc and EAAT1. The protective role of MAG's activation against Glu-mediated toxicity was further confirmed in a murine

model of Multiple Sclerosis, where increasing evidence involved altered Glu homeostasis as a cause of axonal damage. Finally, our results highlight the relevance of an intact axon-myelin communication, expanding our knowledge about the protective properties of myelinating cells in the nervous system.

DAY 2: Wednesday September 30th, 08:30-11:00

Symposium 3 - Room Atlantic

"2015, The International Year of Light: Superresolution microscopies in Neuroscience"

Chair: **Francisco Barrantes**, Instituto Investigaciones Biomédicas, UCA–CONICET, Argentina

"Nanoscopy 2.0. Converging and correlative technologies"

Alberto Diaspro

Department of Nanophysics at the Istituto Italiano di Tecnologia, Genova, Italy

Three-dimensional (3D) fluorescence optical microscopy had a tremendous development in the last thirty years, related to the different converging approaches and technologies. Confocal and multi-photon microscopy pushed the optical sectioning ability of getting 3D information to 3D imaging of thick specimens, including organs and tissues]. The temporal dimension is naturally added towards 4D (x-y-z-t) bioimaging. The discovery andutilization of green fluorescent proteins opened new possibilities[An incredible advance came when unlimited resolution in space was demonstrated. Terms like super resolution microscopy, super or ultra-microscope, optical nanoscopy refer to the possibility of producing images at an unlimited spatial resolution, in principle. One could use the information capacity theory here: sub-diffraction or super resolution can be obtained by encoding information from the saturated spatial channel of the microscope system into the temporal channel and decoding after the collection]. This is the scenario for the new paradigm of fluorescence microscopy that can operate at the 10-50 nm scale surpassing the 200 nm of the optical microscope in x-y and at the 50 nm level along the z-axis also in a dynamic context. We will discuss targeted and stochastic readout methods expanded to multi-photon excitation (MPE)/absorption in a 4D framework. Further advances can be obtained by contamination with information communication alghoritms. Following the recent Nobel Prize in Chemistry awarded to E.Betzig, S.W.Hell and W.E.Moerner, we can say that

Nanoscopy 2.0 is the great immediate challenge. A variety of architectures will be outlined and further variations on the super resolution theme addressed including correlative nanoscopy.

"Visualizing the details of neuronal polarity with fluorescence nanoscopy"

Fernando Stefani
Centro de Investigaciones en Bionanociencias, Buenos Aires, Argentina

Neuronal polarity has been widely studied both in-vitro and, more recently in-situ. However, the precise sequence of events, as well as the cellular and molecular mechanisms behind the generation of axons and dendrites remain largely unknown. Based on the available information, it is currently accepted that at least three factors are involved in neuronal polarization: extracellular stimuli, intracellular signaling cascades, and subcelular reorganization including cytoskeleton NCnd membranous organelles. One of the reasons why vital information remains inaccessible is the fact that many of the cellular components involved, such as microtubuli (MT), microfilaments (MF) or transport vesicles, have dimensions well below the diffraction resolution limit. Although electron microscopy provides the required resolution, its use for the investigation of neuronal polarity has been compromised because the sample preparation procedures are prone to damage key cellular components. More importantly, studies of the dynamics of citosqueleton and membrane traffic, which are essential for the understanding of neuronal polarization, are not possible with electron microscopy. Based on these considerations we have started a collaborative effort to investigate the mechanisms of neuronal polarity using fluorescence nanoscopy. The following aspects of axon development will be investigated:

1) Organization and dynamics of cytoskeleton in growth cones
2) Organization and dynamics of cytoskeleton in axon initial segments (AIS)
3) Post-Golgi membrane transport

We will present our latest results on the investigation of these three aspects of neuronal polarity applying STED and PALM/STORM nanoscopy methods to visualize with super-resolution neurons of primary cultures, grown under experimental conditions that modify the rate and pattern of axonal development.

"Social organization of neurotransmitter receptor nanoclusters"

Francisco Barrantes

Instituto Investigaciones Biomédicas, UCA–CONICET, Argentina

Precise targeting, organization and maintenance of an adequate number of neurotransmitter receptors, key for efficient synaptic transmission, rely on an adequate balance between synthesis, delivery to and removal from the cell membrane. We are interested in the supramolecular organization, dynamics and trafficking of neuronal- and muscle-type receptors and study these proteins using a combination of ensemble averaging methods and single-molecule experimental techniques.

Targeted super-resolution optical microscopy (nanoscopy) techniques like STED (Eggeling, Willig & Barantes, 2013) or STORM and PALM, two variants of single-molecule stochastic localization nanoscopy (Willig & Barrantes, 2014), are increasingly gaining momentum to characterize molecular constituents of the synapse. Application of such techniques to fixed specimens of hippocampal and cerebral cortex neurons has enabled us to image individual, nanometer-sized supramolecular assemblies ("nanoclusters") of nicotinic acetylcholine receptors (nAChRs) beyond the diffraction limit, and characterize their "social habits", i.e. their long-range interactions, which extend to micrometer lengths. In combination with single-particle tracking techniques, it was possible to follow the dynamics of the nAChR assemblies in living cells, and determine their diffusion coefficients. The neutral lipid cholesterol and cortical actin dynamics are found to act synergically to modulate the supramolecular organization and underlying forces governing the architecture of nAChRs at the cell surface.

"Resolft microscopy of living cells in the nervous system"

Ilaria Testa

Royal Institute of Technology, Stockholm, Sweden

RESOLFT fluorescence microscopy is a low light level superresolution technique employing reversibly switchable fluorescent proteins (rsFPs). The improved spatial resolution is accomplished by switching rsFPs such as Dronpa, rsEGFPs and mCherry variants between a dark (OFF) and an active (ON) state multiple times along the sample. The RESOLFT concept was demonstrated with a single ring-shaped beam as well as in a parallelized mode with a periodic line pattern to speed up the acquisition of large field of view. The gentle illumination required for the RESOLFT approach is suitable for live cell imaging especially for long-term recording of protein dynamics in dissociated neurons as well as in brain tissues.

Symposium 4 - Room Topacio

"*Translational approaches to model and treat SNC diseases*"

Chair: **Elena Avale** and **Marcelo Rubinstein**, Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, Dr Héctor N. Torres, CONICET, Argentina

"Levodopa induced dyskinesias: from classical pharmacology to molecular mechanisms"

Oscar Gershanik

Instituto de Neurociencias de la Fundación Favaloro, Instituto de Investigaciones Farmacológicas, CONICET, Argentina

Parkinson's disease (PD) is a neurodegenerative disorder which results from the selective death of nigrostriatal dopaminergic neurons. The administration of L-DOPA is the most effective symptomatic pharmacological therapy. Despite of its benefits, most patients develop side effects known as L-DOPA induced dyskinesias (LID). The current great challenge in PD therapy is to control LID. To reach this goal it is necessary to better comprehend the multiple cellular and molecular mechanisms that take place during LID. Although some protein and gene changes have been described into the dyskinetic striatum, the functions and/or mechanism on which they are involved are not fully understood. In our laboratory we have been analysing some of the molecular intermediates involved in the development of LIDs. We are currently validating a novel target with potential drugability to manage LIDs in PD patients.

"Alzheimer's disease, from a dish to the bed"

Gorazd Bernard Stokin

St. Anne's University Hospital, Brno, Czech Republic, Division of Neurology, University Medical Centre, Ljubljana, Slovenia

Alzheimer`s disease is the most common neurodegenerative disorder characterized clinically by behavioral changes and cognitive decline and pathologically by the aberrant accumulation of amyloid and tau proteins in the form of senile plaques and neurofibrillary tangles, respectively. The major risk factor for the development of Alzheimer`s disease is aging. Considering significant increase in the average age reached by the world population, the incidence and prevalence of Alzheimer`s disease have been continuously growing. This increase in Alzheimer`s disease represents a major burden of today`s society, in particular, in terms of the financial burden it imposes to the healthcare budgets. Despite advances in understanding Alzheimer`s disease, specifically dementia of Alzheimer`s type, several questions remain insufficiently addressed. First, early clinical phenotypes characteristic of Alzheimer`s disease remain poorly defined. Second, although several diagnostic biomarkers of Alzheimer`s disease have been recently proposed, further work is required to identify and validate the most specific and informative ones. Third, mechanisms leading to the development of Alzheimer`s disease remain poorly understood. Without better understanding of these mechanisms it will be difficult, if not impossible, to develop efficient therapeutic strategies to slow down or cure Alzheimer`s disease. Long-lasting focus of Alzheimer`s disease research has been understanding the mechanisms responsible for and consequences of aberrant accumulation of amyloid and tau proteins. To date, despite major effort in deciphering the mechanisms of aberrant protein accumulation in Alzheimer`s disease linked to rare autosomal dominant mutations, the causes of the most frequent sporadic

Alzheimer`s disease remain unclear. Accumulating evidence suggests, however, that development of sporadic Alzheimer`s disease can be the result of impairments in axonal transport. While early studies of impaired axonal transport focused on the analysis of post-mortem Alzheimer`s disease brain tissue and explored animal models of Alzheimer`s disease, current advances in stem cell derived neuronal populations provide an excellent human model to

address questions relevant to the pathogenesis of Alzheimer`s disease in a more physiological setting further.

"What Can Movement Circuitry Tell Us About Obesity?"

Alexxai Kravitz

NIDDK, NIDA, USA

Obesity rates are increasing worldwide. The conventional wisdom for preventing and treating obesity is to "eat less and exercise more". While these solutions are logical from an energy balance perspective, they are very difficult for people to maintain. In short, the clinical literature provides many examples of dieting and/or exercise resulting in robust short-term weight loss (<1 year), which inevitably regresses towards the pre-intervention weight over longer durations. We believe that meaningful solutions for changing these behaviors (or at least understanding why they are so difficult to change), will require an understanding of the brain circuitry that mediates them. I will focus on one question in this talk: Why are obese animals less active than lean animals? Like obese humans, obese mice exhibit greatly reduced levels of physical activity. We have linked this to a deficit in dopamine signalling through the D2 receptor on the striatal indirect pathway medium spiny neurons (iMSNs) of obese mice. Using a combination of genetics and cell-type specific manipulations, we have found that this D2 receptor deficit is both sufficient and necessary for reducing the physical activity of obese animals. In ongoing work, we are investigating how altering the physical activity level of lean and obese animals impacts both energy expenditure and weight gain.

"Phenotypic rescue in a mouse model of tauopathy using trans-splicing RNA reprogramming"

Elena Avale

Instituto de Investigaciones en Ingeniería Genética y Biología Molecular

Dr Héctor N. Torres, CONICET, Argentina

Tauopathies are major neurodegenerative diseases, characterized by the presence of intraneuronal aggregates of the protein tau in insoluble neurofibrillary tangles (NFTs). In the normal brain, Tau is a microtubule-associated protein predominantly expressed in neurons, which participates in a myriad of cellular functions such as microtubule polymerization and stabilization, neurite outgrowth and axonal transport. Human Tau is encoded by the MAPT gene, which comprises 16 exons. Alternative splicing of exons 2, 3 and 10 gives rise to six tau isoforms in the adult brain. Particularly, the alternative splicing of exon 10 (E10) produces tau isoforms with three (3R) or four (4R) microtubule binding repeats. Tau 3R and tau 4R are expressed in equal amounts in the normal adult human brain. Several tauopathies, such as frontotemporal dementia associated to chromosome 17 (FTDP-17), are associated with mutations in the MAPT gene which interfere with exon 10 alternative splicing, leading to an imbalance between the 3R and 4R isoforms, and thus disrupting the normal 3R/4R ratio. Correction of that imbalance between Tau isoforms might represent a plausible therapeutical approach for those tauopathies. In this talk I will summarize our achievements in using an RNA reprogramming strategy to modulate Tau 4R/3R ratio in vivo. We used a mouse model of tauopathy carrying the human MAPT gene, the hTau mouse, which displays an excess of Tau 3R. hTau mice phenotypes include the presence of NFTs and neurodegeneration in the cortex and hippocampus from 9 months old, and cognitive impairment from 12 months old. To restore Tau 3R/4R balance we modulated the inclusion of Tau E10 in hTau mice, by a trans-splicing reaction between an endogenous mRNA and an exogenously delivered RNA pre-trans-splicing molecule (PTM). htau mice rescued by trans-splicing performed normally in cognitive tests and displayed lower Tau aggregates. So far, our results provide evidence for the potential use of RNA reprogramming to correct tau mis-splicing to treat some human tauopathies.

DAY 3: Thursday October 1st, 08:30-11:00

Symposium 5 - Room Atlantic

"Neurophysiology of conscious states"

Chair: **Mariano Sigman**, Universidad Torcuato Di Tella and **Jacobo Sitt**, ICM Research Center, Pitié Salpêtrière Hospital, Paris, France

"Broadband Cortical Desynchronization Underlies the Human Psychedelic State"

Robin Carhart-Harris

University College London, England

Psychedelic drugs produce profound changes in consciousness, but the underlying neurobiological mechanisms for this remain unclear. Spontaneous and induced oscillatory activity was recorded in healthy human participants with magnetoencephalography after intravenous infusion of psilocybin—prodrug of the nonselective serotonin 2A receptor agonist and classic psychedelic psilocin. Psilocybin reduced spontaneous cortical oscillatory power from 1 to 50 Hz in posterior association cortices, and from 8 to 100 Hz in frontal association cortices. Large decreases in oscillatory power were seen in areas of the default-mode network. Independent component analysis was used to identify a number of resting-state networks, and activity in these was similarly decreased after psilocybin. Psilocybin had no effect on low-level visually induced and motor-induced gamma-band oscillations, suggesting that some basic elements of oscillatory brain activity are relatively preserved during the psychedelic experience. Dynamic causal modeling revealed that posterior cingulate cortex desynchronization can be explained by increased excitability of deep-layer pyramidal neurons, which are known to be rich in 5-HT2A receptors. These findings suggest that the subjective effects of psychedelics result from a desynchronization of ongoing oscillatory rhythms in the cortex, likely triggered by 5-HT2A receptor-mediated excitation of deep pyramidal cells.

"Large-scale brain dynamics and connectivity across different statesof consciousness"

Enzo Tagliazucchi
Netherlands Institute for Neuroscience, Netherlands

The human brain is continuously active, even during states such as deep sleep or under anesthesia. It is currently unknown which features of this spontaneous activity represent signatures of conscious awareness and which may correspond to a dynamical and ever-changing baseline. To answer these questions we conduct large-scale multi-modal and non-invasive recordings of brain anatomy and function during different states of conscious awareness including wakefulness, sleep, anesthesia and epilepsy. Analysis of this data consistently reveals localised changes in dynamics underlying states of altered consciousness, including a shift towards less complex temporal fluctuations, loss of spatial coherence and a decoupling of functional and structural connections. These empirical observations can be generally understood by a departure from the critical state in a conceptual model incorporating realistic anatomical connectivity.

"Putting control back into free will"

John Dylan-Haynes
Bernstein Center for Computational Neuroscience, Berlin, Germany

In humans, spontaneous decisions are often preceded by choice-predictive brain signals. We investigate these predictive signals in a series of experiments. In one line of work we used functional magnetic resonance imaging (fMRI) and found that predictive signals arise from multiple regions at a very early stage, several seconds before a conscious decision. This was not only the case for motor decisions but also for abstract decisions related to mental calculation. In another line of work we investigated whether these predictive signals occur unavoidably, as a part of a causal chain leading to an action, or whether they might be under the control of the subject. We addressed this question by testing whether a participant could move while avoiding being predicted from their readiness potential, a movement-related EEG signal. Subjects played a game where they tried to press a button to earn points in a duel with a brain-computer interface (BCI) that had been trained to predict their movements in real-time and to emit stop signals. The topography, amplitude and time course of the readiness potential were not affected by subjects' attempts at being unpredictable, suggesting that the readiness potential itself could not be controlled. Our data furthermore show that movements could be cancelled if stop signals occurred at an early, but not at a late stage of movement preparation. Taken together, our data suggest that subjects cannot elicit movements without generating a stereotypical readiness potential. However, even after onset of the readiness potential, movements can still be cancelled ("vetoed") until a very late stage.

"Imaging neural signatures of consciousness: 'What', 'When', 'Where' and 'How' does it work?"

Lionel Naccache
ICM Research Center, Paris, France

'What' do we call consciousness? 'When' and 'Where' in the brain do conscious states occur, and 'How' conscious processing and conscious access to a given content work? In the present paper, we present a non-exhaustive overview of each of these 4 major issues, we provide the reader with a brief description of the major difficulties related to these issues, we highlight the current theoretical points of debate, and we advocate for the explanatory power of the "global workspace" model of consciousness (Baars, 1989; Dehaene and Naccache, 2001; Dehaene et al., 2006) which can accommodate for a fairly large proportion of current experimental findings, and which can be used to reinterpret apparent contradictory findings within a single theoretical framework. Most notably, we emphasize the crucial importance to distinguish genuine neural signatures of conscious access from neural events correlated with consciousness but occurring either before ('upstream') or after ('downstream').

Symposium 6 ISN Symposium - Room Topacio

"Regulation of neuronal ion channels by G protein coupled receptors"

Chair: **Jesica Raingo**, Instituto Multidisciplinario de Biología Celular, CONICET, La Plata, Argentina.

"Neuronal calcium channels: From one to thousands"

Diane Lipscombe

Brown University, Providence, USA

Cell-specific pre-mRNA processing greatly expands the coding capacity of genes, and the resulting pool of mRNA splice isoforms in specific tissues carries a richer source of information about the function and the state of the cell than the genotype alone. Functional and pharmacological differences among protein isoforms originating from the same gene may be quite subtle but nonetheless of critical physiological significance. I discuss the function of tissue-specific alternative splicing of voltage-gated calcium ion channel pre-mRNAs. The amino acid composition of discrete regions of voltage-gated CaV channels, originating from cell-specific alternative splicing, can vary substantially across isoforms in different tissues. Cell-specific alternative splicing can modify the pharmacological sensitivities of CaV channels to drugs, toxins and G protein coupled receptors.

"Receptors coupled to Gq modulate ion channels by lipid signaling"

Bertil Hille
Washington University, Seattle, USA

Receptors coupled to Gq activate phospholipase C. In the traditional view, they modulate ion channels by generating Ca2+ signals and by activating protein kinase C. Now we understand that they also act by significantly depleting the plasma membrane lipid phosphatidylinositol 4,5-bisphosphate. This rare lipid is a permissive signal needed by many channels. I will discuss the cell biology of phosphoinositide lipids and their regulation of K+ and Ca2+ channel activity.

"Novel clustering of diverse ion channels in neurons mediated by AKAP79/150"

Mark Shapiro

University of Texas Health Science Center, San Antonio, USA

Multi-protein complexes have emerged as a mechanism for spatiotemporal specificity and efficiency in the function and regulation of myriad cellular signals. In particular, many ion channels are clustered either with the receptors that modulate them, or with other ion channels whose activities are linked. Often, the clustering is mediated by scaffolding proteins, such as AKAP79/150. We focus on three different channels critical to nervous function. One is the "M-type" (KCNQ, Kv7) K+ channel that plays fundamental roles in the regulation of excitability in nerve and muscle. It is thought to associate with Gq/11-coupled receptors, protein kinases, calcineurin (CaN), calmodulin (CaM) and phosphoinositides via AKAP79/150. Another channel of focus is TRPV1, a nociceptive channel in sensory neurons that is also thought to be regulated by signaling proteins recruited by AKAP79/150. The third are L-type Ca2+ (CaV1.2) channels that are critical to synaptic plasticity, gene regulation and neuronal firing. We probe complexes containing AKAP79/150 and these three channels using "super-resolution" STORM imaging of primary sensory neurons and heterologously-expressed tissue-culture cells, in which individual complexes can be visualized at 10-20 nm resolution with visible light, breaking the diffraction barrier of physics. We hypothesize that AKAP79/150 brings several of these channels together to enable functional coupling, which we examine by patch-clamp electrophysiology of the neurons. Since all three of these channels bind to AKAP79/150, we hypothesize that they co-assemble into complexes in neurons, together with certain G protein-coupled receptors. This talk breaks new ground into the physiology of signaling in neurons, using several cutting-edge, high-powered approaches that have just recently been developed.

"New regulation of neuronal calcium channels by G protein coupled receptors constitutive activity"

Jesica Raingo

Instituto Multidisciplinario de Biología Ce CONICET, La Plata, Argentina

A long-held tenet of presynaptic voltage gated calcium channels (CaV2.1 and CaV2.2) modulation is that canonical signal transduction mediated by G protein coupled receptor (GPCR) is narrowed to the agonist-induced receptor activation. Here we are presenting compelling support for a novel CaV2 channels regulation by the agonist-independent constitutive activity of a GPCR. We found that constitutive activity of the ghrelin receptor (GHSR1a) and the melanocortin type 4 receptor (MC4R) dramatically decrease CaV2 currents in neurons. This is a newly discovered mode of calcium current influx long lasting control in neurons.


Gador
Al Cuidado de la Vida

MEETING/YOUNG INVESTIGATOR SYMPOSIA

DAY 3: Thursday October 1st, 11:30-13:00

Young Investigator Symposium 1 - Room Atlantic
Chair: **Diego Gelman**, Instituto de Biología y Medicina Experimental, CONICET, Argentina

"The BDNF prodomain (pBDNF) induces neuronal morphological changes associated with neuropsychiatric and neurodegenerative diseases"

Agustin Anastasia

Instituto de Investigación Médica Mercedes y Martín Ferreyra, CONICET, Argentina

Brain-derived Neurotrophic Factor (BDNF), which is the most highly expressed and studied neurotrophic factor in the mammalian brain, is translated as a precursor protein (proBDNF) that is cleaved to two peptides known as the BDNF prodomain (pBDNF) and mature BDNF (mBDNF). There is a common human single nucleotide polymorphism (SNP) in the BDNF gene which leads to a valine for methionine substitution (Val66Met) within the pBDNF sequence. The Val66Met SNP is present in more than 25% of the human population, and is associated with cognitive deficits and increased risk for neuropsychiatric and neurodegenerative disorders. We have recently shown, for the first time, that the Met66 pBDNF is an abundant active secreted ligand in the hippocampus that induces neuronal growth cone retraction (Anastasia et al., 2013. Nature communications 4:2490), dendritic spine collapse and consequently circuitry remodeling in this brain area. Surprisingly, pBDNF is also very abundant in the cerebrospinal fluid of adult humans. Utilizing nuclear magnetic resonance and other biophysical approaches we found that the Val66Met substitution induces structural changes in pBDNF that are responsible for the functional differences. We conclude that the neuronal morphology remodeling induced by the Met66 pBDNF, which is mediated by a receptor complex of SorCS2 and p75NTR, is

a mechanism that contributes to the altered neuronal plasticity in humans with the SNP. Therefore, our research offers a molecular explanation for the increased incidence of neuropsychiatric and neurodegenerative disorders in Met66 human carriers, and provides a new candidate target for therapeutic intervention.

"Recovery of locomotor activities in Spinal Cord Injury: A novel treatment to produce intra-axonal decrease of peroxide levels and reactivation of cytoskeleton dynamics in damaged axons"

Ramiro Quintá

Instituto de Química y Fisicoquímica Biológicas, CONICET, Argentina

The axonal growth cone collapse following spinal cord injury (SCI) is promoted by Semaphorin 3A (Sema3A) signalling via PlexinA4 surface receptor. This signalling triggers intracellular pathways that promote an increase of hydrogen peroxide levels and F-actin destabilization, inducing collapse and inhibition of axonal re-growth. Recently, we demonstrate for first time that Galectin-1 (Gal-1) an endogenous glycan-binding protein, in its dimeric form, promotes functional recovery of spinal lesions by interfering with inhibitory signals triggered by Semaphorin 3A (Sema3A) binding to Neuropilin-1/PlexinA4 complex (Quintá et. al. 2014). However, the intra-cellular mechanism of this process is totally unknown. Therefore, in this study, we successfully demonstrate that, only Galectin-1 (Gal-1) treatment, in its dimeric form but not in the monomeric form promotes the decrease of hydrogen peroxide levels and a re-polimerization of F-actin in the growth cone and in the filopodium of whole neurons. This treatment avoids the hydrogen peroxide production by Sema3A/plexinA4 signalling, and therefore avoids the growth cone collapse by F-actin depolimerization. To promote decrease of hydrogen peroxide levels, Gal-1 in its dimeric form needs in your structure an intact carbohydrate recognition domain. Furthermore, Gal-1 promotes an active endocytosis of PlexinA4 receptor, leaving the neuronal surface less sensitive to Sema3A effects. In summary, our results suggest that Gal-1 in its dimeric form promotes a re-activation of actin cytoskeleton dynamic by decreasing the peroxide levels via internalization of PlexinA4 receptor. This mechanism would explain not only the full axonal re-growth process but also, a "de novo" formation of synapses clustering, a axonal re-mielinization process and functional recovery of locomotor activities in an in-vivo SCI model (acute and chronic).

"A two-pronged approach against Alzheimer's disease neurodegeneration: amyloid-beta synthesis and clearance are both regulated by glial metabotropic glutamate receptor"

Daniela Durand

Instituto de Investigaciones Biomédicas, CONICET, Argentina

Astrocytes are now undoubtedly endorsed as key players in the maintenance of CNS functionality and plasticity and they may aid in the resolution of neuronal damage caused by several injuries. Group II Metabotropic Glutamate Receptors (mGluR) includes mGlu3R and mGlu2R subtypes, among which only mGlu3R is expressed in astrocytes. Increasing evidence suggests that astroglial mGlu3R activation leads to neuroprotective effects. Here we show that mGlu3R activation by LY379268 promotes the 'alpha' or non-amyloidogenic cleavage of amyloid precursor protein (APP) in cultured astrocytes1, which leads to increased release of the neuroprotective soluble fragment sAPPα, while impairing amyloid beta (Aβ) production. This effect is related to the increase in ADAM10 and ADAM17 levels and the reduction in BACE1 expression, and is dependent on PPAR-γ activation1. mGlu3R activation also induces BDNF expression in astrocytes. Furthermore, mGlu3R expression was significantly diminished in hippocampal astrocytes from PDAPP-J20 transgenic mice1, a well-established model of AD, a finding which is consistent with a role for these receptors in avoiding AD progression. We next evaluated the impact of mGlu3R-induced glial soluble neurotrophins on Aβ-challenged hippocampal neurons. Using immunodepleted conditioned media we found that astrocyte-derived sAPPα and BDNF are both involved in neuroprotection exerted by astroglial mGluR3 activation after Aβ insult. Interestingly, mGlu3R activation in both astrocytes and microglia increases the rate of Aβ and latex bead uptake. Altogether these results indicate a double function for glial mGlu3R activation against Aβ neurotoxicity: (i) it promotes α-cleavage of APP and increases sAPPα and BDNF levels, and (ii) it induces amyloid removal from the extracellular space by glial phagocytic mechanisms. These findings suggest that glial mGlu3R activation may hold therapeutic value in AD.

1Durand et al 2014 Neuropharmacol.79:180-189

"Progesterone prevents chronic pain after spinal cord injury"

María Florencia Coronel
Instituto de Biología y Medicina Experimental, CONICET, Argentina

Neuropathic pain develops in nearly 70% of patients with spinal cord injury (SCI). Currently available pharmacotherapy has limited efficacy and adverse side effects. We have recently shown that progesterone (PG), a neuroprotective steroid, may offer a promising perspective in neuropathic pain modulation. After SCI a state of central sensitization is established at the dorsal horn, involving both the hyperexcitability of neurons in the pain pathway, mainly mediated by glutamate NMDA receptor, and the activation of glia cells, with the subsequent release of pro-nociceptive mediators. By using biochemical, immunohistochemical and molecular techniques, we investigated the impact of SCI and PG administration on several markers of neuronal and glial cell activation at the dorsal horn level. We also evaluated the development of allodynia (pain elicited by innocuous stimuli). SCI-animals developed mechanical and thermal allodynia in their hindpaws. Interestingly, rats receiving PG did not display allodynic behaviors. After SCI, a significant increase in the mRNA levels of NMDA receptor subunits (NR1, NR2A, NR2B) and PKCγ, enzyme involved in the activation of NR1, was observed in the dorsal spinal cord. In addition, a marked increase in the number of NR1, pNR1 and PKCγ immunoreactive neurons was detected. In correlation with the observed attenuation of pain, injured animals treated with PG showed reduced expression of all these markers. PG administration also attenuated the injury-induced increase in the number of GFAP-positive astrocytes and OX42-positive microglial cells. Furthermore, PG-treated animals presented significantly lower mRNA levels of the proinflammatory cytokines IL-1β, IL-6 and TNF-α, their receptors, and the proinflammatory enzymes COX-2 and iNOS, as compared to vehicle-treated injured rats. Our results suggest that PG, by modulating neuronal excitability and glial activation triggered after SCI, may represent a useful strategy to prevent neuropathic pain.

Young Investigator Symposium 2 - Room Topacio
Chair: **Rafael Pagani**, Instituto de Fisiología y Biofísica Bernardo Houssay, CONICET, Argentina

"Theta-oscillations in visual cortex emerge with experience to convey expected reward time and experienced reward rate"

Camila Zold

Instituto de Fisiología y Biofísica Bernardo Houssay, CONICET, Argentina

The primary visual cortex (V1) is widely regarded as faithfully conveying the physical properties of visual stimuli. Thus, experience induced changes in V1 are often interpreted as improving visual perception (i.e., perceptual learning). Here we describe how, with experience, cue-evoked oscillations emerge in V1 to convey expected reward time as well as to relate experienced reward rate. We show, in chronic multi-site local field potential recordings from rat V1, that repeated presentation of visual cues induces the emergence of visually evoked oscillatory activity. Early in training, the visually evoked oscillations relate to the physical parameters of the stimuli. However, with training, the oscillations evolve to relate the time in which those stimuli foretell of expected reward. Moreover, the oscillation prevalence reflects the reward rate recently experienced by the animal. Thus, training induces experience-dependent plastic changes in V1 activity that relate to what those stimuli have come to signify behaviorally: when to expect future reward and at what rate.

"VTA and LC control protein synthesis required for long-term memory formation during the behavioral tagging process"

Diego Moncada

Instituto de Biología Celular y Neurociencia, Prof. E. De Robertis, UBA-CONICET, Argentina

Different works have shown that long-term memory (LTM) formation relies on a behavioral tagging (BT) process. In other words, to establish a lasting memory at least two parallel processes must occur: the setting of a learning tag (triggered during learning) that defines where a memory could be stored, and the synthesis of proteins, that once captured at tagged sites will effectively allow the consolidation process to occur. This work focused in studying which brain structures are responsible of controlling the synthesis of those proteins at the brain areas where memory is being stored. It combines electrophysiological activation of the ventral tegmental area (VTA) and/or the locus coeruleus (LC), with local pharmacological interventions and weak and strong behavioral trainings in the inhibitory avoidance (IA) and spatial object recognition (SOR) tasks, in rats. The results presented here show that the VTA is a brain structures responsible of regulating the consolidation of these memories acting through the D1/D5 dopaminergic receptors of the hippocampus to control the synthesis of new proteins required for this process. Moreover, they provide evidence that the LC may be a second structure with a similar role, acting independently and complementary to the VTA, through the β-adrenergic receptors of the hippocampus.

"Dilp8 requires the neuronal relaxin receptor Lgr3 to couple growth to developmental timing"

Andres Garelli

Instituto de Investigaciones Bioquímicas Bahía Blanca, CONICET, Argentina

Developmental stability is the ability of an organism to buffer given traits against environmental and intrinsic perturbations and produce stable genetically determined phenotypes. The processes leading to developmental stability involve physiological, temporal or behavioral adjustments to the developmental program that have been particularly well studied in insects. For instance, if uncoordinated growth occurs in Drosophila imaginal discs, the larval precursors of adult structures, a transient delay in the onset of metamorphosis ensues, allowing extra time for all discs to achieve their specific size. We have recently identified dilp8, a fly specific insulin-like peptide that is produced in damaged imaginal discs and couples tissue growth with developmental timing. Dilp8 transiently delays the onset of metamorphosis by inhibiting the biosynthesis of the molting hormone ecdysone while simultaneously slows down growth of undamaged tissues. Thus, the prolonged larval phase allows tissue regeneration while keeping proportions with unaffected discs and results in proportionate adults. Accordingly, loss of dilp8 increases intra-individual asymmetry(1). However, which molecules and tissues sense and/or transmit this abnormal growth signal remained unknown. We have now found that mutation of Lgr3, a member of the type C1 Leucine-rich repeat-containing G-protein coupled receptors, results in body asymmetries similar to that of dilp8 mutants and the inability to delay development in response to tissue damage. By tagging the endogenous Lgr3 protein with GFP we found that it is expressed and required in a subpopulation of CNS neurons not previously linked to growth control. Our work places Dilp8 and Lgr3 as central players in the interorgan communication system that mediates plasticity to promote developmental stability in Drosophila and reveals a novel neuroendocrine circuit responsive to growth aberrations.

(1)Garelli et al, Science 2012 May 4.

"Muscarinic regulation of dopamine and glutamate transmission in the nucleus accumbens"

Martín F. Adrover

National Institute on Alcohol Abuse and Alcoholism, NIH, Bethesda, USA

Increases in dopamine (DA) concentration in the nucleus accumbens (NAc) are required for reward seeking, motivation and motor control (1). DA is released from the axonal projections of midbrain DA neurons to the NAc and the dorsal striatum, where it regulates synaptic plasticity to influence striatal micro-circuitry (2). Cholinergic transmission in the striatum functions as a key modulator of DA transmission and synaptic plasticity, both of which are required for reward and motor learning. Acetylcholine (ACh) can elicit striatal DA release through activation of nicotinic ACh receptors (nAChRs) on DA axonal projections (3,4). However, it remains controversial how muscarinic ACh receptors (mAChRs) modulate striatal DA release, with studies reporting both potentiation and depression of striatal DA transmission by mAChR agonists (5,6). This study investigates the mAChR-mediated regulation of release from three types of midbrain neurons that project to striatum: DA, DA/glutamate, and glutamate neurons. We found that M5 mAChRs potentiate DA and glutamate release only from DA and DA/glutamate projections from the midbrain. We also show that M2/M4 mAChRs depress the nAChR-dependent mechanism of DA release in the striatum. These results suggest that M5 receptors on DA neuron terminals enhance DA release, whereas M2/M4 autoreceptors on cholinergic terminals inhibit ACh release and subsequent nAChR-dependent DA release. Our findings clarify the mechanisms of mAChR-dependent modulation of DA and glutamate transmission in the striatum.

1. Saddoris et al., 2003, Front Biosci (Elite Ed) 5:273–288.
2. Lerner TN, Kreitzer AC, 2011, Curr Opin Neurobiol 21(2):322–327
3. Cachope et al., 2012, Cell Reports 2(1):33–41.
4. Threlfell, et al., 2012, Neuron 75(1):58–64.
5. de Belleroche JS, Gardiner IM, 1982, Br J Pharmacol 75(2):359–365.
6. Kudernatsch M, Sutor B, 1994, Neurosci Lett 181(1-2):107–112.


ELEA
NEUROCIENCIAS

DAY 2: Wednesday September 30th, 11:30-13:15

Short Talks by Students 1 - Room Atlantic

Chair: **Estela Maris Muñoz**, Instituto de Histología y Embriología "Dr. Mario H. Burgos" and **Pablo Helguera**, Instituto de Investigación Médica Mercedes y Martín Ferreyra, CONICET, Argentina

"Unraveling the Role of GABAergic-Proopiomelanocortin Neurons in the Hypothalamic Control of Energy Balance"

Milagros Trotta

Instituto de Fisiología y Biofísica Bernardo Houssay, CONICET, Argentina

Obesity affects almost half a billion people worldwide. Because of the high prevalence of overweight and the severity of its comorbidities, great effort is made to unravel the neuronal circuits controlling energy balance. Hypothalamic Proopiomelanocortin (POMC) neurons are main regulators of energy balance. POMC deficient patients and mice are hyperphagic and obese. Since there are both GABAergic and glutamatergic POMC hypothalamic neurons, we speculate that both subpopulations have different connections and physiological roles. In order to characterize GABAergic-POMC neurons, we used a reversible knockout mouse model of early-onset obesity that lacks POMC expression in the hypothalamus. In this model, subsequent reactivation of POMC expression specifically in GABAergic neurons can be conditionally achieved. Before treatment at P60, hyperphagic knockout mice were ~60% heavier than their WT littermates, whereas six weeks after POMC rescue this difference dropped to ~22% and food intake completely normalized. Surprisingly, less than 20% of POMC hypothalamic neurons recovered POMC expression in these experiments, all of which are GABAergic cells. Altogether, our results suggest that the subpopulation of GABAergic POMC neurons - is probably the main regulator of food intake among POMC cells. Further experiments are undertaken to identify the target neurons of GABAergic-POMC neurons in order to elucidate the brain circuits in which they are involved.

"ASCL1 regulates late neurogenic events in the ventral neural tube"

Daniela Di Bella

Fundación Instituto Leloir, Argentina

Despite the effort invested in the last decades, the regulatory networks that control lineage specification in the developing central nervous system are not completely understood. We have identified a late-born population of spinal neurons that originate from ventral progenitors while the neuroepithelium is committed towards gliogenesis. These neurons were identified as CerebroSpinal Fluid-contacting Neurons (CSF-cN). The aim of this work is to determine the genetic mechanisms involved in the differentiation of CSF-cN.

Genetic labeling and expression analysis in the mouse spinal cord show that the bHLH-containing proneural protein Ascl1 is expressed in CSF-cN lineage from the progenitor state. Using knock-out mice, we show that Ascl1 is specifically required for CSF-cN development and cannot be replaced by other proneural genes. Cell fate tracings together with Ascl1 mosaic deletion show that Ascl1 regulates CSF-cN differentiation in a cell autonomous manner. We genetically labeled prospective CSF-cN progenitors in Ascl1 mutant background. Based on their morphology, marker expression and electrophysiology, we showed that late neuronal progenitors that fail to acquire CSF-cN identity become ependymal cells that cover the surface of the central canal.

Our results show that Ascl1 is expressed in spinal ventral late progenitors that give rise to CSF-cN and is conferring specific neuronal potential to late ventral progenitors in the amniote spinal cord.

"*Evaluating a possible crosstalk between inhibitory and excitatory calcium signals in inner hair cells of the developing inner ear*"

Marcelo Moglie

Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, Dr Héctor N. Torres, CONICET, Argentina

Altricial rodents do not respond to sound until their second postnatal week. Before the onset of hearing, cochlear inner hair cells (IHCs) fire sensory-independent action potentials sustained by voltage-dependent calcium channels. The influx of calcium triggers the release of glutamate to afferent dendrites of the auditory nerve, determining an excitatory role for calcium ions.

At this stage, IHC are also innervated by efferent cholinergic neurons, projecting from the brainstem. This synapse combines the entry of calcium through α9α10 nicotinic receptors with the activation of nearby SK2, calcium dependent potassium channels, to hyperpolarize and inhibit IHCs. Thus, calcium can have these two contrary roles within a diffusionally compact cell.

Electron-micrographs of IHC exhibited thin near-membrane cisterns juxtaposed to efferent synaptic contacts. Imaging experiments have shown multiple calcium entry hotspots following activation of efferent fibers. These domains would be spatially segregated from those observed after IHC depolarization. In order to understand the physiological implications of such proximity, we have performed whole cell patch clamp recordings of afferent terminals. We found that upon high frequency stimulation of efferent fibers, calcium was capable of eliciting release of glutamate to afferent terminals. Thus, we suggest that intracellular mechanisms in IHC are adapted to prevent crosstalk between these synapses.

"*Kv1.3 is a candidate target to prevent the hypercholinergic state of parkinsonism*"

Cecilia Tubert

Instituto de Fisiología y Biofísica Bernardo Houssay, CONICET, Argentina

Balanced actions of dopamine (DA) and Acetylcholine (ACh) shape striatal function. In Parkinson´s disease (PD) this balance is lost, leading to a hypercholinergic state. The main source of striatal Ach is a small group of striatal cholinergic interneurons (ChIs). Previously we found that ChIs are hyperexcitable in a rat model of PD as a result of a lack of "accommodation". Our aim is to identify currents that regulate ChI accommodation in mouse brain slices. Margatoxin (MgTx), a blocker of Kv1.3 channels, markedly attenuated accommodation in ChIs, as shown by an increase in the number of spikes ($p=0.003$) and a prolonged firing ($p=0.008$) during a depolarizing current step. MgTx also increased spontaneous firing ($p=0.0455$). We have isolated and characterized the MgTx-sensitive current (Imax=1500 pA). Immunohistochemistry in brain sections and PCR of laser dissected ChIs revealed the expression of Kv1.3 channels. Thus, ChIs express a functionally relevant Kv1 conductance. Then we evaluated the influence of endogenous DA on accommodation. Confirming previous findings, fewer ChIs show accommodation in a mouse model of PD induced with 6-OHDA. This hyperexcitability is associated to smaller MgTx-sensitive currents in ChIs of 6-OHDA-lesioned mice compared to sham-mice ($p<0.0001$). Our data show that chronic nigrostriatal lesions reduce the MgTx-sensitive current in ChIs, causing their hyperexcitability, and nominate Kv1.3 channels as potential new targets of antiparkinsonian therapy.

***GDNF/GFRα1 complex is a synaptic organizer required for proper hippocampal circuit development*"**

Dolores Irala

Instituto de Biología Celular y Neurociencia, Prof. E. De Robertis, UBA-CONICET, Argentina

The formation of synaptic connections during nervous system development is a highly regulated process mediated partly by cell adhesion molecules. Previously, we have identified GFRα1 as a glial cell line-derived neurotrophic factor (GDNF)-induced trans- synaptic cell adhesion molecule. In this work, we demonstrate that in the presence of GDNF, GFRα1 mediates postsynaptic assembly and dendrite structural plasticity in hippocampal neurons. In the presence of GDNF, overexpression of postsynaptic GFRα1 leads to an increase in the number of excitatory presynaptic contacts and promoted the assembly of postsynaptic machinery in cultured hippocampal neurons. Postsynaptic differentiation induced by GDNF was markedly reduced in neurons upon GFRα1 downregulation. In agreement with this evidence, ultrastructural analysis of conditional GFRα1-knockout mice showed a reduction in synapse number, alterations in synapse morphology and decrease in the synaptic localization of postsynaptic proteins in hippocampal neurons. Finally GFRα1/GDNF was found to be required for proper hippocampal dendrite development in cultured neurons and in GFRα1-deficient mice. Altogether these data show that GFRα1/GDNF plays a crucial postsynaptic role in the recruitment of excitatory and inhibitory postsynaptic machinery to the sites of synaptic contact as well as in dendrite structural plasticity. Our results indicate an essential role of GDNF and its receptor GFRα1 for proper hippocampal circuit development.

"*Delayed coupling to feedback inhibition during a critical period for the integration of adult-born granule cells*"

Silvio Temprana
Fundación Instituto Leloir, Argentina

Adult neurogenesis provides a particular kind of plasticity that involves the addition of new processing units to pre-established circuits. In higher mammals, including humans, adult neurogenesis is restricted to specific structures, being the most prominent the dentate gyrus of the hippocampus. The functional impact of adult born neurons (newborn granule cells, nGCs) in hippocampal information processing remains unknown. In order to elucidate their precise contribution a lot of effort has been made to characterize their synaptic connections along their development. It has been shown that during their process of maturation nGCs acquire inhibitory inputs that significantly reduce their excitability and at the same time lose their ability to undergo hebbian plasticity via long term potentiation. In this work we combine the usage of optogenetics and synthetic G-coupled receptors to assess the development of their output connectivity. We show that immature nGCs reliably recruit distal targets in the CA3 area but poorly drive proximal circuits responsible of feedback inhibition. As they transition towards maturity they activate local GABAergic interneurons that restrict spiking of the neighboring granule cell layer. Moreover this feedback inhibition impinges only weakly in young cohorts of nGCs. A computational model reveals that the delayed coupling of nGCs to feedback inhibition could be crucial to achieve a fine-grain representation of novel inputs in the dentate gyrus.

"*Involvement of δCaMKII in persistent forms of memory*"

Gisela Zalcman

Instituto de Fisiología, Biología Molecular y Neurociencias, CONICET, Argentina

Calcium/calmodulin-dependent protein kinase II (CaMKII) is an abundant synaptic signaling molecule that is essential for both memory formation and synaptic potentiation. In mammals, CaMKII exists in multiple isoforms that are the product of four closely related genes: α, β, γ, and δ. Little information is known about the role of δCaMKII in memory processes. In a previous study, we showed that in the mouse hippocampus camk2d gene was specifically expressed during the formation of persistent forms of novel object recognition (NOR) memory and that its gene promoter was acetylated during this process on a NF-κB dependent manner. Here, we will present new results in which we show that δCaMKII mRNA expression is increased at different time-points during NOR memory maintenance returning to control levels 20 days after training, once memory retention decays. The increment in its mRNA expression is accompanied by changes in nucleosome positioning on its promoter. Moreover, δCaMKII knock-down after training impairs object recognition memory when assessed one week later. Altogether, our results support a key role for δCaMKII in persistent forms of memory and suggest that δCaMKII may have a sustained expression throughout the "lifetime" of this kind of memory. Furthermore, this is the first work that provides insight information about nucleosome remodeling during memory formation and maintenance.

Short Talks by Students 2 - Room Topacio

Chair: **Mariano Di Guilmi**, Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, Dr Héctor N. Torres and **Nicolás Unsain**, Instituto de Investigación Médica Mercedes y Martín Ferreyra, CONICET, Argentina

"*Altered Corticostriatal Connectivity and Exploration-Exploitation Imbalance Emerge as Intermediate Phenotypes for a Neonatal Dopamine Dysfunction*"

Bárbara Braz

Instituto de Fisiología y Biofísica Bernardo Houssay, CONICET, Argentina

Neonatal dopamine neuron (DAN) lesion in rodents produces hyperactivity and learning deficits and has been proposed as an attention deficit hyperactivity disorder model. However, the core cognitive and physiological intermediate phenotypes underlying this rodent syndrome remain unknown. Here we show that early DAN lesions cause deficits in exploitation of shelter, social and nutritional resources, and an imbalanced exploratory behavior, where local exploration is exacerbated and search behaviors involving sequences of goal directed actions are degraded. In vivo electrophysiological recordings and morphological reconstructions revealed an attenuation of corticostriatal (CS) functional connectivity affecting medial prefrontal inputs more markedly than cingulate and motor inputs, that is accompanied by a contraction of the dendritic arbor of striatal projection neurons. Importantly, the behavioral deficits and the prefrontostriatal disconnection worsen after adolescence in DAN lesioned mice. Thus, DANs are essential during postnatal development for the functional and structural maturation of CS connections. From a bottom-up viewpoint, our findings suggest that neuropsychiatric conditions presumably linked to developmental alterations of the dopaminergic system should be evaluated for deficits in foraging and structural disorganization of the CS system.

"*Is microglia one of the mediators of IGF-1 effects on aged rats*?"

Eugenia Falomir Lockhart

Instituto de Investigaciones Bioquímicas de la Plata, CONICET, Argentina

Microglial cells play an important role in healthy and diseased brain removing apoptotic neurons, establishing transient connections with neuronal synapses and producing neurotrophic factors that modulate neurogenesis during embryogenesis and adulthood. These cells are essential for ensuring neuroprotection in the normal and pathological condition of central nervous system as they are an important sources of neurotrophic factors. It has been described that aging reduces the ability of microglia to provide neuroprotection.

It is well known that IGF-1 plays a physiological role in neuroprotection. In situations involving cytotoxic damage, the microglia increases the production of IGF-1. Previous studies of our group described that intracerebroventricular (ICV) IGF-1 gene therapy induced a significant improvement in motor performance in aged rats. We propose that restorative effects of IGF-1 in motor skills could be mediated by glial cells.

In this study we implemented ICV IGF-1 gene therapy in aged rats and assessed the motor performance pre and 17-days after surgery. Glial cell number and morphology in striatum was determined.

Results: IGF-1 treatment restored motor coordination and limb grip strength in aged rats. Microglia cell number was significantly increased after treatment with IGF-1 (Xm-senil-IGF-I=556.2±30.50 vs Xm-senil-DsRed=359.9±18.08; $p<0.001$), while astrocytes showed not changes. An increase of the active microglia phenotype was observed in experimental rats.

"*Altered maturation through adolescence leads to decreased hippocampal-prefrontal cortex functional connectivity in a mouse model of schizophrenia*"

Rodrigo Alvarez

Instituto de Fisiología y Biofísica Bernardo Houssay, CONICET, Argentina

The glutamatergic hypofunction theory postulates that a dysfunction in NMDA receptors (NMDAr) in cortical interneurons is central in the pathophysiology of schizophrenia. In our previous work, restricted ablation of NMDAr in corticolimbic parvalbumin interneurons during early postnatal development resulted in schizophrenia-like phenotypes in adulthood. To elucidate the pathophysiological changes leading to this phenotype, we placed tetrodes in the mPFC in anesthetized control and mutant, juvenile and adult mice. We found a significant increase in spontaneous firing rate and altered entrainment to cortical local and distant rhythms in mutant juvenile and adult mice. Since juvenile mutant mice lack NMDAr but show no schizophrenia-like phenotype, the above mentioned changes could not explain the behavioral abnormalities of adults. Normal synaptic pruning of local and distant inputs to mPFC occurs during adolescence. We analyzed functional connectivity of the vHP-mPFC pathway before and after adolescence. Mutant adult mice present diminished amplitude of the evoked response in mPFC. We also measured the status of circuit plasticity and found that adult but not juvenile mutant mice are more susceptible to undergo LTD. We propose that early ablation of NMDAr in interneurons leads to an overexcited/uncoordinated cortical circuit that propitiates LTD during adolescence. This results in a decreased functional connectivity in adults that may underlie the schizophrenia-like phenotype.

"*Differential Reactivation Outcomes on a Single US-Contextual Fear Conditioning: a temporal prediction error account*"

Matías Mugnaini

Laboratorio de Psicología Experimental, Facultad de Psicología, Universidad Nacional de Córdoba

Last decades of research on memory dynamic lead to review the fate of a trace after the protein synthesis-dependent consolidation process. It has been demonstrated that once a memory is consolidated, if it is properly reactivated, can enter into a labile state that makes it susceptible to various manipulations, needing to "reconsolidate" in order to persist. Labilization seems to be initiated by a mismatch or temporal prediction error between training and reactivation. However, most research in this topic is conducted with strongly trained memories including several US presentations. In this work we conducted two experiments: in the first one, five groups of rats were fear conditioned to a context in order to achieve moderate levels of conditioned responding. The second experiment tested different reactivation protocols and a control condition. Half of the subjects in each condition were administered with an i.p. injection of saline or midazolam, a fast acting GABA-A positive modulator known to disrupt memory reconsolidation. Results suggest that memories trained with a single unconditioned stimulus also destabilize by a temporal mismatch during reactivation. These results would allow future research with a more sensible memory, able to be strengthened or dampened through various means.

"*Neurochemical phenotypes rescued in Tau Knock-out mice by human Tau re-expression*"

Ana Damianich

Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, Dr Héctor N. Torres, CONICET, Argentina

Tau is a microtubule ¬associated protein predominantly expressed in neurons and involved in many processes, such as microtubule polymerization, stabilization and axonal transport. However, the essential role of Tau in the adult brain has been under debate during the last decade. Based on evidence demonstrating that neither gross behavioural nor neurochemical dysfunctions were observed in TauKO mice, Tau reduction has been proposed as a treatment strategy for tauopathies. However, some recent reports suggest that the lack of Tau might be detrimental in the adult brain, because TauKO mice show mild impairment in fine motor coordination. The aims of this work are to further evaluate the functional role of Tau in motor and cognitive tasks, comparing middle ¬aged wild type and TauKO mice; and to analyze if the expression of human Tau in the TauKO background leads to any phenotypic rescue.

Wild¬Type, TauKO and Htau mice were analyzed in the open field and the rotarod to assess spontaneous locomotion and motor coordination. Cognitive performance was also tested in the novel object recognition (NOR) task and the Morris Water Maze. In addition, immunohistochemical analyses were done to analyze dopaminergic neurons in the SNpc of the three groups and quantified by stereology. Together, our results suggest that lack of Tau has an effect over motor coordination, and that re-¬expression of Tau with a full lenght human Tau transgene might rescue some motor phenotypes.

"Aversive and appetitive memories are simultaneously formed after a single learning session in the crab Neohelice"

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Unlike experimentally controlled situations, animals in nature might be exposed to contradictory information. Situations or places might simultaneously predict desired and undesired consequences. However, at some point the situation has to be categorized as appetitive or aversive, in order to decide if repeat or avoid it in the future. How contradictory information is integrated and how it affects learning and memory has not been yet extensively studied. In the present work we took advantage of the well described aversive and appetitive learning paradigms in the crab Neohelice to explore learning after simultaneous appetitive and aversive experiences associated to the same context. First, we found that two parallel memory traces are formed after simultaneous appetitive and aversive training. Second, we found that the probabilities to express no, one or both learned behaviors depend on the balance between the relative strength of the aversive and appetitive unconditioned stimuli, thus revealing a mutual interference under certain conditions. Finally, we found that the mentioned interferences do not occur during learning or memory formation, rather during memory retrieval. These results suggest that both memories could be actually available to be retrieved upon presentation of the conditioned stimulus, but the access of memory to behavior might be modulated based on specific demands at the moment of retrieval.

"*Hippocampal ERK2 differential activation after memory reconsolidation processes are modulated by α7 nicotinic acetylcholine receptors*"

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We have previously reported not only that hippocampal α7 nicotinic acetylcholine receptors (nAChRs) play a critical role in reconsolidation processes but also that there is a differential activation of extracellular signal-regulated kinases (ERK1/2) after a reconsolidation process in mice trained in an inhibitory avoidance task. Next, we determined whether nAChRs activation/inactivation is able to modulate ERK1/2; responsible of, at least in part, the behavioral changes observed in subsequent tests after memory reconsolidation.

With these in mind, CF-1 male mice were trained in an inhibitory avoidance task using either a mild (0.8 mA, 1s) or a strong (1.2 mA, 1 s) footshock. A reactivation test (T1) was given 48 hours later. Immediately after it, mice were given intra-dorsal hippocampal infusions of choline, an α7nAchRs agonist (Ch, 0.80 g/hippocampus), methyllycaconitine, an α7nAchRs antagonist (MLA, 10.00 ug/hippocampus) or vehicle. Fifteen or forty-five minutes afterwards, the hippocampi were dissected and ERK1/2 activation was determined in nuclear and cytosolic fractions.

Ch or MLA, given immediately after memory reactivation, modified ERK2 pattern of activation depending on both, training conditions and time elapsed after memory reactivation.

Altogether, our results point for the first time to an α7nAChRs-related ERK2 pattern of activation induced during memory reconsolidation modulation.

POSTERS

Cellular and Molecular Neurobiology

P1.-Decreased protein levels of plasticity-related genes Zif268, arc and c-fos in TDP-43- ΔNLS transgenic mice

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Recent studies demonstrated that TDP-43 is a major disease protein in frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS). In these TDP-43 proteinopathies, TDP-43 is redistributed from its normal nuclear localization and form cytoplasmic insoluble aggregates. We generated a new animal model based on the conditional overexpression in the mouse forebrain of a cytoplasmically-localized form of human TDP-43 (hTDP-43-ΔNLS), and recently showed that these mice recapitulate key aspects of FTLD/ALS, including behavioral and cognitive deficits. However, the physiological role of TDP-43 in behavioral responses is not well understood. In this context, we aimed to assess changes in plasticity-related pathways, specifically analyzing well-known gene products involved in neural plasticity (Arc, c-fos, Zif268). Immunofluorescence staining of several brain areas (involved in processing of the behavioral tasks impaired in these mice) revealed a profound decrease of all three genes in transgenic versus control mice. Since these genes are upregulated upon behavioral challenges and are necessary for cognitive processing, we also evaluated the response to exposure to an open field test. TDP-43 transgenic mice display a reduced or absent induction of all three genes in cortical and hipocampal regions. These results suggest a novel TDP-43 driven mechanism underlying the behavioral abnormalities displayed by TDP-43 mice and potentially in human TDP-43 proteinopathies.

Cellular and Molecular Neurobiology

P2.-SUMOylation is a key modulator of glucocorticoid receptor activity in the neuronal context

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FK506-binding protein 51 (FKBP51) is an Hsp90 cochaperone that regulates the activity of the glucocorticoid receptor (GR) and is therefore critical for the stress response. The molecular mechanisms underlying its functional regulation remain elusive. Here, we show that FKBP51 is a novel SUMOylation target. We identify lysine 422 as the major small ubiquitin-like modifier (SUMO) attachment site and PIAS4 as the E3 ligase that enhances its SUMOylation. Most importantly, we show that SUMO conjugation to FKBP51 is required for the inhibition of GR activity. FKBP51 SUMOylation occurs in hippocampal neuronal cells and impacts on GR-dependent neuronal signaling and differentiation. SUMOylation of FKBP51 allows for its interaction with Hsp90 and its recruitment to the GR chaperone complex, and is therefore critical for its inhibitory action on GR hormone binding affinity and nuclear translocation. Our findings establish SUMO conjugation as a novel regulatory mechanism in the Hsp90 cochaperone activity of FKBP51 with a functional impact on GR signaling.

Cellular and Molecular Neurobiology

P3.-Purinergic modulation of synaptic activity at the efferent synapse in mice inner ear

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The inner ear receives innervation from efferent cholinergic fibers, descending directly from the medial olivary complex (MOC) in the auditory brainstem. Acetylcholine (ACh) released by these fibers produces synaptic inhibition by means of a nicotinic receptor with high Ca2+ permeability coupled to a Ca2+-activated K+ channel. MOC fibers directly innervate outer hair cells (OHC), controlling the amplification capability of these cells in the mature cochlea. However, during the first two postnatal weeks, MOC fibers innervate inner hair cells (IHC), controlling an intrinsic electrical activity of these cells. This activity is driven and/or modulated by ATP released from cochlear supporting cells. Here, we investigated the interaction between ATP and MOC inhibition to hair cells.
By recording inhibitory post synaptic currents (IPSC) in P9-11 mice, we showed that ATP decreases ACh release through the activation of presynaptic P2Y receptors. In this work, we evaluated this effect across development (P5-7) and found that ATP-induced inhibition is even stronger at this stage. Finally, because ATP-mediated signaling has been proposed as a mechanism of sensing damage in the cochlea, we studied if ATP modulates the MOC-OHC synapse. We found that ATP decreases IPSC amplitudes in P11-13 mice. This effect was abolished by the non-specific P2 antagonist, suramin. Our findings show an inhibitory role of ATP at the efferent synapse both during development and after the onset of hearing.

Cellular and Molecular Neurobiology

P4.-Characterizing cytoskeleton changes during axonal degeneration

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Axonal fragmentation is a regulated process that requires a growing set of receptors, signaling molecules, proteases and other regulators to disintegrate the axonal compartment. Little is known about the changes (and possible role) in the axonal cytoskeleton during degeneration. In this study we aimed at describing changes to the cytoskeleton during degeneration of sensory neurons axons grown in vitro and induced to degenerate by trophic factor withdrawal ("deprivation") and axon transection ("injury"). We first focused our attention to the F-actin-rich growth cone and found that both in deprivation and injury growth cone collapse (GCC) is almost complete at early time points (3h of deprivation or 1h after injury), indicating an early remodeling of F-actin structures. To our surprise, GCC still took place when axonal fragmentation was prevented by the use of drugs previously shown to prevent or delay degeneration. A global F-actin decrease was evident in every cell compartment during degeneration. Axonal microtubules form tight bundles and we observed early and marked de-bundling of these along degenerating axons. We are going to present preliminary data obtained using super-resolution STORM microscopy in order to unveil structural details with unprecedented resolution. Rather than being the last targets in the degenerative process, the early changes observed suggest that the axonal cytoskeleton has an instructive role in the subsequent fragmentation.

Cellular and Molecular neurobiology

P5.-Shared molecular complexes Between Kidney and Brain: Podocyte Proteins in the Cerebellum

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Nephrin is an immunoglobulin-like transmembrane protein with crucial structural, functional and signalling properties to maintain the glomerular filtration barrier (slit diaphragm). Its discovery was the milestone finding to start the discovery of other elements of this filtration barrier and was shown to be expressed widely in a variety of species. Later on, its extremely limited expression in other organs beyond the kidney was described. Interestingly, nephrin was soon found in a limited manner in the Purkinje cells of the mouse and human cerebellum. Furthermore increasing evidence shows other shared molecules between the podocyte slit diaphragm and neurons. However, the functional role of these proteins in the brain is not well understood.

Here we show results with *Danio rerio* (zebrafish) as model organism. As a first step we studied the exact expression site of components of the slit diaphragm in kidney and brain of zebrafish by immunofluorescence using confocal microscopy.

Our first results indicate that podocin, nephrin, neph3, erbin, beta-catenin and ZO-1 are expressed in a distinct pattern in zebrafish brain. Interestingly, all of them are expressed in the cerebellum. In addition, podocin and nephrin co-localize with parvalbumin7 in Purkinje cells. Thus, our results reveal evidence of shared expression of proteins between the podocytes and neurons and set the basis to future interaction studies and discovery of possible shared functionalities.

Cellular and Molecular Neurobiology

P6.-Low Led Light Effects in Visual and Nonvisual Photoreceptor Cells

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The retina is part of the central nervous system that, in mammals, fulfills two important role; a visual function through rods and cones photoreceptors and synchronization of circadian rhythms to a 24h solar cycle through retinal ganglion cells (RGCs) , specifically through a subpopulation of photosensitive retinal ganglion cells (ifRCGs).

There are numerous works that evaluate the effects of bright light exposure over the retina, however the process of retinal cell death by low intensity LED light stimulus may be different and it is not well characterized yet.

We previously demonstrated that, in albino Wistar rats, exposure to low intensity white LED light (200 lux) produces a significant reduction in the number of rods after 5 days. The mechanism of photoreceptor cell death is through a caspase-3 independent mechanism, the photopigment rhodopsin expression is not alterated nevertheless, it is more phosphorylated in ser334 in LL animals. To evaluate the effects of LED light in nonvisual photoreceptor cells (ipRGC); male albino Wistar rats were exposed for one to seven days to this source of lighting.

We found that low LED light exposure did not alter the number of RGCs. The analysis of expression and localization of melanopsin (CGRif photopigment) showed that it is increased in animals exposed to light (LL8) in relation to control animals and its location varies along the days of LL stimuli, founding exclusively in the soma of CGRif.

Cellular and Molecular Neurobiology

P7.-Altered miRNA expression profile in the hypothalamus of perinatally malnourished mice

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Early life stress -such as malnutrition- during the critical perinatal period modifies cellular differentiation and neurogenesis programs promoting lifetime social and cognitive disturbances. However, the role of miRNAs in the CNS linking malnutrition with behavioral deficiencies has not been described. The hypothalamus emerges as an intriguing target as it plays a crucial role in energy balance, stress response and motivated behaviors.
Here, we used high-throughput sequencing to define to what extent the hypothalamic profiles of miRNA expression are perturbed in perinatally malnourished mice. CF1 dams were fed with normal protein diet (NP) or low protein diet (LP, 40%) during pregnancy and lactation, and the male offspring were analyzed at P56.
We studied the predicted and validated mRNA targets of 18 differentially expressed miRNAs between NP and LP -particularly, miR-124, miR-187 and miR-204. For the prediction, a set of 6 algorithms was used: miRWalk, MicroT4, miRanda, miRMap, RNA22 and TargetScan. Due to differences in sensitivity and specificity of the algorithms, we obtained the intersection of the results in order to enhance credibility and reduce false positives. DAVID tool was used for pathway classification of predicted and validated genes. We found a significant enrichment in the axon guidance pathway, which could have potential consequences in hypothalamic functions. Moreover, several pathways related to cytoskeleton remodeling were also consistently enriched.

Cellular and Molecular Neurobiology

P8.-Electrical manipulation of Dopaminergic Neurons to modulate its survival

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Parkinson's Disease (PD) is characterized by the selective death of nigral dopaminergic neurons, although the causes that determine their death still remain unknown. Several lines of evidence suggest that the peculiar electrical activity of these neurons plays a key role in determining their death or survival.

Blocking electrical activity in vitro is able to induce their specific death, while pharmacological manipulations enhancing their electrical activity induce their survival, through an increase in intracellular Ca2+ levels. On the contrary, there are indications that an excess of Ca2+ signalling, as a consequence of electrical activity, results in a toxic condition for DA neurons.

To put some light on this controversy, the purpose of this study is to manipulate the electrical conditions of DA cells with the use of DREADDs.

We have reproduced an in vitro model of dopaminergic degeneration from embryonic mice, in which we have identified two subpopulations of DA neurons by their expression of Calbindine, a marker mostly expressed in DA neurons from VTA and not Substantia Nigra, and discriminate their response to protective agents, as nicotine. Finally, we transduced these cultures with stimulatory DREADD (M3) lentivirus preventing DA cell death when treated with CNO (Clozapine-N-Oxide) and artificial electrical stimulation was achieved. Further work is still necessary to demonstrate the role of electrical manipulation in death or survival of DA neurons.

Cellular and Molecular Neurobiology

P9.-Contribution of MeCP2 and synaptic activity to hippocampal structural plasticity

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Methyl citosine binding protein-2 (MeCP2) is a chromosomal structural protein involved in the regulation of gene expression. Alterations in MeCP2 levels have been linked to neurodevelopmental and learning disorders. Recent work indicates that removal of MeCP2 in adult animals also induce neurologic defects suggesting a role in the maintenance of synaptic connections in the adult nervous system.

In the adult hippocampus, the infrapyramidal tract (IPT) undergoes dynamic changes in size in response to neuronal activity, and together with adult neurogenesis constitutes important events for pre-synaptic structural plasticity of the hippocampus. In the present work, we aim to define how the lack of MeCP2 interferes with these processes. Using mouse models of MeCP2 deficiency, we found that the volume of the IPT tract increases significantly 2 weeks after kainic-induced seizures in WT mice but not in MeCP2 mutant mouse. However, using BrDU injections and IHC we found no defects in kainic-induced adult neurogenesis in MeCP2 mutant mice, suggesting a defect in the maturation or axonal guidance of the new neurons in the absence of MeCP2.

In addition, we found that BDNF expression was up-regulated at both 6hrs and 2 weeks after kainic exposure in WT mice, but it was increased only after 6hrs in MeCP2 mutant mice. This finding suggests that the sustained increase of BDNF expression may play a role in the dynamic growth of IPT in response to neuronal activity.

Cellular and Molecular Neurobiology

P10.-Synaptic composition and protein acetylation change during Inhibitory avoidance Consolidation in mice.

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It is well known that synapses are plastic structures involved in processes such synaptic plasticity and memory consolidation. Previous studies show that protein composition of the synapse changes during memory consolidation. There is evidence that synaptic protein composition is, at least partly, modulated by Post-Translational modifications (PTM). Furthermore synaptic plasticity and memory consolidation rely in many cases on these PTM of the proteins composing the synapse. In particular, some of the changes that occur at the synapse during these processes involve a reorganization and protein redistribution. Here we use the inhibitory avoidance learning task in mice to study a particular type of PTM, the lysine acetylation, during memory consolidation. We evaluate the changes in synaptic protein composition that occur after training, in correlation with changes in protein acetylation.

Cellular and Molecular Neurobiology

P11.-Selected SNARE proteins are essential for the polarized membrane insertion of IGF-1 receptor and the regulation of initial axonal outgrowth in neurons

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The establishment of neuronal polarity necessitates the addition of new membrane to the axon's plasmalemma. Axolemmal expansion occurs by exocytosis of plasmalemmal precursor vesicles (PPVs) primarily at the axonal growth cone. Previous published data from our laboratory indicates that, in cultured hippocampal neurons, TC10 activation by IGF-1 and recruitment of exo70 to the growth cone plasmalemma are necessary for the regulation of PPVs exocytosis. In contrast, little is known about the SNARE proteins involved in the regulation of PPV fusion with the neuronal plasmalemma at early stages of differentiation. We show here that VAMP2, VAMP4, VAMP7, Syntaxin6 and SNAP23 are expressed by hippocampal pyramidal neurons before polarization. Expression silencing of VAMP4, Syntaxin6 and SNAP23 repressed axonal outgrowth and the establishment of neuronal polarity, by inhibiting IGF-1 receptor exocytotic polarized insertion. In addition, stimulation with IGF-1 triggered the association of VAMP4, Syntaxin6 and SNAP23 to vesicular structures carrying the IGF-1 receptor and over-expression of a negative dominant form of Syntaxin6 significantly inhibited exocytosis of IGF-1 receptor containing vesicles at the neuronal growth cone. Taken together, our results indicated that VAMP4, Syntaxin6 and SNAP23 function are essential for regulation of PPVs exocytosis and the polarized insertion of IGF-1 receptor and required for initial axonal elongation and the establishment of neuronal polarity.

P12.-Triggering Receptor Expressed on Myeloid cells-2 (TREM-2) expression is induced by brain ischemia and DAMPs

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TREM-2 regulates toll-like receptors (TLR) signaling, and thus it is proposed to participate in the fine-tuning of the inflammatory response in professional immune cells like microglia. After brain injury DAMP are released from necrotic cells and activate TLR, but it is unknown whether DAMP or other molecules are TREM-2 ligands. Using an experimental model of focal brain ischemia by unilateral cortical devascularization and astroglial or microglia-enriched cell culture, we studied here the expression of TREM-2 and the cellular consequences of its activation. Our results showed that TREM-2 expression was induced in glial cells from the ischemic penumbra specifically in astrocytes and microglia at early time points after ischemia (3-7 days). In vitro, exposure to LPS or oxygen glucose deprivation also induced TREM-2 expression in astrocytes. The DAMP HMGB1 induced astroglial TREM-2 expression but failed to increase endogenous microglial TREM-2 expression. When TREM-2 was activated by antibody crosslinking in primary astrocytic culture, we observed a partial suppression of TLR-mediated LPS-induced NF-KB activation analyzed by the p65 subunit nuclear localization. We conclude that TREM-2 is expressed in glial cells in vivo after brain ischemia and in vitro. Astrocytes, not only express TREM-2, but also respond with the classical effect of limiting TLR-dependent NF-KB activity thus being an interesting target to limit reactive gliosis. Grants PICT 2012-1424, CONICET, UBACYT.

Cellular and Molecular Neurobiology

P13.-Transferrin enhances neuroblastoma cell line maturation.

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Transferrin (Tf) is a glycoprotein best known for its role in iron delivery, although multiple functions have been attributed to it. As a matter of fact, previous studies conducted in our laboratory in central nervous system have shown that Apotransferrin (iron-free transferrin, aTf) favors proliferation and differentiation of precursor cells to oligodendrocytes (Guardia Clausi et al., 2010; Silvestroff et.al., 2012), and behaves as a trophic factor during oligodendrocyte maturation (Escobar Cabrera et al., 1997; Paez et al., 2005). Transferrin can also modulate proliferation and phenotype activation of microglia in vitro.
In a context of remyelination, glial-axon interaction is essential for the complete repair of lesions. For this reason, we started studies of aTf participation in neuron maturation in vitro, a matter never studied in our lab.
In the present work we analyzed the effects of aTf, added to the culture medium, on Neuro-2a cells (N2a), a mouse neuroblastoma cell line which, once differentiated, shares many properties with neurons.
Viability measured by the MTT assay showed an increase in the presence of aTf in comparison with control. We describe the presence of the Tf receptor in these cells. Tf mRNA is also present but the protein is not expressed under our experimental conditions. Finally, aTf added to the cell culture is internalized and induces neurite outgrowth.

Cellular and Molecular Neurobiology

P14.-Comparative expression analysis of human and chimpanzee nervous system enhancers of the NPAS3 locus

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It was proposed that the phenotypic divergence between human and chimpanzees is largely due to changes in gene regulation. We hypothesize that differences in human behavioral repertoire is due to the acquisition of new expression patterns of preexisting genes. We have recently identified the largest cluster of noncoding DNA elements (named human accelerated elements, HAEs) that show evidence of rapid evolution in the human lineage located within the locus of NPAS3 gene. NPAS3 is a transcription factor that is expressed in the developing nervous system, and its dysfunction has been associated with schizophrenia in humans. In this work, we performed a comparative expression analysis of these NPAS3-HAEs using a transposon-based transgenic assay in stable zebrafish lines using human sequences and their chimp ortholog. We found that whereas, one of the human NPAS3-HAEs function as nervous system developmental enhancers, the chimp orthologs are non-functional, suggesting a gain of function. In addition, three human HAEs are non-functional compared to the chimp ortholog sequence. In order to determine if the enhancer function was lost in the human lineage or gain in the chimp lineage, we selected one of the non-functional HAE and compared with macaque, mice and zebrafish orthologs. We found that the macaque, mouse and zebrafish sequences function as developmental enhancers in the nervous systems probing that DNA differences in the human sequence cause a loss of function.

Cellular and Molecular Neurobiology

P15.-Cell death susceptibility of human glioma cells to Bortezomib treatment is R-CRT regulated

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Previously, we described that calreticulin is posttranslational arginilated (R-CRT) by the enzyme ATE1. Different functions have been proposed to R-CRT as it was found associated with stress granules (SGs) and at the plasma membrane, where it participates in pro-apoptotic events. The apoptosis resistance of different cancer cells to treatment with therapeutic drugs as Bortezomib has been associated with SGs formation. In this study we evaluated if R-CRT modulates the apoptosis susceptibility of different glioma cells treated with Bortezomib. A difference was observed after treatment between HOG and MO59K cell lines, HOG cells showed reduced cell viability and increased levels of cleaved caspase-3 whereas MO59K cells were more resistant to the drug. Also, increased levels of R-CRT were determined in the cytosol and at cell membrane in HOG cells after treatment, but not in the MO59K cells. Additionally, by confocal analysis we measured that only in MO59K cells treated with Bortezomib showed an increased formation of SGs and an augmented cytosolic association of R-CRT to SGs. These results are suggestive that R-CRT up-regulation and its subcellular localization could be involved in caspase-3 dependent cell death response of human glioma cell lines to Bortezomib treatment. Hence R-CRT is a protein that could have an influence on brain cancer treatment.

Cellular and Molecular Neurobiology

P16.-Melanocortin 4 receptor (MC4R) constitutive activity impairs specific voltage-gated calcium channels subtypes

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MC4R regulates food intake and energy expenditure. MC4R is a G protein coupled receptor mostly expressed in the paraventricular nucleus of the hypothalamus and the amygdala. Despite MC4R activity level increases by agonist binding, MC4R displays constitutive activity that is abolished by its endogenous inverse agonist, AgRP. MC4R mutations that modify constitutive activity cause unbalanced food intake and obesity in humans. We recently demonstrated that MC4R activation by agonist can reduce presynaptic CaV2.2 currents, but the neuronal mechanisms activated by constitutive activity are unknown. Hence, we tested if MC4R regulates CaV subtypes activity using the patch clamp technique in HEK293t cells co-expressing each CaV and MC4R. We observed that cells co-expressing MC4R and CaV1.2, CaV1.3 or CaV2.1 have reduced calcium currents and this effect is occluded by AgRP, while CaV2.2 currents are not affected. By pharmacological manipulations we found that Gi/o but not Gs protein is involved in the pathways utilize by MC4R constitutive activity to reduce CaV currents. Moreover, we found that MC4R constitutive activity signaling requires ERK1/2 protein phosphorylation to impair basal CaV1.3 currents. Thus, we conclude that CaV subtype targeted by MC4R and the intracellular pathway involved are different for the constitutive and agonist-evoked modes of activation of MC4R.

Cellular and Molecular Neurobiology

P17.-Neuronal development and axon growth are altered by glyphosate through a WNT non-canonical signalling pathway

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The growth and morphological differentiation of neurons are critical events in the establishment of proper neuronal connectivity and neural function. Developing nervous system is highly susceptible to damage caused by exposure to environmental contaminants. Although Glyphosate-containing herbicides are some of the most used agrochemicals in the world, toxic effects have been reported in mammals. However, its action mechanism on the nervous system remains unclear. Here, we report the impaired neuronal development induced by glyphosate exposure. We observed that the initial axonal differentiation and growth on cultured neurons is affected since after 1 day most glyphosate treated cells remained undifferentiated. Although, they polarized at 2 days in vitro, they developed shorter and unbranched axons and also less complex dendritic arbors compared to controls. To go further, we attempted to identify the cellular mechanism by which glyphosate affected neuronal morphology. Biochemical approaches revealed that glyphosate led to a decrease in Wnt5a, a key factor for the initial neurite development and maturation, as well as a down-regulation of CaMKII. These data suggest that the morphological defects induced by glyphosate could probably be a consequence of a decrease in both Wnt5a expression and CaMKII activity that would likely be reflected in a neuronal dysfunction; therefore it highlights the importance of establishing rigorous control on the use of glyphosate-based herbicides.

Cellular and Molecular Neurobiology

P18.-Neurochemical phenotypes rescued in Tau Knock-out mice by human Tau re-expression

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Tau is a microtubule associated protein predominantly expressed in neurons and involved in many processes, such as microtubule polymerization, stabilization and axonal transport. However, the essential role of Tau in the adult brain has been under debate during the last decade. Based on evidence demonstrating that neither gross behavioural nor neurochemical dysfunctions were observed in TauKO mice, Tau reduction has been proposed as a treatment strategy for tauopathies. However, some recent reports suggest that the lack of Tau might be detrimental in the adult brain, because TauKO mice show mild impairment in fine motor coordination. The aims of this work are to further evaluate the functional role of Tau in motor and cognitive tasks, comparing middle aged wild type and TauKO mice; and to analyze if the expression of human Tau in the TauKO background leads to any phenotypic rescue.

WildType, TauKO and Htau mice were analyzed in the open field and the rotarod to assess spontaneous locomotion and motor coordination. Cognitive performance was also tested in the novel object recognition (NOR) task and the Morris Water Maze. In addition, immunohistochemical analyses were done to analyze dopaminergic neurons in the SNpc of the three groups and quantified by stereology. Together, our results suggest that lack of Tau has an effect over motor coordination, and that re-expression of Tau with a full lenght human Tau transgene might rescue some motor phenotypes.

Cellular and Molecular Neurobiology

P19.-An improved method for primary culture of hypothalamic tanycytes

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Hypothalamic tanycytes are specialized bipolar ependymal cells lining the floor of the third ventricle. Given its strategic location, tanycytes play several key functions acting as a selective blood-cerebrospinal fluid barrier and controlling many neuroendocrine axes. The in vitro culture of this cell population has proved to be difficult due to several factors including their limited amount in vivo, and the particular anatomical location and cellular complexity of their normal environment. Here, we report an improved method for generating primary cultures of rat hypothalamic tanycytes. Ependymal cultures were derived from tissue dissected out of the median eminence region of 10-day old rats and cultured in a chemically defined media. After 10 day in vitro, a significant fraction of the cells in culture exhibited morphological features of tanycytes as observed by phase contrast or scanning electron microscopy. All tanycyte-like cells were immuno-reactive for markers of this cell type, including vimentin, GFAP and DARPP32. Whole-cell patch clamp recordings of these cells showed their resting membrane potential became more negative with increased cell length. Current injections failed to produce spike-like responses. Thus, the current method allows obtaining cultures highly enriched in tanycyte-like cells that resemble in vivo tanycytes in terms of morphology, molecular markers and electrical activity. Supported by PICT2011-2142 and PICTO2013-0065.

Cellular and Molecular Neurobiology

P20.-ASCL1 regulates late neurogenic events in the ventral neural tube

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Despite the effort invested in the last decades, the regulatory networks that control lineage specification in the developing central nervous system are not completely understood. We have identified a late-born population of spinal neurons that originate from ventral progenitors while the neuroepithelium is committed towards gliogenesis. These neurons were identified as CerebroSpinal Fluid-contacting Neurons (CSF-cN). The aim of this work is to determine the genetic mechanisms involved in the differentiation of CSF-cN.

Genetic labeling and expression analysis in the mouse spinal cord show that the bHLH-containing proneural protein Ascl1 is expressed in CSF-cN lineage from the progenitor state. Using knock-out mice, we show that Ascl1 is specifically required for CSF-cN development and cannot be replaced by other proneural genes. Cell fate tracings together with Ascl1 mosaic deletion show that Ascl1 regulates CSF-cN differentiation in a cell autonomous manner. We genetically labeled prospective CSF-cN progenitors in Ascl1 mutant background. Based on their morphology, marker expression and electrophysiology, we showed that late neuronal progenitors that fail to acquire CSF-cN identity become ependymal cells that cover the surface of the central canal.

Our results show that Ascl1 is expressed in spinal ventral late progenitors that give rise to CSF-cN and is conferring specific neuronal potential to late ventral progenitors in the amniote spinal cord.

Cellular and Molecular Neurobiology

P21.-Post-Translational Incorporation Of L-Phenylalanine Into the COOH-terminus of α-tubulin in living cells

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The tyrosination/detyrosination cycle occurring at the C-terminus of α-tubulin is one of the most studied post-translational modifications of proteins. Previous in vitro studies showed that L-phenylalanine (Phe) can be incorporated into tubulin in place of tyrosine. Here, we demonstrate that Phe can be incorporated into the COOH-terminus of tubulin in a reversible fashion in cultured cells and that this incorporation is not due to "de novo" biosynthesis. Phenylalaninated tubulin is able to form microtubules. Phe incorporation occurs in different cell types and it does not interfere with cell viability. We are interested in the incorporation of this amino acid because in patients suffering phenylketonuria, the level of Phe in blood is increased 20-40-fold and the molecular events that lead to the abnormal functioning of the patient brain are not known. Elongation and retraction of neurites in cells of nervous origin (CAD cells) are altered by treatment with high Phe concentration. The rate of elongation and serum induced retraction of neurites seems to be diminished in the presence of Phe suggesting that the dynamics of microtubules and/or the actomyosin system responsible of retraction is involved in the effects induced by Phe treatment. These results allow us to speculate with the hypothesis that the incorporation of Phe into the C-terminus of α-tubulin is responsible or, at least, contributes to the development of the clinical signs of phenylketonuria.

Cellular and Molecular Neurobiology

P22.-The Carboxi-Terminal Domain of Myelin Basic Protein Mediates Microtubules Stability

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Myelin Basic Protein (MBP), an essential component of myelin compact membranes, is capable to stabilize microtubules (Mts) in differentiated oligodendrocytes. As such interaction of MBP with Mts is regulated by calmodulin (CaM), further studies were performed at molecular levels. Four different CaM-binding sites were identified on classic MBP isoforms, localized in the spliced exons and the carboxi-terminal (C-terminal) portion. HeLa cells were transfected with different MBP variants to determine the relevance of these CaM-binding sites on the interaction of MBP with Mts. The colocalization of the different isoforms of MBP with CaM is impaired in those variants whose C-terminal portion is deleted. To analyze which MBP variants are capable to stabilize Mts, cells are exposed to cold temperature. We determined that mainly the MBP isoforms 2, 4 and 6 are capable to preserve a cold stable Mts. Such Mts stabilization is lost when isoforms are deleted in the C-terminal portion. As the tubulin-binding sites of MBP were proposed to be comprised within sequence codified by exon III and IV (common to the different MBP isoforms), these results suggest that the CaM-binding site localized at C-terminal portion of these MBP isoforms significantly modulates the microtubule stabilization. Further studies directed to identify the microtubule binding sites of MBP would help to better understand the regulatory activity of the identified CaM-binding domains onto MBP-Mts interactions.

Cellular and Molecular Neurobiology

P23.-Modulation of TAU isoforms by RNA reprogramming: phenotypic recovery in a mouse model of Tauopathy

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Tauopathies are major neurodegenerative diseases characterized by the presence of intraneuronal aggregates of the protein TAU. TAU is a microtubule-associated protein expressed in neurons, involved in many neuronal processes and is encoded by the MAPT gene. The alternative splicing of exon 10 (E10) produces TAU isoforms with three (3R) or four (4R) microtubule binding repeats; both isoforms are expressed in equal amounts in the normal adult human brain. Several tauopathies are associated with mutations which interfere with E10 alternative splicing, leading to an imbalance between isoforms. Correction of that imbalance might represent a therapeutical approach for tauopathies. We evaluate the phenotypes of mice carrying a human TAU transgene with an imbalance of TAU isoforms (hTau mice). We use RNA reprogramming to modulate the TAU 4R/3R ratio in vivo and analyse if such restoration produces a phenotypic rescue. We delivered into the mouse brain a RNA pre-trans-splicing molecule to create a chimeric mRNA through a trans-splicing reaction to modulate the inclusion of E10. Cognitive performance was tested in the novel object recognition task and the Morris Water Maze. TAU isoforms content was determined by qPCR. The presence of TAU aggregates was analysed by immunohistochemistry and western blot. Htau mice rescued by trans-splicing restored some cognitive and neurochemical phenotypes, indicating that RNA reprogramming is a suitable tool to achieve a relevant phenotypic recovery.

Cellular and Molecular Neurobiology

P24.-Is microglia one of the mediators of IGF-1 effects on aged rats?

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Microglial cells play an important role in healthy and diseased brain removing apoptotic neurons, establishing transient connections with neuronal synapses and producing neurotrophic factors that modulate neurogenesis during embryogenesis and adulthood. These cells are essential for ensuring neuroprotection in the normal and pathological condition of central nervous system as they are an important sources of neurotrophic factors. It has been described that aging reduces the ability of microglia to provide neuroprotection.

It is well known that IGF-1 plays a physiological role in neuroprotection. In situations involving cytotoxic damage, the microglia increases the production of IGF-1. Previous studies of our group described that intracerebroventricular (ICV) IGF-1 gene therapy induced a significant improvement in motor performance in aged rats. We propose that restorative effects of IGF-1 in motor skills could be mediated by glial cells.

In this study we implemented ICV IGF-1 gene therapy in aged rats and assessed the motor performance pre and 17-days after surgery. Glial cell number and morphology in striatum was determined.

Results: IGF-1 treatment restored motor coordination and limb grip strength in aged rats. Microglia cell number was significantly increased after treatment with IGF-1 (Xm-senil-IGF-I=556.2±30.50 vs Xm-senil-DsRed=359.9±18.08; $p<0.001$), while astrocytes showed not changes. An increase of the active microglia phenotype was observed in experimental rats.

Cellular and Molecular Neurobiology

P25.-From behavior to specific genes, and back again

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Through a behavioral screen directed to the circadian neurons in the adult brain, we identi¬fied a gene that we named orsai (osi).Although sequence analysis suggested osi could be a component of the mitochondrial respiratory complex I, our data points to another direction. To characterize osi's function we measured oxygen consumption and ATP levels, and we conclude that osi mutants have an impaired mitochondrial function. Surprisingly, IHC analysis showed OSI is differentially localized in specific tissues: while in gut and trachea is nuclear, in fat body is cytoplasmic; however, was never found in mitochondria. osi mutants die around 72 h AEL as L2 larvae; RNAi-mediated downregulation of osi in an ubiquitous pattern phenocopies the defect. To define the tissue specificity of OSI´s mediated developmental arrest and death, we expressed osi´s RNAi in different expression domains. The most restrictive patterns that caused lethality were btlGal4 and mef2Gal4. When we used osiRNAi in those patterns, we observed additional phenotypes: in tracheas terminal cells are clearly defective; in the gut, lifespan and larval body size differ according to nutrient availability: a restrictive diet enables larvae to live longer and acquire a larger body size; on the contrary, they die early on in the presence of nutritious food. Our results suggest that despite initial assumptions, OSI might play a role in the nucleus, coupling environmental information to basal metabolism.

Cellular and Molecular Neurobiology

P26.-Wnt-Fz signaling regulates dendrite architecture through the co-activation of CaMKII and JNK pathways

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In the nervous system, key processes such neuronal polarization and migration, axon pathfinding, dendrite morphogenesis and synapse formation are regulated by morphogens, among which Wnt proteins stand out. They bind to transmembrane receptors Frizzled (Fz) and also interact with tyrosine kinase receptors, such as Ryk, Ror, and IGF-1R. After the Wnt-receptor interaction, at least three different signalling pathways may be activated: Wnt/β-catenin (canonical pathway), planar cell polarity (PCP) and Calcium pathways (non canonical pathways).
Previously, we observed that Wnt7b-Fz7 affected the architecture of dendrites in hippocampal neurons. To go further, we analyzed the contribution of specific effectors of PCP and Calcium non-canonical pathways and observed that Wnt7b induced the activation of both CaMKII and JNK in Fz7 expressing neurons. In the present work, we link morphological changes in dendritic arborization to CaMKII and JNK activation. Moreover, we analyze a possible co-activation between the non-canonical pathways.

Cellular and Molecular Neurobiology

P27.-Early-life protein malnutrition delays physical and neurological development and causes long-lasting behavioral changes in mice

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Protein malnutrition is in an adverse early-life experience worldwide spread that can interfere with the normal nervous system development and result in cognitive and behavioural alterations. However, little is known about its persistence throughout life and the possibly differential sex incidence. Hence, we decided to study in a murine model the effect of protein malnutrition during the brain mayor growth rate period, gestation and lactation. We studied physical growth and neurodevelopment, and different nervous system functions throughout mouse's life. We made morphological observations and neurological tests during lactation and a behavioural battery test at different ages (6, 22, 39 and 55 weeks).

We found maternal protein restriction delayed offspring physical growth and reflex acquisition. Besides, malnourished female presented an altered juvenile social play. Also, caused a mayor anxiety level that persists along mice life and a reduced exploratory behaviour only in the first half of its life. Working memory was subtly reduced on males throughout life. Moreover, neuromuscular declination was accelerated on malnourished mice. Furthermore, it caused a general adverse effect on mice health which displayed a lower weight during great part of life and a lower survival rate.

These findings show that protein restriction during critical periods of development delay physical growth and neurodevelopment, and also detrimentally program mice behaviour throughout life.

Cellular and Molecular Neurobiology

P28.-Non synonymous polymorphisms (nsSNPSs) in GPM6A´s transmembrane domain coding region: In silico analysis and in vitro study of the effect of point mutations in synapse formation, self-interaction and protein stability

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M6a is a membrane glycoprotein that together with M6b and PLP/DM20 belongs to the proteolipid protein family. We have found that M6a induces neurite and filopodia/spine formation and increases the motility of filopodial protrusions, probably aiding synapse formation, but the mechanism of action remains unknown. We have found that M6a´s transmembrane domains (TMDs) drive M6a induced filopodia formation by its self-association and protein stabilization. Here we further analyze whether 3 nsSNPs present in the coding region of M6a´s-TMD affect synapse formation, TMD self-interaction and protein stability. Cultured hippocampal neurons at 10-12 days in vitro expressing GFP alone or M6a or its mutants fused to GFP were labeled with synaptophysin (pre-synaptic marker) and NMDA-R1 (post-synaptic marker). The number of functional synapses (points of triple colocalization) was quantified using the Image J plugin Puncta Analyser. The number of synapses in M6a expressing neurons was significantly higher compared with GFP expressing neurons. On the contrary, in neurons expressing M6a´s TMD mutants the number of synapses was similar to control. We also showed that these mutants impair M6a stability and self-interaction. In this work we provide evidence that the self-interaction of M6a´s TMDs, as well as the stability and folding of the protein, is critical for filopodium and synapse formation in cultured hippocampal neurons.

Cellular and Molecular Neurobiology

P29.-Effect of prenatal exposure to cannabinoid agonist on ethanol preference in adolescent mice

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The endocannabinoid system is involved in the neurobiological mechanisms of drug addiction. Due to evidence that exposure to cannabinoid agonists during prenatal development affects several neurotransmission systems, and that the adolescent brain is vulnerable to ethanol exposure, we aimed to analyze ethanol preference (EP) in mice prenatally exposed to cannabinoid agonist WIN55212,2 (WIN). For this purpose, we used CB1+/+(wild type, WT) and CB1-/- (KO) mice. Pregnant mice received a daily subcutaneous injection of WIN (0.75 mg/Kg) or vehicle (0.3% Tween 80/saline) from gestational day 5 to the end of pregnancy. When pups reached adolescence, standardized tests were performed with different experimental behavior parameters: an anxiety test (elevated plus maze) and an aerial exploration and locomotor activity test (open field). For EP studies, pups were housed (two per cage) and given the choice to drink either EtOH 6% v/v or water during seven weeks. The volume of EtOH and water consumed and body weight were measured twice a week. WIN prenatal exposure did not produce changes in anxiety tests, although KO mice presented lower anxiety levels than WT. In turn, WT mice prenatally exposed to WIN showed higher EP than WT not exposed to WIN, as from the second week and no differences were found in KO groups. Results suggest that prenatal stimulation of the CB1 receptor could be involved in adolescent EP.
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Cellular and Molecular Neurobiology

P30.-Phenytoin promotes the proliferation of neural precursor cells and oligodendrocyte differentiation

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Phenytoin is an antiepileptic drug that increases the levels of growth factors like fibroblast growth factor-2 (FGF-2) and the expression of epidermal growth factor receptors (EGFR) in oral mucosa. In the adult ventricular-subventricular zone (V-SVZ) exists stem cells that widely expressed receptors of fibroblast growth factor (FGFRs I-V) and the EGFR. These receptors exert regulator functions in proliferation, differentiation and cell survival. Recently, it was reported that oral administration of phenytoin increases the proliferation rate in the V-SVZ. Nevertheless, the cell fate of these cells and the effects of this drug in the phosphorylation of FGFR or EGFR in the V-SVZ was unknown. In this study, we analyzed the cell lineage expanded by phenytoin as well as their effects in the increases of EGFR/FGFR phosphorylation in the V-SVZ. We used male Balb/C mice that received 10 mg/kg phenytoin by oral cannula for 30 days and analyzed the expression of immature and mature cell markers in the V-SVZ. The Phenytoin-treated animals showed an increase in the phosphorilation of FGFR and EGFR and an increases in the number of BrdU+/Sox-2+ and BrdU+/DCX+ cells in the V-SVZ as compared to controls. In conclusion, Phenytoin enhances the phosphorilation of EGFR and FGFR and modifies the cell fate of V-SVZ precursors.

Cellular and Molecular Neurobiology

P31.-Characterization of Membrane glycoprotein M6a endocytic-recycling pathway upon treatment with the neutralizing monoclonal antibody

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Neuronal glycoprotein M6a belongs to the proteolipid protein family PLP. M6a is composed of 278 amino acid residues that form two external loops, a minor EC1 and a major EC2, and four transmembrane domains; the N- and C-terminal regions lye in the cytoplasm. M6a is involved in neurite and filopodia outgrowth and synaptogenesis through unknown mechanisms. To date, no natural ligands or binding partners of M6a have been found. However, a monoclonal antibody against EC2 (mAb) has been used to block M6a function through clustering and possibly by M6a internalization. The membrane protein composition depends on endocytic recycling mechanisms. Interestingly, the PLP endocytic sorting in the oligondendrocyte surface precedes cell differentiation and it was characterized with mAb-internalization assays. Here we used HEK-293 cells and neurons to investigate the subcelular localization of M6a under mAb treatment. We found that M6a colocalizes partly with clathrin in the cell surface. Deconvolution (3D) of confocal z-stacks of each condition showed that after 30 minutes of treatment most of the M6a-mAb complex was present in endosomes and a few of them were positive for Rab5 (early endosome marker). No positive codistribution was observed between M6a and LAMP1 (lysosome marker) after 30 min or 1h. Therefore, we concluded that the reorganization of M6a in the cell surface after mAb treatment leads to its endocytosis. This endocytosis might depend on clathrin and Rab5 GTPase coordination.

Cellular and Molecular Neurobiology

P32.-Selective Oxidation of Alpha-Synuclein on Membrane Interphase

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Parkinson's disease is a progressive neurodegenerative disorder, histologically defined by intracellular aggregates of proteins, α-Synuclein (aSyn) mainly, and lipids. Aggregation of aSyn has been associated with selective loss of dopaminergic neurons, in combination with external factors related to lipid and protein oxidation and mitochondrial malfunction. Early intermediates of aSyn aggregates are thought to be the main "culprits", rather than mature amyloid fibrils. But a comprehensive description of the relationship between protein aggregation and neuronal death is still missing. We decided to evaluate the effects of aSyn oxidation and formation of crosslinked oligomers comparing aSyn free form and the one associated to phospholipid membranes.

aSyn diTyr crosslink was selectively formed using Ru(II) photo-sensitizers in the presence and absence of phospholipid membranes in order to study aSyn´s conformational changes and the role of lipids during oxidative stress modifications of aSyn. The presence of diTyrosine crosslinks was demostrated by fluorescence and westernblot.

Cellular and Molecular Neurobiology

P33.-TGF-beta and Notch pathway in cell fate decisions of adult neural stem cells from the subventricular zone

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Adult neural stem cells (aNSC) are capable of differentiating into neurons, astrocytes and oligodendrocytes throughout life. Notch and transforming growth factor beta 1 (TGF-beta) signaling pathways play critical roles in controlling cell fate. It has been previously reported that TGF-beta is pro-neurogenic on hippocampal and subventricular zone (SVZ) aNSC and that it might interact with Notch pathway in different cellular types. Therefore, the aim of our work was to study the effect of this cytokine on glial differentiation of aNSC from SVZ and its interaction with the Notch pathway.

In order to study TGF-beta and Notch pathway interaction in aNSC, we cultured cells obtained from SVZ of adult Wistar rats. After 48h plating, cells were fixed and immunocytochemically analyzed. Most cells were Nestin- and GFAP-positive, whereas a low percentage of cells expressed oligodendroglial markers such as PDGFR-alpha or NG2, and less than 1% were positive for a neuronal marker. aNSC treatment with TGF-beta during four days significantly increased the percentage of PDGFR-alpha- and NG2- positive cells, demonstrating the glial differentiating effect of this cytokine on neural progenitor cells of the adult SVZ. These results show the impact of TGF-beta on cell fate decisions of aNSC from SVZ. We are currently exploring the effect of TGF-beta on cell phenotype in the presence of a gamma-secretase inhibitor blocking Notch signaling.

Cellular and Molecular Neurobiology

P34.-Notch ligand-selective activation in CNS demyelination-remyelination

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CNS demyelination is a pathological process characterized by myelin loss around axons, while subsequent remyelination solves functional deficits.
This work studies Notch1 ligands differential roles in demyelination-remyelination in a cuprizone (CPZ)-induced demyelination model. Wistar rats were fed a CPZ or control diet for 2 weeks and sacrificed 7d before (-7d), upon (0d), and 7, 14 and 21d (+7d, +14d and +21d) after CPZ withdrawal. For each survival time, F3/contactin (F3) and Jagged1 levels were assessed by Western blot in the subventricular zone (SVZ) and corpus callosum (CC) and ligand-expressing cells were immunohistochemically characterized. Primary neurosphere cultures (NC) from SVZ were carried out to evaluate SVZ participation in CC remyelination. Cultured cell populations were characterized by specific markers and Notch activation, F3 and Jagged1 expression were determined.
An increase in F3 expression was detected at -7d, +7d and +21d in CC and at +14d in SVZ of CPZ animals as compared to controls. Cells expressing F3 were NG2+ and Olig2+. Also, an increase in Jagged1 expression was observed at -7d in GFAP+ cells. NC showed higher OPC proliferation in response to injury at 0d and +7d. Notch activation was found in NG2+ OPC, together with a larger percentage of Jagged1-expressing cells. F3 was found in NG2+ but went undetected in GFAP+ cells. Finally, co-immunoprecipitation assays proved F3 binding to Notch, thus confirming its role as a non-canonical ligand.

P35.-Doxycycline modifies α-synuclein Aggregation Pathway Yielding a Lacking Toxicity Novel Oligomeric Species

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The dopaminergic neuronal loss, observed in Parkinson disease, has been linked to the pathological aggregation of α-synuclein and its transcellular traffic in the dopaminergic system. The neuroprotective properties of tetracyclines in Parkinson's disease animal models have been reported. However, the interaction between members of this antibiotic family with α-synuclein has not been reported yet. In the present work we explore the mechanism by which doxycycline is able to exert a protective effect against α-synuclein mediated toxicity. According to NMR studies, doxycycline cannot interact with monomeric α-synuclein but, by using fluorescence and infrared spectroscopy together with electronic microscopy, we demonstrate the ability the antibiotic to interact with certain oligomeric species of α-synuclein leading to the formation of novel species structurally and morphologically different from the toxic ones. Moreover, these species are not capable of altering the permeability of model membranes neither diminishing the viability of dopaminergic cells shedding light into the mechanism of the neuroprotection conferred by tetracycline in Parkinson´s disease models. In addition we demonstrate that doxycycline may also inhibit the seeding effect of α-synuclein oligomers on the native protein in vitro. This study represents a milestone in the assessment of the feasibility of using doxycycline as a therapeutic agent in the treatment of neurodegenerative disease.

Cellular and Molecular Neurobiology

P36.-Oligodendrocyte maturation through gestational iron deprivation: the road not taken

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Iron deficiency (ID) affects oligodendrocyte (OL) maturation causing hypomyelination which continues in adulthood even after normal iron diet reinstatement. Our fundamental aim is to elucidate the role of iron during OL maturation and myelination processes. To describe the whole population of OL, we have now focused on four aspects: a) OL morphological architecture, which reflects OL maturation, b) the timing of dysmyelination in different brain areas, c) the expression pattern of markers for different stages of OL lineage along ontogenetic myelination and d) OL interaction with different cell types within normal CNS cytoarchitecture. To this end, we used an eGFP::CNPase transgenic mouse experimental model, whose green-fluorescent OL-lineage-committed cells (CNPase-positive cells) allow the visualization and analysis of single OL morphology and population features. Pregnant mice were fed ID diet (4mg/g/kg) from gestational day 5 until pup weaning (post-natal day 21, P21). CNPase-positive cell features were analysed at P15 and 30 in different brain areas. Control animals evidenced an increase in OL complexity during ontogenetic development. In turn, ID animals exhibited fewer CNPase-positive cells, with prevalence of immature OL, as tested by specific markers and confirmed by a decrease in MBP expression. We conclude that low iron availability does not affect cell lineage specification but expands an arrested OPC population, which is responsible for hypomyelination.

P37.-Hypoxia-Ischemia Induces Retinal Damage

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Introduction. A hypoxic event leads to an overall diminished cellular antioxidant capacity and causes several damages to cellular components (3). The retina is a tissue that undergoes high levels of stress due to its exposure to elevated oxygen levels and different light intensities (1), making it particularly susceptible to a hypoxic-ischemic insult. Our aim is to observe and establish the changes that this tissue undergoes after a hypoxic event related to pathologies such as retinopathy of the premature that usually follows perinatal asphyxia.

Methods. Histological techniques, Immunohistochemistry, Immunofluorescence, Electron microscopy, and Western blot were used to determine the outcome of hypoxia-ischemia in retinal tissues obtained from Sprague Dawley rats previously exposed to a global model of hypoxia-ischemia.

Results. Retinal tissues exposed to hypoxia-ischemia showed changes in layer width, nuclear membrane disorganization in ganglion cells, alterations in NeuN levels in different retinal layers, increased GFAP levels, and oxidative damage to DNA.

Conclusions. Hypoxia-ischemia affects retinal morphology, increases astrogliosis and induces cell damage. These findings are important to further establish the alteration that the retinal tissue undergoes after hypoxia as a basis for additional biochemical analysis.

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Cellular and Molecular Neurobiology

P38.-GDNF/GFRα1 complex is a synaptic organizer required for proper hippocampal circuit development

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The formation of synaptic connections during nervous system development is a highly regulated process mediated partly by cell adhesion molecules. Previously, we have identified GFRα1 as a glial cell line-derived neurotrophic factor (GDNF)-induced trans-synaptic cell adhesion molecule. In this work, we demonstrate that in the presence of GDNF, GFRα1 mediates postsynaptic assembly and dendrite structural plasticity in hippocampal neurons. In the presence of GDNF, overexpression of postsynaptic GFRα1 leads to an increase in the number of excitatory presynaptic contacts and promoted the assembly of postsynaptic machinery in cultured hippocampal neurons. Postsynaptic differentiation induced by GDNF was markedly reduced in neurons upon GFRα1 downregulation. In agreement with this evidence, ultrastructural analysis of conditional GFRα1-knockout mice showed a reduction in synapse number, alterations in synapse morphology and decrease in the synaptic localization of postsynaptic proteins in hippocampal neurons. Finally GFRα1/GDNF was found to be required for proper hippocampal dendrite development in cultured neurons and in GFRα1-deficient mice. Altogether these data show that GFRα1/GDNF plays a crucial postsynaptic role in the recruitment of excitatory and inhibitory postsynaptic machinery to the sites of synaptic contact as well as in dendrite structural plasticity. Our results indicate an essential role of GDNF and its receptor GFRα1 for proper hippocampal circuit development.

Cellular and Molecular Neurobiology

P39.-Brain responses after a distant chronic spinal cord injury

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The impact of spinal cord injury (SCI) has long focused on motor deficits. Cognitive deficits have been reported after SCI and its prevalence has been underestimated. Here we examined effects of chronic thoracic SCI on the activation of microglial cells in the hippocampus and the effects on SGZ neurogenesis. We used a clip compression model of SCI and performed two kinds of lesion, one moderate and one severe in rats. We studied by immunohistochemistry the number of ramified and bushy microglial cells (Ox-42 + cells) and the number of doublecortin positive cells (an index of neurogenesis) in the hippocampus. Our results shown that 60 days after injury (60 dpi) there was an increased in the number of activated busy microglia at hippocampus hilus in the moderate and severe SCI models. In addtion, the density of activated busy microglia was up regulated only in the severe model of SCI at the stratum radiatum of the CA1 and CA3 region of the hippocampus. The number of ramified microglia cells did not change in any studied group. Next, we explored neurogenesis at the SGZ of the hippocampus, and we observed that doublecortin + cells were down regulated 60 dpi after SCI compared to intact rats. Our results suggested that chronic brain neuroinflammation could occur after chronic SCI, likely related to sustained microglial activation. Microglial activation could reduce neurogenesis at the hippocampus and be related to cognitive deficits reported in patients.

Cellular and Molecular Neurobiology

P40.-Towards Unmasking the Pathophysiology of Epilepsy using a New Cellular Model based on Human iPSCs

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Epilepsy is one of the most common and disabling neurologic conditions, characterized by an enduring predisposition to generate epileptic seizures. Those seizures are paroxysmal alterations of neurologic function caused by the excessive, hypersynchronous discharge of neurons in the brain, that becomes apparent when there is a distortion of the normal balance between excitation and inhibition. For a better understanding of the pathophysiology of the disease, we developed a cellular model of epilepsy by differentiating iPSCs lines obtained from patients with an epileptic syndrome classified as a "benign focal epilepsy of childhood" (BFEC) and healthy individuals. After validating those cell lines, the functionality of the corresponding neurons was characterized. A delay in the development of electrophysiological properties was observed, with patients´ neurons being more excitable than controls. These neurons not only exhibited a lower action potential threshold, but also a marked tendency towards an increase of voltage-dependent K+ (rapidly inactivating and non-inactivating) currents, after 12 weeks of differentiation. Our observations suggest that patients´ neurons present a developmental delay, explaining the occurrence of seizures in children and being in accordance with the progression of BFECs, which are resolved by the end of adolescence, when the inhibitory circuitry matures. Further analysis would be necessary to elucidate the exact mechanisms underlying BFECs.

Cellular and Molecular Neurobiology

P41.-Delayed postconditioning with cobalt chloride activates pro-survival pathways in a murine model of perinatal asphyxia

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Perinatal asphyxia (PA) is an obstetric condition associated to a high morbimortality rate (incidence of 1 to 5/1000 born alive) and a significant risk factor for various neurodevelopmental disorders. A substantial body of evidence supports that a repetitive series of mild ischemic events applied after a main hypoxic-ischemic event (post-conditioning) activates various neuroprotective mechanisms, favoring neuronal survival and preserving memory and learning processes altered by PA. Cobalt chloride (CoCl2) might work as a post-conditioner since in normoxia it triggers transcriptional changes mimicking the organism response to a hypoxic event. Therefore, we studied this compound as a possible neuroprotective therapy. To that end, Sprague Dawley 7-day-old male rats were subjected to a hypoxia-ischemia (H-I) model by ligation of the right common carotid artery and a subsequent acute asphyxia by exposure to a 100% N2 atmosphere for 3 minutes. 24 hours later 60mg/kg of CoCl2 or vehicle were subcutaneously administered. After 1, 4 and 24 h, hippocampi were removed and the expression of HIF-1alfa, G3PDH Bcl2, Bax and P-AKT were analyzed by western blot. Postconditionated animals showed: an increase in Hif-1α expression and G3PDH at 1 h and 24 h after the acute treatment and a higher Bcl2/Bax ratio and P-AKT expression at 24 h post treatment. This data suggests that CoCl2 postconditioning activates pro-survival pathways in a murine model of PA.

Cellular and Molecular Neurobiology

P42.-Dual actions of HMGB-1 on neuroinflammation are dependent on the cellular micro-environment

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Brain injury produces a release of DAMP (Damage Associated Molecular Patterns) from the lesion core to the extracellular space activating brain innate immunity. The protein HMGB-1 is a main DAMP discharged by necrotic neurons and it is proposed to signalize through receptors TLR-2, TLR-4 and RAGE to induce an inflammatory response mediated by astrocytes and microglia.
In this work, we used an in vitro approach to study the role of HMGB-1 in reactive gliosis and to analyze the astroglial-microglial interaction. In hippocampal mixed neuro-glia culture containing all glial types and neurons, HMGB-1 induced neurodegeneration and astroglial conversion into a fibrillar reactive phenotype. Using astrocytes-enriched culture, we revealed that HMGB-1 activates the inflammatory transcriptional factor NF-kB in a time and concentration-dependent manner. When we dissected the HMGB-1 effects on the different cell types using conditioned media we observed that astrocytes exposed to HMGB-1 released factors induce microglial conversion to a mixed microglia M1/M2 activation profile. However, on neurons cultures, direct HMBG-1 application did not dramatically affected neuronal survival whereas conditioned media from astrocytes exposed to HMGB-1 increased neuronal survival. Based on these experiments, we conclude that HMGB-1 released by necrotic neurons after brain injury may have either a neurodegenerative or protective effect depending on the cellular micro-environment where it is released.

Cellular and Molecular Neurobiology

P43.-Comparative study of the functional properties of cochlear and neuronal cholinergic nicotinic receptors across different tetrapod species

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Nicotinic acetylcholine receptors (nAChRs) are members of the Cys loop superfamily of ligand-gated ion channels. They can be classified into muscle, neuronal and epithelial. The neuronal receptors assemble from the combination of different α2 –α8 and β2 –β4 subunits and the epithelial receptor of cochlear hair cells from α9 and α10 subunits. Unlike the rest of nAChR subunits, phylogenetic analysis has shown that mammalian α10 has been under positive selection and acquired several non-synonymous substitutions in its coding region. We propose that these acquired changes is the basis for functional diversity across species in the case of α9α10 compared to neuronal nAChRs, which can assemble from the wide variety of subunits expressed in the central nervous system. Thus, we hypothesize that the biophysical properties of the α9α10 nAChRs should vary across different vertebrate species, but those of neuronal nAChRs should be conserved. We compared the properties of recombinant rat, chicken and frog α9α10 and those of α7 neuronal receptors expressed in Xenopus laevis oocytes. Whereas rat, chicken and frog α9α10 nAChRs differ in their desensitization rate, Ca2+ permeability, Ca2+ modulation and current-voltage relationship, neuronal α7 nAChR exhibit similar channel properties. These results suggest that the peculiar evolutionary history of the α10 subunit resulted in differential functional properties of the α9α10 nAChR across species.

Cellular and Molecular Neurobiology

P44.-DMT1 is expressed and required for adequate maturation in oligodendrocytes

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Myelination requires oligodendrocyte precursor cells (OPCs) to experiment phenotypic changes in order to accomplish a maturation program that finally yields mature oligodendrocytes (OLGs). Divalent Metal Transporter 1 (DMT1) is a multi-metal transporter whose participation in OLG maturation and myelination remains unknown.

Our work shows that only DMT1 isoform B is present in OPCs. In vitro experiments demonstrate that DMT1 is upregulated during OPC development. Western blot and RT-PCR performed on primary oligodendrocyte cultures show low levels of DMT1 in OPCs but higher quantities in mature OLGs. Co-localization experiments revealed that MBP, CC1, O1 and O4 positive cells express high levels of DMT1, while PDGFrα positive cells show low expression of this transporter. In vivo experiments showed that DMT1 contents increase in the corpus callosum, cortex and cerebellum during myelination. Furthermore, DMT1 is highly expressed by OLGs during the development of the corpus callosum and cortex in the EGFP::PLP mice. Interestingly, the spinal cord shows decreasing levels of DMT1 content during the time points analyzed. siRNA mediated silencing of DMT1 in primary cultures of OPCs proved that reduced levels of DMT1 impairs OPC development. DMT1 knockdown in turn induces a decrease in the proportion of myelin-protein-expressing OLGs and an increase in cells retaining immature oligodendrocyte markers. Our data suggest that DMT1 is an important for proper oligodendrocyte maturation.

Cellular and Molecular Neurobiology

P45.-Urokinase-type plasminogen activator (uPA) promotes the axonal outgrowth in retinal ganglion cells

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Urokinase type plasminogen activator (uPA) is a serine protease which facilitates growth cone movement by local proteolysis of extracellular matrix. Recently we have demonstrated that the interaction between uPA and its receptor (uPAR) induces the neuronal migration and neuritogenesis by both proteolytic activity and nonproteolytic events throughout the activation of intracellular signaling in migrating neurons of the developing chicken Optic Tectum.
In this work we investigated whether the uPA:uPAR complex participates in the axonal growth of retinal ganglion cells (RGC). We performed cultures of retinal explants from chicken embryos of 7 days. Some explants were treated with 10 nM of uPA. By immunocytochemistry we indentified the presence of uPAR in the axonal growth cones of RGC. On the other hand we performed time-lapse experiments with control and experimental explants. The cultures were photographed during 3 hours and we measured the length of the axons each 30 minutes in both control and uPA stimulated explants.
The results showed that after 3 hours, the axons exposed to uPA stimulation grew 118% ($p<0.05$) more than the control axons. The growth rate was also higher in uPA-stimulated axons in relationship with control ones (78,04 □m/h vs 44,24 □m/h respectively, $p<0.05$). In summary we could determine that uPA:uPAR system promotes the axonal growth of RGC during the development of the retina.
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Cellular and Molecular Neurobiology

P46.-Reactive astrocytes secrete exosomes that induce motor neuron death. Implications for ALS

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In the neurodegenerative disease amyotrophic lateral sclerosis (ALS), reactive astrocytes undergo alterations in gene expression that modify their neurotrophic properties inducing the death of surrounding motor neurons. Astrocyte conditioned media from ALS models is toxic to motor neurons but the exact nature of the neurotoxic agent is unknown. Our hypothesis is that extracellular vesicles, such as exosomes, might carry this neurotoxic agent. We compared the effects of exosomes derived from SODG93A-expressing rat astrocytes and IL- 1B/LPS stimulated wild-type astrocytes on the survival of isolated spinal motor neurons. Exosomes derived from both types of astrocytes were characterized by electron microscopy and analysis of their differential protein content. Purified embryonic motor neurons were incubated with the different fractions of astrocyte-conditioned media: Both SOD1G93A-expressing and IL1B/LPS-stimulated astrocyte-conditioned media were toxic for motor neurons. Purified exosomes from both types of astrocytes were sufficient to induce the death of motor neurons while the exosome-free astrocyte conditioned media did not induce motor neuron death. These results support the hypothesis that exosomes play a significant role in ALS spreading, through other mechanisms besides the familial-linked mutated SOD. We are currently searching for candidate molecules responsible for the exosome-mediated neurotoxicity in these different models of astrocyte reactivity.

Cellular and Molecular Neurobiology

P47.-Phytotherapic potential of infusive plant extracts on overweighed mice: Brain exposure to polyphenols and oxidative risk in adolescent females.

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Biomedical potential of phytochemicals depends on their ability to modulate redox balance and mechanisms, which are involved in the development of chronic noncommunicable diseases (NCDs). Among them, obesity is a major cause of human morbidity and mortality worldwide, thus bioprospecting studies in flora are encouraged to find bioactive molecules. In consequence, the aim of this work was to establish effects of extracts (100 mg/Kg/d) from infusions of Lantana grisebachii Stuck. (Verbenaceae), Aspidosperma quebracho-blanco Schltdl. (Apocinaceae) and Ilex paraguariensis St.-Hil. (Aquifoliaceae) in adolescent female mice with overweight, after 15-day oral treatment. Weight gain, total polyphenols and oxidative markers (superoxide anion, hydroperoxides and lipoperoxides) were measured by colorimetric techniques in different brain regions (telencephalon, diencephalon, midbrain, brainstem and cerebellum), with results being compared by ANOVA ($p<0.05$). A. quebracho-blanco significantly decreased weight gain and led to significant concentration of polyphenols in brainstem ($p<0.02$). However, it increased levels of superoxide anion ($p=0.0029$) and lipoperoxides ($p=0.0280$) in this region. L. grisebachii reduced levels of oxidative markers differentially in each brain region ($p<0.05$). I. paraguariensis was oxidant in midbrain and cerebellum, although it was antioxidant in brainstem ($p<0.05$). Also, the three treatments were antioxidants in the telencephalon ($p=0.0029$). Overall, the A. quebracho-blanco extract showed potential activity on overweight, although it was accompanied by collateral oxidative stress in the brainstem, where polyphenols were increased. This should be considered to propose its phytomedicinal use and to design future studies with their derivatives.

Cellular and Molecular Neurobiology

P48.-Cuprizone effects on proliferation and differentiation of neural stem cells

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Cuprizone (CPZ) is a copper-chelating agent which induces demyelination in mice. Although its neurotoxic mechanism is still unknown, it was shown in vivo that CPZ produces astrogliosis, microglial activation and loss of oligodendrocytes that progressively lead to demyelination and neuronal degeneration.
Neural stem and progenitor cells (NSC/NPC) are able to generate all neural cell types and can be cultured as Neurospheres (NS). Depending on culture conditions, NS can be maintained in a proliferative and undifferentiated sate or alternatively they can be forced to differentiate into neurons, astrocytes and oligodendrocytes. Here, by using NS cultures, we evaluate CPZ effects on NSC/NPC survival and proliferation, cell migration and cell differentiation.
Although NSC/NPC survival was not affected at lower CPZ doses, we detected a reduction in NSC/NPC viability at higher CPZ concentrations. Cell proliferation was not affected in the presence of CPZ at any tested concentrations. Contrarilly, differences in cell migration patterns were found in CPZ treated cultures in comparison to control cells.
CPZ treatment of dissociated NS during differentiation process did not change neurons or astrocytes proportions. However, oligodendroglial differentiation was completely abolished when cells were exposed to low CPZ doses. The detection of oligodendroglial precursor cells in these conditions, suggests that CPZ has an inhibitory effect on oligodendroglial maturation.

P49.-Sensing Light by Horizontal Cells in the Chicken Retina: A New Player in the Photoreceptive System

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Retinal ganglion cells (RGCs) expressing the photopigment melanopsin (Opn4) display intrinsic photosensitivity. In the chicken retina, two Opn4 genes, the Xenopus (Opn4x) and the mammalian (Opn4m) orthologs were described. Opn4m was shown to be restricted exclusively to the RGCs whereas Opn4x was found in the GC layer and optic nerve at embryo day 8 (E8), but by E15 mostly in Prox1 (+) horizontal cells (HCs). The aim of this work was to obtain HC primary cultures and to characterize their potential intrinsic photosensitivity. Disaggregated chicken retinas at E15 were subject to a discontinuous 1-4% bovine serum albumin (BSA) gradient. Only the 2.5% BSA phase contained most cells displaying PROX-1 and Islet-1 positive immunoreactivity with a typical HC morphology. Moreover, by immunopanning against Opn4x of the 2.5 % phase we obtained HC cultures strongly expressing Opn4x. In addition, HCs expressed different components of the non-visual phototransduction cascade. When HC cultures were exposed to light to assess intrinsic photosensitivity by calcium imaging, we found positive light responsiveness in cells exposed to different light intensities as compared with controls kept in the dark. This study strongly suggests the existence of a novel type of photosensitive cells in birds, Opn4x (+) HCs localized to the inner retina likely implicated in novel connections between the outer and inner retina.
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Cellular and Molecular Neurobiology

P50.-*In vitro* modeling of the broad spectrum of astrogliosis

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Astrocytes are complex, highly heterogenic cells found throughout the CNS, which make numerous contributions to normal function in healthy CNS. Their broad spectrum of activation profiles in response to insults is still poorly understood. Astrogliosis is a gradated continuum, ranging from diffuse mild or moderate astrogliosis to severe astrogliosis with scar formation. Therefore, the existence of assorted in vitro models seems essential for a better comprehension of the cellular pathways involved in different scenarios. In the present work, we used 2D or 3D in vitro models to analyze astrocyte's response to different stimuli. In a 2D glial scar model, involving meningeal cells, astrocytes forming the scar had increased expression of TLR-2 and TLR-4, two receptors related to innate immunity. However, we didn't see increased incorporation of a reporter nanoparticle, which is more avidly up taken by other activated astrocytes. For 3D astrocyte cultures we used a peptide nanofiber scaffold, were astrocytes grew in a more realistic environment, and survived for several weeks with excellent viability and in vivo like morphology. In this context, we were able to produce astrogliosis in response to certain DAMPs, or to leukocytes, but unable to obtain a glial scar. These results show different astrocytic responses, according to context, and indicate that the 3D model is a more realistic versatile approach for the study of astrogliosis dynamic responses. PICT2012-1424, CONICET, UBACYT

Cellular and Molecular Neurobiology

P51.-Does voltage-gated calcium channels (CaV) activity inhibition by constitutive growth hormone secretagogue receptor type 1a (GHSR1a) activity depend on CaVβ auxiliary subunit?

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GHSR1a is the receptor of ghrelin, which participates in neural circuits involved in physiological functions such as food intake, stress and memory. One of its main features is its constitutive activity (CA) that seems to be relevant due to its expression in brain areas with restricted access to ghrelin. We have shown that GHSR1a CA impairs presynaptic CaV (CaV2.1 and CaV2.2) activity by reducing their surface expression. We are now investigating which structures of CaV are the targets of GHSR1a CA. We found that GHSR1a CA also inhibits other channels with different pore-forming subunits: the high voltage activated (HVA) CaV, CaV1.2 and CaV1.3, but not a low voltage activated (LVA) CaV: CaV3.2. All the HVA CaV tested have in common that CaVβ auxiliary subunit modulates their surface expression, degradation and gating, so we included CaVβ in such experiments, while we did not when we tested the LVA CaV, which was not affected by GHSR1a CA. Thus, we hypothesize that CaVβ may be the target of GHSR1a CA. We found that GHSR1a is able of inhibiting all CaV subtypes only when CaVβ is included in the channel complex, while the presence of CaVα2δ auxiliary subunit is not required. We are currently testing several mutated CaVβ at key aminoacids that could be targeted by protein kinases activated by GHSR1a CA. Our results point to a new function of CaVβ auxiliary subunit as a joint factor that controls CaV trafficking and the impact of G protein-coupled receptors activity on CaV.

Cellular and Molecular Neurobiology

P52.-Regulation of Neuronal Developing by Growth Factors: "In vivo" studies.

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The cerebral cortex, experiences a rapid expansion at early stages of development, resulting in the formation of six horizontal layers, each containing distinct neuronal populations exhibiting different biochemical and functional characteristics. This development requires the coordinated of several processes, such as symmetric and asymmetric division of progenitor cells at the ventricular zone (VZ).Neuronal migration to fill specific areas of the neo-cortex, and specification neuron polarization leading to dendritic and axonal development. Using "in utero" electroporation (IUE) we studied the consequences of blocking two different receptor-growth factor systems on cerebral cortex formation: i)The IGF-1 receptor (IGF-1R) system involved in neuronal differentiation and the establishment of polarity¸ and ii)transforming growth factor β (TGF-β)-TGF-β receptors (TGF-βR), also shown to be important for the establishment of neuronal polarity. The result shows that knocking down the IGF-1R in arrested cortical neurons at the VZ and resulted in heterotopic like structures. This phenotype was partially rescued by over expressing P110, a kinase known to act downstream of the IGF-1R. Silencing the TGF-βR resulted in a subpopulation of neurons delayed in the intermedia zone (IZ) of the cortex and a small cohort of late born neurons abnormally stalled at the upper layer V. The results demonstrate the critical participation of the two receptors at different stages of neuronal developed.

Cellular and Molecular Neurobiology

P53.-Intermediate filament expression in the cervical spinal cord of rats after intraparenchymal injection of kainic acid

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Pathological features of many neurodegenerative diseases include synaptic loss, dendrite retraction and neuronal degeneration and death. It was recently reported that neuronal intermediate filaments (IF) play a central role in damage-response mechanisms by activating a developmental program to differentiate neurons and to establish synaptic connections. The goal of the present work was to evaluate IF immunohistochemical changes induced by an intraparenchymal injection of kainic acid (KA) into the rat cervical spinal cord. Male rats were injected with 1 mM of KA at the C5 segment. Saline-treated animals were used as sham and non-operated rats as control group. Rats were sacrificed either at days 1, 2, 3 or 7 post-injection (pi). Anti-neurofilament (NF) and anti-vimentin (VIM) antibodies showed positive labeling for neuronal projections and ependymal and endothelial cells in control group, respectively. Besides, anti-GFAP labeled all glial cells. Sham rats showed anti-VIM and anti-NF positive labeling of perikarya for the first two days pi. Perikarya of KA-treated animals showed positive labeling for VIM and NF throughout the experiment, whereas GFAP reactivity was observed only for the first two days pi. These results suggest that in the perikarya of treated rats there is an unconventional IF expression, which may respond to the neuronal damage induced by the mechanical injury and the KA to reestablish synaptic connections lost during the neurodegenerative process.

Cellular and Molecular Neurobiology

P54.-The polarized transport of Syntaxin 6 mediated by KIF5C is essential for the exocytosis of IGF-1 receptor and establishment of neuronal polarity

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The formation of a polarized neuron, containing one long axon and several branching dendrites, requires the action of two interrelated processes, specification of the axon and axonal elongation. Regarding the regulation of initial axonal outgrowth, a particularly early event that occurs in neurons that have not yet exhibited a discernible axon (stage 2 of differentiation) is the segregation of activatable IGF-1 receptors (IGF-1r) in a single neurite(Sosa et al., 2006)). This event is critical for the outgrowth of the future axon. In order to become activatable, the IGF-1r needs to be inserted to the neuron plasmmalema. This occurs by exocytotic incorporation of vesicles containing IGF-1r with the participation of the exocist complex (Dupraz et al., 2009) and the SNAREs proteins VAMP4, Syntaxin 6 and SNAP23 (Grassi et al., 2015). Besides polarization of the IGF-1r, two early polarity events are known: i) The accumulation of stable microtubules at one neurite (the future axon-Witte et al., 2008), and ii) The preferential transport to one neurite of the microtubular motor KIF5C (Jacobson et al., 2006) which shows a preference to interact with axonal (stable) over dendritic microtubules. Our results show that these three early polarity events described above are interdependent phenomena necessary for the regulation of initial axonal outgrowth, essential for the establishment of neuronal polarity.

Cellular and Molecular Neurobiology

P55.-EphA4 activity stimulates retinal stem cells proliferation and regulates their sorting in vitro

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The chicken embryonic retina presents multipotent stem cells in the ciliary zone (CZ).
We showed that stem cells express molecules of the Eph-ephrin system. We investigated whether the EphA4 expressed in retinal neurospheres regulates proliferation of stem cells obtained from CZ and whether these stem cells express positional information.
We cultured dissociated cells obtained from chicken embryonic nasal and temporal CZ, favoring neurospheres formation. We added BrdU to detect proliferating cells and compared nasal and temporal control neurospheres with nasal and temporal neurospheres treated with Kyl peptide (specific inhibitor of EphA4).
We compared the distribution of BrdU + cells, the ratio of BrdU + cells /Hoechst + nucleous, the level of activated EphA4 and the expression of topographic markers (FoxG1 and ephin-A5 for nasal retina).
It was shown that: 1) BrdU + cells concentrate in the peripheral zone of neurospheres; 2) partial inhibition of EphA4 activity reduces the proportion of proliferating cells by reducing its density in the peripheral zone, changing their peripheral location to a more homogeneous distribution. 3) It was also shown that nasal neurospheres express higher levels of FoxG1 and ephrin-A5 such as the case for nasal retina.
Results show that EphA4 activity not only increases stem cells proliferation but also favors sorting of proliferating cells. Besides, they show that stem cells express positional information.
Supported by UBA and CONICET

Cellular and Molecular Neurobiology

P56.-Myelin-associated glycoprotein modulates apoptosis of motoneurons during early postnatal development via NgR/p75NTR receptor-mediated activation of RhoA signaling pathways

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Myelin-associated glycoprotein (MAG) is a minor constituent of nervous system selectively expressed on the periaxonal myelin wrap involved in axon-glia communication. The aim of this study was to analyze a possible modulatory role of MAG on MNs apoptosis during postnatal development. A time course study showed that Mag-null mice suffer a loss of MNs during the first postnatal week. Also these mice exhibited increased susceptibility in an animal model of p75NTR-dependent MNs apoptosis induced by nerve-crush injury, which was prevented by treatment with a soluble form of MAG (MAG-Fc). The protective role of MAG was confirmed in in vitro models of p75NTR-dependent MN apoptosis using the MN1 cell line and primary cultures. Lentiviral expression of shRNA sequences targeting Nogo-receptors NgRs on these cells abolished protection by MAG-Fc. Analysis of RhoA activity using a FRET-based RhoA biosensor showed that MAG-Fc activates RhoA. Pharmacological inhibition of p75NTR/RhoA/ROCK pathway, or overexpression of a p75NTR mutant unable to activate RhoA, completely blocked MAG-Fc protection against apoptosis. The role of RhoA/ROCK signaling was further confirmed in the nerve-crush model, where pre-treatment with a ROCK inhibitor blocked the pro-survival effect of MAG-Fc. These findings identify a new protective role of MAG as a modulator of apoptosis of MNs during postnatal development and highlight the relevance of the protective effects of myelin on neurons

Cellular and Molecular Neurobiology

P57.-Constitutive Hippocampal Cholesterol Loss Underlies Poor Cognition in Old Rodents: effect on receptor trafficking and in epigenetic mechanisms

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Cognitive decline is one of the many characteristics of aging. Reduced long-term potentiation (LTP) and long-term depression (LTD) are thought to be responsible for this decline, although the precise mechanisms underlying LTP and LTD dampening in the old remain unclear. We previously showed that aging is accompanied by the loss of cholesterol from the hippocampus, leading to PI3K/Akt phosphorylation which in turn perturbs the normal cellular and molecular responses induced by LTD. Electrophysiology recordings in brain slices of old mice and in anesthetized elderly rats demonstrate that the reduced hippocampal LTD associated with age can be rescued by cholesterol perfusion.

Later, we found that a memory-inducing stimulus triggered by NMDA application induced the transcription of Bdnf promoters by H3K27Me3 demethylation and H3K27Me3 phosphorylation leading to displacement of EZH2, the catalytic subunit of Polycomb Repressor Complex 2. Our data also showed that the fast transient expression of Bdnf promoters after neuronal stimulation is dependent on acetylation of histone H3K27 by CREB/pCBP complex. Interestingly, these epigenetic mechanisms controlling Bdnf induction are impaired in old neurons and rescued by cholesterol replenishment. Accordingly, cholesterol replenishment in old animals improves hippocampal-dependent learning and memory in the water maze test.

Cellular and Molecular Neurobiology

P58.-Cross talk between Bone Marrow Mononuclear Cells and immune system cells in Peripheral Nervous System remyelination

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Wallerian degeneration (WD) is a pathophysiological process characterized by demyelination and axonal degeneration. Immediately after injury, the interruption of Schwann cell (SC) and axon dialogue generates SC dedifferentiation. Along with fibroblasts, these SC secrete cytokines such as IL-1β, IL-6, TNFα, GM-CSF, CCL2 and CCL3, which promote immune cell infiltration as from 24h post injury. Among other cell types, hematogenous macrophages and the few resident ones take over as the dominant leukocyte population playing a critical role in ensuring complete WD through debris phagocytosis and contributing to axonal regrowth through IL-10, TGFβ and trophic factor secretion. Bone marrow mononuclear cell (BMMC) transplant has recently become relevant in multiple pathologies, including peripheral nervous system (PNS) lesions. To test the hypothesis that BMMC effects involve immunomodulatory actions, the present work evaluated BMMC remyelination ability and cytokine mRNA levels in adult rats undergoing sciatic nerve crush. Epifluorescent, confocal, optical and electronic microscopy and Western blot analyses showed axonal recovery and remyelination promoted by cell transplant. As regards cytokine secretion, qPCR studies performed 24h post injury proved BMMC transplant to promote a decrease in IL-1β levels compared to non-transplanted rats. Macrophage polarization assays are now under way in order to further confirm a cross talk between BMMC and immune system cells in PNS remyelination.

Cellular and Molecular Neurobiology

P59.-Astroglial alterations from early to late stages in a model of Alzheimer's disease: evidence of autophagy involvement in amyloid- β internalization

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Alzheimer's disease (AD) is the principal cause of dementia in the elderly. One histopathological hallmark of AD is the presence of senile plaques, extracellular deposits principally composed of amyloid-β (Aβ) peptides, which are normally surrounded by reactive glia. We studied early alterations in hippocampal astrocytes and their progression during the degenerative process in PDAPP transgenic mice, a model of AD. At 3 months of age, before plaques formation, we found less number of GFAP+ astrocytes in the stratum radiatum under CA1 ($p<0.05$), suggesting early glial dysfunction and low neuronal support. At 15 months of age, astrocytes around plaques exhibited strong LC3 immunoreactivity, suggesting an increased autophagic flux. Moreover, autophagosome-like vesicles were detected in hippocampal astrocytes surrounding dystrophic neurites in PDAPP mice by electron microscopy. In vitro experiments were conducted to determine if astrocytes were capable of internalizing Aβ into autophagic vesicles. In rat astrocytoma C6 cells exposed to Aβ fibrils, LC3+ vesicles colocalized with Aβ peptides. In C6 glioma cells with impaired autophagy by expression of mutated ATG5 gene, the Mander's overlap coefficient between Aβ and LC3 was significantly decreased ($p<0.001$). Our results provide evidence of plaque-independent alterations in hippocampal astrocytes and suggest that astroglial autophagy could play a role in processing Aβ fibrils at advanced stages of AD.

Cellular and Molecular Neurobiology

P60.-Optogenetic corticostriatal circuit modulation: validation of a new lentiviral vector carrying channelrhodopsin-2

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Tools that permit the activation of specific neuronal groups allow testing their contribution to specific behavioral outcomes. One of these tools is optogenetics. Our goal was the construction of a lentiviral vector capable of expressing Channelrhodopsin-2 (ChR2), a light-sensitive (~450nm) nonspecific cation channel fused to the reporter protein EYFP. An advantage of lentiviruses over other vectors is that infections of non-replicating cells result in the insertion of the virus DNA into the host cell genome, allowing a long-term and stable expression of a transgene. To ensure the cellular specificity of the expression we inserted a STOP cassette flanked by LoxP sites between ChR2-EYFP and the ubiquitous neural promoter synapsin that would prevent the expression of the ChR2 in “non-Cre cells“. To test the specificity of the lentiviral vector we performed an in vitro transduction of cholinergic-like cell line Neuro-2a co-infecting or not with another lentiviral vector which expressed Cre recombinase. We also tested the specificity in vivo by microinjecting the vector in the primary motor cortex or striatum of transgenic mice that express Cre recombinase under the control of the parvalbumin or choline acetyltransferase promoters. To test our capacity to modulate the activity of ChR2 expressing cells we will perform whole cell recordings of EYFP+ cells while illuminating with different patterns of blue light. This is to our knowledge the first optogenetic tool developed locally.

Cellular and Molecular Neurobiology

P61.-Three-dimensional reconstruction of degenerated corticospinal tracts from TDP-43 transgenic mice using clearing technique

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Mislocalization and aggregation of the protein TDP-43 are hallmark features of the neurodegenerative diseases amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). We have previously shown in mice that inducible overexpression of a cytoplasmically-localized form of TDP-43 (TDP-43-ΔNLS) in forebrain neurons evokes neuropathological changes that recapitulate several features of TDP-43 proteinopathies. Moreover, we recently described profound behavioral deficits in these mice, including motor, cognitive and social phenotypes. Some of these alterations were reversed upon transgene repression. Here, we describe the use of a novel approach to study the anatomo-pathological correlates of these changes, combining a cost-effective unsectioned brain/spinal cord clearing technique, fluoroRuby staining, one-photon confocal microscopy and three dimensional reconstruction to study the morphological changes in motor cortex (MC) and corticospinal tract (CST) of TDP-43 transgenic mice. Our immediate goals are to study the degree of CST degeneration as well as MC integrity and to analyze if the reversible motor phenotypes can be associated with axonal regeneration and sprouting in these structures. The application of this combined approach allows for evaluation of the damage caused by TDP-43 manipulation along the entire affected pathway, and to help elucidating the mechanisms underlying the motor phenotype recovery after transgene suppression, with implications for ALS/FTD.

Cellular and Molecular Neurobiology

P62.-Galectin-1 circumvents lysolecithin-induced demyelination through the modulation of microglial polarization and phagocytosis

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Galectin-1 (Gal-1), a member of a highly conserved family of animal lectins, binds to both N- and O-glycans decorating cell surface glycoconjugates. Current evidence supports a role for Gal-1 in the pathophysiology of multiple sclerosis (MS), one of the most prevalent chronic inflammatory diseases. Previous studies showed that Gal-1 exerts neuroprotective effects by promoting microglial de-activation in a model of autoimmune neuroinflammation and induces axonal regeneration in spinal cord injury by interfering with inhibitory signals triggered by Sema3A.
Seeking for a model that could link myelination, oligodendrocyte responses and microglia activation, we used a lysolecithin (LPC)-induced demyelination model to evaluate the ability of Gal-1 to preserve myelin without taking part in T-cell modulation. Our results demonstrate that Gal-1 treatment after LPC-induced demyelination induced a significant decrease in the demyelinated area and promoted a more efficient remyelination process, concomitantly with an attenuated oligodendroglial progenitor response reflecting less severe myelination damage. Moreover, there was a decrease in the area of microglial activation with a shift toward an M2-polarized microglial phenotype and diminished astroglial activation. Mechanistically, Gal-1 was observed to mainly target activated microglia, leading to an increased phagocytic capacity. This study supports the use of Gal-1 as a potential treatment for demyelinating diseases such as MS.

Cellular and Molecular Neurobiology

P63.-Long Lasting Cerebellar Alterations After Perinatal Asphyxia In Rats

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The developing brain may be particularly vulnerable to hypoxia as a consequence of perinatal asphyxia (PA), being cerebellar circuitry particularly susceptible, as the cellular makeup and the quantity of inputs change quickly during days and weeks following birth.
Short-term cerebellar alterations using a rat model of global PA at the time of birth have been previously reported but whether such alterations remain in adulthood has not been conclusively determined yet. For this reason, and given the crucial cerebellar role in determining connectivity patterns in the brain, the aim of our work is to unveil long-term cerebellum histomorphology following a PA insult.
Morphological and cytological neuronal changes and glial reaction in the cerebellar cortex were analyzed at postnatal 120 (P120) following injury performed at birth. As compared to control, PA animals exhibited: an increase in molecular and granular thickness, both presenting lower cellular density; a disarrayed Purkinje cell layer presenting a higher number of anomalous calbindin-stained cells; focal swelling and marked fragmentation of microtubule-associated protein 2 in Purkinje cell dendrites and, an increase in glial fibrillary acidic protein expression in Bergmann cells and the granular layer. In conclusion, we demonstrate that PA produces long-term damage in cellular histomorphology in rat cerebellar cortex which could be involved in the pathogenesis of cognitive deficits observed in both animals and humans.

Cellular and Molecular Neurobiology

P64.-Toll-like receptors TLR2, TLR4 and the DAMP HMGB-1 are involved in reactive gliosis and neuronal survival

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Reactive gliosis is a generic glial response to brain injury that may have beneficial or detrimental effects for neuronal survival. TLR are innate immunity receptors activated by HMGB1 and other DAMPs released by necrotic cells after brain injury and it is supposed that they are involved in the reactive astrocytes conversion to the proinflammatory-neurodegenerative phenotype.
In this work we studied the role of TLR2, TLR4 and HMGB-1 in the astroglial conversion to the reactive phenotype and neuronal survival. First, we demonstrated that TLR2 and TLR4 are expressed in the periphery of brain ischemic lesions induced by the cortical devascularization model. Then, we showed that HMGB1 exposure (50-500 ng/ml) induce astrocytic stellation, NF-kB activation and, surprisingly, facilitated the expression of antinflammatory genes in primary astrocytic culture. Conditioned media from HMGB1-exposed astrocytes (ACM-H) was used to treat primary cortical neuronal cultures. Neuronal viability was assayed by an enzymatic assay (MTT) and ACM-H showed to improve neuronal survival. ACM-H also showed a pro-synaptogenic effect when synaptic puncta were quantified by synapthophysin immunocytochemistry. On primary cultured microglia, ACM-H induced an M2-like profile identified by a panel of genes. Our results show that HMGB1 induces reactive gliosis on astrocytes and, contrary to it was previously supposed, produces an anti-inflammatory and pro-survival effect.
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Cellular and Molecular Neurobiology

P65.-Guillain Barré Syndrome-associated anti-glycan antibodies halt axon regeneration by inhibiting microtubule assembly via RhoA-ROCK-dependent inactivation of CRMP-2

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Axon regeneration is a response of injured nerve cells critical for the nerve repair in human acute immune neuropathies such as Guillain Barrè Syndrome (GBS). Clinical studies associate the presence of anti-ganglioside antibodies (anti-Gg abs) with poor recovery in GBS. Passive transfer of mAb (GD1a/GT1b, clone 1B7) in an animal model halts axon regeneration. Cultures of dissociated DRG neurons (DRGn) demonstrated that anti-Gs inhibits neurite outgrowth via activation of RhoA/ROCK-dependent pathways. The aim of this work is to study the molecular basis of the inhibitory anti-Gg abs-effect on neurite outgrowth. Time-lapse video microscopy was used to study their dynamics at growth cones (GC) in DRGn cultures together with immunofluorescence analysis of stable/dynamic microtubules (MT). Studies of 1B7-triggered signaling events included activity of small GTPase RhoA and its downstream target Collapsin response mediator protein-2 (CRMP-2). 1B7-treated DRGn cultures display reorganization of cytoskeleton´s components such as a rapid loss of filopodia and collapse of lamella followed by a delayed in MT retraction. MT but not lamella alterations were preceded by increase in RhoA activity. Y-27632 and C3 treatment did not reverse alterations in lamella. Electroporation of animals with a mutant CRMP-2 T-555A overcome 1B7-induce axon regeneration inhibition. Our results suggest that anti-Gg abs induce halt in axon regeneration by RhoA/ROCK-dependent and independent signaling pathways.

Cellular and Molecular Neurobiology

P66.-Brain-derived neurotrophic factor protects astrocytes from cell death

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Brain-derived neurotrophic factor (BDNF) is a neurotrophin that promotes neuronal survival and inhibits apoptosis of neurons and oligodendrocytes. Nevertheless, little is known about BDNF effects on astrocytes. We have previously shown that BDNF increases astrocyte viability through ERK and Akt pathways. Now, we studied the effects of BDNF on astrocyte death. Primary cultures of rat astrocytes were incubated for 24 h with or without BDNF 50 ng/ml and apoptosis was induced by serum deprivation. We found that BDNF blocked the decrease in cell viability induced by serum deprivation. BDNF decreases the percentage of hypodiploid (Sub G1) astrocytes and the percentage of TUNEL-positive astrocytes induced by serum deprivation. The BDNF receptor (TrkB) antagonist, ANA12, blocked the protective effects of BDNF. We also treated astrocytes with 3-nitropropionic acid (3NP), a toxin widely used as an in vitro model of Huntington's disease. 3NP decreased astrocyte viability in a dose-dependent manner. BDNF abolished the inhibitory effect of 3NP on astrocyte viability. Next, we treated PC12 neurons with 3NP to induce neuronal death. Conditioned medium from BDNF-treated astrocytes blocked the decrease in PC12 neuron viability induced by 3NP. These results indicate that BDNF protects astrocytes from different insults by engaging TrkB, and that BDNF-treated astrocytes have neuroprotective actions in a model of Huntington disease.

Cellular and Molecular Neurobiology

P67.-Relevance of axonal transport in the developing brain: a forgotten road in growth and axonal guidance

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Axonal pathfinding decisions rely on intercellular attractive and repulsive guidance cues which are read by receptors located in the navigating growth cones. Recently, the endocannabinoid (eCB) system has been identified as an important regulator of brain wiring during development with relevant roles in axonal outgrowth and pathfinding. eCBs mediate the motility and directional turning of axons by activating CB1 cannabinoid receptors (CB1R) in its growth cone. Although cargo delivery are essential in developing neurons for molecule presentation during elongation and guidance of axons, the axonal transport system that mediate this delivery of guidance receptors remain mostly unknown. To test the hypothesis that CB1R function depends on kinesin-1 mediated anterograde delivery for proper axonal guidance, we use mice lacking the kinesin light chain 1 (klc1) subunit of the anterograde motor kinesin-1. To test this hypothesis in vivo, we performed axon-tracing experiments with DiI in klc1-/- mice and found several axonal guidance defects. Interestingly, we revealed the dependency of KLC1 motor for normal CB1R dynamics and positioning by live-cell imaging of CB1-GFP transfected neurons. Finally, we demonstrated that KLC1 is required for the correct growth cone collapse and axonal outgrowth-induced by CB1R agonists. Altogether, these results suggest that KLC1-mediated transport of CB1R is required for a normal eCB signaling in the axonal pathfinding of the developing brain.

Cellular and Molecular Neurobiology

P68.-Opposite cues contained in spaced stimuli provide precision in the magnitude of the structural synaptic plasticity

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Synaptic plasticity produced by specific temporal patterns of activity is essential for behavioral readjustment. Exists plenty evidence on how spaced patterns of training enhance memory and skills acquisition. However, little is known about how synapses encode and decode information from these activity patterns.
Here we analyzed events of synaptic plasticity (new synaptic boutons) in motoneurons after a variety of protocols of stimulation in transgenic fruit flies by fluorescence microscopy.
Our findings showed that spacing and repetition during stimulation are relevant cues for structural synaptic plasticity. Surprisingly, these two parameters appear to have opposite effects on synaptic plasticity. The inter-stimulus intervals actively inhibit synaptic plasticity presumably by activity-dependent Ras activation, whereas repetition, overcomes such inhibitory effect.
The integration of the information carried by the pulses of stimulation and the inter-stimulus intervals modulates the magnitude of synaptic plasticity. Noteworthy, this integration in the spaced protocol elicited a high fidelity synaptic plasticity response (lower Kurtosis and Asymmetry), which was never produced by massed stimulations. We found our model as a powerful tool for studying how stimulus are integrated in time by a neuron. Increasing knowledge in this mechanisms could contribute to understand more complex processes like memory and skills acquisition by spaced patterns.

Cellular and Molecular Neurobiology

P69.-Control of Neurogenic vs Astrogliogenic Fate in A Restricted Spinal Cord Progenitor Domain

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Despite of the high heterogeneity in morphology and function of astrocytes, their development is not completely understood. By using mouse genetics, we studied a group of ventral progenitors of the embryonic spinal cord -called p0-, which expresses the transcription factor Dbx1. After producing V0 Interneurons (V0-IN), this progenitor pool generates a subset of astrocytes, which we named vA0, that radially migrate to populate a defined region of the spinal cord. Mosaic labelling of vA0 demonstrated that a single progenitor domain produces both protoplasmic and fibrous astrocytes. Dbx1 controls the specification of these astrocytes, as in its absence vA0 is augmented at the expense of V0-INs. We next evaluated if Notch pathway modulates early decisions among p0 progenitors. Presenilin1 (Psen1) mutants, which have reduced Notch signalling, have fewer p0-derived glial cells, while V0-INs are increased. Impairing Notch pathway only at neurogenic stages, but not later, showed similar results. Additionally, gliogenic precursors are increased in Dbx1 mutants and reduced in Psen1 mutants. When we analyzed the Notch signalling pathway in Dbx1 mutants, we surprisingly found that, while wild type p0 domain expresses Delta1 ligand, this domain is converted into Jagged1 positive in the absence of Dbx1. Our results suggest that the type of Notch ligand expressed in p0 and directed by Dbx1 controls the differentiation of p0 progenitors by biasing neurogenic vs astrogliogenic fate.

Cellular and Molecular Neurobiology

P70.-Bone marrow and adipose tissue mesenchymal stem cells: two ways to promote neuroregeneration in the peripheral nervous system

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Neurodegenerative diseases include both central and peripheral nervous system (PNS) disorders such as multiple sclerosis and type II diabetes respectively.

Research in stem cells has recently become an important tool to accelerate regeneration in the PNS and finally achieve full functional recovery of injured nerves. Pluripotent stem cells from bone marrow and adipose tissue are suitable candidates for regenerative therapies, as they are easily obtained and cultured and, even more relevant, they can be used for autologous transplant.

In our laboratory, we used a model of reversible degeneration promoted by 8-second compression of the rat sciatic nerve. Isolated bone marrow stem cells (BMSC) and mesenchymal stem cells from epididimal adipose tissue (MSAD) were evaluated through flow cytometry and immunocytochemistry. In parallel, cells were labeled with a rhodamine derivative fluorophore for systemic transplant studies.

MSAD demonstrated a higher reproduction rate than BMSC. Isolated BMSC and MSAD were characterized by multipotent cell marker expression and the absence of Schwann cell marker expression, which makes them good candidates for transplantation studies.

Epifluorescence and confocal microscopy analysis proved the arrival of both cell types exclusively to the injured nerve and their participation in the demyelination-remyelination process.

Further experiments are necessary to determine which of these populations best suits peripheral regenerating strategies.

Cellular and Molecular Neurobiology

P71.-A nervous system enhancer underwent accelerated evolution in primates and shows heterochrony during brain development in transgenic mice

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The lineage leading to humans underwent an expansion in cortical brain size which is, at least in part, responsible for the particular cognitive abilities of primates and particularly of humans. Aiming to identify the genetic elements that underlie brain evolution in primates, we found a 400 bp region that shows signatures of acceleration in the primate lineage and that we have named AANC. In contrast, the gene that is regulated by AANC shows a very high conservation from insects to mammals and shows no sign of acceleration in the primate lineage. This gene is key for neurogenesis during the development of the central nervous system (CNS).

Using a transgenic reporter assay in mouse, we tested the ability of the murine AANC to act as an enhancer in the CNS during mouse development. We found that mouse AANC drives, in a very specific manner, reporter expression to the ventricular zone of the neocortex during neurogenesis. In order to understand the biological meaning of the DNA changes that occurred in AANC in primates, we conducted transgenic reporter assays in mouse using the human AANC. We conclude that human AANC can expand the spatial and temporal expression of the reporter activity compared to mouse AANC.

This works shows that AANC is a strong brain developmental enhancer which expanded its temporal and spatial expression pattern in primates and help us to understand genetic and molecular mechanisms that could be important in primate brain evolution.

Cellular and Molecular Neurobiology

P72.-Delayed axonal development of dentate granule cells born in aging mice

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Neural progenitor cells of the adult hippocampus can differentiate and develop into fully functional dentate granule cells (DGCs). During aging low levels of neurogenesis have been reported. In recent work from our laboratory, analysis of dendritic tree length and spine density indicated that an aged niche imposes a largely decreased rate of maturation of new DGCs. We investigated if this effect could also be exerted on axonal development. To address this problem we injected a GFP-expressing retrovirus in 2 (2M) and 5 month-old (5M) mice to visualize DCGs born in adult hippocampus. We then analyzed the number of secondary branches including contacts in the hilus. During the first weeks of axonal development the 5M group presented a lower number of secondary branches compared to 2M mice. However, the number of hilar contacts remained constant. This observation suggests that aging affects axonal branching in DGCs born in aging mice. We then asked if aging modifies functional connections between newborn DGCs and their postsynaptic targets in the hilus. To answer this question we used a retrovirus expressing the synthetic g-coupled receptor hM3Dq to activate newborn DGCs in the aged hippocampus in vivo, and quantified c-Fos+ cells in the hilus. Mature adult-born DGCs from both 2M and 5M groups were able to activate similar numbers of postsynaptic targets. This indicates that despite the delayed axonal development, they both reach the same level of connectivity upon maturation.

Cellular and Molecular Neurobiology

P73.-Delayed neuronal maturation in the middle-aged hippocampus

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Aging is one important factor associated to decreased rates of neuronal production in the adult hippocampus. In this work we hypothesize that the aging hippocampus may alter neuronal development. To test this hypothesis we injected a retrovirus expressing GFP in the dentate gyrus of five and eight-month-old (5M and 8M) mice and analyzed neuronal morphology at different ages. Dentate granule cells (DGCs) in 5M mice display immature features when compared to neurons of the same age in young mice. Moreover, DGCs born in 8M mice show a more dramatic delay in maturation during their first weeks of development. Loose patch recordings showed that 6-week-old DGCs of 5M can fire action potentials in response to stimulation of the perforant path. We then explored different factors that could modulate the rate of maturation of DGCs during aging. Running reversed the effects of aging on neurons born in 5M and 8M mice. Next we used receptors activated solely by synthetic ligands (RASSLs) to incrase activity developing DGCs in five-month-old mice, which caused an acceleration of their maturation. These results indicate that both extrinsic and intrinsic activity play an important role in neuronal development in aging mice.

Cellular and Molecular Neurobiology

P74.-Unraveling the Role of GABAergic-Proopiomelanocortin Neurons in the Hypothalamic Control of Energy Balance

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Obesity affects almost half a billion people worldwide. Because of the high prevalence of overweight and the severity of its comorbidities, great effort is made to unravel the neuronal circuits controlling energy balance. Hypothalamic Proopiomelanocortin (POMC) neurons are main regulators of energy balance. POMC deficient patients and mice are hyperphagic and obese. Since there are both GABAergic and glutamatergic POMC hypothalamic neurons, we speculate that both subpopulations have different connections and physiological roles. In order to characterize GABAergic-POMC neurons, we used a reversible knockout mouse model of early-onset obesity that lacks POMC expression in the hypothalamus. In this model, subsequent reactivation of POMC expression specifically in GABAergic neurons can be conditionally achieved. Before treatment at P60, hyperphagic knockout mice were ~60% heavier than their WT littermates, whereas six weeks after POMC rescue this difference dropped to ~22% and food intake completely normalized. Surprisingly, less than 20% of POMC hypothalamic neurons recovered POMC expression in these experiments, all of which are GABAergic cells. Altogether, our results suggest that the subpopulation of GABAergic POMC neurons - is probably the main regulator of food intake among POMC cells. Further experiments are undertaken to identify the target neurons of GABAergic-POMC neurons in order to elucidate the brain circuits in which they are involved.

Cellular and Molecular Neurobiology

P75.-Ghrelin Uptake By The Blood-Cerebrospinal Fluid Barrier Occurs In A Ghrelin Receptor-Independent Manner

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Ghrelin is a 28-amino acid octanoylated peptide hormone secreted from the stomach. It has been suggested that one of the mechanisms by which ghrelin reaches the brain involves crossing the blood-cerebrospinal fluid barrier at either the choroids plexus and/or the hypothalamic tanycytes that line the floor of the third ventricle. However, the mechanisms mediating the transport of ghrelin into the brain are currently unknown. The goal of this study was to get insights into the system mediating ghrelin uptake at the blood-cerebrospinal fluid barrier. We found that both the ependymal cells of the choroids plexus and the hypothalamic tanycytes display fluorescent-ghrelin tracer uptake in vivo. Interestingly, we failed to find ghrelin receptor gene expression within these cell types or an increase of pERK levels, a downstream target of ghrelin receptor signaling, by ghrelin or the fluorescent-ghrelin tracer. In addition, we found that ependymal cells of the choroids plexus and the hypothalamic tanycytes of a ghrelin receptor deficient mice show similar patterns of tracer uptake as seem in wild type mice. Thus, we conclude that ghrelin uptake in the blood-cerebro spinal barrier occurs in a ghrelin receptor-independent fashion. Supported by PICT2011-2142, PICTO2013-0065 and 11/X605.

Cellular and Molecular Neurobiology

P76.-Peripheral remyelination: evidence of a temporal window for Bone Marrow Mononuclear Cell transplantation

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We have previously described the migration of bone marrow mononuclear cells (BMMC) to the demyelinated area when systemically transplanted immediately after rat sciatic nerve crush. Once BMMC arrive at the ipsilateral nerve, some of them colocalize with Schwann cell and nerve fiber markers and accelerate the regeneration process.

In this context, the aim of the present work was to determine the temporal window during which BMMC are able to migrate to the injured nerve and whether this migration is also accompanied by a beneficial effect. To such end, adult Wistar rats were submitted to sciatic nerve crush and transplanted with BMMC at different survival times post crush. Five days after transplantation, animals were sacrificed and immunohistochemical, Western Blot and functional studies were performed.

Results show that BMMC were able to migrate to the ipsilateral nerve up to 28 days post crush, although the peak of migration occurred when BMMC were transplanted 7 days post crush, a crucial point in the demyelination process. In terms of MBP levels, cell transplantation promoted a faster recovery than in non-transplanted animals. Functional studies are being carried out to determine whether morphological recovery is accompanied by a functional one.

The confirmation of a temporal window for BMMC treatment would open a promising field to evaluate the possible application of BMMC in therapies for neurodegeneration processes.

P77.-Viral vectors designed for NMDA receptor subunits silencing

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N-methyl-D-aspartate receptors (NMDAR) are found in the post-synapses of neurons in the central nervous system (CNS). These receptors are formed by two constitutive subunits (GluN1) and two variable ones: GluN 2 A-D or GluN3 A-B, which determine the receptor´s subtype and characteristics. In the hippocampus, GluN2A and GluN2B are NMDAR most abundant regulatory subunits.It was suggested that memory acquisition is related to either an increase of NMDAR, a variation of its subtype's relative abundance in the hippocampus, or both. In our laboratory, we showed that GluN1 and GluN2A expression increases temporarily 70 minutes after plasticity induction.To further study NMDAR's role in learning, our group generated adeno-associated viral vectors (AAV) carrying green fluorescent protein gene (GFP) and short-hairpin RNA (shRNA) against GluN2A (shN2A), to knock-down this NMDAR subunit. Another vector, carrying a similar, scrambled sequence (shSc) was built as a control.AAV's expression was assayed in neuroblastoma cells (Neuro-2a) with the shSc AAV, allowing to determine an optimal multiplicity of infection (MOI) of 105. After essays in primary cultured neurons, optimal MOI was established in 104. No differences in GFP expression were observed after 6 or 7 days post infection (DPI). On day 8, GFP quantification was restricted by a significant decrease in cell viability.Currently, experiments are being made to assess the effect of shN2A on its target messenger RNA and its expression.

Cellular and Molecular Neurobiology

P78.-Protective effects derived from oligodendrocyte-neuron interaction via Myelin-Associated Glycoprotein (MAG): mechanisms against glutamate induction of oxidative stress

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The Myelin-Associated Glycoprotein (MAG) is localized in myelin sheaths where it functions in glia–axon interactions. MAG Crosslinking at the cell membrane of OLs using an anti-MAG mAb can initiate signaling events such as protein phosphorylation. Previous findings suggest that one such event is related to increased resistance to glutamate excitotoxicity. Preliminary evidences showed that MAG crosslinking protected cerebellar organotypic cultures against an excitotoxic glutamate concentration. In addition MAG activation was effective in two animal models implying glutamate overload (MOG-induced Experimental Autoimmune Encephalomyelitis and Stroke model). In an attempt to identify the mechanism of OL protection, we found that MAG activation can affect glutamate dynamics on OLs. Besides, it can increase OL glutathione: one of the most important antioxidant defenses of the cell. This increase is time-dependent and relies on Xc- cysteine/glutamate antiporter activity. Glutathione rise is related to activation of PKC, one of MAG substrates on OLs cytoplasm. Altogether these results allow us to propose OLs as critical white matter extracellular glutamate modulators. Identifying receptors and mechanisms that increase clearance of extracellular glutamate and promote survival of OLs could be a critical point to understand and target excitotoxicity-related diseases, opening a new opportunity for therapeutic intervention to mitigate consequences of demyelinating diseases.

Cellular and Molecular Neurobiology

P79.-CK2-mediated reduction in Fast Axonal Transport by a cytoplasmic form of Prion Protein

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Prion diseases (PrD) are fatal progressive neuropathies characterized by specific neuronal and synaptic dysfunction and loss, associated accumulation of pathogenic forms of Prion Protein (PrP). Loss of synaptic connectivity occurs early in PrD. Several studies point to a specific domain in the PrP sequence as the major trigger of the disease features. However, neither the exact segment of the PrP involved nor the pathological mechanism of its action has been described. Reductions in fast axonal transport (FAT) has been associated with dying back neuropathies and mutations in functional domains of kinesins or dyneins lead to neurodegeneration. Nontheless, mutations in motor proteins have not been described in PrD. An alternative explanation is that alterations in FAT regulatory pathways result in a reduce delivery of critical synaptic components, leading to failure of synapses and loss of neurons following a dying back pattern. Here we evaluated the effects of the PrP 106-126 peptide domain on kinesin-1 and dynein-based FAT. Ex-vivo and biochemical experiments reveal that abnormal casein kinase 2 (CK2) activation mediates PrP induced inhibition of both anterograde and retrograde FAT. Significantly, PrP-mediated FAT inhibition can be prevented by a specific CK2 inhibitor. In summary, our results suggest reduction of FAT induced by an imbalance in specific kinase activities in neurons by PrP may represent an early, critical step for initiation of neuronal pathology.

Chronobiology

P80.-Role of G Substrate in the photic signaling pathway of the circadian clock

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Circadian clocks drive rhythms in physiology and behavior, providing a selective advantage by enabling organisms to synchronize to 24h environmental day using light-dark transitions as the main phase-shifting signal. In mammals, light input from the retina reaches the master circadian clock in the hypothalamic suprachiasmatic nucleus (SCN) through glutamatergic afferents. Both the phase-shift induced, and the pathway activated, depends on the zeitgeber time (ZT) at which light is applied (ZT12 is defined at locomotor activity onset). Light pulse (LP) at ZT18 causes phase-advances by activation of the nitric oxide-guanylate cyclase-protein kinase G (NO-GC-PKG) pathway, leading to enhanced circadian gene transcription by still unknown components. Previous studies show that PKG interacts with G substrate (GS), which has been identified in SCN and cerebellum. We found that both GS and its phosphorylated form (pGS) have a restricted distribution at the hamster SCN, having GS levels a circadianrhythm peaking at ZT18 . Increased SCN-levels of pGS were found after a LP delivered to hamsters at the same ZT. In addition, treatment with KT-5823, a specific inhibitor of PKG, decreased this photic induction of pGS levels, while sildenafil, a strong activator of PKG, increased pGS photic levels. These results provide evidence that photic NO-GC-PKG pathway involves downstream phosphorylation of GS by PKG to synchronize the SCN clock.

Chronobiology

P81.-Aging modifies the oxidative status and the circadian expression of BDNF and its TrkB receptor in the hippocampus

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Hippocampus plays a key role in memory and learning and is especially susceptible to oxidative stress. We previously observed BDNF and TrkB mRNA as well as antioxidant enzymes rhythms are modified in the hippocampus of aged rats. We hypothesize that aging, thorough the modification of the oxidative pattern, can alter 24h-rhythmicity. To test that, we measured the levels of BDNF and TrkB proteins, lipid peroxidation (LPO), protein carbonylation (PC) and total antioxidant capacity (TAC) throughout a 24h cycle in the hippocampus of young and aged rats. Holtzman 3- and 22-months old rats were maintained under constant darkness conditions during 15 days before the experiment. BDNF and TrkB proteins as well as TBARS (LPO), PC and TAC were determined in samples isolated at 4h-intervals. BDNF and TrkB levels varied ($P<0.05$) in a 24h period, with maximal levels at circadian time (CT) 18:19±1:25 and 13:28±1:31, respectively, in young rats. Aging increased amplitude ($p<0,05$) and shifted BDNF rhythm acrophase (04:45±0:50, $p<0,01$); on the other hand, it abolished the TrKB oscillation. LPO, PC and TAC data didn't fit to a cosine curve in young rats, however, we observed a significant variation between the different time points ($p<0,01$). Aging abolished the LPO and TAC variation and changed PC higher concentration from CT 14 to CT 6 ($p<0,01$). Alteration of oxidative status, BDNF and TrkB circadian rhythmicity could be, among others, the molecular basis of aging-associated cognitive decline.

Chronobiology

P82.-Redox regulation of photic synchronization of the mammalian circadian clock

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Since life in earth has been developed under a 24h day, virtually all organisms have developed a circadian system to adapt to this oscillating ambient. Light/dark cycle is the most important phase-shifting cue for the synchronization of the circadian rhythms. In mammals, the hypothalamic suprachiasmatic nucleus (SCN) is considered the master clock receiving light information from the retina through a glutamatergic synapse. In turn, Nitric Oxide Synthase (NOS) is activated for bifurcating pathways depending on circadian time (CT) of stimulation: light-pulse induced advances of wheel-running activity rhythm at CT18 (CT12 is activity onset) with the activation of guanylate cyclase (GC), or ryanodine receptor II for delaying pulse at CT14. Recent studies highlight the importance of the co-evolution of a circadian redox system together with canonical circadian components. We have evidenced a decrease in hamsters' SCN reduced/oxidized glutathione rate (key components of the redox system) at CT18 (but not at CT14). Also, only changing the neuronal redox state by intracerebroventricular injections of the antioxidant L-N-acetyl-cysteine induced phase-shifts at CT14; however it decreases the light-induced shifts at both CTs. Thus, neuronal redox state could be modulating the synchronization of the clock. Also, since s-nitrosothiol type of NO donors potentiate both advances and delays, while non-s-nitrosothiol only advances, we hypothesize a different NOS activity between this CTs.

Chronobiology

P83.-Testing the functional role of putative synaptic contacts of core pacemaker neurons

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Our laboratory is interested in characterizing the neuronal circuitry involved in rhythmic behavior of Drosophila melanogaster. We have previously shown that the sLNvs, a key group of circadian neurons commanding locomotor activity behavior, undergo structural remodeling of their axonal terminals in a circadian fashion. By means of a GRASP screen we have identified several neuronal clusters contacting the sLNvs across the day.
Currently, we are testing the functional role of these neuronal clusters on the control of locomotor activity, by means of overexpressing different ion channels that will either hyperpolarize or depolarize their cell membrane. In some of the neuronal clusters, constitutive overexpression of the KIR2.1 channel was lethal. In other cases, it reduced behavioral rhythmicity. To eliminate the possibility that constitutive overexpression yielded developmental defects we induced for a short time the activation of TRPA1, a depolarizing, temperature-activated ion channel, in an adult-specific manner. Interestingly, activation of a subset of the putative synaptic partners triggered behavioral defects suggesting that they are part of the circuit controlling locomotor activity. Additionally, we performed whole-brain immunohistochemistry in order to begin to characterize these novel circadian-relevant clusters. These results suggest that additional clusters (beyond the well-characterized clock neurons) are part of the Drosophila circadian network.

Chronobiology

P84.-Melatonin modulates interval timing in rats: effect of pinealectomy

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Interval timing is a complex cognitive process that involves the estimation of time within the seconds-to-minutes range. On the other hand, the circadian system controls physiological and behavioral functions with periods close to 24 h. We have previously reported that short-time perception in mice is influenced by the circadian pacemaker, with dopamine signaling as a link between both temporal systems. In this work we evaluated the involvement of melatonin in the circadian modulation of interval timing, as well as the interaction between this hormone and dopamine function.
We report that melatonin-depleted rats showed an impairment in their ability to time short intervals as compared to controls. Moreover, melatonin administration restored time estimation accuracy in pinealectomized rats. We also demonstrate that circadian desynchronization caused a transient impairment in this cognitive task. Furthermore, melatonin depletion increased striatal dopamine availability, which was reverted by external melatonin administration. Taken together, our findings add further support to the notion that the circadian system regulates interval timing, probably by using melatonin as an output to regulate dopaminergic functions in structures that are important for interval timing mechanisms.

Chronobiology

P85.-Analysis of circadian locomotor activity behavior in Caenorhabditis elegans

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C. elegans is a model extensively used for research in genetics, neural control and sensory transduction, as well as an emerging model in the field of chronobiology. One of the most common variables that are recorded to assess circadian rhythmicity is locomotor activity. Here we show different methods for it acquisition, as well as the methods we have developed for circadian analysis.

We have employed three different methods for the study of locomotor activity of C. elegans: a) infrared beam interruptions that allows to record under different light and temperature conditions; b) long-term video tracking, which allows the analysis of speed, acceleration, number of turns and distance travelled for each single nematode; in addition, short video tracks enabled us to test the response of different mutant strain to acute light stimulation; c) automated recording of the distribution of C. elegans populations in agar plates, including the response to different acute and chronic stimuli.

Finally, we developed a statistical package to analyze locomotor activity and other circadian outputs. We first determined how the pre-processing of a signal can influence the analysis, In addition, a combination of ANOVA for circular statistics and Q factor were used to analyze data with simultaneous phase shifts and amplitude changes. Finally we developed an algorithm to quantify period drifts. In summary, we now have powerful tools to analyze circadian rhythmicity in this fascinating model.

Chronobiology

P86.-Aging modifies the circadian expression of antioxidant enzymes in cerebellum

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Cerebellum functions decline in older adults and are susceptible to oxidative stress. Previously, we observed oscillating 24h-patterns of catalase (CAT) activity and GSH levels are modified in the cerebellum of aged rats. The objectives of this study were: 1)to analyze the CAT and GSH peroxidase 4 (GPX4) expression as well as the clock factor, BMAL1, protein levels throughout a 24h period in the rat cerebellum and 2) to investigate whether circadian rhythms of antioxidant enzymes and BMAL1 are modified in aged rats. Holtzman rats from young (3-months old) and aged (22-months old) groups were maintained under constant darkness conditions, during 15 days before the experiment. CAT and GPx4 mRNA as well as BMAL1 protein levels were determined in cerebellum samples isolated every 4 h during a 24h period. We observed BMAL1 protein vary significantly throughout a 24h period in young and aged rats. However, aging phase shifted BMAL1 protein rhythmicity (CT 08:48±00:42 vs CT 18:02±01:05, p<0,001) and reduced the rhythm mesor (0,97±0,001 vs 1,07±0,008 , p<0.001). We also observed GPx4 mRNA circadian rhythm. In this case, aging reduced the rhythm's mesor (1,37±0,04 vs 1.87±0.03, p<0.001). However, we didn´t observe a 24h- variation in CAT expression, neither in young or aged animals. Aging affects circadian rhythms of antioxidant enzymes and BMAL1 clock factor in a different way. This might indicate other factors are mediating circadian regulation of antioxidant enzymes expression.

Chronobiology

P87.-Effects of peripheral immune activation on the master circadian oscillator: role of Ccl2 chemokine.

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The mammalian circadian system is mainly originated in a master oscillator located in the suprachiasmatic nuclei (SCN) in the hypothalamus. Previous reports from our and other groups have shown that the SCN are sensitive to systemic immune activation during the early night, through a mechanism that relies on the action of proinflammatory factors within this structure. Chemokine (C-C motif) ligand 2 (CCL2) is induced in the brain upon peripheral immune activation, and it has been shown to modulate neuronal physiology. In the present work we tested whether CCL2 might be involved in the response of the circadian clock to peripheral endotoxin administration. The CCL2 receptor, C-C chemokine receptor type 2 (CCR2), was detected in the SCN of mice, with higher levels of expression during the early night, when the clock is sensitive to immune activation. Ccl2 was induced in the SCN upon intraperitoneal lipopolysaccharide (LPS) administration. Furthermore, mice receiving an intracerebroventricular (Icv) administration of a CCL2 synthesis inhibitor (Bindarit), showed a reduction of LPS-induced circadian phase changes. In addition, we tested the possibility that CCL2 might also be involved in the photic regulation of the clock. Icv administration of Bindarit could not modify the effects of light pulses on the circadian clock. In summary, we found that CCL2, acting at the SCN level is important for the circadian effects of immune activation.

Chronobiology

P88.-Cellular and functional characterization of the GABAergic system in the adult rat pineal gland

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Circadian modulation of organismal physiology and behavior facilitates adaptation to environmental changes. One of the principal regulators of the mammalian circadian timing system is the pineal gland (PG), which is the main source of circulating melatonin. The indole rhythm is under sympathetic regulation via local norepinephrine (NE) release at night from the nerve ends of neurons located in the superior cervical ganglia (SCG). In this work we studied the influences of the GABAergic system in the physiology of the adult rat PG. The GABAergic innervation showed a marked tropism for blood vessels. Moreover, a subpopulation of interstitial cells immunoreactive for cytoplasmic vimentin, GAD65, GAD67 and GABA was clearly identifiable. The spatial and temporal distribution of the α1 subunit of the ionotropic GABAA receptor and the B1 subtype of the metabotropic GABAB receptor, implicates GABA in the presynaptic modulation of GABAergic and sympathetic nerve fibers, in vascular tone regulation, and in the maintenance of the quiescent status of potential Pax6+ precursors in the adult PG. In vivo and in vitro pharmacological manipulations of GABAA receptors altered pineal serotonin levels, suggesting an inhibitory effect of GABA on the synthesis and secretion of NE-induced melatonin. Our results, together with previous bibliographic data, allow us to propose a hypothetical model of melatonin rhythm modulation by the local GABAergic system in the adult rat pineal gland.

Chronobiology

P89.-Sleep and synaptic plasticity in the fruit fly Drosophila melanogaster

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Sleep deprivation, caused either by environmental or physiological factors, is likely to interfere with the synaptic homeostasis processes associated to sleep. The aim of this project is to describe sleep deprivation-associated changes of synaptic plasticity in the large lateral ventral neurons (lLNvs). These neurons intersect the sleep and circadian circuits of Drosophila and, interestingly, have been described to undergo synaptic changes under different environmental conditions, including situations where sleep pressure is imposed to the organism. However, this phenomenon has not been systematically analyzed yet. We are studying the levels and distribution of different synaptic markers after sleep deprivation, in particular we are assessing; 1) peptidergic neurotransmission; 2) classical neurotransmission; 3) axonal structural plasticity and 4) and dendritic structural plasticity. In parallel, we are analyzing synaptic plasticity when sleep is disturbed by genetic manipulation of lLNvs excitability. Future experimental lines would delve into the role of predicted GABAergic inputs that mediate homeostatic sleep regulation into the phenomenon of synaptic plasticity studied. Information obtained from this research project will help produce a model of synaptic plasticity in the context of sleep deprivation. Moreover, it will aid in the understanding of how arousal neurons process information and translate it into its neuronal outputs.

Chronobiology

P90.-Fast Feedback Inhibitory Transmission Among Circadian Clocks Keeps the Network in Tune

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The circadian network in the adult Drosophila brain relies on 150 neurons organized in clusters. One of them, the small lateral neurons ventral (sLNvs), is a major pacemaker since it defines the temporal organization of locomotor activity. Nevertheless, all independent yet interconnected oscillators operate coherently to provide flexibility to the network. The small and large LNvs release the PDF neuropeptide. While the relevance of PDF in the synchronization of the circadian network is well established, the role of fast neurotransmission has only recently been addressed. We showed that LNvs release glycine onto other circadian clusters and postulated that it organizes the firing pattern of clock neurons. Reducing glycine availability within LNvs alters PDF release, and remodeling of the sLNv terminals, suggesting that LNvs are also glycinergic targets. RNAi-based downregulation of a glycine receptor subunit reduced the consolidation of diurnal activity and increased nocturnal activity, decreasing overall rhythmicity. Moreover, glycine application abrogates firing of both s- and l-LNvs, consistent with an inhibition mediated by a ligand-gated Cl- channel. Furthermore, an antibody for human glycine alpha1 receptor (GlyR) stained both s- and l-LNvs neurons, providing further support to the notion that LNvs express GlyR. We propose that glycine is a fast feedback inhibitory signal that modulates the output of the sLNvs, and thus provides coherence to the circadian network.

Chronobiology

P91.-A dual luminescent-fluorescent reporter tool reveals robust circadian rhythms in C. elegans

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In this work, we present the development and biotechnological application of a bioluminescence-based reporter in the model organism C. elegans that allows for real time monitoring of gene expression in live animals for long periods of time without affecting their physiology. We applied this reporter to study the circadian expression of sur-5, which encodes a protein with high similarity to H. sapiens acetoacetyl-CoA synthetase, in adult nematodes under LD (light:dark) and WC (warm:cold) cycles. With different assays and entrainment conditions we show that C. elegans exhibits robust sur-5 circadian expression rhythms, with a free running period of 23.51 ±0.54 h (n=12, Lomb-Scargle), and that it also complies with classic circadian characteristics: temperature compensation (Q10= 1.098, n=77, Δ=4°C) and resynchronization after a forced 6 h phase change of the entrainment conditions. In addition, we have determined part of the pathway needed for photic entrainment, since mutant strains of two components involved in light/temperature signal transduction exhibit a decrease in entrainment ability. While 75.32% percent of N2 populations are able to entrained to the LD:WC cycle, the number was reduced to 44.94% in TQ1101, and further decreased to 29.17% in strain PR671 ($p<0.01$, Fisher's Exact test in both cases).

In summary, our novel tool allows us to extend the use of C. elegans as a powerful model to investigate circadian biology and opens the door for new experimentation in the search for the molecular circadian clock of this species.

Chronobiology

P92.-Cell autonomous and non-autonomous mechanisms relevant for the remodelling of axonal terminals of pacemaker neurons in *Drosophila melanogaster*

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A number of years ago we reported that pigment dispersing factor (PDF) neurons, which are essential in the control of rest-activity cycles in Drosophila, undergo circadian remodeling of their axonal projections (Fernández et al., Plos Biol, 2008). We then proposed that such remodeling could provide an additional means of transmitting time of day information in addition to differential neurotransmitter release (i.e., PDF). Axonal arborizations display higher complexity during the day and less so at night, and this structural plasticity ensures changes in the degree of connectivity (Gorostiza et al., Curr Biol, 2014). In this work we characterize the molecular mechanisms that underlie these structural changes in PDF neurons, under the hypothesis that this process would allow the molecular clock to modulate the output of the pacemaker circuit. We examined how downregulation or overexpression of specific clock genes affects structural plasticity, to define whether the cell-autonomous circadian clock accounts for a full structural effect. In addition, we characterized PDF´s impact on this structural plasticity, through the analysis of consequence of RNAi-mediated silencing at different times in the day. To delve further into the mechanisms underlying PDF´s function we attempted to define the source of the neuropeptide through cluster-specific downregulation.

Chronobiology

P93.-Diurnal variations on the speed and quality of human decisions

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Human behavior and physiology exhibit diurnal fluctuations. These rhythms can be entrained by light and social cues, with vast individual differences in individual´s chronotype. Whether diurnal preferences determine decision making in real-life situations has both practical and theoretical implications. However, answering this question has remained elusive due because of the difficulty of rating precisely the quality of a decision in real-life scenarios. Here we investigate diurnal variations in decision-making as a function of an individual´s chronotype capitalizing on a vast repository of human decisions: web-based chess servers. In a chess game, every player has to make around 40 decisions using a finite time budget and both the time and quality of each decision can be accurately determined. We found reliable diurnal rhythms both in performance and response time, the two main properties of decisions. Performance fluctuations depended both on the interaction between daytime and diurnal preferences, with higher performance during the preferred time of each chronotype. Instead, response times show a marked diurnal variation, which is independent of chronotypes, with all individuals making faster decisions from noon to evening. Our results show diurnal rhythms in human behavior and cognitive function under real life conditions, finding changes in decision making processes relaying both in daytime and individual chronotypes.

Chronobiology

P94.-Cholinergic Transmission in the Circadian Pacemaker of *Drosophila*

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The central circadian oscillator of Drosophila melanogaster is composed of about 150 neurons organized in clusters defined by their anatomical location. Among them, the lateral ventral neurons (LNvs) are central in the temporal organization of the fly´s daily locomotor activity. The LNv cluster includes two groups of neurons, the large and small LNvs. These neurons release a neuropeptide called Pigment Dispersing Factor. While the relevance of PDF released by the sLNvs in the synchronization of the circadian network is well established, the role of fast neurotransmission is still under debate. LNvs release glycine. Notwithstanding, excitatory cholinergic transmission also seems to be implicated. To uncover the role of acetylcholine in the LNvs, we downregulated the expression of the vesicular acetylcholine transporter. We found that the temporal organization of locomotor activity is changed under these conditions. Flies display less consolidated diurnal and increased nocturnal activity. A reduction in the levels of acetylcholine vesicular transporter produces a loss of about 40% in the rhythmicity of the population. We are in the process of defining which group of LNvs is responsible for the behavioral phenotype and also whether reducing acetylcholine availability affects additional sLNv outputs. We postulate that LNvs make use of both fast excitatory and inhibitory signals in favor of organizing a coherent firing pattern in the circadian circuit.

Chronobiology

P95.-Neuroimmunology of circadian rhythms during chronic lung inflammation

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Daily environmental changes have imposed a selective pressure for life driving the development of a mammalian clock, which resides in the hypothalamic suprachiasmatic nuclei (SCN). The principal signal that adjusts its activity is the light-dark cycle. Bidirectional interactions between the immune and the circadian systems have been under intensive study in recent years, under physiological or pathological conditions. In particular, bronchial asthma symptoms are more severe at night. Lung function exhibits a circadian rhythm with a maximum during the afternoon and a minimum in the early morning. In order to study the response of the circadian system to a chronic inflammation we used a murine model of airway inflammation, induced by the repetitive intranasal inoculation of ovalbumin (OVA). First we confirmed that cytokine levels were increased in the lung of mice treated with OVA. We also observed that mice subjected to this protocol exhibited an increased interdaily variability, less rhythmicity of their locomotor activity and a higher quotient between the length of the subjective night and the day, compared to control animals. Finally, we found that the levels of the cytokine TNF-α and the chemokine CCL2 changed in the SCN only after six weeks of treatment. In conclusion, here we show that chronic lung inflammation can affect circadian locomotor activity and that the bidirectional interaction between the immune and circadian systems can be mediated by TNF-α and CCL2.

Chronobiology

P96.-The BMP pathway modulates the interactions of the neural network that drives circadian behavior in Drosophila

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Circadian behavior is controlled by an endogenous clock that enables organisms to adapt to the daily environmental changes, driving various aspects of their physiology and activity. In Drosophila this clock relies in a cluster of neurons, the sLNvs and lLNvs, which release the PDF neuropeptide onto other circadian clusters and thus set their period. To improve our understanding about the role of the pathways involved in the communication among different circadian clusters as well as identify additional components involved in sending or receiving information relevant for synchronization of the circadian network a missexpression screen was carried out. This screen identified an insertion that produces a long period phenotype. The affected gene is a positive regulator of the bone morphogenetic protein (BMP) pathway, a highly conserved retrograde signaling pathway that influences synaptic connectivity, ultimately controlling transcription. While activation of different members of the signaling cascade triggers a long period phenotype, ligand downregulation (dpp, gbb, actb, scw, daw, myo and mav) in a broad circadian domain generates arrhythmicity. Next we attempted to define the source of each specific ligand within the circadian network. In this context, we are characterizing the contribution of the BMP cascade, as well as it ligands, in different clusters and their relative relevance in the communication with the sLNvs through RNAi- mediated downregulation.

Chronobiology

P97.-Neuroimmune communication between the circadian clock and tumor development in mice

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Circadian disruption related to shift-work and jet-lag has been established as a health hazard in both humans and animal models. The mechanisms by which these conditions lead to such a wide range of deleterious effects are still unclear. It has been shown that circadian disruption increases the incidence of different types of cancer. Our purpose is to determine the effect of chronic jet-lag (CJL) in tumor development, focusing in the role of the immune system. We injected subcutaneously B16 cells in C57bl/6 mice housed under CJL (6 h advances every 2 days) or normal light (LD 12:12) conditions. We compared tumor latency and growth, survival and variations in immune and circadian variables. Our results show that the tumor growth rate was significantly higher, while survival and latency was lower under CJL condition. However, early tumor angiogenesis was not affected. In CJL conditions, tumor levels of pro-inflammatory cytokines Interleukin (IL)-6, IL-1β and Tumor Necrosis Factor (TNF)-α lost their LD expression patterns (which tend to be higher during the day). In addition, tumor expression of clock genes Per1 and 2 were decreased in this group. Moreover, animals under CJL schedule showed an increase in TNF-α and the chemokine CCL2 levels in the hypothalamic suprachiasmatic nucleus at night. In summary, we conclude that the CJL schedules increased the rate of tumor development in mice and circadian modifications in immune variables may be implicated in this association.

Cognition, Behavior, and Memory

P98.-Deficits in temporal processing in an animal model of autism

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Temporal processing in the seconds-to-minutes range, known as interval timing, is a crucial cognitive function that requires activation of cortico-striatal circuits via dopaminergic-glutamatergic pathways. Interval timing is altered in disorders associated with pathological dopaminergic function, including schizophrenia, Parkinson's disease, and Huntington's disease. It has been reported that children and adults with autism spectrum disorders (ASD) are impaired in their ability to accurately perceive time. In the present study, a mouse model of autism - which involves prenatal exposure to valproic acid (VPA) at gestational day 12.5 - was evaluated for its ability to acquire timing responses in simultaneously trained 15-s and 45-s peak-interval (PI) procedures. In the PI procedure, subjects are first trained on a fixed-interval (FI) schedule of reinforcement and then transitioned to the PI protocol in which unreinforced probe trials are introduced. With repeated experience on probe trials, subjects learn to respond at a time closer to the expected time of reinforcement, producing a Gaussian-shaped response function. Our results indicate that both male and female VPA-mice showed significant impairments in timing accuracy and precision compared to control (saline) groups. These deficits in temporal processing in a mouse model of autism are consistent with previous results in humans, and provide a useful tool for further behavioral and pharmacological studies.

Cognition, Behavior, and Memory

P99.-Temporal Processing in Huntington's Disease

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The perception of the passage of time is a fundamental component of cognition. Duration discrimination within the seconds-to-minutes range, known as interval timing, is involved in fundamental behaviours such as foraging, decision-making and learning, via activation of cortico-striatal circuits. Deficits in timing functions have been reported in disorders associated with pathological dopaminergic function, including Parkinson's disease and schizophrenia. Moreover, variability in temporal processing (TP) has been described in small samples of Huntington's disease (HD) patients where abnormalities in TP may be critical for movement deterioration. In the present work, we assessed temporal cognition in two different experiments: a peak-interval (PI) time production and a reaction time task in patients with molecular diagnosis of HD and controls matched by age, sex and educational level. The time production task was a variant of the human peak-interval task and consisted in the production of 3, 6 and 12s target intervals. In the reaction time task, participants were asked to respond as quickly as possible to black circles appearing on a white screen. Results indicate a significant decline in timing function in HD patients. Indeed, significant differences in the PI task were found, with worse performance in HD compared to controls. Moreover, reaction time was longer in HD patients vs. controls. Our results support the notion that timing functions are impaired in HD.

Cognition, Behavior, and Memory

P100.-Two-trial long-term memory in the crab Neohelice

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The repetitive presentation of a visual danger stimulus (VDS) in contingency with a light-driven context elicits the decay in the escape response of the crab Neohelice granulata. Following the training, animals retain the low response levels 24 hs later during a test session. This long-term memory (LTM) paradigm, known as Contextual Pavlovian Conditioning (CPC), has been exhaustively studied. Recently, we developed a new device to measure the crab´s escape response that allows more detailed measurements and a thorough description of the behavior. Moreover, it allows a reduction in the number of individuals tested. Using this device, we were able to validate the previously obtained results in our lab and to reduce the number of animals needed by half. Additionally, we have made progress in studying the effect of intensity and dynamics of VDS presentation on memory retention. 24 h-LTM was observed after a 2-trial training session in spite of the different inter-trial intervals used (e.g. 3; 45 or 60 minutes). We are currently seeking experimental conditions (e.g., water absence, total time spent on device, etc.) under which 2 trials are capable of eliciting LTM, as these results disagree with previous reports. These findings open the possibility to hound deeper into the molecular mechanisms that allow memory formation under such flexible training situations.

Cognition, Behavior, and Memory

P101.-Recognition effects in a pair-association task measured in reaction times (RTs) and event related potentials (ERP)

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Experiments with humans and non-human subjects during a recognition task tested by a delayed match to sample- (DMS) or a delayed paired association- (DPA) tasks with artificial images have shown characteristic brain activity. The purpose of the present experiment was to compare the ERP activity in DMS and DPA between conditions (same/different, pair/non-pair) with fractal stimuli in human subjects. Twelve healthy subjects were evaluated. We registered the EEG activity in 32 channels; the traces were analyzed "off-line". All subjects reached an 80% (or above) performance in both tasks. Reaction times in all 12 subjects were significantly different with slower responses in non-pairs condition in DPA as compared to same condition in DMS. The ERP recording in 6 subjects showed several task-related components: Late components (P2 and LPC) were notably different between the first and the second stimulus of each pair in both tasks. Moreover, there were differences between the LPC between task conditions, with a positivity in non-pairs as compared to pairs (DPA). This difference was not present between the conditions in DMS and its topography was predominantly centro-parietal. These findings suggest that the late positive component observed during non-pair condition in DPA would correspond with the “rectification” process of the pre-activated sensory-motor network, similar to those observed in P600 components during linguistic tasks with syntactic anomalies.

Cognition, Behavior, and Memory

P102.-Normative values of Verbal Learning Test Spain Complutense (TAVEC) in population of the City of Córdoba

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The episodic memory impairment is one of the most common cognitive deficits in Mild Cognitive Impairment (MCI) and is often the first symptom of Alzheimer's Disease (AD). The aim of this study is to present the normative values of Verbal Learning Test Spain Complutense (TAVEC) in population of the city of Cordoba (45 to> 74 years, n = 120) publishing descriptive statistics (mean and standard deviation) for the different reference groups. Methodology: The demographic analysis was performed taking into account different age groups, sex and educational level. The sample selection was made through the Unidad de Neuropsicología y Neurorehabilitación del Sanatorio Allende (UNR) and the Centro Integral de Apoyo Terapéutico y Estimulación Cognitiva (CIATEC). Data was obtained through registration of neuropsychological assessments made during the period between 2008 and 2015 in community population. Such assessments consist of a qualitative interview with the patient, the application of TAVEC and behavioral self-administered questionnaires. Sample excluded all individuals with a history of neurological or psychiatric disorders. Conclusion: TAVEC is a useful for detecting the usual cognitive deficits in MCI and AD instrument. The normative values of the test population of the City of Cordoba clinically facilitate the development of early diagnosis.

Cognition, Behavior, and Memory

P103.-Selective ablation of striatal cholinergic interneurons before adolescence, but not during adulthood, results in impulsive behavior

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Adolescence is a developmental stage characterized by distinctive physical, cognitive and emotional features, including increased novelty seeking, skills consolidation, and emergence of risk-taking behaviors. The basal ganglia are critical in consolidating these behaviors during this developmental stage. Striatal cholinergic interneurons (SCI) are key regulators of the striatum, the main input nucleus of the basal ganglia. SCI have been proposed as detectors of behaviorally-relevant salient events and their function may be critical for behavioral response selection. Interestingly, SCI complete their development during adolescence, however their role in sculpting striatal function in relation to reinforcement-learning during this period has not been assessed. We developed a transgenic mouse system allowing the inducible ablation of SCI at pre-adolescent stage or in adulthood. Instrumental learning was evaluated in control and SCI-lesioned mice during adulthood in a reward-value discrimination task followed by a delay discounting paradigm using automated operant conditioning chambers. We found that SCI ablation does not impact in reward-value discrimination but leads to increased impulsivity only if SCI ablation is achieved before adolescence. Furthermore, adult mice with SCI juvenile lesions present a different learning strategy. Our results support a role of SCI in striatal maturation during adolescence in relation to reward value and behavioral response selection.

Cognition, Behavior, and Memory

P104.-Time-course of cognitive processes during proverb reading

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As we read our brain performs a series of processes that allow the recognition of words in different levels of analysis: grapheme decoding, conversion to phonemes, meaning acquisition, context integration, etc. In most of sentence-reading EEG studies, it is proposed that the integration processes occur only at long latencies after each word was seen, presenting differences in N400 and P600 potentials. However, these times are not consistent with results from eye movements studies, where interaction of context-dependent variables (such as predictability of the word) and the word recognition itself are visibles in shorter times (before 300ms). In the present study we aimed to analyze the time-course and the interaction of the context with word recognition in Proverbs, as a extreme case of context-dependent processing. To achieve this goal, EEG recordings were made on participants during foveated word-by-word reading of three types sentences: Proverbs and high and low predictability sentences. As expected we found an early effect of the variables associated with word recognition (frequency on the lexicon) and later effect for integration with the context (predictability). On the other hand, both sentence type and the word position in the sentence also have effects on the ERPs, that could not be explained by a predictability effect alone.

Cognition, Behavior, and Memory

P105.-Acute Administration of CRH Antagonist Do not Prevent Memory Impairment in Sleep Deprived Animals

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In a stressful event, the hypothalamic-pituitary adrenal axis (HPA) is activated causing the release of corticosterone (CORT) hormone in rodents. Paradoxical Sleep Deprivation Protocol (PSD) is associated with memory impairment and an increase in CORT. Our goal was to verify the effect of the blockage of CRH receptors (CP-154.526 antagonist) in the contextual fear-conditioning task (CFC) in PSD animals. Eighty Wistar males rats, three months old were divided into two groups: PSD or CTL. The PSD group was submitted to 96 hours of PSD in the modified multiple platforms method. The CTL group was kept in their house-cages. At the end of the PSD period, the animals were divided into four groups: administered with antagonist of CRH (PSD+CP, CTL+CP) or vehicle solution (0.5% carboxymethyl cellulose in water) (PSD+VEI; CTL+VEI). Thirty minutes after the treatment, the animals were then submitted to the CFC training, where they received five-foot shocks of 0,8mA, 1s duration, with 30s of interval among them. Forty-eight hours after the training session, freezing behavior was recorded in the conditioning box for 5 minutes, with no foot shock. ANOVA and post hoc analysis showed that freezing time on the CFC test from the third minute on was significantly lower in PSD than in CTL animals ($p < 0.05$). We found no effect of treatment or interactions related to this effect. We concluded that the impairment on the acquisition of aversive memory induced by PSD is not prevented by acute administration of the CRH antagonist. In addition, the CRH antagonist did not promote significant changes in the acquisition of aversive memory in animals CTL group.

UNIFESP Committee for Research Ethics. (#972753)

P106.-Implementation of a program for diagnosis and monitoring of cognitive impairment, supported by a system of tele-assistance, in Caldas department, Colombia

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Mild cognitive impairment is defined as a greater cognitive loss than expected for age and educational level, which does not interfere significantly with the activities of daily living. The prevalence, according to several epidemiological studies, ranges between 3% and 19% in adults over 65 years. (Gauthier et al, 2006, p.1262)

According to statistics projections, it is expected that by 2020 there will be in Colombia around 6.5 million old people, marking an increase of 39.2% compared to 2011, people who may potentially suffer from some kind of cognitive impairment (CI).

The diagnosis of CI is challenging in the early stages due to the lack of awareness of the early symptoms by the patient and their relatives, moreover, issues regarding adequate access to health care services delay the diagnosis, due to difficulties of coordination between primary care and specialized care, availability of diagnostics methods and others.

In this work we address this issue by showing the implementation of a system of tele-assistance in several medical specialties, enabling opportune control of this entity in the place of origin of the patient ensuring a proper diagnosis, treatment and rehabilitation. In addition, it was designed an educational program for patients, caregivers and professionals from several fields on the CI and its intervention through information and communication technology.

P107.-Promoting independence and functionality of older adults diagnosed with mild cognitive impairment through an educational program for their family and caregivers

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Mild Cognitive Impairment (MCI) is considered an intermediate state between normal aging and dementia, which is characterized by the presence of subjective memory alterations but without functional impairment, prevalence is higher in older adults and increases with age. According to statistics projections in 2013 10.5% of the Colombian population were over 60 years and this is expected to continue growing, creating new challenges in mental health care for this age group.
MCI may progress to dementia and in the course of the disease, the patient becomes more dependent when executing their daily activities and need the support of a caregiver, so it is necessary to implement preventive strategies to stop the evolution of the disease in order to maintain maximum patient autonomy. With this work, it is proposed an educational program guided with information and communication technologies, on the subject of cognitive impairment and home management of the disease, aimed at families and caregivers of patients with MCI that allows them to provide supportive care to the patient. The objective is to stimulate their cognitive functions through simple activities, keep them socially active and properly handle patient personality changes. At the same time, the educational program teaches self-care for the caregiver in order to prevent overloading in activities of daily living and have protective habits against cognitive decline.

Cognition, Behavior, and Memory

P108.-Mice lacking dopamine D2 autoreceptors as an animal model for neuropsychiatric disorders

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Dopamine (DA) D2 receptors expressed in DA midbrain neurons (autoD2R) regulate cell activity and DA release. Thus, it is likely that variations in D2 autoreceptor levels would play a role in prevalent human pathologies associated with DAergic circuits, namely ADHD, OCD and Tourette's syndrome. Our lab has generated mice selectively lacking autoD2R and demonstrated their critical importance in regulating DA neurotransmission. To study the consequences of this alteration and its possible contribution to neuropsychiatric disorders, we tested our mice in multiple behavioral experiments to identify different possible endophenotypes following an integrated perspective, in agreement with the 2008 Strategic Plan of the National Institute of Mental Health of the USA. Within this framework, psychiatric disturbances are not regarded as rigid compartments elicited by a unique single cause and showing an invariant outcome, but rather as a malleable constellation of symptoms originated by multifactorial and/or unidentified alterations. Our initial results show that autoD2RKO mice have early onset hyperlocomotion and increased stereotyped movements, overly rigid grooming sequences, reversal learning deficits and excessive compulsive-like behaviors. This combination of phenotypes leads us to propose that changes in autoD2R levels may shape a variety of mental disorders sharing a common substrate and demonstrated the importance of broad spectrum investigation approaches in neuroscience.

P109.-Emotional symptoms in patients with subjective memory complaints without cognitive impairment

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At the present a significant number of adults attend to neurology services with subjective memory complaints. However, a high percentage of them do not show objective impairment to corroborate these failures. The aim of this study was to determine whether patients who present subjective memory complaints without cognitive impairment have emotional symptoms of depression, anxiety and stress. The analysis was performed on a sample of 40 people between 20 and 69 years and with subjective memory complaints that following the completion of a comprehensive neurocognitive assessment and neuroimaging studies could confirm that there was no neurological cause for their subjetive complaints and had no criteria for the diagnosis of cognitive impairment. It was carried out a descriptive analysis and Spearman correlation analysis. It was found that 50% of the sample had depressive symptoms. From this group, 15% presented only depression, 10% showed depression and stress, 5% depression and anxiety and 20% depression, anxiety and stress. 5% of the population showed anxiety only. The remaining 45% presented no emotional symptoms. A significant correlation ($p < 0.001$) between depression, anxiety and stress was found. Conclusion: Population with subjective memory complaints but without an objective cognitive impairment presented a high prevalence of emotional symptoms, particularly depressive symptoms.

Cognition, Behavior, and Memory

P110.-Whose chair is it, anyway? Weighing ownership heuristics to resolve third-party disputes over property

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As Rousseau pointed out over 250 years ago, property is the cornerstone of civil society. Early on in our lives we learn rules that apply to the acquisition and transfer of property, and that we ought to respect other people's property. Nevertheless, the principles of ownership develop during our lifetime and, moreover, are shaped by our cultural environment. The importance of understanding how we think, act and, finally, how we legislate and judge according to these notions cannot be overestimated.
In this work, we began to evaluate how Discovery, Fabrication, Occupation, and Transfer, four different heuristics for ownership acquisition, interact in the resolution of third-party claims of property. Participants were asked to resolve conflicts of ownership after reading or listening to stories regarding two children who disputed over an object. First, we tested how the weight on adults' judgments of the aforementioned heuristics depended on their actual merit (e.g. how long did it take to fabricate an object). In a second experiment, we evaluated whether the relative weighing of these different heuristics changed from early childhood to adulthood.
Fabrication came out from our results as the strongest ownership heuristic, dominating all remaining heuristics. Transference strength showed a tight dependence on the formality of the transfer operation. This is the first study in which heuristics of acquisition of property are evaluated to this extent.

Cognition, Behavior, and Memory

P111.-Activation of medial prefrontal cortex dopamine receptors induces a persistent aversive behavior

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Medial prefrontal cortex (mPFC) and its dopaminergic (DA) signaling through dopamine receptors have been involved in cognitive, emotional and motivational processes. The primary source of DA to the mPFC is the ventral tegmental area (VTA) and it has been shown that aversive stimuli activate synapses selectively from the VTA to the mPFC. Here we use a non-aversive and non-rewarding modified conditioned place paradigm in rats, to analyze if the dopaminergic system in the mPFC is necessary and sufficient to signal aversion in the processing of a contextual memory. The activation of the D1/D5 dopamine receptors immediately after the exposure to a context, produces an aversive behavior when the animals are re exposed to that context 7 days, but not 24 hours after the training. In addition the activation of this long-lasting behavior seems to be through the activation of D5 dopamine receptors. The aversive effect is reversed by the association of this context with a reinforcing agent such as cocaine. Also, this effect is not observed when the D1/D5 receptors are activated in the hippocampus, other structure important for this memory. These experiments suggest that dopaminergic signaling in the mPFC would be involved in determining the aversive property of a persistent memory.

Cognition, Behavior, and Memory

P112.-Absence of EEG gamma oscillations coherence under a pharmacologic psychosis model

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During cognitive processes there is an extensive interaction between various regions of the cerebral cortex and subcortical regions. The extent of these interactions can be examined by means of a mathematical algorithm called "coherence". Ketamine is an (NMDA) receptor inhibitor which is used as psychosis model in subanesthetic doses. The present study was conducted to analyze the EEG coherence in the gamma frequency band under ketamine and compare it with the gamma coherence during AW, QW, NREM and REM sleep.
Cats and rats were implanted with electrodes in various cortices to monitor EEG activity. Ketamine (5-15 mg/kg i.m. in cats, 50 mg/kg i.p. in rats) was administrated and animals were recorded for 4 hours. The coherence values within the gamma frequency (30-45 Hz) from pairs of EEG recordings were analyzed. A decrease in coherence in the gamma frequency occurred among all cortical regions after 3 minutes of ketamine administration, and lasted for more than 10 to 40 minutes. This gamma coherence was similar to REM sleep gamma coherence, and lower than QW, NREM and wakefulness gamma coherence. Gamma power was similar to wakefulness and REM sleep. We conclude that subanesthetic doses of ketamine decreases the extent of gamma band interactions between different cortical areas in a similar manner to REM sleep. his phenomenon could explain alterations of cognitive functions and consciousness induced by ketamine.

Cognition, Behavior, and Memory

P113.-Learning strategies to ameliorate memory deficit in a Drosophila model of Noonan syndrome

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Intellectual disability and long-term memory (LTM) deficit has been detected in a group of genetic disorders with enhanced RAS-ERK1/2 signaling known as RASopathies. Studies in patients and animal models for RASopathies such as Noonan syndrome (NS) and Neurofibromatosis type-1 (NF1) showed that memory deficit can be restored. In NF1 mutant mice and patients memory was rescued by additional training, whereas in a Drosophila model of NS the memory deficit was restored by a longer spacing between trials of training.

What is the relationship and mechanisms of those strategies for memory improvement?

We used multiple training sessions and two-trial training sessions with longer spacing to examine the relation between those strategies in Drosophila. This experiments and genetic manipulation of RAS activity in mushroom bodies showed that both strategies share common properties, including RAS requirement to induce 24hr LTM, without affecting other memory components at 24 hr or immediate memory.

Moreover, transgenic fruit fly expressing a mutant allele of Ras, which models NS alleles showed a 24hr LTM deficit. Interestingly, additional training trials or additional spacing between trials restore memory deficit but did not provide a full memory rescue. These observations supported the concept that memory problems in RASopathies might be based on impaired ability to make use of the repetition and the spacing effect.

Cognition, Behavior, and Memory

P114.-Transient changes in GluN1 and GluN2A NDMAR subunits expression after habituation

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NMDA receptors (NMDAR) play a critical role in synaptic plasticity, are required for memory encoding and "storage" (Paoletti et al., 2013). NMDAR are heterotetramers composed by 2 GluN1 obligatory subunits and 2 regulatory subunits, being GluN2A and GluN2B the major regulatory subunits in CNS regions involved in cognitive functions. We have shown that about 1 hour after memory acquisition of a hippocampus depending task (open field [OF] habituation, GluN1 and GluN2A expression transiently increased in the hippocampus. We have analyzed NMDAR subunits levels in Wistar rats after 5 minutes exploration of an unique OF, in central structures other than the hippocampus, at different ages (30, 60 and 90 days old). There was an increase in GluN1 and GluN2A levels in the hippocampus about 1 hour after the OF session, though neither in the cerebral cortex nor in the amygdala; that increase was reversible since the levels were not significantly different from controls 24 h later, in all the ages assessed. We analyzed two other groups of rats (30 and 90 days old) that were exposed to a second OF session (test). The 2nd OF session was performed either 24 h or up to 2 month after the 1st session. GluN1 and GluN2A levels increased in the prefrontal cortex 60 minutes after the test, either 24 hs or 2 months later. Our results suggest that the transient increase and rebalance of specific NMDAR subunits in structures involved in memory processing could be related to the memory tracing.

Cognition, Behavior, and Memory

P115.-Influence of Video Games in the Working Memory and Visual Attention of Student of Sports Training Career

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It is known that physical activity, usully shows a fairly healthy behavor, due to the improved capabilities concentration, stress reduction and rates of aggression and helps prevent obesity the interestin thing is tat within exercisers and non A stereotype is knows and handedalmost like a recurring myth in these social groups which are divided between those who are athletes and Nerds first as a person with high physical and exceptional abilities in games involving the same, but litter intellectual capacity and the second high ability to be good at video games, but poor physical perforance, both wrapped in a stereotype. This paper tries to testing the possible influence of)commercial= gae on cognitive abilites of people who constantly play video games and instead, play constant physical activity. This document only males occur Degree in Sport Training UACJ in a sample of n= 18 using a quasi-experimental methodology where two groups were used Control and experimental n=9 n=9, using the Trail Making Test as an indicator of visual attention and test for early detection of Alzheimer's disease as it indicated consisting of working memory. The results indicate that it is likely that the stimulus control group had a higher livel of significance in the performance of cognitive activities, while the experimental group showed thet cognitive level is lower, showing that the stimulus created by the game more fluid create a cognitive level.

Cognition, Behavior, and Memory

P116.-Micro-Positron Emission Tomography Study of Memory Reconsolidation

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After learning, the new information is encoded in neural circuits. For that encoding, neuronal plastic changes occur to stabilize the memory trace, a process termed consolidation. If some cues of the learning event are presented to the subjects after the memory is fully consolidated, the memory trace can become labile and requires a process of re-stabilization called reconsolidation. For the past decades, labilization/reconsolidation processes has been studied from behavioral, cellular and molecular approaches but no studies have emerged to elucidate neural circuits subserving memory dynamics during these processes. Here, we studied the mouse brain from a functional perspective using small-animal Positron Emission Tomography (PET). The main objective was to study functional dynamics after the labilization/reconsolidation phases of memory in contextual fear conditioning in mice. We found differences in glucose consumption mainly in ectorhinal cortex, hippocampus and amygdala in re-exposed animals compared to non-re-exposed animals. Moreover, animals that only evocated but did not labilized the trace showed differences with mice that labilized and reconsolidated. The differences in glucose consumptions showed a marked temporal and spatial course, and were context-specific. This work opens new insights in the dynamics of activation of different brain areas during memory reconsolidation.

P117.-Peer tutoring in changing children's models of the Earth

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Teaching is believed to be fundamental to maintain and develop culture and a natural cognitive ability we are all exposed to and participate in. Surprisingly, teaching abilities have been much less studied than learning skills by neuroscience and cognitive neuroscience.

Children have shown capable of teaching and of engaging in peer tutoring, i.e. teaching between individuals with similar interests, age and contexts. We thus propose this peer-mediated instruction may provide the conditions for conceptual change to occur; a goal often difficult to achieve.

First, we conducted a tightly scripted face-to-face interview with 7- to 8-year-old children from two second-grade courses. The interview, based on previous work by Vosniadou et al (Vosniadou, 1992), was aimed to elicit verbal responses and drawings so as to reveal the Earth models held by these children.

Then, in a separate session, some children were matched with a partner holding a different model of the Earth and one of them was instructed to explain the other they should agree on a common drawing. Other children, instead, revised their knowledge on their own. Finally, the initial interview was repeated after the intervention to evaluate response changes.

As expected from previous work by Vosniadou et al for children of this age, we found diversity in the Earth models held by them. We hypothesize children who engaged in interaction with another will show greater response changes than those who did not interact.

Cognition, Behavior, and Memory

P118.-Retrosplenial cortex is necessary for consolidation and expression of object recognition memories

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Over the last few years, the interest in the retrosplenial cortex (RSC) function on memory has increased. Recently, our laboratory has demonstrated that RSC is required for the formation, storage and expression of aversive memories using the inhibitory avoidance (IA) paradigm. Moreover, we have shown that the RSC and hippocampus (Hp) interact for long-lasting IA memory storage. To further analyze the role of RSC in memory processing, we used two different variants of the object recognition paradigm to evaluate which components of the information are processed in RSC, one variant with spatial cues (SOR) to study "where", and a non-spatial task (Y-OR), to study "what". Inactivation of RSC immediately, but not 3 h after training, impaired memory consolidation tested 24 h later in both variants of the task. In addition, we found that inactivation of RSC 15 min before test at 24 h also impaired memory retrieval in both variants. On the contrary, we found no effect after inactivation of the hippocampus in the same conditions. These results indicate that RSC is involved in object recognition memory formation and expression, and it is processing different components of information to be store.

Cognition, Behavior, and Memory

P119.-Pharmacological Blockade of GABA-A receptors in the Basolateral Amygdala Complex Prevented the Disrupting Effect Of Midazolam on Fear Memory Reconsolidation. Influence of D-Cycloserine

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Consolidated memories can enter into a labile state after retrieval, requiring a restabilization process defined as reconsolidation. This process can be interfered by agents such as protein synthesis inhibitors or benzodiacepines. However, there are conditions that constraint the occurrence of labilization/reconsolidation process. For instance, memory age, strength of training among others. Previous studies shown that stress prior to contextual fear conditioning generates a memory resistant to the disrupting effect of Midazolam (MDZ) on reconsolidation. Furthermore, it is known that stress leads to reduced GABAergic transmission in Basolateral Amygdala (BLA), promoting LTP and fear memory formation. Interestingly, these effects are mimicked by intra-BLA administration of Bicuculine (BIC), a GABA-A receptor antagonist. Therefore, this study was aimed to evaluate if BIC intra-BLA prior to fear conditioning generates resistance to the effect of MDZ in a similar way to the resistance generated by stress. As expected, infusion of BIC mimics the effect of stress in generating a memory insensitive to MDZ interference. Additionally, this resistance is reverted by systemic administration of D-Cycloserine, an NMDA receptor co-agonist, prior to memory reactivation. These results demonstrates that BLA hyperexcitability leads to the formation of resistant memories, and that the onset of the labilization phase can be promoted by activation of NMDA receptor at the moment of retrieval.

Cognition, Behavior, and Memory

P120.-The dynamic nature of the reconsolidation process and its boundary conditions

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The reconsolidation process is the mechanism by which consolidated memories are updated in strength or content. Memory age and strength are boundary conditions for reconsolidation process. We investigated the dynamics of such conditions, anticipating that the boundary conditions of the reconsolidation process are subject to change rather than immovable. Aversive emotional events such as stress affect different memory process in several ways. Here we showed in Experiment 1 and 2, that stress prior the acquisition of a neutral declarative, improves acquisition and enhance consolidation. In Experiment 3, a strong memory initially acquired under stress pass through the reconsolidation process and could be interfered by new learning after the presentation of a reminder. In Experiment 4 and 5, we revealed that seven days after training the reconsolidation of a strong-old memory could be interfered by new learning after a reminder presentation and that two successive presentation of the reminder strengths memory. As a whole, the results suggest that the fate of a neutral declarative memory acquired after a social threat result in a strong memory, and under this condition the reconsolidation process per se and the strengthening function is active on older memories. Boundary conditions vary rather than being permanent, showing the dynamic nature of the reconsolidation process.

Cognition, Behavior, and Memory

P121.-Ethanol Consumption in Adolescents Rats Resulting from Selectively Bred, High and Low Ethanol Consumers, Lines

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We aimed to generate two lines of Wistar rats with high or low predisposition for ethanol intake, by selective breeding of high and low ethanol consumers during adolescence, a developmental stage in which ethanol use disorders emerge and are likely determined. These lines should be a valuable tool to analyze the relationship between motivational sensitivity to ethanol and ethanol intake during the initial generations. In this study, we present data of ethanol intake and its preference of the first two generations derived from our short-term selection process. Results indicated that in F1 generation, ethanol intake was significantly greater in High Alcohol Consumers (HAC) than in Low Alcohol Consumers (LAC) animals. Visual inspection of the data from F2 generation suggests a similar pattern of intake as observed in F1 generation, but differences between HAC and LAC intake patterns did not attach significance. These results suggest a rapid emergence of differences in ethanol intake following a short-term selective breeding that takes into account the expression of this variable within a short time frame of development (i.e., adolescence). Even we were not able to observe differences in ethanol intake between HAC and LAC animals of F2, this model provides a useful tool for the analysis of behavioral and neural determinants of alcohol intake trajectories.

Cognition, Behavior, and Memory

P122.-Retrieval and reconsolidation of fear memory can be interfered independently

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Consolidated memories might become temporally unstable if reactivated under propper conditions. After destabilization memories need to reconsolidate in order to persist and be available for retrieval. Previous work from our group has demonstrated that fear memory reconsolidation can be interfered with an appetitive experience. Here we wanted to determine if an appetitive experience could also affect fear memory retrieval. By combining fear memory reactivation and an appetitive or neutral expirience in different orthers and time intervals we found that retrieval and de reconsolidation can be affected in separate ways. Furthermore we confirmed this by using Midazolam as amnestic agent. This results suggest that memory destabilization is a dissociable process from memory retrieval.

Cognition, Behavior, and Memory

P123.-Development of a retrieval-induced forgetting paradigm in rodents to model adaptive forgetting in the mammalian brain

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Over a century of memory studies have presumed that forgetting was the product of passive mechanisms such as decay and interference. In the last two decades, however, studies on Retrieval-Induced Forgetting (RIF) have demonstrated the existence of active mechanisms of adaptive forgetting, such as the inhibitory control. Despite this, the lack of animal models precluded the understanding of the neurobiological mechanisms underlying these processes. Using spontaneous object recognition, we developed a paradigm that allowed us to observe retrieval-induced forgetting in rats. We have shown that forgetting an item associated with a particular context occurs under conditions which cause competition between memory traces (two pairs of objects that share a context as an evocation cue). We used local pharmacological inactivation to show that this kind of forgetting requires the activity of the medial prefrontal cortex (mPFC) in rats; structure homologous to the human dorsolateral prefrontal cortex (DLPFC). By using c-Fos imaging, we also observed that mPFC activation by retrieval practice occurs only during the first practice sessions, providing evidence that, as for humans, forgetting is adaptive also for rats. These results are consistent with the idea that the RIF occurs via a top-down inhibitory control mechanism exerted by the mPFC on structures where memory traces may be stored.

Cognition, Behavior, and Memory

P124.-Effect of alpha-MSH on the reconsolidation of a contextual fear memory; melanocortinergic receptors and mechanisms involved

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Melanocortins are important modulators of the immune response, and they have diverse and important effects in the central nervous system. Between them, a-MSH can modulate the consolidation and reconsolidation of a contextual fear memory during inflammatory processes. However, the effect of a-MSH on these processes, in physiological conditions, is not clearly established yet. The objective of the present study was to determine the effect of a-MSH on the reconsolidation of a contextual fear memory, the melanocortinergic receptors involved and possible molecular mechanisms. Our results showed that the intrahippocampal administration of a-MSH after memory reactivation can increase the percent of freezing during the test. This effect would be specific to the reconsolidation process because it is only observed when the memory was reactivated. The effect of a-MSH is mediated by the activation of the MC4R. Our results also demonstrated that the treatment with a-MSH didn't produce changes in the expression of zif-268, a transcription factor specifically involved in memory reconsolidation. Our results demonstrated that a-MSH has an effect on memory reconsolidation through the activation of MC4R.

Cognition, Behavior, and Memory

P125.-Measuring Emotions in the Hotel Experience

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Introduction. The merger of the application of the theories of Economics and the neuroscientific findings is called Neuroeconomics. The present research work applied finds neuroeconomics based on satisfaction of purchase and loyalty of purchase on the research of tourism services with the purpose of characterizing those elements of tourism enterprises that can help to activate brain regions associated with generating a pleasant experience of customers. Objective. Characterize the elements of visual interest in a hotel by the Eye-Tracking system and describe patterns of electrical activity of brain associated with the evocation of the emotions experienced during the stay of the subject at the hotel. Development. Attentional foci of visual type by means of Eye Tracking in-situ measurement: customer runs for 15 minutes the facilities and activities of interest offered by the hotel, as they are rescued the main points of visual interest through Eye-Tracking System. Send client to the same hotel, without invasive techniques of any kind, that you experience during a couple of days the complete purchase of services offered by the hotel. Registration of brain activity through EEG: the customer will see a video that evokes the major infrastructures and activities that previously lived in the hotel, while the video is projected.

P126.-Neuroprotective role of Oleoylethanolamide in a rat model of perinatal asphyxia

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Perinatal asphyxia (PA) is still associated with neurological morbidity and implies overactivation of inflammatory response. The absence of an established treatment for PA encourages research on neuroprotective mechanisms. N- acylethanolamides have exerted anti-inflammatory actions via PPARα activation in several models of brain injury. However, its role in perinatal hypoxic brain injury remains still unknown. The aim of our study is to evaluate the neuroprotective role of Oleoylethanolamide (OEA) treatment in 30 days-old asphyctic rats.

A rat model of global severe PA was performed. The sample (N=24) consisted of male Sprague Dawley rat pups and was divided into 4 groups: asphyctic rats undergoing to vehicle treatment (PA20), asphyctic rats subjected to treatment with OEA (PA20 OEA10), control of cesarean delivery undergoing to vehicle treatment (C+), control of cesarean delivery subjected to treatment with OEA (C+ OEA10). The respective treatment was performed within the first hour of life by subcutaneous injection. Rats were subjected to behavioral tests.

Asphyctic rats treated with OEA (10 mg/kg) showed an improvement in exploratory locomotion in Elevated Plus Maze test.

OEA may exert a neuroprotective effect in perinatal asphyxia. Further studies are being carried out in our laboratory to determine if OEA might have some protective effects on synaptic structure and functions.

Cognition, Behavior, and Memory

P127.-Alpha-melanocyte stimulating hormone (α-MSH) increase fear memory expression through melanocortin 4 receptor (MC4R)

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Melanocortins have important effects at central nervous system. In particular, alpha-melanocyte stimulating hormone (α-MSH) through MC4R, mediates antipyretic and neuroprotective actions. Although it has not been concretely demonstrated it has been suggest that α-MSH also could modulate learning and memory processes. We previously establish that α-MSH prevents the amnesia induced by inflammatory conditions. However, the effect of α-MSH on memory consolidation has not been clearly elucidated. In the present work, we show that intrahippocampal injection of a high dose of α-MSH after contextual fear conditioning produce a significant increase of freezing levels compare with control group indicating that the neuropeptide would be a positive modulator of memory consolidation. Conversely, the lowest doses did not produce changes on freezing percentage. Treatment with HS014, a specific MC4R antagonist, prevents the effect of α-MSH on fear conditioning suggesting that α-MSH increases fear memory expression through this receptor. We also demonstrated that fear conditioning produce ERK2 activation, a MAPK critically involved in memory consolidation, 60 min after training. The α-MSH administration anticipates this effect producing an increase in pERK2 levels 15 min after conditioning in dorsal hippocampus. α-MSH and MCR4 seem to be important modulators of cognitive processes and therefore could be promising targets for pharmacological intervention in several conditions.

Cognition, Behavior, and Memory

P128.-Early BDNF/c-Fos cascade in the retrosplenial cortex is required for the persistence of a long-lasting aversive memory.

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During the past few years there has been growing interest in the role of the cortex in memory processing. In the present work, we studied the early posttraining participation of retrosplenial cortex (RSC) in the formation and storage of a long-lasting memory, and the molecular changes that take place in this brain region during memory storage. We found an increase in c-Fos levels in the anterior part of the RSC (aRSC) after inhibitory avoidance (IA) training. Interestingly, this increase was associated with memory durability, since blocking c-Fos expression using specific antisense-oligonucleotides (ASO) impaired long-lasting retention 7 days after training without affecting memory expression 2 days after training. In addition, we showed that BDNF is one of the upstream signals for c-Fos expression required for memory persistence. We found that injection of BDNF around IA training into aRSC was sufficient to establish a persistent memory and that this effect was prevented by c-fos ASO infusion into the same structure. These findings reveal an early posttraining involvement of aRSC in the processing of a long-lasting aversive memory and also some of its key molecular components necessary for this process.

Cognition, Behavior, and Memory

P129.-Bacterial LPS induces sickness behavior in honey bees

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During an infection, animals suffer several changes in their normal physiology and behavior which can include lethargy, appetite loss and reduction in grooming and general movement. These sets of alterations are known as sickness behavior and although they have been extensively believed to be orchestrated primarily by the immune system, a relevant role for the central nervous system has also been established recently.
The aim of our work is to develop a simple animal model to study how both of these systems interact during an infection. We administered a bacterial lipopolysaccharide (LPS) into the thorax of honey bees to mimic a bacterial infection, and then we evaluated a set of stereotyped behaviors of the animals.
First, we showed that this immune challenge reduces the locomotor activity of the animals in a narrow time window after LPS injection. Furthermore, bees exhibit a loss of appetite 60 and 90 minutes after injection, but not 24 hours later. We also demonstrated that LPS injection reduces spontaneous antennal movements in harnessed animals, which suggests a reduction in the motivational state of the bees. Finally, we showed that the LPS injection diminish the interaction between animals, a crucial behavior in social insects.These findings prove the honey bee as a useful insect model for the study of the sickness behavior.

Cognition, Behavior, and Memory

P130.-Aversive and appetitive memories are simultaneously formed after a single learning session in the crab Neohelice

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Unlike experimentally controlled situations, animals in nature might be exposed to contradictory information. Situations or places might simultaneously predict desired and undesired consequences. However, at some point the situation has to be categorized as appetitive or aversive, in order to decide if repeat or avoid it in the future. How contradictory information is integrated and how it affects learning and memory has not been yet extensively studied. In the present work we took advantage of the well described aversive and appetitive learning paradigms in the crab Neohelice to explore learning after simultaneous appetitive and aversive experiences associated to the same context. First, we found that two parallel memory traces are formed after simultaneous appetitive and aversive training. Second, we found that the probabilities to express no, one or both learned behaviors depend on the balance between the relative strength of the aversive and appetitive unconditioned stimuli, thus revealing a mutual interference under certain conditions. Finally, we found that the mentioned interferences do not occur during learning or memory formation, rather during memory retrieval. These results suggest that both memories could be actually available to be retrieved upon presentation of the conditioned stimulus, but the access of memory to behavior might be modulated based on specific demands at the moment of retrieval.

Cognition, Behavior, and Memory

P131.-Hippocampal ERK2 differential activation after memory reconsolidation processes are modulated by α7 nicotinic acetylcholine receptors

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We have previously reported not only that hippocampal α7 nicotinic acetylcholine receptors (nAChRs) play a critical role in reconsolidation processes but also that there is a differential activation of extracellular signal-regulated kinases (ERK1/2) after a reconsolidation process in mice trained in an inhibitory avoidance task. Next, we determined whether nAChRs activation/inactivation is able to modulate ERK1/2; responsible of, at least in part, the behavioral changes observed in subsequent tests after memory reconsolidation.

With these in mind, CF-1 male mice were trained in an inhibitory avoidance task using either a mild (0.8 mA, 1s) or a strong (1.2 mA, 1 s) footshock. A reactivation test (T1) was given 48 hours later. Immediately after it, mice were given intra-dorsal hippocampal infusions of choline, an α7nAchRs agonist (Ch, 0.80 g/hippocampus), methyllycaconitine, an α7nAchRs antagonist (MLA, 10.00 ug/hippocampus) or vehicle. Fifteen or forty-five minutes afterwards, the hippocampi were dissected and ERK1/2 activation was determined in nuclear and cytosolic fractions.

Ch or MLA, given immediately after memory reactivation, modified ERK2 pattern of activation depending on both, training conditions and time elapsed after memory reactivation.

Altogether, our results point for the first time to an α7nAChRs-related ERK2 pattern of activation induced during memory reconsolidation modulation.

Cognition, Behavior, and Memory

P132.-Analysis of neurocognitive profiles of young adults and healthy older adults

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Introduction: typical neuropsychological profiles provide features that can guide the specialists towards a differential diagnosis of cognitive impairment in adulthood. The objetive of this study was to compare neuropsychological profiles among young adults and healthy older adults. Methodology: The analysis was performed using the data of neuropsychological evaluations conducted between years 2012 and 2015 at the Unidad de Neuropsicología y Neurorehabilitación del Sanatorio Allende and Centro Integral de Apoyo Terapéutico y Estimulación Cognitiva (CIATEC). Two age groups were considered for analysis: 45-65 years (N = 44) and 66 years and older (N = 30). Scores on the different neuropsychological testing were compared using t-test mean difference. Results: A significant decrease in age-related attentional processes (p <0.01), verbal episodic memory (p <0.01), processing speed (p <0.01), semantic verbal fluency (p <notes 0.01), name (p <0.05) and visuospatial abilities (p <0.05) was found. Conclusion: This study demonstrated the existence of cognitive changes associated with normal aging and the existence of neuropsychological differences in terms of age is established. Consistent with the literature, verbal episodic memory, processing speed and attention processes were the functions found more decreased in the older age group.

Cognition, Behavior, and Memory

P133.-The emotional tagging: stress and the promotion of durable memories.

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Long -term memory (LTM) formation requires protein synthesis. We have demonstrated that a weak learning task which only induces short-term memory (STM) can be stabilized into LTM if another closer event provides the necessary proteins. It was postulated that these proteins could be capture at tagged sites induced by the weak learning task by a process referred to as behavioral tagging. Thus, we decided to investigate whether an acute stress can promote the formation of LTM from a weak training by providing the proteins necessary for its consolidation. For this purpose, rats were subjected to a weak training (spatial object recognition –SOR) that induces only STM and, at different times close to training, they were exposed to a stressful situation (an elevated plataform-EP- for 30 min). Our results show that acute stress experienced one hour after SOR training promotes its LTM formation. We studied the dependence of this phenomenon on protein synthesis and also on glucocorticoids (GR) and mineralocorticoids receptors (MR) activity in the dorsal hippocampus. The local infusion of Emetine, Anysomicin, Mifepristone or Spironolactone before the exposure to the EP shows that promoting effect of stress on SOR -LTM formation depends on protein synthesis and GR-MR activity in the dorsal hippocampus. Finally, we present data aimed to investigate the role of stress induced by exams on the promotion of LTM in students.

Cognition, Behavior, and Memory

P134.-Different Behavioral Phenotypes Observed in Adult Mice with Selective Ablation of Striatal Cholinergic Interneurons at Different Postnatal Stages

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It is known that basal ganglia (BG) dysfunctions are involved in neuropsychiatric disorders like Tourette Syndrome (TS), which is characterized by the presence of tics and rituals. We have previously shown that striatal cholinergic interneurons (SCI) can be selectively ablated using a new transgenic mouse system, mimicking what was observed in postmortem studies of TS patients. TS symptoms diminish in intensity or mutate as patients progress through adolescence. Whether SCI regulate TS-related behaviors in an age dependent manner has not been examined before. SCI were ablated in mice before and after adolescence. As adults, the mice were subjected to extensive behavioral testing, including tasks designed to evaluate social-directed repetitive behaviors. SCI adult-lesioned mice showed an increase in exploratory behaviors in novel contexts, an increase in object-oriented, but not self-oriented perseverative behaviors along with a ritualistic-like persistence in social exploration. In contrast, mice injected before adolescence and tested during adulthood only showed a hyperlocomotor response. These results indicate that a reduction of SCI produces perseverative and ritualistic behaviors, with strong impact on social interaction and exploration of the environment, having a differential influence at different postnatal stages. Overall, the data suggest that a reduction of SCI might contribute to generate symptoms of perseveration in neuropsychiatric disorders, like TS.

Cognition, Behavior, and Memory

P135.-Hemiellipsoid bodies: neural plasticity in the crab's "mushroom bodies"

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The corpora pedunculata (or mushroom bodies) are complex paired structures in the brain of various invertebrate species and vastly studied in insects. Since their first description in the mid-1850, the corpora pedunculata were considered as higher-order brain centers involved in sensory integration and memory. Although morphologically diverse, a corpora pedunculata common ground plan was described across different invertebrates. Moreover, it has been proposed that the mushroom bodies and the vertebrate pallium evolved from the same structure in a common ancestor circa 600 million years ago. In crustaceans, neuropils sharing a similar ground pattern with the corpora pedunculata are the Hemiellipsoid bodies (HBs), which have been proposed to have an evolutionary common origin. Our group's works in the crab Neohelice granulata shows morphological and immunohistochemical studies that parallel the results for well described HBs in other crustaceans, allowing an accurate identification of this neuropil in this crab (see poster Shkedy:au). Here we evaluate, in the crab N. granulata, context-signal memory related neural plasticity of the intrinsic neurons of the crab's HBs by in vivo calcium imaging. We found neuronal responses to mechanical and visual stimulation and stimulus specific changes during and after training. These results provide the first in vivo physiological evidence that support the idea that the HBs, the crustaceans' mushroom bodies, are involved in memory processes.

Cognition, Behavior, and Memory

P136.-The immediate early gene Arc is required for pattern separation non-spatial memories in the perirhinal cortex

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Successful memory involves not only remembering information over time but also keeping memories distinct and less confusable. The ability to separate the components of memories into distinct representations relies on pattern separation, a computational process by which differences are amplified to disambiguate similar events. Despite the importance of this mnemonic function, the molecular mechanisms necessary for the behavioral manifestations of this process remain unknown. Although pattern separation in a spatial domain has been localized to the dentate gyrus of the hippocampus, this cognitive function is thought to take place also during processing of other types of information. The perirhinal cortex (PRH) is involved in the acquisition and storage of object memories, and is crucial for the resolution of tasks with ambiguous features. Thus, we hypothesized that this structure is involved in pattern separation of object memories. In this work, we used a PRH-dependent task and manipulated the load of pattern separation during encoding. We showed that consolidation of pattern-separated object memories depends on the expression of the gene Arc in the PRH during a restricted time window, and that interaction between Arc and BDNF is necessary for successful pattern separation. These findings suggest that Arc, an immediate early gene known to regulate synaptic plasticity and mediate memory formation, is involved in the molecular mechanisms underlying non-spatial pattern separation.

Cognition, Behavior, and Memory

P137.-Cognitive scores on tests of verbal episodic memory: analysis of its prevalence in population with different profiles of cognitive impairment

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The memory failure is a common concern in the adult population. Such failures can respond to various sources and present a differential clinical pattern. The aim of this study is to describe and compare different production errors in population with normal aging, Mild Cognitive Impairment (MCI), Alzheimer's Disease (AD) and mixed dementia, with the Verbal Learning Test Spain Complutense (TAVEC). Methodology: The sample consisted of 144 adults between 45 and 90 years, divided into four groups according to their diagnosis (Healthy N: 60; DCL N: 44; EA, N: 24, mixed dementia N: 16). It was carried out a descriptive analysis and a one-factor ANOVA with mean difference.

The data provide meaningful evidence of clinical markers that differ in all populations analyzed. A mean difference was evident in the number of intrusions (F: 12.95 Next:, 000; F: 23.64 Next:, 000) and even more of false positives (F: 34.74, sig:, 000) in people with MCI and AD. In subjects with DTA it is where the means of false positives and intrusions usually peak. However, in relation to the amount of perseverations no significant difference was found in average (F: 1,015 sig: 388). Discussion: The verbal episodic memory tests provide clinical markers that collaborates in conducting differential diagnoses in adults and identifying early signs of possible evolution of DCL to different types of dementia.

Cognition, Behavior, and Memory

P138.-Serotoninergic system mediate the resolution of memory tasks that needs control of interference during retrieval phase

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Serotonin (5-HT) plays a role in the control of impulsive an other types of behavior. Increasing evidence shows that 5-HT is involved in learning and memory processes. However, role of 5-HT in recognition memory is poorly understood in particular in conditions that cause interference between memory traces. We have shown that blocking 5-HT2AR or activating 5-HT1AR in the medial prefrontal cortex (mPFC) of rats impairs the resolution of an object in context task (OIC) in which the animal has to recognize a novel combination of a familiar object and a familiar context. We postulated that both receptors might play a role in the resolution of OIC. If this is the case, then an imbalance of 5-HT2AR or 5-HT1AR signaling might alter the mnemonic function of mPFC. We tested this hypothesis using a genetically modified mice that present a global disruption of 5-HT2AR (htr2a-/-).We exposed htr2a -/- and htr2a+/+ to the OIC task. We found that htr2a -/- mice showed a deficit in the resolution of the OIC task similar to the one observed in rats when 5-HT2AR was blocked in mPFC during memory retrieval. Infusion of a selective 5-HT1AR antagonist in the mPFC before the retrieval session rescued the discrimination deficit observed in htr2a-/- mice without affecting the performance in htr2a+/+ mice. These result suggest that 5-HT modulates mPFC function through 5-HT1AR and 5-HT2AR and that in the absence of 5-HT2AR, 5-HT1A signaling affects the ability of the mPFC to control memory interference.

Cognition, Behavior, and Memory

P139.-Differential Reactivation Outcomes on a Single US-Contextual Fear Conditioning: a temporal prediction error account.

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Last decades of research on memory dynamic lead to review the fate of a trace after the protein synthesis-dependent consolidation process. It has been demonstrated that once a memory is consolidated, if it is properly reactivated, can enter into a labile state that makes it susceptible to various manipulations, needing to "reconsolidate" in order to persist. Labilization seems to be initiated by a mismatch or temporal prediction error between training and reactivation. However, most research in this topic is conducted with strongly trained memories including several US presentations. In this work we conducted two experiments: in the first one, five groups of rats were fear conditioned to a context in order to achieve moderate levels of conditioned responding. The second experiment tested different reactivation protocols and a control condition. Half of the subjects in each condition were administered with an i.p. injection of saline or midazolam, a fast acting GABA-A positive modulator known to disrupt memory reconsolidation. Results suggest that memories trained with a single unconditioned stimulus also destabilize by a temporal mismatch during reactivation. These results would allow future research with a more sensible memory, able to be strengthened or dampened through various means.

Cognition, Behavior, and Memory

P14O.-Fear memory recall induces an anxiogenic-like behavior in ethanol withdrawn rats: reversion by memory reconsolidation interference

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Withdrawal from chronic ethanol consumption promotes the formation of a robust and resistant fear memory. Here, we examine the anxiety-like effect of fear memory recall in ethanol-dependent (ETOH) rats. Male Wistar rats were made dependent via an ethanol containing liquid diet (6% v/v) for 14 days. Contextual fear conditioning (FC) was performed on day 3 of withdrawal. Seven days after, rats were re-exposed to fear training context and tested in the elevated plus maze (EPM) 30min later. A significant decrease in the percentage of time spent on the open arms (%TA) and in the number of open arm entries (OAE) was induced by fear memory recall only in ETOH rats. Next, we evaluated whether interfering memory reconsolidation could affect recall-induced anxiety-like behavior in the EPM. We have demonstrated that D-cycloserine (DCS)/Propranolol (PROP) administration in combination with memory retrieval impair of fear memory reconsolidation in ETOH rats. Therefore, seven days after FC, rats received DCS (5mg/kg, ip) 30 min before a 5-min context re-exposure and immediately after, were injected with PROP (10mg/Kg, ip) or SAL. The following day, memory retention was evaluated and then, rats were tested in the EPM. ETOH animals treated with DCS/PROP displayed levels of %TA and number of OAE similar to CON animals. Our results suggest that fear memory retrieval promotes an anxiety-like behaviour in ETOH rats and its consequence is prevented by the interference of reconsolidation process.

Cognition, Behavior, and Memory

P141.-Stroop interference before aversive and neutral images in Ciudad Juarez population

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Several studies suggest that the interaction with violent stimuli presented in the media (especially in videogames) cause an increase in violent behavior. To study this phenomenon, neuropsychology has had to adapt its tools to generate experimental studies in which the subject becomes involved in stressful situations (but controlled) to verify the potential impact of violent stimuli.
One test used was the Stroop test, with which found that exposure to violent or non-violent stimuli has effects for activation or inhibition of reactions and / or violent behavior, even with randomly selected people.
Using a quasi-experimental methodology random assignment of participants to violent images and neutral stimuli, this study sought to see the level of attentional interference by Stroop Test, followed by applying a series of 10 images, whether violent or neutral stimuli, segmented in 10 seconds each, followed by a blank image of three seconds before starting with the next picture. The results indicated, by means of descriptive data, experimental group that has less interference than the control. However, no statistical significance was found, so it is recommended to seek higher sampling conditions and greater control for variable and subject.

Cognition, Behavior, and Memory

P142.-Involvement of the anterior and posterior insular cortex in memory consolidation of contextual and tone fear conditioning

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The insula or insular cortex can be divided into two functionally heterogeneous subregions. Nevertheless, no work, to the best of our knowledge, had investigated the specific role of each subregion of the insular cortex on consolidation of different fear conditioning tasks.
In our study, 3-month-old male Wistar rats underwent stereotaxic surgery for implantation of bilateral guide cannulae aimed directly above the anterior or posterior insular cortices. For the training session, the rats were individually placed into a conditioning box. After 120 seconds of free exploration, a tone was delivered for 30 seconds and it ceased with a footshock. Immediately after, each rat received microinjection of muscimol (GABAergic agonist) or saline (control) into the intended insula subregion.
We performed the contextual fear-conditioning test 48 hours after training and, 24 hours later, the same animals were submitted to tone fear-conditioning test. To summarize our results, there were no difference between groups in the contextual fear-conditioning test. However, the rats that had received muscimol into the anterior and posterior insula showed less freezing behavior in the post-tone period during the tone fear-conditioning test.
This data highlight evidence that the insula as a whole is part of the neural circuitry involved in consolidation of the tone fear conditioning task - a model for emotional memory.

Cognition, Behavior, and Memory

P143.-Effects of scopolamine on a modulator treatment in frustration

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The consummatory successive negative contrast (cSNC) paradigm is an animal model for the study of frustration in which acceptance of 4% sucrose is assessed in animals that had been exposed to 32% sucrose. These downshifted animals usually exhibit significantly less sucrose acceptance than animals that always received the 4% sucrose solution. On the other hand, exposing Wistar rats to a novel situation, as the exploration of an open field (OF), prior to the first downshift trial (T1) generates memory impairment on frustration. The opposite pattern was observed when the OF was applied prior to the second trial (T2) as it generates an accentuation of frustration. With the aim of investigate the implications of cholinergic system in the phenomenon, scopolamine (a cholinergic antagonist) was administered 20 minutes before or immediately after the OF. The drug blocked the effect of OF in T1 and T2, when it was injected previously and also immediately after. These results provide new information on functional and pharmacological dissociations during the first and second trials of cSNC and also show the role of the cholinergic system in the phenomenon.

P144.-First evidence of a behavioral tagging process acting in memory reconsolidation

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Behavioral tagging (BT) is a process underlying long-term memory (LTM) formation. It consists on two parallel and complementary mechanisms: the setting of a learning tag (LT) that defines where a memory will be stored, and the synthesis of proteins (PRPs), that once captured at tagged sites, stabilize the mnemonic trace into a LTM. Thus as long as PRPs and LTs coexist in the same substrate, proteins can be supplied by any event. Interestingly, BT also underlies memory extinction; one of the processes triggered by retrieval. But, retrieval can also trigger reconsolidation. As one its principal functions would be memory updating, it is worthwhile asking if the new mnemonic engram, set during reconsolidation, is established through a BT process.

To study this, we combined protocols capable of inducing reconsolidation in the inhibitory avoidance and spatial object recognition tasks, with pharmacological interventions and the exploration to a novel open field (OF). We show that reconsolidation blockade, induced by the inhibition of protein synthesis during retrieval, can be rescued by previous exploration to a novel OF that provides the PRPs. Moreover, other amnestic evens such as the inhibition of PKA or the exploration to a novel arena after retrieval cannot be rescued by providing PRPs through OF exploration.

As a whole, our result show for the first time that BT underlies memory reconsolidation.

Cognition, Behavior, and Memory

P145.-Dorsal striatum D1-expressing neurons are involved in sensorimotor gating on prepulse inhibition test

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Startle is an involuntary, widespread, musculature contraction when an animal is surprised by an intense sensory stimulus. In general, the stimuli are noise bursts and the startle response is called Acoustic Startle Response (ASR). This reflex can be modulated, i.e. when a weak stimulus is presented previous the starling one, decreasing the reflex amplitude Prepulse Inhibition (PPI). The PPI sensorimotor gating is impaired in many psychiatric disorders such as Obsessive Compulsive Disorder, Schizophrenia, Parkinson and others. Besides PPI disability these disorders also share common neural substrates dysfunctions, particularly in forebrain structures innervated by dopaminergic nuclei. In this study, the contribution of Dorsal Striatum (DS) and its dopaminergic transmission on PPI sensorimotor gating were investigated. In experiment 1, temporary inactivation of DS with Muscimol (0.5μg/0.5μl), a GABAA receptor agonist, did not change PPI neither ASR. In experiment 2, the infusion of D1like receptor antagonist (SCH23390; 0.04 and 0.4μg/0.4μl) decreased PPI response (0.4μg/0.4μl) but did not affect ASR or locomotor activity. On the other hand, in experiment 3, the D2like receptor antagonist microinjection (Sulpiride; 100 and 250ng/1μl) did not disturb PPI neither ASR but decreased the locomotor activity during the open field test (100ng/1μl). These results point to an important DS role, probably mediated by direct basal ganglia pathway, on modulation the sensorimotor gating.

Cognition, Behavior, and Memory

P146.-Electrophysiological Correlates of Stimulus Equivalence Classes: A Semantic Priming Study

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The paradigm of Stimulus Equivalence Classes (SEE) has proven to be relevant for the investigation of semantic processes of learning (Wulfert & Hayes, 1988). Barnes-Holmes et al. (2005) showed that the presentation of stimuli pairs related and unrelated by equivalence generated a potential similar to the N400. The objective of this research was to evaluate the differences in the physiological measures and in the behavioral responses in the different conditions (Trained, Symmetry and Equivalence) and type of relation (Related and Unrelated). 52 subjects received training in SEE and were then tested in a semantic priming task and a matching to sample task with electroencephalographic register. During this phasem reaction times were compared between pairs of stimuli (prime-target) related (stimuli belonging to the same class) and unrelated (stimuli belonging to different classes). A repeated measures ANOVA with permutations was made with reaction times as dependent variable comparing Condition (Trained, Symmetry and Equivalence) and Relation (Related and Unrelated). A principal effect of Condition and a principal effect of Relation were found. Regarding the electroencephalographic register, a negative potential was observed between 200 and 400 milliseconds and a positive potential was observed between 400 and 600 milliseconds after the target stimulus.

Cognition, Behavior, and Memory

P147.-Winner or loser? Reduced memory ability after winning a fight in the crab Neohelice granulata”

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It has been demonstrated in both animal models and humans that social stressful events can change the properties of memory processes. In this sense, a relationship between social rank and memory has been proposed in several studies. Thus, the aim of this study was to analyze the role of an agonistic experience, as the event which induced social stress, on the contextual Pavlovian conditioning (CPC) in the crab. Each experiment consisted of an agonistic phase and a memory one. During the former, matched pairs of male crabs were staged in a 10-min encounter and the dominant or subordinate condition of each member of the dyad was determined. Immediately after the encounter crabs were trained to acquire CPC and tested 24h later. Results showed that the agonistic encounter can modulate memory according to the dominance condition; in such a way that memory retention of subordinates results higher than that of dominants. Further, this difference in memory retention is maintained even when increasing the intensity of the US. Importantly, a comparison between the acquisition phase of dominants and subordinates did not reveal any divergences. Thus, we propose that the agonistic experience differently modulates the crab’s memory consolidation. Future experiments will allow us to address the mechanisms of this modulation, and determine whether this result can be expanded to other memory phases, such as the reconsolidation, and to other learning tasks.

Cognition, Behavior, and Memory

P148.-Long-term overexpression of the neurodegenerative disease-related protein TDP-43 leads to time-dependent behavioral decline in transgenic mice

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Frontotemporal Dementia (FTD) and amyotrophic lateral sclerosis (ALS) are two neurodegenerative diseases associated to mislocalization and aggregation of TAR DNA-binding protein 43 (TDP-43). To investigate the time course of this proteinopathy, we used as a model transgenic mice conditionally overexpressing human wild-type TDP 43 protein (hTDP-43-WT). We previously characterized and evaluated the behavioral phenotype (including motor, cognitive and social performance) 1-month after transgene induction, when mice display an impairment in cognitive and social domains in the absence of motor abnormalities. In the present study we analyzed the behavior of mice after long-term (12 months) transgene induction, performing a battery of tests to determine if the phenotypes worsen as the animals age. Our results reveal a decreased performance on the rotarod test and in the hanging wire test, indicating a motor phenotype that was absent in younger mice. In addition, long-term hTDP-43-WT expression led to hiperlocomotion in the open field test. Regarding the cognitive and social phenotypes established early on, they are still present in older mice, including altered social interaction and Y-maze tests. Our findings demonstrate a time-dependent emergence of a motor phenotype in older hTDP-43-WT transgenic mice, recapitulating aspects of clinical FTD presentations with motor involvement in human patients, and providing a complementary model for studying TDP-43 proteinopathies.

Cognition, Behavior, and Memory

P149.-Signatures of awareness in the restingstate brain activity dynamics. An EEG/fMRI study of similarities and differences between wakefulness, anesthesia and disorders of consciousness

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At rest, the brain is traversed by spontaneous functional connectivity patterns. Two hypotheses have been proposed for their origins: they reflect a continuous stream of ongoing cognitive processes or a random fluctuation shaped by a fixed anatomical connectivity matrix. I will show that both sources contribute to the shaping of resting-state networks, yet the level of consciousness modulates the respective contributions.

I will show that wakefulness is characterized by a sequential exploration of a repertoire of functional configurations. These dynamical states are often dissimilar to the underlying anatomical connectivity structure, and comprising positive and negative correlations among brain regions. Conversely, under anesthesia functional connectivity patterns inherit the structure of anatomical connectivity, exhibit fewer small-world properties, and lack negative correlations. This is also the case for disorders of consciousness patients that show fluctuations between low and high efficiency information integration states but only the probability of the high efficiency states parametrically increases with the clinical level of consciousness.

These results reconcile theories of consciousness with observations of long-range correlation in the unconscious brain and show that a rich functional dynamics might constitute a signature of consciousness, with potential clinical implications for the detection of awareness in anesthesia and brain-lesioned patients.

Cognition, Behavior, and Memory

P150.-Visual distance perception is calibrated by auditory spatial information related to the size of the room

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In this work we studied if the Visual Distance Perception (VDP) is modulated by information related to the perceived size of a room obtained through the auditory modality (higher reverberation times are consistently associated with larger rooms). Our hypothesis is that in environments with few visual cues (e.g. a dark room), the PVD is calibrated by the auditory spatial information related to the size of the room. To this end, we conducted PVD experiments in two dark rooms with extremely different reverberation times (an anechoic chamber and a reverberation chamber) in order to generate the sensation in test subjects of being in two rooms with different actual sizes. Interestingly, the results show that only the participants who reported having musical training perceive the reverberation chamber as larger than the anechoic. In addition, only the participants of this last described subgroup perceived visual targets at significantly greater distances in the reverberant than in the anechoic room. This result supports the hypothesis reported in previous studies that the context influences the PVD (first related to the size of the room), and also shows that the visual perception of space can be modulated by acoustical cues, particularly through reverberation time.

Cognition, Behavior, and Memory

P151.-Dorsal medial prefrontal cortex contributes to conditioned taste aversion memory consolidation and retrieval

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The medial prefrontal cortex (mPFC) is known for its role in decision making and memory processing, including the participation in the formation of extinction memories. However, little is known regarding its contribution to aversive memory consolidation. Here we demonstrate that neural activity and protein synthesis are required in the dorsal mPFC for memory formation of a conditioned taste aversion (CTA) task and that this region is involved in the retrieval of recent and remote long-term CTA memory. In addition, both NMDA receptor and CamKII activity in dorsal mPFC are needed for CTA memory consolidation, highlighting the complexity of mPFC functions.

Cognition, Behavior, and Memory

P152.-Involvement of δCaMKII in persistent forms of memory

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Calcium/calmodulin-dependent protein kinase II (CaMKII) is an abundant synaptic signaling molecule that is essential for both memory formation and synaptic potentiation. In mammals, CaMKII exists in multiple isoforms that are the product of four closely related genes: α, β, γ, and δ. Little information is known about the role of δCaMKII in memory processes. In a previous study, we showed that in the mouse hippocampus camk2d gene was specifically expressed during the formation of persistent forms of novel object recognition (NOR) memory and that its gene promoter was acetylated during this process on a NF-κB dependent manner. Here, we will present new results in which we show that δCaMKII mRNA expression is increased at different time-points during NOR memory maintenance returning to control levels 20 days after training, once memory retention decays. The increment in its mRNA expression is accompanied by changes in nucleosome positioning on its promoter. Moreover, δCaMKII knock-down after training impairs object recognition memory when assessed one week later.
Altogether, our results support a key role for δCaMKII in persistent forms of memory and suggest that δCaMKII may have a sustained expression throughout the “lifetime” of this kind of memory. Furthermore, this is the first work that provides insight information about nucleosome remodeling during memory formation and maintenance.

Cognition, Behavior, and Memory

P153.-Therapeutic potential of human mesenchymal stem cells in a sporadic Alzheimer rat model

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Alzheimer disease (AD), the most common form of dementia, is characterized by the degeneration of neurons and reduced brain glucose metabolism, as well as by the progressive decline of cognitive function. Our objective is to develop therapeutic strategies that allow preventing and/or overcoming the degenerative changes in the brain with experimental AD. In this context, cell therapy emerges as a promising therapeutic approach. First, we set up a model of sporadic AD with the intracerebroventricular injection of streptozotocin (STZ-icv) in young male rats. Second, we explored the therapeutic effect of mesenchymal stem cells (MSC) in this animal model. Three experimental groups were used: (N=6/group): intact, STZ and STZ+MSC. STZ and STZ+MSC groups received 3 mg/kg STZ-icv and, 24 hours after, STZ+MSC group received 1x105 human bone marrow derived-MSCs. We assessed hippocampus-dependent learning and spatial memory by the Barnes maze test and recognition memory by Novel Object Recognition test (NOR). After 24 days, we proceeded to the euthanasia. STZ group showed less preference for goal region in the Barnes test ($P=0.054$ vs. intact), and in NOR test they explored 43% familiar vs. 57% new object (not significant). MSC treated animals have shown an improvement in their spatial memory ($P<0.01$ vs. STZ), and recognition memory (36% familiar vs. 64% new object, $P<0.05$). In this first study we concluded that cell therapy ameliorates cognitive deficits in this AD model.

Computational Neuroscience

P154.-Mathematical modeling of reactive gliosis using in silico Bayesian method

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Reactive astrogliosis (RA) is a general, graded, glial response to brain injury. Reactive astrocytes are characterized by an increase of the cellular volume and secretion of pro-inflammatory molecules. Until now it is unknown which are the signals that initiate/propagate RA, but it is proposed that diffusion of damage associated molecules pattern (DAMP) from the necrotic core; calcium waves propagated by glial gap junctions or dramatic changes in extracellular milieu (i.e. ATP levels) are likely to be involved. We here evaluated two main paradigms to understand the progression of RA using mathematical modeling. Model I is a simple mechanism that contemplates the diffusion of DAMP from the necrotic core as the main responsible for the RA. Model II is more complex mechanism involves a subsequent signal carried out by soluble mediators secreted by the proximal astrocytes acting as amplifiers of the signal. Starting with GFAP-stained brain sections of animals subjected to cortical devascularization analyzed by Sholl analysis and confocal 3D reconstruction, we studied the morphology of the cortical astrocytes at 3 and 7 days post ischemic injury. Then, we applied a Bayesian Computational Modeling approach by building a parametric mathematical model using partial differential equation for diffusion of DAMP and soluble mediators coupled with a Markovian Model for the signal-triggered RA. Finally, we tested both models and found strong evidence for model II with soluble mediators.

Computational Neuroscience

P155.-Mean-field modeling and interpretation of electrical impedance spectra of neural tissues

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Microelectrode arrays are important tools in the study of the electrical behavior of excitable cells. These devices detect passive and active electrical responses from cells to external stimuli and thus provide quantitative information about relevant biophysical parameters, such as action potential occurrence times or the degree of electrical coupling between cells and microelectrodes. In this work we present a model to infer the electrical properties of excitable tissues from electrical impedance spectroscopy (EIS) measurements. The model, which was developed by assuming that the tissue under study is an element with mean electrical characteristics, was particularly designed to take into account those aspects that arise when the electrode size is reduced to cellular and sub-cellular dimensions. By comparing model calculation with published experimental data (EIS measurements obtained from metallic microelectrodes), several biophysical parameters were determined, such as the mean resistivity of the cell layer, the capacitance of cell membranes, and the presence of tissue inhomogeneities, among others. The close correspondence between experimental data and model predictions provides significant evidence about the validity of our modeling strategy. As a result, the developed model allows to study the physical principles underlying a number of biomedical applications, including pharmaceutical screening, toxin detection and implantable neuroprosthetics.

Computational Neuroscience

P156.-Cooling HVC explained by a circular model of birdsong production

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A mathematical model is studied to describe the variation of pressure patterns during song production in domestic canaries (Serinus canaria), as the telencephalic nucleus HVC is cooled. By working with average activity of different neural populations, in a frame of a circular model[1], we propose that motor gestures emerge from the combined activity of two different timescales. The input activity arriving at the nucleus in charge of the expiratory gestures are composed of (1) sparse activity at significant motor instances of an initiate nucleus, and (2) a synchronized, but delayed, activity in telencephalic center (HVC). We manipulate thermally this nucleus. Two main features observed in the experiments[2] (the stretching and breaking of certain types syllables) can be explained by our model.

[1] Alonso R. G., Trevisan M. A., Amador A., Goller F. and Mindlin G. B. - A circular model for song motor control in Serinus canaria. Frontiers in Computational Neurocience 2015. Vol 9 – 41.

[2] Goldin, M. A. and Mindlin, G. B. - Evidence and control of bifurcations in a respiratory system. 2013. Chaos 23:043138.

Computational Neuroscience

P157.-Characterization of delta to theta transitions in the Hippocampus and Lateral Habenula following mechanic or optogenetical stimulation in anesthetized rats

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Based on experimental data, the correlation between the activation of the Diagonal Brocca Band (DBB) and the transition from delta to theta state is sought. The experiments have considered two different stimulations, exogenous and endogenous (pulse wave), namely an optogenetic activation of DBB and a nociceptive stimulus given by a tail pinch. The light stimulation is a pulse wave parameterized by its frequency, pulse width and duration. In addition, the local field potential (LFP) of the Lateral Habenula (LHb) and the hippocampus (HPC) have been recorded in order to determine the switch between states. The experimental data has been classified to obtain the following information: 1) Relation between the frequency content of the LHb and/or the HPC and the anesthesia effect on the specimen (delta power); 2) For the same input, how delta power influences the transitions from delta to theta and theta to delta, e.g. the dwell time in theta, excitation length to achieve a switch, etc; 3) Relation between the input stimulation and the switch from delta to theta.

The method used here is based on a Fourier analysis and signal processing techniques applied to the experimental data. The final objective is to establish a correlation between the input signal characteristics (frequency, pulse width, duration) and delta power with the transitions from delta to theta in both the LHb and HPC.

Computational Neuroscience

P158.-Perturbations in a mathematical model for finger tapping

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Finger tapping is one of the most simple tasks to study sensorimotor synchronization. In this task a subject is instructed to tap in synchrony with a periodic sequence of brief tones, and the time difference (called asynchrony) between each response and its corresponding stimulus is recorded. Despite its simplicity, this task helps to unveil interesting features of the underlying neural system and the error correction mechanism responsible for synchronization. Perturbation experiments are usually performed to probe the subject response, for example in the form of "step change" when an unexpected change in the interstimulus interval occurs. The asynchrony is the usual observable in such experiments and it is chosen as the principal variable in many mathematical models (maps) which attempt to describe the phenomenon.
In this work we show that although asynchrony is perfectly described in operative terms, it is not well defined as a map variable when perturbations of the stimulus period are considered. We introduce an alternative variable and a mathematical model that takes into account the perturbation, and show that our proposal is relevant to understand the interpersonal synchronization and the synchronization to a non-periodic stimulus.

Computational Neuroscience

P159.-Functional specificity of rat vibrissal primary afferents

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In this study we propose to quantify the peripheral vibrissal system specificity through its neuronal responses. Receiver operating characteristics (ROC) curve analyses were used, which required the implementation of a binary classifier (artificial neural network) trained to identify the applied stimulus. The training phase consisted of the observation of a predetermined amount of vibrissal sweeps belonging to two experimental situations. Our results suggest that specificity of peripheral vibrissal system, quantified through neuronal responses, varies according to the perceived stimulus, and that it can be quantified through an ROC curve analysis. Furthermore we found that such specificity can be determined by an binary classifier after observing at most 5 vibrissal sweeps.

Computational Neuroscience

P160.-Study of the basal ganglia network dynamics with applications to closed-loop deep brain stimulation paradigms

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The application of electrical pulses on specific neuronal groups using implantable devices is an established therapy (e.g. DBS: Deep Brain Stimulation) for the treatment of advanced stages of motor disorders, such as Parkinson disease. Currently, the DBS technique is implemented through open-loop schemes which are mainly based on heuristic approaches and do not allow on-line optimization of the monomorphic stimulation pattern. Closed-loop DBS schemes extract relevant features from the electrical activity of the network in order to determine the optimal parameters for the adaptive stimulation signal. Thus, the closed-loop DBS paradigm is aimed to overcome the limitations inherent to the presently used open-loop DBS methods.
This work focuses on the study of the relationship between parkinsonian states and relevant features observed in the electrical signals from the basal ganglia network (BG). A rate model of the direct and hyperdirect loops of the BG was implemented including the level of dopaminergic activity. Besides, the one-dimensional architecture of the model allows the study of relevant phenomena related to the spatial scales involved in the DBS technique (e.g. effect of the electrodes for sensing and stimulating the network). The role of the direct and hyperdirect loops on the selection of the motor plan is analyzed using analytical and numerical tools. Moreover, we describe a novel closed-loop DBS scheme based on artificial neural networks and supervised learning.

Motor Systems

P161.-Neural coding of timbre in birdsong

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Given two sounds of same pitch, loudness and duration, timbre is the acoustical property responsible for sound's identity. Timbre is a multidimensional attribute that is most elusive to describe. In humans, some of these dimensions have been identified and explored in psychoacoustics experiments, but an objective way to measure it is yet to be determined.
In this work, we studied the contribution of attack time to the sound identity. Attack time is a well-established dimension of timbre: it is the time it takes for the sound envelope to reach its maximum.
Zebra finches (Taeniopygia guttata) present a remarkable opportunity to study timbre in complex vocalizations, as they present a wide syllable repertoire and they have a large number of syllables with a rapid attack time and a slower decaying envelope. In addition, previous experimental evidence showed that telencephalic neurons in HVC respond in a highly selective fashion to auditory presentations of the Bird's Own Song (BOS) while not responding to the reverse song (REV). Therefore, we used HVC neural activity as a measure of BOS recognition. We generated a modified BOS in which the sound envelope of each syllable was reversed (MOD). This effectively switches the attack and decay times of each syllable while maintaining the rest of the acoustical properties intact. We have found that these changes in the attack time lead to a decrease in the neural response of HVC units, unveiling a neural representation of timbre in HVC.

Motor Systems

P162.-Midbrain circuits for defensive behavior

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The periaqueductal grey (PAG) is a central part of the circuitry that elicits defensive behaviors in aversive situations. Previous research has shown that the PAG is organized into functionally distinct columns, with the dorsolateral and lateral (dl/lPAG) columns producing flight and the ventrolateral column (vlPAG) being responsible for freezing. However, little is known about functional roles of neuronal subpopulations within these regions in the expression of active and passive defensive behaviors. We used optogenetics, neuronal recordings, and tracing techniques to characterize circuits important for innate and fear-evoked freezing and flight. Active defensive behaviors were evoked by optical activation of dl/lPAG glutamatergic cells, while freezing was observed after activation of vlPAG glutamatergic cells or inhibition of GABAergic cells in the PAG. Freezing behavior correlated with both enhanced as well as reduced single-unit activity in the PAG. Slice recordings and rabies tracing revealed preferential connectivity of GABAergic CEA inputs onto GABAergic vlPAG cells, and local GABAergic input onto glutamatergic vlPAG cells. Our data suggests that inhibitory input from the amygdala onto GABAergic cells of the PAG produces freezing by disinhibition of glutamatergic vlPAG outputs. Specific optogenetic activation of glutamatergic vlPAG output to the magnocellular nucleus of the medulla resulted in freezing behavior. In summary, we here present novel insights into the circuit mechanisms underlying defensive behaviors.

Motor Systems

P163.-7-Nitroindazole and nitrergic signaling after dorsolateral striatal lesion by 6-OHDA in rats

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Objective: In the present study, we investigated nitrergic alterations in caudate-putamen, after a mild right dorsolateral striatal (DLS) 6-OHDA lesion (4mg/ml/2x2µl) in adult male Wistar rats, and subsequent development of motor changes. Methods and Results: Sham or lesion groups was divided in vehicle and 7-Nitroindazole (7NI-15mg/kg/ip/5 days). Locomotor activity was evaluated by computerized actometers, and rotational, stereotyped behavior with amphetamine (5 mg/kg/sc), but no difference between 6-OHDA/7NI and 6-OHDA/vehicle ($P>0.05$). Amphetamine induced ipsilateral turns, significant difference was found between 6-OHDA/Vehicle (19.48±2.84) and 6-OHDA/7NI (10.02±0.4)[$F(3,19)=20.15, P<.001$]. Histological analysis showed a loose of TH+ fibers in the DLS in 6-OHDA/vehicle, it being that the treatment with 7-NI moderately did attenuate the loss of TH+fibers [$F(3,19)=8.24; P<0.001$]. Increased dorsolateral nNOS expression was observed in 6-OHDA/vehicle [$F(3,19)=6.19; P=0.003$], also showed an increased NADPH-diaphorase staining in ventrolateral striatum (VLS)[$F(3,19)=3.49; P=0.032$], but no in DLS ($P>0.05$). Conclusions: Important result was the stereotyped behavior in 6-OHDA/vehicle correlates with increased NADPH-diaphorase activity in VLS. This effect can be reduced by 7-NI treatment. Furthermore, VLS is accepted to be an equivalent of the putamen in primates and humans, and can be correlates with early clinical stages of Parkinson disease.

Motor Systems

P164.-Spatial perturbations in a finger-tapping task

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Finger tapping is a paradigmatic task to study sensorimotor synchronization. In this task the subject must tap with his finger in synchrony with a periodic sequence of brief tones, as in keeping pace with music. The temporal differences (asynchronies) between the occurrence time of every stimulus and the occurrence time of the corresponding responses are recorded. A usual experimental manipulation is the shortening or lengthening of the period of the sequence (tempo change). In this work we make a novel perturbation: we change the spatial conditions of the task by changing the force field the finger is subjected to. Our aim is to characterize the motor adaptation behavior and to decide whether a purely temporal (spatial) perturbation has spatial (temporal) effects. Recent models of motor timing and motor production of spatiotemporal patterns propose that temporal and spatial aspects of a task in this time range (hundreds of milliseconds) are intrinsically coupled. Our long term aim is to elucidate whether temporal and spatial components of the internal model of motor control are entangled or can be adjusted independently.

Motor Systems

P165.-Role of ASIC channels after excitotoxic damage induced by kainic acid in a model of spinal injury.

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Excitotoxicity, a major contributor to spinal injury, has strong impact on neuronal loss and locomotor function. Acid-sensing ion channels (ASICs) exert an important role for pH sensor in pathologies associated with acidosis and neurodegeneration. We have shown that kainate applied for 1 h largely destroys neurons with widespread effect on various spinal regions (Mazzone et al, 2010). Our present objective was to enquire if the glutamate analogue kainate could modulate the expression of ASICs and locomotor function. Mouse organotypic spinal slices (22 DIV) were treated with kainate (0.01 or 0.1 mM) for 1 h, and then washed for 24 h prior to analysis. The gene analysis by RT-PCR demonstrated that kainate (0.01 mM) increased the expression of ASIC1a, ASIC1b, ASIC2 and ASIC3. A more potent excitotoxic stimulation reduced mRNA levels of ASIC1a and ASIC2. Indeed, 4',6-diamidino-2-phenylindole (DAPI, 3.5 μM), a powerful ASICs inhibitor, decreased mRNA expression levels. Immunohistochemistry indicated a much larger loss of neurons using kainate followed by delayed DAPI and amiloride (100 μM) treatment. Electrophysiological recording from isolated spinal cords, showed that fictive locomotion, slowed down by kainate, was fully abolished by continuous application of DAPI and amiloride. Our data indicate that kainate-mediated excitotoxicity in spinal cord preparations was associated with an early rise in ASIC expression, and was important for the locomotor network function.

Motor Systems

P166.-Effect of the interstimulus interval in the kinematics of the finger movement during a finger tapping task

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When humans synchronize to an external periodic stimulus the brain has to interpret afferent information from sensory organs (e.g. eyes, ears) in order to execute a motor task that is coordinated with the stimulus. In this work we concentrate in the motor side of the problem and explore the kinematics of the finger movement during a finger tapping task under a sensorimotor synchronization paradigm. We want to investigate the effect of the tempo of the sequence on the trajectory of the finger, and for that we performed a finger tapping experiment where the task consisted of tapping on a force sensor with the index finger, synchronizing this taps with a periodic isochronous auditory stimulus (short tones of 50 ms duration and a pitch of 440 Hz). The kinematics were studied by means of a digital accelerometer attached to the finger. We find that when the finger raises, the magnitude of the acceleration and the rising time depends on the tempo. For slow tempos the time trace of the finger's acceleration remains mostly invariant and independent of the stimulus period, but as the tempo increases and gets closer to the synchronization rate limit, the finger raises earlier and with a higher acceleration.

Motor Systems

P167.-Intraoperative cortical recordings during speaking tasks

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Subdural electrocorticographic (ECoG) recordings in patients performing a speaking task are associated with event-related broadband gamma activity in a high frequency range (HG) (>70 Hz) [1]. Some works suggest the utility of this HG activity for mapping language cortex, by comparing its neuroanatomical distribution with the one obtained with electrical cortical stimulation (ECS), which remains the standard for predicting functional impairment after surgery for epilepsy, tumour or vascular malformations.[2] We hypothesize that ECoG recordings could be used to complement ECS during surgical intervention to map language cortex by an on-line analysis of HG activity during intra-operative speaking tasks. We asked 4 patients implanted with subdural multi-electrode superficial arrays to do an overt single-phoneme repetition task study. One of them was an epileptic patient that completed the study over many sessions during monitoring, and the others had brain tumours and completed a single, short session intraoperatively. Here we show our preliminar results and compare the performance of the different cortical recordings.

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Motor Systems

P168.-Surface electromyographic recordings applied in small animals. An approach for movement disorders quantification.

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Surface electromyography (EMG) and kinematic measurements enable the objective quantification of neuromuscular function and movement in patients suffering Parkinson's disease or another movement disorders. However, neuromuscular activity has never been characterized in animal models by using this useful technique. Instead of that, subcutaneous EMG recordings have commonly been used with the subsequent pathological and time limitation problems. In this work we describe the use of subcutaneous and surface electrodes to obtain recordings either from healthy rats as from those that were treated with 6-OHDA. Experimental studies in neurodegenerative disorders require the implementation of different behavioral tests to verify and quantify the illness progression. Our results demonstrate that the use of surface electrodes could be a feasible technique to characterize neuromuscular activity because of its reliability and its easy implementation.

Motor Systems

P169.-Sensory and motor aspects of temporal processing during finger tapping tasks

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We propose a sensorimotor synchronization experiment (finger tapping) to decouple in different degrees the temporal perception and motor aspects of the task. We ask the subjects to tap in synchrony with a periodic sequence of auditory stimulus (tones of 50 ms duration, initial period 500 ms) that makes an unexpected tempo change (perturbation sizes: -50 ms, 0 ms and +50 ms). In condition 1, the subject must synchronize from the beginning of the sequence trying to keep synchrony if a tempo change appears (normal tapping). In condition 2, the subject is instructed to start tapping only if —and immediately after— perceiving a tempo change. In condition 3, the subject starts tapping when a light turns on (the light turns on simultaneously with the period modification). Condition 4 is the same as condition 1, but the subject must keep his finger in permanent contact with the sensor (isometric tapping). Preliminary data show asymmetries between the results obtained in conditions 1 and 3, presumably due to differential features in sensory and motor timing.

Neural Circuit Physiology

P170.-Altered maturation through adolescence leads to decreased hippocampal-prefrontal cortex functional connectivity in a mouse model of schizophrenia

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The glutamatergic hypofunction theory postulates that a dysfunction in NMDA receptors (NMDAr) in cortical interneurons is central in the pathophysiology of schizophrenia. In our previous work, restricted ablation of NMDAr in corticolimbic parvalbumin interneurons during early postnatal development resulted in schizophrenia-like phenotypes in adulthood. To elucidate the pathophysiological changes leading to this phenotype, we placed tetrodes in the mPFC in anesthetized control and mutant, juvenile and adult mice. We found a significant increase in spontaneous firing rate and altered entrainment to cortical local and distant rhythms in mutant juvenile and adult mice. Since juvenile mutant mice lack NMDAr but show no schizophrenia-like phenotype, the above mentioned changes could not explain the behavioral abnormalities of adults. Normal synaptic pruning of local and distant inputs to mPFC occurs during adolescence. We analyzed functional connectivity of the vHP-mPFC pathway before and after adolescence. Mutant adult mice present diminished amplitude of the evoked response in mPFC. We also measured the status of circuit plasticity and found that adult but not juvenile mutant mice are more susceptible to undergo LTD. We propose that early ablation of NMDAr in interneurons leads to an overexcited/uncoordinated cortical circuit that propitiates LTD during adolescence. This results in a decreased functional connectivity in adults that may underlie the schizophrenia-like phenotype.

Neural Circuit Physiology

P171.-A local feedback circuit mediates integration of adult-born dentate granule cells promoted by experience

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Adult-born dentate granule cells (GCs) develop and integrate into the local networks in a process that lasts several weeks. It has been shown that network activity modulates maturation of adult-born neurons. Here we show that developing GCs display a critical period previous to the onset of glutamatergic synaptogenesis whereby they are prone to modulation by activity. A brief (48 h) exposure to enriched environment within this critical period is sufficient to accelerate maturation and functional integration of new GCs. Accelerated integration was also found upon brief direct activation of new GCs during the critical period using the synthetic G-coupled receptor hM3Dq. In addition, mature GCs were activated by means of the hM3Dq receptor to test the influence of local circuits on developing GCs. Indeed, activation of mature GCs by the synthetic ligand accelerated GC integration. In agreement with recent works we propose that parvalbumin-expressing GABA interneurons (PV) are responsible of controlling neuronal maturation. Interestingly, ex-vivo activation of PV interneurons induced a postsynaptic response in developing neurons. Our results suggest that during cognitive tasks, GCs activate PV interneurons, which, in turn, promote functional recruitment of new cohorts of developing GCs.

Neural Circuit Physiology

P172.-Altered Corticostriatal Connectivity and Exploration-Exploitation Imbalance Emerge as Intermediate Phenotypes for a Neonatal Dopamine Dysfunction

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Neonatal dopamine neuron (DAN) lesion in rodents produces hyperactivity and learning deficits and has been proposed as an attention deficit hyperactivity disorder model. However, the core cognitive and physiological intermediate phenotypes underlying this rodent syndrome remain unknown. Here we show that early DAN lesions cause deficits in exploitation of shelter, social and nutritional resources, and an imbalanced exploratory behavior, where local exploration is exacerbated and search behaviors involving sequences of goal directed actions are degraded. In vivo electrophysiological recordings and morphological reconstructions revealed an attenuation of corticostriatal (CS) functional connectivity affecting medial prefrontal inputs more markedly than cingulate and motor inputs, that is accompanied by a contraction of the dendritic arbor of striatal projection neurons. Importantly, the behavioral deficits and the prefrontostriatal disconnection worsen after adolescence in DAN lesioned mice. Thus, DANs are essential during postnatal development for the functional and structural maturation of CS connections. From a bottom-up viewpoint, our findings suggest that neuropsychiatric conditions presumably linked to developmental alterations of the dopaminergic system should be evaluated for deficits in foraging and structural disorganization of the CS system.

Neural Circuit Physiology

P173.-Participation of glia in the post-natal refinement of the olfactory bulb sensory map

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The olfactory system of rodents is host to a special type of glia, the olfactory ensheathing cells (OECs), which evidence a high degree of gap junction connectivity. In addition, in the olfactory bulb post-natal refinement of the topographic innervation from olfactory sensory neurons occurs, yet no studies exist in regards to post-natal changes in glial network architecture. We propose that a simultaneous refinement of glial connectivity occurs, and like the neuronal counterpart, it depends on sensory experience. Moreover, OECs are attributed to providing the appropriate environment for olfactory sensory neuron turnover and regeneration, partly through expression of neurotrophins. The neurotrophin BDNF allows the expression of axonal competence for the establishment of stable connections in the olfactory bulb. Therefore, our hypothesis is that BDNF released from olfactory ensheathing cells participates in the post-natal maturation of the sensory map. Our approach is the generation of an in vivo mouse model that applies the Cre-lox technology to delete coding regions for BDNF in OECs with time control. We use morphological, histochemical, electrophysiological and genetic techniques to evaluate whether the maturation of olfactory circuits and/or glial networks are deficient in these mice. We show preliminary expression profiles of connexins 30 and 43, as an early approach to glial connectivity, and raise the question whether there is a connexin switch during refinement.

Neural Circuit Physiology

P174.-Setting-up in-vivo optogenetic stimulation of VTA dopaminergic neurons

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Mesolimbic and mesostriatal dopaminergic transmission signals the occurrence of an unexpected reward and plays a role in the association between rewards and predictive cues. The ventral tegmental area (VTA) is composed mainly of dopaminergic neurons, but GABAergic and glutamatergic neurons have been described too. Added to its neurotransmitter diversity, axonal terminals of VTA neurons have been found in different brain areas as the nucleus accumbens, dorsal striatum, olfactory tubercles, prefrontal cortex, amygdala and hippocampus. In support to this anatomic diversity, deregulation of dopaminergic transmission is associated not only to reward seeking pathologies, but also to anxiety states and cognitive disorders. In order to study the role of specific VTA dopaminergic subpopulations we have tuned up optogenetic stimulation of dopaminergic cells in freely moving mice. Rewarding effect of the in-vivo stimulation was assessed by Real Time Place Preference (RTPP) experiments. Posterior histological analysis showed high levels of channelrhodopsin expression in dopaminergic neurons of the VTA. Neuronal activation was verified by activation of the early gene c-fos revealed by immunostaining performed on coronal brain slices. Ongoing studies are under development to evaluate the effect of specific dopaminergic projections on behavior by optogenetic stimulation of neuronal axons.

Neural Circuit Physiology

P175.-Neuronal regulation of stress response in C. elegans: Role of the neurotransmitter tyramine

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In nature, animals are frequently exposed to physiological and environmental challenges. The individual cellular response to these unfavorable conditions should be finely coordinated in multicellular organisms. The neural control of the systemic stress response was first evidenced in the free-living nematode C.elegans. However, the identity of the systemic neural signal that integrates stress perception with the response in non-neuronal tissues remains unknown.
Our analysis of the C.elegans neuronal wiring diagram reveals that the circuits activated upon exposure to stressful situations converge in the only tyraminergic neuron, RIM. Tyramine is the invertebrate counterpart for adrenaline. Here we found that tyramine-deficient animals are resistant to thermal stress, starvation and pathogen infection. Moreover, these mutant strains exhibit molecular hallmarks of stressed worms, such as autophagy and lypolisis induction, even when they are grown under favorable conditions. Our results suggest that inhibition of the basal release of tyramine is a neuroendocrine signal required for a coordinated triggering of the stress response in C. elegans. This study contributes to a better understanding of the neurohormonal signaling that controls the systemic processes in multicellular organisms.

Neural Circuit Physiology

P176.-Dynamics of excitatory and inhibitory circuits in piriform cortex and its modulation

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The piriform cortex (PC) serves as point of anatomical convergence for olfactory bulb output neurons, conveying information about distinct odorant features extracted from the periphery. The spiking of pyramidal neurons can be driven directly by afferent inputs from the lateral olfactory tract (LOT), by intrinsic associational fiber inputs (ASSN), or the combination of both. Activity arriving from LOT or ASSN not only produces excitation but also recruits inhibitory circuits. The contribution of each of these pathways on the population activity is not known in detail. We test if the response of Layer II neurons depends on the differential contribution of ASSN and LOT inputs through differential recruitment of inhibitory circuits, in PC slices. In addition, we use optogenetics to stimulate afferents coming from other brain regions as the basolateral amygdale (BLA) and study the modulation of the excitation/inhibition dynamics in PC neurons. Preliminary results show that the relationship between E/I under LOT stimulation is quite linear for different stimulation intensities but with a slope on which outweighs inhibition. Something similar happens with ASSN. Stimulation of BLA primarily produce inhibition in PC. Interestingly, simultaneous stimulation LOT + BLA generates similar excitatory currents as LOT stimulation only, but inhibitory currents summate in an arithmetic manner, indicating that the population of inhibitory interneurons that recruits LOT and BLA are different.

Neural Circuit Physiology

P177.-Amygdala in Social Interaction

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Social behaviors encompass a complex set of conducts, which are impaired in several psychiatric disorders such as depression, autism spectrum disorder (ASD), schizophrenia and social anxiety disorder. Many efforts have been dedicated to understand social behavior, however the underlying neuronal circuits are poorly understood. A large body of research indicates that the amygdala is a key brain structure for emotional processing and memory, in particular fear learning. Although the amygdala has so far been mostly investigated in the context of fear responses, there are several studies implicating this brain area as an important structure involved in patients with ASD. Recently, some studies revealed a direct role of the amygdala during social interaction. Our aim is to dissect the contribution of defined amygdala cell types and circuits to social behavior. To this end, we employ a combination of cell-type specific targeting, optogenetics and single unit recordings techniques in freely behaving mice to determine the activity and causal involvement of defined amygdala circuits and projections during social interaction.

Neural Circuit Physiology

P178.-Nicotinic α4 Receptor-Mediated Cholinergic Influences on Food Intake and Activity Patterns in Hypothalamic Circuits

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Despite much data supporting a role for nicotine-mediated suppression of appetite, little is known about the mechanism of action of the endogenous neurotransmitter, acetylcholine (ACh), on this effect. To shed light on the hypothalamic circuits governing ACh regulation of appetite, we investigated the influence of (nAChRs) expressing the α4 subunit in rats. Immunocytochemical analysis revealed the expression of nAChR α4 subunit at different populations of hypothalamic neurons (orexin/hypocretin (HO), melanin concentrating hormone (MCH), oxytocin, and tyrosine hydroxylase (TH)-containing neurons). We found that antagonizing the α4β2 nAChR locally in the lateral hypothalamus with DHβE, an α4 nAChR antagonist with moderate affinity, caused an increase in food intake after a 12 hours fast, compared to saline-infused rats. Systemic DHβE (2 mg/kg) administration similarly increased food intake. In these animals a subpopulation of OH neurons showed elevated activity compared to controls and MCH neuronal activity was overall lower as measured by expression of cFos, the immediate early gene marker for neuronal activity. No differential activity patterns where observed in the other populations of neurons analyzed. These results indicate that various neurochemically distinct hypothalamic circuits are under the influence of α4β2 nAChRs and that cholinergic inputs to the lateral hypothalamus can affect satiety signals through activation of local α4β2 nAChR-mediated transmission.

Neural Circuit Physiology

P179.-Analysis of Local Field Potential and Single-unit Firing Patterns during Spontaneous Seizures in Human Hippocampus and Insular Cortex

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We studied the spatiotemporal scale of focal epilepsy and the spread of epileptogenesis. We used wide-bandwidth electrophysiological intracranial recordings using clinical macro (cM)- and research microelectrodes (rM) in patients with epilepsy.

We analyzed 23 spontaneous seizures of 2 patients with insular epilepsy (IE) and frontal epilepsy (FE). For further analysis, we only included 11 seizures that showed a local field potential (LFP) with single units (SU) activity during ictal period and at least within 15 min before seizure onset. LFP and SU were recorded across multiple days. In IE case, microelectrodes were localized within the epileptogenic zone (EZ) in posterior ínsula. However, seizures recorded from cM were not simultaneously observed in adjacent rM. LFP during seizures did not showed epileptiform discharges and FR remained constant or decreased. In FE case, rM were outside of EZ (hippocampus) and when seizures spread to the hippocampal area, cM recorded epileptiform discharges that were simultaneously observed on LFP. In this case, a marked increase in FR was observed. The areas involved directly in the seizure or its propagation that showed hypersynchronous discharges on LFP, presented an increased in FR. While LFP did not show epileptiform discharges in spite of being part of EZ, FR only show low-level. These findings could have important implications for how we localize seizure activity and how we map its propagation.

Neural Circuit Physiology

P180.-L-DOPA induced dyskinesia in a mouse model of Parkinson's disease and its impact on striatal medium spiny neuron activity

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Medium spiny neurons (MSNs), the main striatal projection neurons, are key to action selection. Direct pathway MSNs (dMSNs) convey signals that promote actions, while indirect pathway MSNs (iMSNs) inhibit competing actions. Normally, dopamine (DA) promotes movement by favoring cortical action on dMSNs and lessening cortical drive onto iMSNs. In Parkinson's disease (PD), where nigrostriatal dopaminergic neurons degenerate, iMSNs prevail over dMSNs. Most pharmacological treatments for PD target dopaminergic transmission and when used chronically may cause structural and functional changes in MSNs. In fact, chronic treatment with L-DOPA, a DA precursor, causes abnormal and incapacitating movements known as dyskinesia in up to 50% of the patients after 5 years of treatment. A long-standing hypothesis proposes that dyskinesia is due to overactivity of dMSNs, yet the effects of L-DOPA on dMSNs and iMSNs have not been studied before. Here we explore the electrophysiological effects of L-DOPA on MSNs in a mouse model of L-DOPA-induced dyskinesia (LID). Transgenic mice showing MSN type-specific expression of fluorescent proteins are lesioned with 6-hydroxidopamine to model PD and later LID is induced. Then, using in vivo juxtacellular recordings, MSN responsiveness to motor cortex stimulation is assessed before ("off") and following ("on") an acute L-DOPA challenge. Only for dMSN we found a significant difference between the on and off response, linking LID to dMSN overactivity.

Neural Circuit Physiology

P181.-Neuronal activity of the striatum in a rewarded learning task

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In rewarded learning tasks the subject has a goal and explores the environment preferentially selecting the behavior that maximizes the chances of achievement. In the presence of cues that had been previously associated to the reward, the expectancy of reaching the goal can drive the subject's actions. The striatal activity conveys information about the reward and, presumptively, about the outcomes of the actions in the context. Here we recorded striatal unitary activity of freely moving adult rats all throughout the learning stages of the training of a multiple-trial task. Briefly, each trial starts with a nose-poke after which a visual cue is delivered. Water-deprived rats are rewarded with a drop of water in 50% of the trials only with the completion of eight or more licks. After exiting the nose-poke port they must wait for two- seconds to initiate a new trial. First, we classified the neurons into different neuron types (i.e. principal cells, interneurons) according to the characteristics of their action potentials (such as peak-valley width, inter-spike interval), firing rate and firing pattern. We found neurons that respond to different events of the task, like the visual cue, the entrance to and the exit from the nose-poke port. Besides, there were neurons that increased or decreased their activity tonically while the animal is within the port. As behavioral control is immature during adolescence, we plan to study unitary activity in younger subjects.

Neural Circuit Physiology

P182.-Administration of glyphosate to pregnant rats induce alterations of electrocortical activity in their offspring

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We tested the electrical activity of the cerebral cortex in rats exposed in utero to glyphosate (GLYP) (N-Phosphonomethyl glycine, monoisopropylamine salt) dosed at 35 mg/kg subcutaneously to mothers every other day of pregnancy. Control pregnant rats were injected with saline (SAL) with the same scheme. Rats offspring (ages 20-40 days) were anesthetized with 1.5 mg/kg intraperitoneal urethane. Stainless steel electrodes were inserted extradurally 4 mm left and right from midline at Bregma, with a reference electrode at the midline, 10 mm rostral to Bregma. Electrical activity from these electrodes was amplified, digitized and analyzed with Scope 3.9 and Chart 4.2 software (AD-Instruments). One forepaw (FP) was stimulated with, 0.5 Hz frequency, 0.1 msec duration, supramaximal pulses for elicitation of somatosensory evoked activity (SEP). We found a statistically significant ($P<0.05$) decrease in the 0 to 2 Hz band and increase in the 2 to 4 Hz band in the power spectrum of the electroencephalogram (EEG) contralateral to the stimulated FP in the GLYP compared the SAL group. Initial SEP latency was shorter in GLYP (5.5±0.6 msec, n=19) than SAL (8.9±0.6 msec, n=18, $P<0.001$) in the cortex contralateral to the stimulated FP. An early wave (12.6±0.9 msec peak latency) appeared in the GLYP homolateral cortex and was absent in SAL. In conclusion, administration of GLYP to pregnant rats induced faster EEG rhythms and facilitated SEP activity in their offspring.

Neural Circuit Physiology

P183.-Neuromodulators in the processing of afferent inputs in the dentate gyrus

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Neurogenesis in the adulthood continuously provides the dentate gyrus (DG) of the hippocampus with pools of granule cells (GC) which integrate into the preexisting network. The maturation process of this newborn neurons is well characterized and is similar to the maturation of GC during development. It has been shown that newly born GC are necessary for many types of memory but how these neurons contribute to the hippocampal function is under intense investigation.

As inputs arrive to DG, they activate both excitatory and inhibitory neurons, and the excitation to inhibition (E/I) balance results in a pattern of population activity. Immature 4 week old GC have specific processing features, as they exhibit a higher E/I balance compared to mature GC. Thus, even though this population of neurons represents only 3-6 % of the total GC, their contribution to processing could be important due to their higher activity, their higher spiking rate and their higher plasticity. Neuromodulatory circuits projecting to the DG could modulate E/I balance in GC, providing a new level of plasticity for information processing.

Using electrophysiological techniques, we evaluated the effect of serotonin and acetylcholine in the DG input processing, both during adult neurogenesis and during development. Our results suggest that neuromodulators can selectively affect one population of GC in the DG , modifying their relative contribution to the output signal towards CA3.

Neural Circuit Physiology

P184.-Differential feed-forward inhibition generates division of labor of developing and mature granule cells in the adult hippocampus

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Adult neurogenesis provides the dentate gyrus (DG) of the hippocampus with constantly renewing pools of immature granule cells (GC) with unique properties. In the present work we address the specific contribution of immature GC to encoding of information arriving to the hippocampus. We injected a retrovirus to express RFP in dividing hippocampal cells and recognize immature four week old GC (4wpiGC) in acute hippocampal slices four weeks later. We stimulated the medial perforant path with a monopolar electrode with trains at physiological frequencies (1 Hz, 10 Hz, 20 Hz and 40 Hz) and recorded activation from 4wpiGC and mature GC with loose-patch. Results show that spike trains arriving to the DG at different frequencies are channeled into two populations of neurons with variable frequency-filter gains and temporal fidelity: Immature GC respond to a wider range of afferent stimuli arriving at 1-40 Hz, whereas mature GC are less effective in following higher frequencies; mature GC, on the other hand, show a higher temporal fidelity than 4wpiGC. Whole cell recordings of stimuli evoked excitation and inhibition indicate that activation differences are mainly dictated by feed forward inhibition, which predominantly restricts mature GC and time locks their spiking. Thus, adult neurogenesis provides pools of GC that escape form feed-forward inhibition and can reliably transit incoming frequency, while mature GC are precise at informing the beginning of the stimulus.

Neural Circuit Physiology

P185.-Selective ablation of connexin 43 in olfactory ensheathing cells is associated to a reduction of sensory input

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The olfactory epithelium produces olfactory sensory neurons (OSNs) throughout life. Permissivity for OSN turnover is attributed to glial cells, olfactory ensheathing cells (OECs) which can promote axon growth in vitro and when transplanted in vivo to injury sites. However the mechanism rests unknown due to the limited knowledge of OEC physiology.
We previously reported that OECs are gap junction-coupled and express connexin 43 (Cx43). We propose that OSN incorporation to the circuit depends on functional OEC networks involving Cx43. To test this, we used inducible Cre-Lox technology to delete Cx43 sequences in OECs of adult mice to: 1) characterize electrophysiological indicators of OEC coupling and 2) evaluate indicators of sensory input. OECs with reduced expression of Cx43 showed smaller amplitude membrane currents, lower conductance and lower sensitivity to a gap junction blocker. Also, bulbs of mice with Cx43 deletion displayed lower numbers of tyrosine hydroxylase positive interneurons, indicating a weaker sensory input. Finally, to assess the effect of OEC disconnection on olfactory function we performed a habituation-dishabituation test. Preliminary results suggest a reduced sensitivity to opposite-sex social odors in Cx43-deleted mice. In summary, our results support that Cx43 is a key mediator of OEC coupling, and present a model to understand the role of OEC connectivity on the maintenance of the olfactory pathway and to explore the regenerative potential of OECs.

Neural Circuit Physiology

P186.-Codification of reward's subjective value at the Nucleus Accumbens

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Every action has a purpose. But, how could we evaluate if the result of an action, either to obtain a reward or harm's avoidance, worth the effort? Our future behavior will depend on this assessment. Rewards are associated with different types of costs (such as delays or uncertainty about its obtaining). These costs affect the perceived value of the rewards (subjective value). The Nucleus Accumbens (NAc) has been implicated in evaluation and decision-making processes, as an interface between motivation and action. This nucleus receives projections from motor cortices, assosiative cortices involved in decision-making, limbic areas (as the hippocampus and amygdala) and from dopaminergic neurons. NAc neurons are sensible to the reward's type and volume and they change their response to a stimulus according to the reward's value associated to it. The aim of this project is to understand how this area encodes the subjective value of a reward whose acquisition requires different efforts and how this influences the behavioral response. In order to achieve this we carried out electrophysiological studies of NAc activity of adult male Long Evans rats, while the rats were performing a behavioral test with two different volumes of rewards and different delivery's delays.

Neural Circuit Physiology

P187.-Delayed coupling to feedback inhibition during a critical period for the integration of adult-born granule cells

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Adult neurogenesis provides a particular kind of plasticity that involves the addition of new processing units to pre-established circuits. In higher mammals, including humans, adult neurogenesis is restricted to specific structures, being the most prominent the dentate gyrus of the hippocampus. The functional impact of adult born neurons (newborn granule cells, nGCs) in hippocampal information processing remains unknown. In order to elucidate their precise contribution a lot of effort has been made to characterize their synaptic connections along their development. It has been shown that during their process of maturation nGCs acquire inhibitory inputs that significantly reduce their excitability and at the same time lose their ability to undergo hebbian plasticity via long term potentiation. In this work we combine the usage of optogenetics and synthetic G-coupled receptors to assess the development of their output connectivity. We show that immature nGCs reliably recruit distal targets in the CA3 area but poorly drive proximal circuits responsible of feedback inhibition. As they transition towards maturity they activate local GABAergic interneurons that restrict spiking of the neighboring granule cell layer. Moreover this feedback inhibition impinges only weakly in young cohorts of nGCs. A computational model reveals that the delayed coupling of nGCs to feedback inhibition could be crucial to achieve a fine-grain representation of novel inputs in the dentate gyrus.

Neural Circuit Physiology

P188.-Simultaneous micro patterned excitation of light activated molecules

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Channelrhodopsin excitation of neural targets brings light to structural and dynamic properties of neural systems. Two main approaches are currently being used to illuminate the brain tissue: massive illumination and 2 photon excitation. Here, we propose the use of a programmable liquid crystal array as a spatial light modulator for creating two dimensional micro excitation patterns. Our method allows us to create arbitrary spatiotemporal light patterns which can be projected onto the tissue simultaneously, at 2.3 um resolution
and at 60 frames per second.
A laser diode (445 nm) is projected onto the liquid crystal array, which in turn reflects, through the microscope objective (10 x), the programed image onto the sample. We tested the system using fluorescein (F7250, Sigma-Aldrich) and a low noise CMOS camera. Fluorescence images matched with the programed patterns, showing almost not noticeable distortion.
As its ability to project complex illumination patterns onto cortical brain tissue (including real images), our method can be used not only to discover fast dynamical properties of the neural system but as a tool to provide differential excitation for brain computer interfaces.

Neural Circuit Physiology

P189.-Optogenetic mapping of GABAergic interneurons controlling integration and function of adult-born dentate granule cells

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The adult dentate gyrus produces newborn granule cells (nGCs) throughout life. This process is known to be involved in learning and memory. Recently, it was shown that immature (4-week-old) nGCs can efficiently drive distal CA3 targets but poorly activate proximal interneurons responsible for feedback inhibition (FBI), which is strongly recruited by adult-born GCs only upon maturation. We are now studying how nGCs of different ages become integrated in the pre-existing circuit of the dentate gyrus, particularly identifying individual populations of GABAergic interneurons responsible for these feedback loops. Parvalbumin-expressing basket cells (PV cells) are a major population within these GABA interneurons (around 25%) and are known to be involved in perisomatic inhibition. We have combined optogenetics and acute slice electrophysiology to activate PV cells and nGCs at different stages of maturation and study their connectivity in both directions, interneuron to nGCs and viceversa. We have collected preliminary evidence demonstrating that PV cells are major targets of nGCs at the mature stage, and that in turn PV cells contact mature GCs. This approach is currently being combined with markers of other interneuron subtypes (such as the somatostatin-expressing GABA cells) to obtain a complete spatiotemporal map of neuronal connectivity in the dentate gyrus that encompasses the dynamic changes imposed by the continuous integration of nGCs.

Neurochemistry and Neuropharmacology

P190.-Consequences of SAL administration in voluntary ethanol consumption and subsequent locomotor activity in perinatally lead-exposed rats

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Developmental exposure to low lead (Pb) doses induces elevated voluntary ethanol intake in rats, an effect that we attribute to central acetaldehyde (ACD) accumulation, which is considered reinforcing. Furthermore, this metabolite reacts with dopamine (DA) to form salsolinol (SAL) inducing DA release. Based on previous evidence of ACD involvement in the reinforcing effects of ethanol in Pb-exposed animals, we here postulate that SAL may mediate these differential effects. To this end, SAL (10 mg/kg i.p.) or vehicle was administered at the end of the voluntary ethanol intake test (2-10% for 28 days) to register voluntary ethanol consumption and subsequent locomotor activity. In addition, a group of animals that were not submitted to the ethanol protocol was included as control. The results indicate that SAL administration failed to modify ethanol consumption, but evidenced a statistically significant increase in locomotor activity in Pb exposed animals that consumed ethanol and were injected with SAL, indicating a heightened response to the stimulant effects of ethanol. These results provide further support to the hypothesis that ACD (and thereby SAL) is involved in the heightened ethanol-related effects observed in the Pb-exposed animals.

Neurochemistry and Neuropharmacology

P191.-Chronic restraint stress facilitates the acquisition of cocaine self-administration

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Clinical evidence supports the idea of individuals that suffer stressing events along their lives are vulnerable to developing substance use disorders (SUDs). Here, we attempted to mimic how exposure to chronic stressful life events can create a vulnerability to developing SUDs. Sprague-Dawley rats were exposed to chronic restraint stress (2 hs daily) during seven days. A week after the last stress session, all animals were anesthetized and implanted with indwelling jugular catheters. Seven days after surgery, rats began daily 2 hs cocaine self-administration (SA) sessions (fixed ratio 1), in which one response on the active lever yielded one intravenous cocaine infusion (0.2 mg/infusion, followed by a 5 s time-out period), paired with a white cue light above the active lever and a discrete tone cue. An inactive lever was also available throughout each session. Rats were allowed ten days to reach SA criterion, which was defined as the first day animals obtaining more than ten infusions of cocaine. Our results point out a facilitation of the acquisition of cocaine SA as well an augmented intake of cocaine in pre-stressed animals as regard to control unstressed animals. This behavioral facilitation induced by chronic stress on cocaine SA was proved by quantifying the ratio of response on the active lever and the amount of cocaine infusions, these findings constitute a starting platform to study the mechanisms underpinning the comorbility between stress and SUDs.

Neurochemistry and Neuropharmacology

P192.-GABAA receptors mediating phasic and tonic inhibitory neurotrasmission in the retina and the hippocampus are targets of redox signalling

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Redox regulation is a key factor in the modulation of cellular pathways, including synaptic neurotransmission. In contrast to irreversible oxidative damage, redox signalling is mediated by modifications of target proteins at particular redox-sensitive thiols that operate as redox-switches producing reversible changes on protein function (neurotransmitter receptors, voltage-gated ion channels and transporters). As redox status significantly fluctuates during normal and pathological conditions, the study of endogenous and pharmacological redox dependent synaptic modulation is fundamental for understanding the mechanisms controlling neuronal activity.

We demonstrated that GABAA receptor function can be directly modulated by endogenous redox agents, through reversible thiol modification of cysteine residues located to extracellular or cytoplasmic domains. Either phasic GABAA receptors (mediating the fast component of GABAergic neurotransmission elicited by GABA release) as tonic GABAA receptors (which mediate the slow component elicited by ambient GABA), both in retinal bipolar cells and hippocampal CA1 pyramidal neurons, are sensitive to modulation exerted by redox agents. We identified a GABAA receptor variant carrying an intracellular sensor for reactive oxygen species (H2O2) capable to induce allosteric transitions that modulate channel activity. We also found that changes in ascorbic acid and nitric oxide levels can be detected at the cys-loop to produce similar effects.

Neurochemistry and Neuropharmacology

P193.-Exploring Fyn as a novel molecule in Levodopa induced dyskinesias

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The administration of L-DOPA is the most effective symptomatic pharmacological therapy for Parkinson's disease (PD). Despite its benefits, most patients develop side effects known as L-DOPA induced dyskinesias (LID). To control LID in PD therapy is necessary to better understand the multiple cellular and molecular changes that take place during LID. Some protein and gene changes have been reported within the dyskinetic striatum, but the mechanisms in which they are involved are not fully understood. Pleiotrophin and its receptor RPTPz/b are up-regulated as a consequence of dopaminergic cell loss and L-DOPA treatment. RPTPz/b interacts with PSD95 at the postsynaptic density complex and regulates the protein kinase Fyn, a key molecule involved in synaptic plasticity and cytoskeleton stability. We found an increase in the number of Pleiotrophin(+) neurons and that Fyn is highly phosphorylated in the striatum of dyskinetic rats. We performed behavioral tests and determined abnormal involuntary movements (AIMs) in a model of LID in both Fyn knock-out (KO) and WT mice. Dopaminergic denervation was confirmed by immunodetection of nigral and striatal tyrosine hydroxylase. In addition, the levels of molecular markers involved in LID were determined by Western blot. Fyn KO mice showed a significant reduction in the development of AIMs in relation to WT controls. Our data suggest that Fyn might be involved in the development of LID, and its role as a potential target to control AIMs.

Neurochemistry and Neuropharmacology

P194.-CB2 agonist improves motor activity and reduces tissue damage after cerebral ischemia

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The cannabinoid receptor type 1 (CB1R) is expressed in neurons and glial cells in many brain regions, where they play neuromodulatory roles in synaptic transmission, while cannabinoid receptor type 2 (CB2R) is mainly expressed by cells of the immune system.
Cannabinoids can exert anti-inflammatory effects by inhibiting the proliferation of lymphocytes and inducing their death by apoptosis.
The aim of this work is to evaluate the potential neuroprotective effects of CB2R agonist treatment in a mouse model of middle cerebral artery occlusion (MCAo).
We evaluated the effect of CB2R agonist (JWH015) and antagonist (AM630) in C57Bl/6 mice subjected to focal brain ischemia by MCAo. 3, 24 and 48 hours after MCAo, mice received JWH015 4mg/kg, AM630 1mg/kg. To assess motor activity, neural deficit score and motor tests were performed 1 day before and 3 and 7 after MCAo. At 7 days (D7) post-lesion, mice were fixed and their brains processed for immunohistochemistry for GFAP, MAP-2 and lectin. JWH015 reduced infarct area, neuronal dendrite loss, astroglial hypertrophy and hyperplasia. In contrast, AM630 increased these parameters of damage. Motor test data analyzed showed a progressive deterioration to D7 in ischemic animals and the CB2R agonist treatment produces an improvement in motor activity.
The results suggest that CB2R agonists may be involved in neuronal survival and the regulation of neuroprotection during focal cerebral ischemia in mice by reducing inflammatory response.

Neurochemistry and Neuropharmacology

P195.-The serotonin effect in the neuromuscular function of Echinococcus granulosus and other cestodes: Potential role of serotonergic GPCRs

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Cestode parasites are a diverse group of organisms, many of them are cause of neglected zoonoses with major impact in local health and global economy. The adecuate nerve function is essential for the parasitic way of life and is a target for cestocide drugs. Genomic and transcriptomic data of E. granulosus (Tsai et al., 2013) and experimental resuls showing the motor response to serotonin (5-HT) of the larval stage (Camicia et al., 2013) suggest the existence of serotonergic GPCRs in this parasite. In this work, we propose the existence of an important role for 5-HT in the neuromuscular function of cestode parasites and that the observed stimulatory effect could be mediated by serotonergic GPCRs. The bioinformatics search of this kind of receptors in cestode parasites such as Mesocestoides corti, Taenia solium, E. granulosus and Echinococcus multilocularis resulted in the interesting finding of conserved sequences with amino acid identity with serotonergic GPCRs. The addition of 5-HT to the larval stages of M. corti and Taenia crassiceps resulted in the stimulation of the motility measured by light scattering and imaging techniques in a dose dependent mode. The motility curves showed a specific response to the neurotransmitter according to the species in question and this result suggests the intervention of different types of serotonergic GPCRs for each species. The identification of survival genes will be of great impact for the development of cestocide drugs.

Neurochemistry and Neuropharmacology

P196.-Flavone and chalcone derivatives as promising AChE and BuChE inhibitors for Alzheimer's disease treatment

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Alzheimer's disease (AD) is an age related neurodegenerative disorder associated with neuropathological and neurobehavioral changes accompanied by memory and cognitive impairments. The development of drugs with therapeutic potential in AD is one of the major targets in neuroscience. It has been shown that AChE activity is reduced in patients in the late phase of AD, whereas expression and concentration of butyrylcholinesterase (BChE) is compensatory and is increased. Inhibitors of acetylcholinesterase (AChE), such as galantamine, rivastigmine and donepezil, are prescribed to patients in the early stages of AD. The inhibitory effects of flavonoids on both AChE and BuChE have attracted great interest among researchers. We studied the effect of a wide range of natural and novel flavone and chalcone derivatives on murine AChE and both human and murine BuChE.

Different grade of inhibition was observed depending on the compound and the enzyme tested. The most promising results were obtained for 3,3-dibromoflavanone (100 µM) that showed 100% AChE inhibition. Flavone; chrysin; 6-methylflavone; 6-methoxy-3'-bromoflavone; 6,3'-dimethylflavone; 3'-methylflavone; 3-bromoflavone; 6-hydroxy-3´-bromoflavone and 3,3-dibromoflavanone (100 µM) showed >75 % BuChE inhibition. Meanwhile the chalcone derivatives tested showed <75 % inhibition on both enzymes. The effectiveness of some of the most active compounds will be evaluated in mice in behavioral tests that assess learning and memory.

Neurochemistry and Neuropharmacology

P197.-Brain ALDH expression is reduced in developmentally-lead-exposed animals after voluntary ethanol intake

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Perinatal lead (Pb)-exposure induces higher ethanol intake in adolescent animals compared to non-exposed controls, likely due to brain ACD accumulation which is considered reinforcing. We here sought to determine whether ALDH expression in limbic regions (prefrontal cortex -PfC, caudate-putamen –CP, and nucleus accumbens -NAc) is modified by developmental Pb exposure. Thirty five-day old male Wistar rats were offered with ethanol (2-10% v/v) or water during 28 days. Two additional groups were included as controls: 35 and 63 day-old animals that have not consumed ethanol (non-ethanol groups). At the end of the study all the animals were perfused, the brain fixed, and immunohistochemistry performed for ALDH abundance. The results evidence that ALDH expression was not affected by perinatal Pb exposure, given that both control and Pb-exposed adolescent animals showed comparable cell counts in the three regions analyzed. However, ethanol intake induced a dramatic reduction in the Pb-exposed group's ALDH positive cells in all three regions when they were assessed at the end of the ethanol intake test. This effect was blunted in the NAc and CP from animals not submitted to the ethanol consumption protocol, while the PfC data showed a raise in cell count number in the Pb-exposed rats that deserves further consideration. The results indicate lower ALDH expression in key brain regions that could led to brain ACD accumulation and consequent higher ethanol intake in Pb-exposed animals.

Neurochemistry and Neuropharmacology

P198.-New drug-resistant seizure model in mice to evaluate P-gp non-substrate anticonvulsant drugs

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About 30% of epileptic patients do not respond to clinically established AED plasma concentrations; therefore to identify new AEDs and incorporate models of refractory epilepsy (RE) into its development is a necessary event. We have developed a drug-resistant seizure model in Swiss mice associated with P-glycoprotein (P-gp) overexpression with low mortality (20%) and high performance of antiepileptic drug resistant animals for using in the early stages of pre-clinical trials. 3-mercaptopropionic acid (MP, 36 mg/kg, i.p.) was administered to mice (25-30 grs.) during 23 consecutive days, showing generalized clonic crisis. Day 24th 80% of animals were resistant to the P-gp substrate drug phenytoin (18 mg/kg, i.p.) an effect that was reversed with the P-gp blocker nimodipine (3.5 mg/kg, i.p.). Furthemore mice showed resistance to phenobarbital (P-gp substrate drug); although P-gp non-substrate antiepileptic drugs like carbamacepine, levetiracetam and diazepam induced anticonvulsant effect. The resistance are strongly associated with P-gp overexpression which was observed by western blot in cerebral cortex (34%), hippocampus (86%) and striatum (30%). Immunohistochemistry assays showed increase in P-gp expression in the same brain regions. This model is an useful tool for in vivo screening of new anticonvulsant drugs selected as P-gp non-substrate by virtual screening in our laboratory.

Neurochemistry and Neuropharmacology

P199.-Tolerance to diazepam is associated with different alterations of GABAA receptors in rat cerebral cortex

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The clinical use of benzodiazepines is limited by the development of tolerance. The aim of this work was to investigate the mechanism of tolerance by performing behavioral tests in combination with biochemical studies. To this end, we administered chronic treatments of diazepam to rats for 7 or 14 days. Tolerance to the sedative effects of diazepam was detected after the 7- and 14-day treatments, whereas tolerance to the anxiolytic actions of the benzodiazepine manifested following only the 14-day treatment. The cerebral cortical concentrations of diazepam did not decline after the chronic treatments, indicating that tolerance was not due to alterations in pharmacokinetic factors. The uncoupling of GABA/benzodiazepine site interactions and an increase in the degree of phosphorylation of the GABAA receptor gamma 2 subunit at serine 327 in the cerebral cortex were produced by day 7 of diazepam treatment and persisted after 14 days of exposure to the benzodiazepine. Thus, these alterations could be part of the mechanism of tolerance to the sedative effects of diazepam. An increase in the percentage of α1-containing GABAA receptors in the cerebral cortex was observed following the 14-day treatment with diazepam but not the 7-day treatment, suggesting that tolerance to the anxiolytic effects is associated with a change in receptor subunit composition. Our results suggest that tolerance to the sedative and anxiolytic effects of diazepam is mediated by different mechanisms.

Neurochemistry and Neuropharmacology

P200.-Blocking the development of cocaine induced sensitization by Lithium Chloride: long terms effects and Wnt signaling pathway involvement

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Wnt factors are cistein rich secreted proteins that interact with their receptors: Frizzled, Ryk, and Ror. Because of the interaction, Dishevelled (DVL) is activated, and consequently, one of three pathways: Wnt/B-catenin, Planar Cell Polarity, or Wnt/calcium pathways. Wnt signaling pathways are essential for development of the mammalian brain.
However little is known regarding its role in adulthood. Recent results from our lab showed that a decrease of B-catenin in Prefrontal Cortex is a cocaine-induced neuroadaptation required for the development of behavioral sensitization. We also showed that Lithium Chloride (LiCl) is capable of blocking the development of cocaine sensitization while increasing B-catenin levels. Thus, in order to elucidate if those changes in B-catenin during cocaine treatment have an impact on the behavioral manifestations that happened 3 weeks later (i.e. expression of sensitization), we administered LiCl i.p. before each cocaine injection. Then, we measured locomotor activity on Day 1 and 7 of the treatment as well as 21 days later. Animals were sacrificed 24hs after last injection and brain areas were dissected. So far our results showed that systemic LiCl injections might as well blocking the expression of cocaine-induced sensitization by modifying B-catenin levels in different brain areas.

Neurochemistry and Neuropharmacology

P201.-Striatal interneuron changes in hemiparkinsonian and L-DOPA-induced dyskinetic mice

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The administration of L-DOPA still remains as the preferred treatment for Parkinson´s disease despite its propensity to induce severe motor complications, known as L-DOPA-induced dyskinesias (LID). Striatal interneurons strongly modulate striatal output, and changes in striatal microcircuits may contribute to basal ganglia dysfunction in a number of movement disorders.

We determined changes in number and activity of the different populations of striatal interneurons in a mouse model of hemiparkinsonism and L-DOPA induced dyskinesias. C57BL6 mice were injected with 6-hydroxydopamine (6-OHDA) or vehicle in the medial forebrain bundle and treated daily with a dyskinetogenic dose of L-DOPA or saline solution. 6-OHDA lesion led to changes in the number of parvalbumin (PV) + and calretinin (CR) + interneurons. CR+ but not PV+ cell density returned to normal levels after L-DOPA treatment. No change was observed in cholinergic or somatostatin (SST)+ interneurons. 6-OHDA lesion led to an increased c-fos expression which was further increased after L-DOPA treatment. While no PV+ nor CR+ interneurons showed c-fos expression in any condition, ~47% of SST+ and ~7% of cholinergic interneurons where c-fos positive in LID but not in 6-OHDA animals. Our findings suggest that changes in striatal interneurons contribute to the maladaptive state that occurs after dopamine depletion. In addition, chronic treatment with L-DOPA fails to reestablish the initial physiological landscape.

Neurochemistry and Neuropharmacology

P202.-Cannabinoid CB1 Receptors within Nucleus Accumbens Shell Are Not Involved in Stress-Induced Reinstatement in Extinguished Cocaine–Conditioned Animals

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Endocannabinoid system, primarily through their actions at CB1 receptor (CB1R), is implicated in drug relapse. Previous results from our lab demonstrated that in extinguished cocaine-conditioned animals, evaluated in a conditioned place preference test (CPP), the administration of AM251, a CB1R antagonist, or ACEA, a CB1R agonist, into the Core of the Nucleus Accumbens (NAc) abrogated or facilitated restraint stress-induced reinstatement of cocaine-CPP responses, respectively. In order to compare the involvement of both NAc compartments, extinguished cocaine-conditioned Wistar rats were microinjected into the Shell of NAc with ACEA (0.01fmol/side), AM251 (10ug/side) or vehicle, and subsequently assigned to the following treatments: 1) Stressed Animals (SA): 15 or 30 min-restraint exposure, depending on the experiment, and 2) Control Animals (CA). The intra-Shell administration of CB1R antagonist or agonist did not modify the restraint stress-induced reinstatement of cocaine-CPP responses as previously observed after intra-Core administration of CB1R ligands. These findings support the hypothesis of the preferential influence of CB1R within NAc Core, but not Shell, in the reinstatement of cocaine seeking behavior. Future studies will attempt to identify a possible glutamate dependent mechanism underpinning the effects of CB1R ligands on the restraint stress-induced reinstatement of cocaine-CPP responses.

Neurochemistry and Neuropharmacology

P203.-2’-Hydroxy-5’-methyl-3’-nitrochalcone: a novel chalcone with antinociceptive effects

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Chalcones (1,3-diaryl-2-propen-1-ones) belong to the flavonoid family. Chemically, they consist of open-chain flavonoids in which the two aromatic rings are linked by a three-carbon α,β-unsaturated carbonyl system. Chalcones have been reported to possess many pharmacological activities, including anti-inflammatory, antimicrobial, antifungal, antioxidant, cytotoxic, antitumor and anticancer actions.
The aim of this work was to synthesize, by aldol condensation, a series of chalcones and evaluate their potential effect on the Central Nervous System (CNS). The binding capacity of these compounds to receptors present in synaptosomal membranes of rat brain related to anxiety disorders, depression and pain was evaluated by displacement of labeled specific ligands: [3 H] FNZ (binding site for benzodiazepines, in the GABAA receptor), [3H] 8-OH-DPAT (serotonin 5-HT1A) and [3H] DAMGO (μ-opioid). One of the most active compound in the binding inhibition of [3H] DAMGO, was 5’-methyl-2’-hydroxy-3’-nitrochalcone, which presented a Ki value of 13.5± 6.9 μM. In acute chemical and thermal models of nociception in mice, it exerted antinociceptive action without showing sedative, anxiolytic, antidepressant and motor incoordination effects. Blockade in vivo assays revealed that μ opioid receptors are involved in its mechanism of action.

Neurochemistry and Neuropharmacology

P204.-Functional role of the duplicated α7 nicotinic receptor subunit

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The α7 nicotinic receptor subunit gene, CHRNA7, codes for a subunit that forms the homomeric α7 receptor, which is involved in learning and memory. Exons 5-10 of CHRNA7 were duplicated upstream interrupting another partial duplication of the gene ULK4, called FAM. The product of the resulting chimeric gene (CHRFAM7A), dupα7, is a receptor subunit that lacks part of the ACh binding site. We here combine cell expression and electrophysiological recordings in HEK cells to understand the functional role of the dupα7 subunit. Incorporation of dupα7 cDNA during cell transfection with α7 cDNA reduces surface α-BTX labeling, indicating reduced number of α7 binding sites, and in turn, suggesting a negative modulatory role. To determine if dupα7 can assemble into functional receptors we used, as a reporter of receptor stoichiometry, an α7 subunit (α7LC) carrying mutations in determinants of ion conductance. α7LC forms functional receptors but single-channel openings cannot be detected due to their low conductance. Co-expression of α7LC with dupα7, which by itself does not form functional receptors, allows detection of single-channel openings elicited by ACh. This result unequivocally indicates that α7 and dupα7 subunits assemble into functional heteromeric receptors. The analysis shows that a minimum of two α7 subunits is required for forming functional receptors. Our results contribute to the understanding of the functional significance of the partial duplication of the α7 gene.

Neurochemistry and Neuropharmacology

P205.-JMV4957 impairs constitutive activity of ghrelin receptor (GHSR1a).

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GHSR1a is a G protein coupled receptor with constitutive activity (CA) that modulates neuronal circuits that control appetite. Thus, its inverse agonists are potential therapeutic agents that could lower the set point for hunger. Substance P analog (SP) has been extensively used in research to reduce GHSR1a CA but this peptide is highly unspecific. Therefore, there is a great interest in developing specific GHSR1a inverse agonists. Here we present a compelling study of JMV4957, a new compound recently synthetized with potential GHSR1a inverse agonist properties.

We first tested if JMV4957 is capable of binding GHSR1a in HEK293t transfected cells, and found that it has a high binding affinity (Ka=28 nM). Next we explored the inverse agonist effect of JMV4957 and found that this compound induced a 20% reduction of the basal inositol phosphate production with a EC50=35nM in HEK293t cells expressing GHSR1a. Moreover, we explored the effect of JMV4957 on the previously described inhibitory effect of GHSR1a CA on voltage-gated calcium channels basal currents (ICa) and surface channel expression. We found that pre-incubation with JMV4957 avoided this effect in the same manner as SP. We also performed imaging experiments and found that JMV4957 recovers channel surface density to control values in cells transfected with GHSR1a and GFP tagged channels. We are currently testing JMV4957 effect on ghrelin-induced food intake on mice.

Neurochemistry and Neuropharmacology

P206.-Noise-induced rat hippocampal oxidative changes were inverted after rearing in an enriched environment. Correlation with behavioral alterations.

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Hippocampal-related behavioral alterations were observed in noise-exposed rats. However, a correlation with hippocampal oxidative status was only evaluated in animals exposed at 15 days.
Therefore, the aim of the present work was to test if behavioral alterations induced by noise exposure in younger animals might be also related with hippocampal oxidative changes. The possible prevention of these changes through the use of an enriched environment (EE) was also assessed.
7-days-old rats were exposed for 2 h to white noise (95-97 dB). After weaning, rats were transferred to an enriched cage with toys, a wheel, tunnels and ramps, whereas other groups stayed in standard cages. After one week, different behavioral tests were performed and levels of Trx-1, a member of the family of the antioxidants thioredoxins, were also evaluated.
Results show that Trx-1 levels were decreased in exposed animals and increased after EE rearing. Moreover, whereas noise-exposed animals showed an increase in risk assessment behavior (RAB), with no changes in associative memory (AM), EE rearing restored RAB and increased the performance in AM.
These findings suggest that an oxidative imbalance might be triggered after noise exposure that might underlie RAB alteration. The increase in AM performance in exposed animals reared in an EE might be correlated with a more reduced cellular milieu, suggesting that EE could be a useful strategy that might allow animals to cope with an unfavorable condition

Neurochemistry and Neuropharmacology

P207.-Temperature Dependence of the Enhancementof α7 Nicotinic Receptor Activity by Potential Therapeutic Compounds

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Alpha 7 nicotinic acetylcholine receptors (nAChRs) are widely distributed throughout the central nervous system, mainly in hippocampus and cortex, and are implicated in several neurological disorders such as Alzheimer´s disease and schizophrenia. Enhancement of α7 nAChR activity by positive allosteric modulators (PAMs) is a promising therapeutic strategy to improve cognitive deficits. PAM activity has been evaluated mainly at the macroscopic level, and PAMs have been classified as type I, which increase α7 currents, and type II, which cause also a profound decrease in desensitization and/or reactivate desensitized receptors. In addition, most in vitro studies have been performed at room temperature whereas preclinical studies and clinical use require physiological temperatures. To cover these limitations we evaluated potentiation of human α7 at the single-channel level. Our results revealed that both types of PAMs enhance open-channel lifetime and produce activation episodes of successive opening events. By analyzing their activities at a physiological temperature, we found that potentiation decreases with respect to room temperature. However, both PAM types show different sensitivity to temperature, suggesting distinct mechanisms by which they induce sustained activation. Overall, temperature dependence analysis emerges as a key requisite during evaluation of potential clinical applications of PAMs.

Neurochemistry and Neuropharmacology

P208.-A novel α-hydroxyamide, N-propyl-2,2-diphenyl-2-hydroxyacetamide, with anticonvulsant, antidepressant and anxiolytic action

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Epilepsy is a common group of neurological disorders whose hallmark is unprovoked seizures that can be distressing, harmful and fatal. In patients with epilepsy, anxiety and depression are the most frequent psychiatric comorbidities but they often remain unrecognized and untreated. Science has focus its efforts to find drugs that can be active as anticonvulsant/antidepressant/anxiolytic minimizing their side effects. Our group has expertise in the synthesis of new molecular entities with anticonvulsant, antidepressant and anxiolytic profiles. We found that microwave radiation has simplified and improved the synthesis of α-hydroxyamides, with good yields, shortened reaction times and carried out without solvents or catalysts. All α-hydroxyamides synthetized showed anticonvulsant activities and were not neurotoxic. From these, N-propyl-2,2-diphenyl-2-hydroxyacetamide showed the most remarkable antidepressant effect in the forced swimming and tail suspention tests (0.3-30 mg/kg, i.p.), and anxiolytic action in the plus maze test (3-10 mg/kg, i.p.) in mice. Studies of its mechanism of action, by means of its capacity to act via the GABAA receptor ([3H]flunitrazepam binding assay), the serotonin 5-HT1A receptor ([3H] 8-OH-DPAT binding assay) and the Na+ channel (NaCh) (using veratrine, a voltage-NaCh agonist in vivo) demonstrated that its effects are not likely related to 5-HT1A or GABAergic pathways and its antidepressant-like effects could be due to its NaCh blocking properties

Neurochemistry and Neuropharmacology

P209.-Role of cofilin in nucleus accumbens core during the cross-sensitization between chronic stress and cocaine

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Several evidences support the idea of a proactive influence of stress on drug-addiction. Studies from our laboratory revealed that repeated stress alters the capacity of a subsequent cocaine injection to modulate dendritic spine morphology, actin dynamics and AMPAR expression in the nucleus accumbens (NA) core. We have demonstrated that the inhibition of actin polymerization in the NA prevents stress cross-sensitization with cocaine. Thus, the main goal of this project is to evaluate the impact of the actin cytoskeleton in the changes underling the facilitatory influence of cocaine after chronic stress. For this purpose we have generated a lentivirus containing a short hairpin RNA (shRNA) specific to cofilin, to inhibit its expression in NA, and explore its function during cross-sensitization between stress and cocaine. Thus, Wistar rats will be exposed to chronic restraint stress two hours daily during 7 days. Stressed and control animals will be administered with an intra-accumbens injection of lentiviral vector 20 days (day 8) before a challenge with cocaine administered 3 weeks after the final stress (day 28), when behavioral sensitisation to cocaine was evaluated. Our preliminary data suggests that the inhibition of cofilin is sufficient to prevent the expression of cross-sensitization between stress and cocaine. Future studies will attempt to identify the upstream signaling pathways regulating cofilin activity during stress induced cross sensitization to cocaine.

Neurochemistry and Neuropharmacology

P210.-Effects of Adenosine A1 and A2a Receptors Modulation on Müller Cell Activation in Light Induced Retinal Degeneration

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Light induced retinal degeneration (LIRD) resembles retinal degenerative diseases and is a useful model to search for neuroprotective drugs. The modulation of adenosine A1 and A2a receptors have been proved to be neuroprotective in acute retinal injury, and in diverse CNS pathologies. The aim of this work was to evaluate the potential neuroprotective effect of A1 and A2a agonists and antagonists on Müller cell (MC) activation using the model of LIRD.
Sprague Dawley rats were intravitreally injected in one eye with one of the following drugs: CPA (A1 agonist); DCPCX (A1 antagonist); CGS 21680 (A2a agonist) or SCH 58261 (A2a antagonist). Contralateral eyes were injected with respective vehicles as control. Then, rats were submitted to continuous illumination (12000 lux) during 1 day. Retinas were processed by GFAP immunocytochemistry. GFAP immunoreactive areas were quantified using Fiji image analysis, and data were statistically analysed using Student´s t test. Animals treated with CPA showed a diminution in GFAP expression ($P< 0.0001$). The same trend was seen after SCH 58261 treatment. On the opposite, eyes treated with DCPCX and with CGS 21680 showed a rise in GFAP ($P<0.05$ and $P<0.001$, respectively).
Our results suggest that the activation of MCs is controlled by adenosine mediated transmission. As MCs have pro and antiapoptotic effects, we sustain that adenosine receptor modulation is a plausible therapeutic strategy being A1 agonist and A2a antagonist drugs neuroprotective.

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Neurochemistry and Neuropharmacology

P211.-Evaluation of neural connectivity between parvalbumin-expressing interneurons and medium spiny neurons in the lesioned striatum of dyskinetic mice

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Dyskinesias are a debilitating side-effect of chronic L-DOPA treatment in Parkinson's disease (PD). Abnormal stimulation of dopaminergic receptors by L-DOPA correlates with long-term functional synaptic changes in striatal medium spiny neurons (MSNs) deprived of dopaminergic innervations which may contribute to the development of dyskinesias induced by L-DOPA (LID). Parvalbumin (PV) striatal interneurons modulate and control differentially the activity of MSNs expressing either D1R or D2R and therefore contributing to the imbalance between these pathways both in PD and LID. However, the state of the connectivity between these cell populations in the dyskinetic condition remains elusive.

To establish whether the development of LID modifies the connectivity of PV interneurons with MSNs we perform an animal model of LID using transgenic mice expressing red (tomato) or green (EGFP) fluorescent markers in MSNs containing either the D1R or D2R promoter. PV was determined by inmunofluorescence on fixed striatal tissue sections using Cy5 as fluorochrome. In these animals we are analyzing the number of synaptic contacts of PV positive terminals on D1 or D2 MSNs by confocal microphotography. We expect that this work will provide evidences about the structural alterations taking place in the striatum after L-DOPA treatment, leading to a new pathophysiological condition in basal ganglia circuits, and whether these structural adaptations are directly related to the development of LID.

Neurochemistry and Neuropharmacology

P212.-Allosteric inhibition of the GABAAρ1 receptor function by L-cysteine

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L-cysteine (L-cys) is a rate-limiting precursor for the glutathione synthesis in neurons that act either as neuroprotector or neuromodulator. Additionally, L-cys produces excitotoxic effects, which have been implicated in the pathogenesis of neurological disorders such as Parkinson´s and Alzheimer´s disease. However, the role of L-cys in the SNC still is far from being entirely understood.

It has been also reported that L-cys can modulate the activity of ionic channels, for example is a redox modulator of calcium channels in rat peripheral nociceptors and a low potency agonist of the NMDA receptor in cultured rat hippocampal neurons. We have previously reported that L-cys inhibited the function of homomeric GABAAρ1 receptors. Now we extend this characterization by studying the underlying mechanism of action.

Homomeric GABAAρ1 receptors were expressed in Xenopus laevis oocytes and GABA-evoked Cl- currents recorded by two-electrode voltage-clamp in the presence or absence of L-cys. Inhibition by L-cys was dose-dependent, reversible and voltage independent. DR curves for GABA were shifted to the right in the presence of L-cys and no effects were observed at saturating GABA concentrations. L-cys inhibition was insensitive to the irreversible cysteine alkilating agent NEM. The present results suggest that redox modulation is not involved during L-cys actions and that this intermediate metabolite more likely acts as a competitive antagonist of the GABAAρ1 receptors.

Neuroendocrinology and Neuroimmunology

P213.-Participation of GABA transporters in immune response and neuro-immune communication

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The nervous and the immune systems (NS and IS respectively) are physically and physiologically connected. Recently, expression of neurotransmitter system components in immune cells and synthesis and receptors of cytokines in NS cells were described. We previously reported that a complete GABAergic system is functionally expressed in human lymphocytes. Now, we are focusing on GABA transporters (GATs). Four GAT subtypes (GAT 1-3 and BGT-1) were described in human NS. We studied GAT mRNA levels in activated and resting lymphocytes (with and without the mitogen phytohemagglutinin (PHA), respectively). GAT-2 and BGT-1 mRNAs were detected in most of activated cells. Moreover, incubation with PHA also increased [3H]GABA uptake. To evaluate the physiological role of GATs we determined cell proliferation by PHA in the presence of nipecotic acid (NA), a GAT inhibitor. Cell proliferation was negatively modulated by NA. We also analyzed GABA levels in lymphocyte cultures. We could only detect GABA in supernatant from activated cells. Despite its typical role in the synapse where they mediate cellular uptake of GABA, under certain conditions GATs can reverse and release GABA. This secretion is vesicle independent. We propose that this mechanism could be involved in GABA release in lymphocytes. Establishing the role of endogenous GATs in immune response and as a link between NS and IS will provide new therapeutic targets for the treatment of diseases that could affect both systems.

Neuroendocrinology and Neuroimmunology

P214.-Fast-Refeeding-Induced Hyperphagia Requires Ghrelin Signaling

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Animals refed after fasting display a robust hyperphagia, which aims at restoring the energy balance. Interestingly, hyperphagia persists even after animals have reached their energy needs if fasting is severe. The mechanism regulating the magnitude of the compensatory events of hyperphagia are currently unclear. Here, we tested the long-term eating behavior of mice exposed to a fast-refeed paradigm and also analyzed the dynamic of the ghrelin system -the only known hormone able to increase food intake- under these circumstances. In addition, we tested the eating behavior after fast-refeeding in mice lacking the ghrelin receptor. We found that previously fasted wild-type mice display a significant increase of the total food intake that continues for 4 days after refeeding. Fasting increases both ghrelin plasma levels and the ghrelin binding in some, but not all, hypothalamic nuclei. This binding was particularly increased at the GABAergic terminals within the hypothalamic arcuate nucleus. Notably, ghrelin binding and sensitivity to exogenous ghrelin administration remained increased even after 4 days of refeeding. In contrast, ghrelin receptor deficient mice exposed to a fast-refeed paradigm failed to increase the total food intake after refeeding. We conclude that ghrelin signaling in GABAergic terminals increases under fasting and that this readjustment of the ghrelin system is involved in the fast-refeeding-induced hyperphagia. Supported by PICT2011-2142 and PICTO2013-0065

Neuroendocrinology and Neuroimmunology

P215.-Microglia shape the pineal gland

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Microglia are the phagocytic cells of the CNS. In this work we studied their distribution and role in the developing and adult rat pineal gland (PG) under normal and inflammatory conditions. We found that microglial cells with active ameboid morphology invade the PG from the early stages of organogenesis onwards. The number of microglial cells per PG and their phagocytic capacity based on the lysosomal marker ED1/CD68 increased towards adulthood. Microglia were in very close contact and in some cases engulfing Pax6+ precursor cells, nerve fibers and blood vessels, but not mature pinealocytes. We used Pax6, an essential determinant of PG genesis, and the microglial marker Iba1 to analyze cell-cell interactions. While Pax6+ cells decreased throughout PG development, the proportion of phagocytosed precursors increased. Microglia were challenged in the adult PG by superior sympathetic ganglionectomy (SCGx) and decentralization (SCGd). Both procedures increased the number of microglia and their phagocytic capacity compared to the sham group four days after surgery. These results illustrate the responsiveness that these cells have to relatively subtle changes in the surrounding microenvironment. In conclusion, we postulate that microglia have a key role in helping to regulate morphogenesis and function in the PG by phagocytosing precursor cells, remodeling blood vessels and pruning sympathetic nerve fibers; activities that could be carried out in collaboration with astrocytes.

Neuroendocrinology and Neuroimmunology

P216.-Early behavioral and physiological alterations in a mouse model of autism

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Autism is a neurodevelopmental disorder characterized by impaired sociability and stereotypic behaviors. Individuals also have auditory symptoms, i.e. hypersensitivity to loudness of sound with abnormal behavioral reactions to environmental sounds. We hypothesized that there is a developmental critical window in which maturation and consolidation of the neural systems responsible for these symptoms typically occur.

The administration of VPA at GD 12.5 results in autism-related behaviors in adulthood. The aim of this work is to characterize early alterations in this model. We found a delay in the apparition of the righting reflex and in the acoustic startle response. To further characterize this last deficit, we measured the auditory brainstem response (ABRs), which reflect synchronized discharges from neurons along the auditory pathway and found an elevated threshold profile at P14-P16 and P21-P23 in VPA mice. This shows that the maturation delay observed leads to a lasting alteration in hearing function.

We observed other alterations in the brain at P21: VPA animals show higher GFAP+ density and activated microglia in the hippocampus, but fewer activated microglial cells in the cerebellum.

In summary, we found that mice prenatally exposed to VPA show altered physiological responses at the third postnatal week. Current experiments are testing whether this period is a critical period for autism related behaviors.

Neuroendocrinology and Neuroimmunology

P217.-Interleukin-1β-induced memory reconsolidation impairment is mediated by a reduction in glutamate release and AMPA phosphorylation. α-melanocyte-stimulating hormone prevented these effects.

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The immune system is an important modulator of learning, memory and neural plasticity. Interleukin 1β (IL-1β), a pro-inflammatory cytokine, significantly affects several cognitive processes. Previous studies of our group have demonstrated that the intrahippocampal administration of IL-1β impairs reconsolidation of contextual fear memory. This effect was reversed by the melanocortin alpha-melanocyte-stimulating hormone (α-MSH). The mechanisms underlying the effect of IL-1β on memory reconsolidation have not been established yet. Our results demonstrate that IL-1β produced a significant decrease in the glutamate release from dorsal hippocampus synaptosomes after reactivation of the fear memory. Examination of the cytosolic Ca2+ using Fluo-3AM revealed that the inhibition of glutamate release could be attributed to a reduction in voltage-dependent Ca2+ influx. Also, western blot analysis demonstrated that IL-1β reduced the expression and phosphorylation of GluR1 AMPA subunit. The intrahippocampal administration of α-MSH can modulate these effects. Our results establish a possible mechanism involved in the detrimental effect of IL-1β on memory reconsolidation and also that α-MSH may exert a beneficial modulatory role in preventing IL-1β effects.

P218.-Brain serotonin in a social fish: distribution and effects of an L-tryptophan-enriched diet

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Serotonergic neurons are present in all vertebrates and, even though they are few and scattered within the brain, many behaviors and physiological processes are regulated by their activity. In particular, serotonin (5-HT) is known to be associated with agonistic behavior, stress and reproduction. This monoamine synthesis depends on the amino acid L-tryptophan (TRP) and so, brain 5-HT levels can be indirectly augmented by incorporating TRP in the diet. Cichlasoma dimerus is a local cichlid fish that exhibits notorious asymmetries between subordinate and dominant animals in respect to aggression, stress, and reproductive chance. Considering this, we first aimed to morphologically describe C. dimerus' brain serotonergic system. Secondly, we evaluated the effects of a TRP-supplemented diet on brain serotonergic activity, cortisol, sex steroid hormones and growth in isolated specimens. C. dimerus' brain 5-HT-immunoreactive neurons were found to be located in three main areas, pretectum, hypothalamus and raphe, with no clear differences between males and females. Animals fed with TRP-enriched diets exhibited 1.6 times higher forebrain serotonergic activity and a significant 3.3 times reduction in their relative cortisol levels, with no effects on sex steroid plasma levels or growth parameters. In conclusion, TRP supplement was a successful method to increase 5-HT turnover and reduce stress, with no significant consequences on sex steroid levels or growth.

P219.-The mushroom bodies of crabs: the hemiellipsoid body

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The hemiellipsoid body (HB) is a neuropil located in the lateral protocerebrum of crustaceans. HB is a multi-sensorial integration center where some memory and learning data processing are proposed to take place. Two related structures, mushroom bodies (MB) in insects and pallium in vertebrates appear to share a common origin (Tomer et al 2010). MB is a higher order integration center involved in cross-sensory integration and memory formation. HB has been scarcely studied in true crabs, Brachyura crustaceans, like Neohelice granulata, where several memory processes have been profusely studied. Golgi staining show that, like the MB in insects, the HB globulli cells project to a tract that is subdivided into lobes and claw cells are present (Strausfeld 2015). This neuropil lacks of calyces, a common feature in aquatic arthropods. We used immunohistochemistry methods to explore the expression patterns of neuronal markers previously described in both HB and MB. To explore their function, in vivo assays were made by applying Calcium Green (see poster Maza et al.). Results reveal the expected patterns for allatostatine, synapsin and CAMKII (Harzsch et al. 2012). CAMKII and Calcium green stain facilitate the detection of the globulli cells cluster and allow visualization of its tracts leading to the HB. These findings match the morphological patterns described for the HB and MB in other arthropods and further support the common origin of both structures.

Neuroendocrinology and Neuroimmunology

P220.-Effects of dietary restriction on the hippocampus and behavior of adult mice

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Dietary restriction (DR) has been associated with a reduction of age-associated diseases and to an increase in life expectancy in humans and animal models. Dietary restriction was shown to promote neuronal survival and functionality in the context of neurodegenerative diseases and normal aging. In the present work we aimed at studying the effect of DR on behavioral and neural parameters in 8-month old healthy mice. Daily food consumption was restricted to 60% for 6 weeks and behavioral tests and immunohistochemistry were conducted. We found decreased number of entries to open arms in the elevated plus maze in DR mice in comparison with controls, suggesting a reduction of anxious-like behavior. Mice in DR presented a decreased number of doublecortin (DCX) positive cells in the subgranular zone of the hippocampus, indicating impaired adult neurogenesis. In a parallel study, PDAPP-J20 transgenic mice, model of Alzheimer's disease, suffered 100% mortality when exposed to DR. These results lead us to propose a less severe DR protocol for upcoming studies. We plan to study glial and neuronal parameters in hippocampus and amygdala in response to DR and, concomitantly, to evaluate cellular and molecular pathways involved in an in vitro model of cell starvation.

Neuroendocrinology and Neuroimmunology

P221.-The tyramine receptor TYRA-3 is involved in the neural coordination of oxidative stress response in the model organism C. elegans

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The nervous system plays a pivotal role in the coordination of systemic stress response in multicellular organism. However, the molecular and cellular bases of this modulation are poorly understood. Oxidative stress constitutes one of the most complex forms of systemic stress and plays a major role in the pathogenesis of conditions such as diabetes, cancer and neurodegenerative disorders. In addition, strong evidences have linked increased oxidative stress with aging. Our previous results have related the neuroendocrine tyraminergic signaling, the invertebrate counterpart for adrenergic transmission, and the systemic response to starvation and thermal stress in C. elegans. By analyzing the survival of different C. elegans mutant strains, we evaluated the role of this aminergic signaling in the iron-induced oxidative stress response. Our results suggest that inhibition of tyramine release in the nervous system is essential for mounting an appropriate systemic response to oxidative stress. Moreover we identified the GPCR TYRA-3 as the receptor involved in this neural modulation. We are now evaluating both, the specific cells where TYRA-3 expression is required as well as the molecular pathways underlying this regulation. Our work will contribute to identify cellular and molecular mechanisms involved in neuronal coordination of systemic stress response, leading to a better understanding of the integration between stress sensory perception and the response in non-neuronal cells

Neuroendocrinology and Neuroimmunology

P222.-Immune responses in a model of autism spectrum disorder (ASD)

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It has been proposed that autoimmunity may play a role in the pathogenesis of ASD. However, immune findings in ASD patients are often inconsistent likely due to the heterogeneous, behavior-defined subject groups. Rett Syndrome is an ASD caused by mutations in Methyl Cytosine Binding Protein 2 (MeCP2) and mouse models of Rett have been widely used for studying ASDs. The main goal of our project is to use this monogenic model of ASD, which shows a highly reproducible pathologic phenotype, in order to evaluate the role of altered immunity in the pathogenesis of this disorder. To this end we first evaluated the autoimmune response in the context of the experimental autoimmune encephalomyelitis (EAE). Male MeCP2 WT and MT mice, 9 weeks old, were immunized with MOG 35-55 peptide, scored daily for EAE signs and sacrificed at 11 days post induction (dpi; acute stage) or at 56 dpi (chronic stage). When compared WT-EAE animals with MT-EAE animals, we found that MeCP2 MT mice showed accelerated onset of the disease and more severe clinical scores. Coronal sections of spinal cord were subjected to IHC to analyze the occurrence of activated microglia (Iba-1 and CD11-b) and pro-inflammatory cytokines, and to evaluate the presence of cell infiltration. Our results showed a more severe neuroinflammation in the absence of MeCP2; further studies will determine whether Mecp2 affects the generation of autoimmunity and /or the regulation of neuroinflammation.

Neuroendocrinology and Neuroimmunology

P223.-Role of the cerebellum in the modulation of sociability in the mouse

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Autism spectrum disorder (ASD) is group of complex disorders of brain development. These disorders are characterized, in varying degrees, by difficulties in social interaction, verbal and nonverbal communication and repetitive behaviors. The exact causes of these disordes remain unclear. Recently the cerebellum has emerged as one of the key brain regions affected in autism. To evaluate the role that the cerebellum plays in behaviors related to autism, we used a mouse model of ASD. Mice prenatally exposed to valproic acid (VPA) at gestational day 12.5 (GD 12.5) showed reduced social interaction in adulthood and signs of neuroinflammation in the cerebellum. Moreover, the injection of an inflammogen in the lobule VII of the cerebellum of adult mice caused reduced social interaction and an increase in activated microglia. These effects were absent when we injected the lobule IV/V, suggesting that specific structures are involved in the modulation of sociability. Our working hypothesis is then that lobule VII is implicated in the regulation of sociability in mice. The aim of this work is to study the structure and the funtion of the lobule VII in the cerebellum in different stage of development in mice. To this aim, we quantified the density of Purkinje cells in the cerebellum of VPA and control animals, at different postnatal ages. We hope that this study will help us understand the role of the cerebellum in the regulation of the sociability in a mouse model of ASD.

Neuroendocrinology and Neuroimmunology

P224.-Altered mitochondrial membrane lipid profile in the hippocampus of chronic ovarian hormone-deprived rats and its putative association with mitochondrial dysfunction

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Functional mitochondria are key for a healthy brain and their dysfunction is involved in age-related disorders. Ovarian hormone loss during reproductive senescence is associated with mitochondrial dysfunction, brain energetic deficits and increased risk of neurodegeneration. Also, mitochondria functional integrity is highly dependent on their membrane lipid composition, mainly cardiolipin content and the nature of its acyl chains, parameters that are also affected during aging.
The aim of this work was to study the putative association between previously shown mitochondrial dysfunction and their membrane lipid profile induced by chronic ovarian hormone deprivation in the hippocampus, a highly hormone-responsive area primarily affected during aging. We analyzed membrane fatty acid composition and phospholipid content in hippocampal mitochondria from long-term ovariectomized or sham-operated Wistar rats. Mitochondria from OVX rats showed higher levels of polyunsaturated fatty acids with similar cholesterol levels. Interestingly, they also showed lower cardiolipin content containing a higher proportion of saturated acyl side-chains, indicating abnormal cardiolipin molecular species and suggesting alterations in its remodeling.
Our results indicate that OVX rat mitochondria exhibit a lipid membrane composition comparable to an aging phenotype. This lipid profile could account for mitochondrial dysfunction in the hippocampus of chronic ovarian hormone-deprived animals.

Sensory Systems

P225.-Mathematical modeling of the electromechanic processes that underlie the vibrissal system mechanotransduction: A study over the passive perception of tactile information

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In the present study we are proposing a mathematical model able to predict the afferent discharges of the afferent vibrissal nerve. Vibrissal passive stimulation has been used as the model signal input. The mechanisms of mechanotransduction within follicle-sinus complex were implemented by using the model of Lottem and Azouz (2011), which uses the rigid body model principle and predict neuronal responses evocated by unidirectional passive stimulation. In this work we have modelling the vibrissal system considering the changes in the neuronal responses due to the directional sensibility. The simulations obtained have been validated with experimental recording of the afferent activity of the vibrissal nerve evocated by vibrissae passive stimulation in different directions. To fit the experimental data to the modeling results, we implemented a simplified model for electrophysiological activity in a bundle of myelinated nerve fibers. Finally, the result demonstrated that it is possible to predict the afferent nerve discharges in passive conditions by using the model proposed.

Sensory Systems

P226.-Development ofaA New Experimental Model of Primary Optic Neuritis

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Optic neuritis (ON) involves inflammation, demyelination, axonal injury in the optic nerve, retinal ganglion cell (RGC) loss, and visual dysfunction. We investigated the ability of a single injection of bacterial lipopolysaccharide (LPS) directly into the optic nerve to induce functional and structural alterations compatible with ON. For this purpose, optic nerves from male Wistar rats were injected with vehicle or LPS. At several time points post-injection, we analyzed: i) visual pathway function (visual evoked potentials (VEPs), ii) anterograde transport from the retina to its projection areas, iii) consensual pupil light reflex (PLR), iv) optic nerve structure, v) microglia/macrophage (by Iba-1- and ED1-immunostaining), vi) astrocytes (by glial fibrillary acid protein-immunostaining), vii) axon number (by toluidine blue staining), vii) demyelination (by myelin basic protein immunoreactivity and luxol fast blue staining), viii) optic nerve ultrastructure, and ix) RGC number (by Brn3a immunoreactivity). LPS induced a significant and persistent decrease in VEP amplitude and PLR, a deficit in anterograde transport, and an early inflammatory response consisting in an increased cellularity, and Iba-1 and ED1-immunoreactivity in the optic nerve, which were followed by changes in axonal density, astrocytosis, demyelination, and axon and RGC loss. These results suggest that the microinjection of LPS into the optic nerve may serve as a new experimental model of primary ON.

Sensory Systems

P227.-The angle of light polarization as a source of visual contrast in Neohelice granulata

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Polarization vision is used by many different species in vital tasks, such as orientation, navigation, prey detection and communication. Neohelice granulata, like other crabs, lives in an environment rich in polarization information. A prior experiment showed that a looming stimulus with the same intensity and spectral light composition that the background, but a 90º difference in the angle of polarization of light evoked the animal's escape response. Thus, this species is able to detect moving stimuli using only polarization cues.

Our goal now was to assess if Neohelice senses differentially stimuli polarized with different angles. To achieve this, we presented looming stimuli in a modified LCD screen, placed to one side of a sphere where the animals could walk freely. The monitor was mounted on a rotating device. The stimuli held a 90º polarization contrast with the background, but by rotating the ensemble, the two main polarization vectors entered the crab's eye at different angles.

We quantified the escape response and found it to be greater for those stimuli for which the two E-vectors' angles were vertical and horizontal. This result suggests that Neohelice's photoreceptors have a maximal sensitivity for vertically and horizontally polarized light. This interpretation is consistent with anatomical studies that demonstrate an orthogonal disposition of the rabdomere's microvilli in the decapod's eyes.

Sensory Systems

P228.-Codification of visual cues by parallel channels in the third optic ganglion of an arthropod

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Visual scenes comprise enormous amounts of information from which nervous systems extract and process behaviorally relevant cues. Here, we study how visual information is segregated in high order visual centre in a highly visual crab. Crab's optic lobe encompasses three serially arranged ganglia built from repetitive neuronal columns transected by horizontal neuronal layers.

Studies in flies show that outer horizontal layers of the second optic ganlgion differentially codify light-On/Off stimuli while layers of the third ganglion codify the four cardinal directions of optic flow motion. Here we study through calcium imaging if the three horizontal input layers of the crab´s third optic ganglion differentially codify light-On/Off stimuli or motion direction.

On and Off step changes in light intensity evoked similar calcium peak responses and similar times of recovery of the signal in the three input layers. However, both peak and times of recovery differed between the On and Off stimuli. To study the codification of motion direction we presented 14 black edges translating horizontally in one direction and the 15th one in the opposite direction, thus, we expected to reveal the strata involved in coding direction. The calcium response in the 15th trial was similar for the three input strata. However, this response is significantly higher than the response to a 15th with no change in direction. Our results suggest the existence of deferential visual coding at crab's lobula.

Sensory Systems

P229.-Activity of the α9α10 nAChR inversely correlates with the magnitude of acoustic injury.

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Noise induced hearing loss (NIHL) has become a major public health problem. In order to address the role of the efferent olivocochlear system in NIHL we made use of a mouse model in which the α9 nicotinic receptor subunit bears a mutation and leads to enhanced medial efferent activity (Chrna9L9'T knock-in (KI)) in addition to one lacking the a9 subunit of the nicotinic receptor (Chrna9 knockout (KO)).
We exposed WT, Chrna9L9'T KI and Chrna9 KO mice to loud sounds (1-16 kHz, 100 dB SPL, 1hr) and measured auditory brainstem responses (ABR), which reflect synchronized discharges from neurons along the auditory pathway. We tested outer hair cell function by recording the distortion product otoacoustic emissions (DPOAEs). Large auditory threshold shifts were found one day after exposure in WT and Chrna9 KO mice. However, one week later, thresholds returned to normal in WT, whereas the Chrna9 KO ears did not recover. In contrast, Chrna9L9'T KI mice were resistant to the same noise exposure. Finally, we used immunohistochemistry to visualize efferent neurons and found a reduction in the number of terminals after trauma in WT mice. Immunofluorescence against Ctbp2, a protein located in inner hair cell (IHC) ribbon synapse, revealed a decrease in the number of ribbon synapses per IHC after acoustic trauma in WT mice. These findings suggest a key role of α9α10 nAChRs in the efferent-mediated noise protection.

Sensory Systems

P230.-Dynamic of neuronal degeneration and regeneration in the olfactory epithelium of Xenopus laevis triggered by axotomy

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The olfactory epithelium (OE) has the unique capacity to regenerate the olfactory receptor neurons (ORNs) throughout life. The neurogenesis involves proliferation of neural progenitors (NP) in the basal layer of the OE. This is followed by progressive differentiation in which the neuronal precursors migrate to the middle layer where finally the ORNs somas remain. In the present work, we studied the dynamic of neuronal degeneration in the OE after bilateral axotomy of the olfactory nerves in premetamorphic tadpoles of Xenopus laevis, and the subsequent regeneration. We found that axotomy induced neuronal death by apoptosis between the first 24 and 48h post injury. In concordance, there was a progressive decrease of mature ORNs marker OMP until it was absent 72h post injury. On the other hand, neurogenesis started 24h post injury with increased number of proliferating NP. The differentiation process was already evident 48h post injury by the increase of Gap 43 positive immature neurons. Mature ORNs were replenished 21 days post injury and the olfactory function was recovered, indicating that new ORNs were integrated to the olfactory bulb glomeruli. Taken together, our work is the first sequential analysis of the processes triggered by axotomy in the OE. Moreover, we demonstrated correlation between morphological and behavioral events. Furthermore, this supports the amphibian OE as a model to studying apoptosis, neural progenitor proliferation and neuronal differentiation.

Sensory Systems

P231.-Visual input is necessary to achieve retinal neuroprotection in an enriched environment

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Enriched environment (EE) is defined as a complex combination of inanimate and social interaction. In EE, several animals are housed in big cages, with frequently changing objects, thus stimulating exploratory conduct, voluntary physical exercise, enhanced visual and cognitive functions, and social interaction. However, the relative contribution of each of these components to the effects of EE is still controversial. Different groups have described the effects of the environment on visual plasticity during development. However, up to now, there was no information on the effect of the environment on the retina of adult animals, originally considered a non-plastic tissue. For the first time, we have shown that the exposure to EE protects the retinal function and histology from acute unilateral retinal ischemia and diabetic retinopathy in adult rats. Then, we aimed to dissect the contribution of social, motor and visual stimuli to the neuroprotection induced by EE. When ischemia was bilaterally induced, the protection triggered by EE was abolished, suggesting that the visual input, likely regardless of social and/or motor components, was a necessary condition within EE to achieve retinal neuroprotection. These results suggest that, at least for the visual pathway, visual stimuli and, probably, its central processing, could account for the retinal neuroprotection induced by EE, which could become the first evidence of the visual contribution in the protective effects of EE.

Sensory Systems

P232.-The exposure to enriched environment prevents retinal ischemic damage

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Ischemia is a key component of several retinal diseases that are leading causes of irreversible blindness. At present, there are no effective strategies to prevent retinal ischemic damage. We have demonstrated that the exposure to an enriched environment (EE) after retinal ischemia reduces functional and histological alterations induced by ischemia. EE constitutes a strategy that boosts exploratory, visual, and cognitive activities, social interaction and voluntary physical exercise. In this context, the aim of the present work was to analyze the effect of EE housing before acute retinal ischemia damage. For this purpose, adult male Wistar rats were exposed to standard environment (SE) or EE for 3 weeks before retinal ischemia. EE consisted of big cages housing 6 animals and containing several food hoppers, wheels and different objects repositioned once/day and fully substituted once/week. Ischemia was induced by increasing intraocular pressure to 120 mm Hg for 40 min. After ischemia, both groups were housed in SE for 3 weeks, and subjected to electroretinography (electroretinogram, ERG) and histological analysis. In animals previously housed in SE, ischemia induced a significant decrease in ERG a- and b-wave amplitude, and retinal ganglion cell (RCG) loss, whereas the exposure to EE significantly prevented these alterations. These results suggest that the EE housing, a non-invasive strategy, could reduce retinal vulnerability to ischemic damage.

Sensory Systems

P233.-Neural basis of intensity invariance in olfaction

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Numerous animals rely on olfaction to extract ecologically important information from the environment. At olfactory sensory neurons, the different odors are encoded as combinatorial patterns of finite number of receptors. Yet in natural conditions, meaningful odors are present at different concentrations that produce differences in both intensity and combination of receptors activation. Thus, a central problem is how the system is able to recognize the same odor across different concentrations in spite of different input patterns. In this project we work on the hypothesis that local inhibition at processing of the olfactory information provides the gain control that stabilizes odor identity irrespective of odor intensity. We use honey bees as model animal for studying odor generalization across concentrations and to understand the neural computations that underlie generalization. In olfactory learning experiments we observed an asymmetric behavioral generalization from high to low concentrations and to a less degree from low to high concentration. Using calcium imaging we measured the neural representation of high and low odor concentrations in the antennal lobe. The results are analyzed in terms of the algorithm used by postsynaptic neurons that link combinatorial patterns with odor identity.

Sensory Systems

P234.-Can you see it? Polarized light stimuli startle goldfish

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Aquatic environments are rich in polarized light patterns, creating a background polarization field against which objects, which diffuse or differentially reflect polarized light, can be viewed. To an animal with a visual system sensitive to polarized light such as common goldfish, Carassius auratus, these cues could provide valuable information about its environment and be used for navigation and object detection.
To test to which extent their polarization sensitivity has behavioral significance, we recorded their startle response (C-start) in response to an expanding disc (loom) in two situations: 1) intensity contrast, where a black disc expanded on a white background and 2) polarized light contrast, where the background light was linearly polarized and the loom was elliptically polarized. These last stimuli were projected with a modified LCD screen. The question under scrutiny is if stimuli where the only source of contrast is the polarization angle between object and background (no intensity contrast) are conspicuous enough to trigger a startle reaction.
Preliminary results show that polarized light contrast stimuli are salient enough to trigger a C-start although with a smaller probability (40% vs. 100% for intensity contrast stimuli). The results are discussed in the context of the different sources of visual information available and the relative contributions of different properties of light for threat detection.

Sensory Systems

P235.-Searching for the optomotor response processing center in the crab Neohelice

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The crab Neohelice offers significant methodological advantages for the study of general principles of visual information processing, including the possibility of recording intracellularly the response to visual stimuli of neurons in the intact animal. So far our studies focused on neurons that participate in detection of external objects moving. When a rotational movement of the visual panorama occurs, animals, from invertebrates to humans, tend to stabilize the movement through compensatory movements of the eyes or your whole body. This is known as the optomotor response. Our knowledge about the mechanisms involved in the perception of visual flow and the optomotor response derives significantly from research in flies. In Diptera, the lobula plate is an elongated neuropil found in the optic lobes. It was concluded that the tangential neurons present in this neuropil are involved in the execution of optomotor responses and the control of the flight. Our histological results in Neohelice show the existence of a neuropil alike the lobula plate of flies: similar connections within the optic lobe and the presence of tangential neurons fed by columnar inputs. The presence of a comparable lobula plate supports the theory that there is a close phylogenetic relationship between crustaceans and insects. To further prove the role of the lobula plate in crabs, we are performing intracellular recordings to explore the physiological properties of its tangential neurons.

P236.-Transcranial Ultrasound Axicon-Driven Activation of the Motor Cortex in Anaesthetised Mice

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In this work we study the acoustic characteristics of ultrasonic transducers for transcranial transmission of focused ultrasound with the addition of axicon lenses. We made a comparison between this combination and a standard transducer directly coupled for transmitting pulsed ultrasound through the skin and skull into the intact brains. We implemented an ultrasonic propagating model based on a k-space pseudo-spectral scheme in 2D that takes in consideration the acoustic velocities and densities of the liquid in the transducer-lens interface, the lens material and the biological tissue.

The setup used in this case was: 140° Axicon lens–Epoxy resin, 0.45MHz–Ø28mm transducer, vacuum oil, Stand-off: 30mm.

The motor cortex of CF-1 mice (n=3) was then stimulated with pulsed ultrasound (160c/p, 1kHz PRF, 300 pulses) having an ISPTA = 87mW/cm². Evoked motor responses in different body segments could be clearly observed, including whiskers, hindlimb, forelimb and tail.

Axicon lens produce a narrow beam at the focus, compared with non-focused transducer. In this case we have a focus, with a -6dB drop, smaller than 2.0 mm in diameter which is approximately five times better than the lateral spatial resolution offered by other more conventional noninvasive brain stimulation methods. Out of the focus the sound pressure decreases with a very steep slope. Based on those observations, it is reasonable to expect that brain regions <1.0 mm may be accurately targeted for neurostimulation.

Synaptic Transmission and Excitability

P237.-Mimicking Human Myasthenic Syndromes in C. elegans: Evaluation of Function and Drug Modulation of Nicotinic Receptors

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In humans, gain-of-function mutations in the muscle nicotinic receptor (AChR) lead to slow-channel congenital myasthenic syndromes (SCCMS), characterized by slow decay of endplate currents, destabilization of the closed channel and prolonged activation episodes of AChR. Our goal is to use the free-living nematode C. elegans to generate models of these human syndromes. To this end, we first generated transgenic worms expressing mutant L-AChRs at 9' position of the M2 segment, which has been shown to form the gate of the ion channel in vertebrates. Electrophysiological recordings of L-AChRs from muscle cells of these transgenic worms show an increase of 11- to 14-fold of the open-channel lifetime and decreased desensitization rate with respect to wild-type, as expected for a gain-of-function mutation. We found that quinidine sulfate, a long-lived open-channel blocker of the human AChR used for the treatment of SCCMS, also reduces the open duration of the mutant C. elegans L-AChR. These results show that it is possible to mimic in C. elegans the molecular and functional changes observed in human AChRs as well as their responses to therapeutic drugs. We next generated mutant strains with L-AChRs mimicking gain-of-function mutations that lead to severe slow-channel CMS to be used as models of these human neuromuscular disorders for drug screening and development of therapeutic strategies.

Synaptic Transmission and Excitability

P238.-MLC phosphorilation and pH affects vesicle release mode at the mouse neuromuscular junction

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Carbonic anhydrase (CA) is an enzyme that regulates pH inside and outside the cell. Acetazolamide (AZ) is a specific inhibitor of CA that is used to treat several diseases like epilepsy or ataxia, but its mechanism of action is still unknown

We combined fluorescence and electrophysiological techniques at ex vivo levator auris longus neuromuscular junctions (NMJ) from mice, in order to study if AZ modulates synaptic transmission.

We have previously shown that AZ reduced significantly the fluorescence transients in vesicle load and unload studies with FM 2-10 dye in bicarbonate buffer (BB). In contrast we observed that AZ induces only a 20.12 % reduction quantal content of transmitter release at 0.5 Hz and no changes in the time course of EPP´s amplitudes at 50 Hz frequency stimulation. These results suggest a change on vesicle fusion mode. To clarify this possibility, we applied in the presence of AZ, a fast quencher, bromophenol blue, able to get through a small fusion pore, before the unload phase. We observed similar results in control and AZ treated muscles supporting the idea that AZ may shift vesicle recycling to a fast (kiss and run) mode.

Inmunohistochemical studies revealed a small activation of MCKL enzyme by AZ and a more intense activation when is combined with stimulation.

These data suggest a relation between pH changes and synaptic transmission vesicle recycling mediated by MKCL activation.

*Both authors contributed equally to this work

Synaptic Transmission and Excitability

P239.-Strength of the efferent olivocochlear system modifies the normal activity of a central auditory nuclei

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The auditory system in many mammals is immature at birth but precisely organized in adults. Spontaneous activity in the inner ear comes into play to guide this process. This activity is modulated by an efferent pathway that descends from the brain. In this work, we used a mouse model with enhanced medial efferent activity (Chrna9L9'T, KI) to understand the role of the olivocochlear efferent system in the correct establishment of auditory circuits.

We measured auditory brainstem responses, which represents synchronized activity of neurons along the auditory pathway. Wave I amplitude (activity of cochlear nerve fibers) was the same for WT and KI. However, wave III (activity of synapses within the MNTB) was smaller in the KI suggesting a central dysfunction. In order to analyze this functional observation, we studied the underlying mechanism on brain slices containing the medial nucleus of the trapezoid body (MNTB). Several electrophysiological properties measured in current-clamp (resting membrane potential, input resistance, action potential amplitude, number of spikes during a current pulse injection) and in voltage-clamp mode (Ih current) were topographically organized along a medio-lateral axis in WT. However, these tonotopic differences were abolished in the KI. Our preliminary data suggest that the efferent pathway could be involved in the refinement of the tonotopic map along the auditory pathway.

Synaptic Transmission and Excitability

P240.-Effect of purinergic receptor antagonists on high K+- and electrically-evoked ACh secretion at mammalian neuromuscular junctions (MNJ)

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At mammalian NMJ, ATP and its metabolite adenosine decrease ACh release by activation of presynaptic P2Y12-13 and A1 receptors (R), respectively. Our aim was to analyze the effect of endogenous purines on K+- and electrically-evoked ACh secretion. In phrenic-diaphragm preparations (CF1 mice), we studied the action of selective antagonists of P2Y12-13R (2 µM AR-C69931MX) and A1R (0.1 µM DPCPX) upon MEPP frequency (10, 15, 20 mM K+) and on EPP amplitude when the nerve was stimulated at 0.5, 5 or 50 Hz (train or bursts). AR-C69931MX induced an increase in MEPP frequency at 10, 15 and 20 mM K+ whereas DPCPX did not modify asynchronic secretion at 10 mM K+, but provoked a significant increase at 15 and 20 mM K+. On the other hand, AR-C69931MX and DPCPX did not change the amplitude of the first EPP in any of the studied frequencies. At 50 Hz (750 pulses or 5 bursts of 150 pulses), the antagonists diminished the declination of EPP amplitude that normally occurs during repetitive stimulation. There was not a significant difference at 0.5 and 5 Hz. We suggest that depolarization of motor nerve endings by high K+ concentrations or 50 Hz-stimulation generates endogenous ATP/ADP and adenosine able to modulate ACh secretion. These findings raise the possibility that selective P2Y12-13R and A1R antagonists can be used therapeutically to assist in the treatment of neuromuscular disorders such as myasthenia gravis, in which the prejunctional depression can produce life-threatening effects.

Synaptic Transmission and Excitability

P241.-Functional changes in synaptic transmission at the mouse medial olivocochlear (MOC)-inner hair cell synapse during neonatal development

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Inner hair cells (IHC) are innervated by MOC fibers since birth to the onset of hearing (postnatal day 12, P12). At P9-11 ACh release is supported by P/Q- and N-type VGCC and negatively regulated by L-type VGCC coupled to BK channels. The transient nature of the MOC-IHC synapse suggests there might be changes in the properties of synaptic transmission throughout this period.

Short term synaptic plasticity (STP) is the change in synaptic strength (facilitation/depression) as a consequence of preceding activity. We have shown that stimulation trains at 10, 40 and 100 Hz led to depression at P9-11 whereas at P6-7 the 10 Hz train led to facilitation and the 40 and 100 Hz trains to facilitation followed by depression. We now found that at P4 synapses trains at 10, 40 and 100 Hz caused a 1.5±0.7; 2.2±0.6 and 2.0±0.6-fold increase, respectively, in the ratio between the amplitude of the tenth and the first evoked synaptic current, indicating that at this stage there is facilitation even at the highest frequencies tested. Consistently, both the quantal content (0.3±0.1 P4; 0.8±0.1 P6-7; 1.6±0.2 P9-11 $p<0.001$) and the ready releasable pool of vesicles (1.6±0.2 P4; 4.7±0.9 P6-7; 10.7±1.9 P9-11), $p<0.003$) increased during development. Moreover, we found changes in the types of VGCC coupled to release at the different stages. Our results show that the MOC-IHC synapse undergoes significant changes in the STP pattern and in the VGCC that support release.

Support: UBA&ANPCyT to EK and ABE

Synaptic Transmission and Excitability

P242.-Evaluating a possible crosstalk between inhibitory and excitatory calcium signals in inner hair cells of the developing inner ear

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Altricial rodents do not respond to sound until their second postnatal week. Before the onset of hearing, cochlear inner hair cells (IHCs) fire sensory-independent action potentials sustained by voltage-dependent calcium channels. The influx of calcium triggers the release of glutamate to afferent dendrites of the auditory nerve, determining an excitatory role for calcium ions.

At this stage, IHC are also innervated by efferent cholinergic neurons, projecting from the brainstem. This synapse combines the entry of calcium through α9α10 nicotinic receptors with the activation of nearby SK2, calcium dependent potassium channels, to hyperpolarize and inhibit IHCs. Thus, calcium can have these two contrary roles within a diffusionally compact cell.

Electron-micrographs of IHC exhibited thin near-membrane cisterns juxtaposed to efferent synaptic contacts. Imaging experiments have shown multiple calcium entry hotspots following activation of efferent fibers. These domains would be spatially segregated from those observed after IHC depolarization. In order to understand the physiological implications of such proximity, we have performed whole cell patch clamp recordings of afferent terminals. We found that upon high frequency stimulation of efferent fibers, calcium was capable of eliciting release of glutamate to afferent terminals. Thus, we suggest that intracellular mechanisms in IHC are adapted to prevent crosstalk between these synapses.

Synaptic Transmission and Excitability

P243.-Kv1.3 is a candidate target to prevent the hypercholinergic state of parkinsonism

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Balanced actions of dopamine (DA) and Acetylcholine (ACh) shape striatal function. In Parkinson´s disease (PD) this balance is lost, leading to a hypercholinergic state. The main source of striatal Ach is a small group of striatal cholinergic interneurons (ChIs). Previously we found that ChIs are hyperexcitable in a rat model of PD as a result of a lack of "accommodation". Our aim is to identify currents that regulate ChI accommodation in mouse brain slices. Margatoxin (MgTx), a blocker of Kv1.3 channels, markedly attenuated accommodation in ChIs, as shown by an increase in the number of spikes ($p=0.003$) and a prolonged firing ($p=0.008$) during a depolarizing current step. MgTx also increased spontaneous firing ($p=0.0455$). We have isolated and characterized the MgTx-sensitive current (Imax=1500 pA). Immunohistochemistry in brain sections and PCR of laser dissected ChIs revealed the expression of Kv1.3 channels. Thus, ChIs express a functionally relevant Kv1 conductance. Then we evaluated the influence of endogenous DA on accommodation. Confirming previous findings, fewer ChIs show accommodation in a mouse model of PD induced with 6-OHDA. This hyperexcitability is associated to smaller MgTx-sensitive currents in ChIs of 6-OHDA-lesioned mice compared to sham-mice ($p<0.0001$). Our data show that chronic nigrostriatal lesions reduce the MgTx-sensitive current in ChIs, causing their hyperexcitability, and nominate Kv1.3 channels as potential new targets of antiparkinsonian therapy.

Synaptic Transmission and Excitability

P244.-A point mutation in the α9α10 nAChR alters short-term synaptic plasticity of medial olivocochlear- hair cell (MOC-HC) synapses

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IHCs convey acoustic information to the central nervous system while OHCs are responsible for the mechanical amplification of sound. IHCs receive a transient MOC innervation since birth to the onset of hearing, while MOC fibers synapse onto OHCs from the first postnatal week throughout adulthood. The MOC-HC synapse is inhibitory and mediated by α9α10 nicotinic receptors (nAChRs). We analyzed the properties of synaptic transmission of a knock-in mouse (Kin) with a point mutation in the α9 nAChR subunit (L9´T) that prolongs MOC inhibition (Taranda et. al 2009). Synaptic currents (IPSCs) were recorded in IHCs and OHCs of isolated mouse cochleas at postnatal day 9-13 during electrical stimulation of MOC fibers. In previous studies we showed that high frequency stimulation causes synaptic depression in MOC-IHC synapses, whereas it causes facilitation in MOC-OHC synapses. We found that in both wt and Kin IHCs, 100Hz-trains applied to the MOC fibers caused depression of IPSC amplitudes (S10/S1: 21% and 10% in Kin and wt mice, respectively) whereas 10Hz-trains caused depression only in Kin mice (S10/S1:60%). Accordingly, the ready releasable pool size was smaller in Kin mice (wt:3.7±0.9 Kin:2.7±0.8). Preliminary experiments in OHCs show that high frequency stimulation (40-80 Hz) caused 3-fold more facilitation in Kin than in wt mice. These results show that a modification in the postsynaptic nAChR alters the short term plasticity pattern of MOC-HC synapses.

Support UBA & ACyPT

P245.-Organize 12 school visits and entertain 400 kids in 2 weeks and live to tell the story: What we learned from "Neuroscience of the Senses" during BAW 2015 in Córdoba Elementary schools

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Thanks to a grant from the Argentinean Society for Neuroscience Research (SAN) we participated in Brain Awareness Week 2015 with classroom activities in 4th grade. "Neuroscience of the Senses" explores the function of the brain with respect to the five senses by means of games and hands-on demonstrations. The project started with preparing the materials to take to the classrooms, the instructor´s manual and contacting recipient schools. 14 instructors were split in couples to reach classrooms of 12 elementary schools imparting more than 30 classes. The instructors were mainly advanced PhD students from the public-outreach group "Jóvenes Investigadores de Neurociencias", as well as some investigators from Instituto Ferreyra. Two instructors would arrive at their designated school with a box bearing all necessary materials and equipment for the class, which in some cases included carrying a microscope and a projector. Instructors were previously trained in group sessions with others prospective instructors and the help of a manual that also included a suggested script. The ultimate goal is to build a periodic activity for BAW and to help colleagues to repeat it in their cities. During the poster presentation we are going to share more details of the activity and the wonderful experience of interacting with the most curios creature in our galaxy: 10 years-old kids of H sapiens sapiens. This could not have been done without the help from SAN, INIMEC, SECYT-UNC, CONICET AND JIN.

P246.-Brainazategui: the first BAW experience at Buenos Aires south metropolitan area.

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The Berazategui-BAW was carried out on March 20-21 in Berazategui, Buenos Aires. On Friday 20th, a "Neuro-Fair" was held consisting in stands curated by scientists from different neuroscience labs from the Buenos Aires area, covering topics such as sleep, memory, visual and auditory perception, biological rhythms, development of the nervous system, and brain anatomy, among others. The stands and displays were specifically designed for a high-school level audience. Groups of students received an introductory talk about the importance and scope of neuroscience research. In the afternoon, the exhibition was opened to the general public and several popularization lectures were presented by researchers from the National University of Quilmes, covering themes such as perception, chess and neurosciences, compared brain anatomy, and common myths in neuroscience. On Saturday, the activities were taken to the town's central pedestrian avenue, where an auditorium of a hundred chairs was mounted for three special stand-up presentations: "Neuro-magic" by Dr. Andrés Rieznik, "Neuroscience with your hands" by Dr. Rodrigo Laje, and "That damn memory" by Dr. Pedro Bekinschtein. A selection of stands was also offered to the street public. We estimate that the event reached an audience of over 1000 people, an outstanding number being this the first BAW event in the province. The importance of having reached some hundreds of young students (i.e. potential neuroscientists) cannot be overstated.

P247.-Brain Awareness Week II in La Plata: “The Chemical Brain”

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This time we focus on the different features of the brain, and in particular, the chemical aspects related with its function. We organized a continuous exhibit where we offered a wide range of activities designed to spark the public's curiosity and reflection about neurosciences and its importance to human health, arranged in a circuit of different stations. The 1st station consisted in a brief introductive talk. The 2nd one consisted in a participative demonstration of brain samples from different species -frog, cockroach, cow, rat and mouse- and in observation of neurons in brains slides with microscopes. In the 3rd station we made live measurements of the electric neural activity of cockroaches, using a Spikerbox and a laptop. The 4th station focused on the effect of chemicals (ethanol and vinegar) on the behavior of fruit flies. In the last station public experimented with taste and smell senses by performing very simple tests. At times, we also carried out a representation of neurotransmission using a giant neuron model. We also invited Dr. Zaratiegui, a recognized psychiatrist and a reference in psychopharmacology, who gave an interesting and general public oriented talk on common psychotropic drugs. The event was a very enjoyable and successful experience, with great feedback from the broad range of attending public. La Plata BAW has had a great impact in our community as we have been convoked by many local and national organizations several times during the year.

P248.-Working in Neurosciences today. BAW 2015 IBCN

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The International Brain Awareness Week (BAW) took place from March 16th to 20th in the Institute of Cell Biology and Neuroscience "Prof. E. de Robertis" (IBCN), Facultad de Medicina, Universidad de Buenos Aires. The activities were sponsored by the Sociedad Argentina de Investigación en Neurociencia (SAN) and were aimed to the general public.
The speakers exposed the following topics: Neurodegenerative Diseases, Epilepsy, Fetal Alcohol Syndrome, Addictions, Development of the Visual System, Research in Argentina, Animal models in research, Stroke, Developmental Neurobiology, Memory and Learning, Behaviour and Epilepsy, Techniques in Electronic Microscopy.
The activities were broadcasted on radio, TV and newspapers (Clarin and Pagina/12). The number of participants exceeded 200 people. The audience consisted of secondary and university students, employees such as therapeutic assistants, radiologists, nutritionists, doctoral fellows of CONICET and psychologists.
Attendees were very enthusiastic, which was demonstrated by the large number of questions raised during the discussions. Since there are few opportunities for the general public to interact with researchers, people suggested that this kind of activities should be maintained in the future.

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