Latin American Training Program 2018

"From molecules to behavior - the quest for new treatments of neuropathologies"

Assemble a local organizing committee to develop the course content, identify faculty, and select fifteen Fellows.

1. Andrés Chávez
2. Pablo Moya
3. Agustín Martínez
4. Carlos González
5. Patricio Orio
6. Ana María Cárdenas
7. Kathleen Whitlock
8. Ramón Latorre
9. Alan Neely
10. Helmuth Sánchez
11. Chaiyu Chiu
12. Tomás Pérez-Acle
13. Danilo González
14. John Ewer
15. Oliver Schmachtenberg

Course Director: **Juan C. Sáez**

Latin American Training Program 2018
"From molecules to behavior - the quest for new treatments of neuropathologies"

Preliminary program

Sunday, August 26th.

17:00-19:00. Reception for graduate students and postdoctoral fellows

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Monday, August 27th.

MODULE 1. FROM ION CHANNELS TO NEURONAL NETWOK ACTIVITY

Chair: John Ewer

9:00-9:10 "Opening remarks to the school by the director of the CINV"- **Ramón Latorre**

9:10-10:00 "Developmental Neurogenetics and Behavior using Zebrafish as a Model System". **Kathleen Whitlock.**

10:00-11:00 "Illuminating chemical synapses to dissect neural circuits in the brain". **Chiayu Chiu.**
11:00-11:30 Coffee Break

11:30-12:30 "Fundamental mechanisms of neuronal excitability". **Ramón Latorre**

12:30:14:30 Lunch Break

14:30-17:00

Hands on

Laboratory Rotation

Tuesday, August 28th

Chair: Agustín Martínez

9:00-10:00 "Axonal translation and trafficking of plasma membrane proteins". **Andrés Couve"**

10:00-11:00 "Functional membrane protein synthesis by isolated squid giant axons" **Ramón Latorre**

11:00-11:30 Coffee Break

11:30-12:30 "The visual system: From the retina to the brain". **Francois Paquet-Durand**

12:30:14:30 Lunch Break

14:30-17:00 Hands on

Rotation in different labs.

Wednesday, August 29th

Chair: Oliver Schmachtenberg

9:00-10:00 "Synaptic Integration". **Diamond, Jeffrey**

10:00-11:00 "Signal transduction mechanisms beyond the dopamine receptor". **Angus Nairn.**

11:00-11:30 Coffee Break

11:30:12:30 "Serotonin and synaptic transmission at a central synapse" **Andrés Chávez**

12:30:14:30 Lunch Break

14:30-15:30 "Protons and Acid Sensing Ion Channels (ASICs) role in synaptic transmission" **Osvaldo Uchitel**

15:30-18:00 Hands on

Laboratory Rotation

Thursday, August 30th

Chair: Chiayu Chiu

9:00-10:00. “Protein phosphorylation and dephosphorylation in the central nervous system”. **Angus Nairn.**

10:00-11:00. "A Spike Timing-dependent plasticity rule for single, distributed, and clustered dendritic spines". **Roberto Araya.**

11:00-11:30 Coffee Break

130:00-12:30. “Visual Computation in the Retina”. **Jeffrey Diamond**

12:30:14:30 Lunch Break

14:30-15:30 “The diversity of neurodegenerative mechanisms: Apoptosis, Necrosis, or what?” **Francois Paquet-Durand.**

15:30-18:00 Hands on

Laboratory Rotation

Friday, August 31st

Chair: Juan C. Sáez

9:00:10:00. “Input transformation by dendritic spines of pyramidal neurons”. **Roberto Araya.**

10:00-11:00. “ NO signaling in the retina”. **Oliver Schmachtenberg**

11:00-11:30 Coffee Break

11.30-12:30. “Cannabinoid signaling in the retina”. **Andrés Chávez**

12:30:14:30 Lunch Break

14:30-15:30 “From basic research to clinical translation: What do you need to make it happen?” **Francois Paquet-Durand.**

15:30-18:00 Hands on

Student presentation and discussion of projects to be developed during the following two weeks.

Saturday, September 1st

MINISYMPOSIUM ON ION CHANNELS

Chair: Patricio Orio

9:00-9:30 "Gating mechanisms of H^+ channels". **Carlos González**

9:30-10:00 " Fine tuning the activation of calcium ion channels". **Alan Neely**

11:00-10:30 "Regulation of connexin hemichannels by Ca^{2+}". **Helmuth Sánchez**

10:30-11:00 Coffee Break

11:00-11:30 "Voltage gating regulation of hemichannels"**. Isaac García**

11:30-12:00 "Regulation of intercellular communication by Cx-Cx interactions". **Agustín Martínez**

12:00-12:30 "Exploring the molecular elements for temperature detection in the cold receptor TRPM8 channel"**. Karen Castillo**

12:30:14:30 Lunch Break

15:00-17:00 Students presentations I

17:00-17:30 Coffee Break

17:30-19:00 Student's presentations II

Monday, September 3rd

Chair: Ana M. Cardenas

9:00-10:00 Hemichannel trafficking by using TIRF microscopy". **Agustín Martínez**

10:00-11:00. "Regulation of glial connexin-based channels". **Juan C. Sáez**

11:00-11:30 Coffee Break

11:30-12-30 "TRPV1 regulate synaptic transmission". **Andrés Chávez.**

12:30:14:30 Lunch Break

14:30-16:30

2 hours Methodological section: interactive with students participation: how to use steady state and presteady-state kinetics to study ion channels (will work if students remember how to use simple math from solving first degree equations to differential equations). Two examples will be used: the activation of a ligand-gated channel and ball and chain channel inactivation. (**Dr. Laurent Counillon**)

16:30 and on Hands on

Tuesday, September 4th

Chair: Juan C. Sáez

9:00-10:00 "Neurotrasmitter transporters in health and disease". **Pablo Moya**

10:00-11:00 "The neurochemistry of vision in normal and diseased eyes". **Monica Acosta**.

11:00-11:30 Coffee Break

11:30-12:30 "The retina as an earlier biomarker of degeneration: Some case studies". **Monica Acosta**

12:30:14:30 Lunch Break

14:30-17:00 Hands on

Wednesday, September 5th

MODULE 2. FROM GENES TO NORMAL AND PATHOLOGICAL BEHAVIOR

Chair: Pablo Moya

9:00-10:00 "From molecules to behavior: a fly´s perspective of the logic underlying the molecular clock". **Fernanda Ceriani.**

10:00-11:00. "Drosophila in the study of biogenic amines, olfaction, psychiatric and neurodegenerative diseases. Can this fly?" **Jorge Campusano.**

11:00-11:30 Coffee Break

11:30-12:30 "How does the **molecular clock control physiology and behavior?" Fernanda Ciriani.**

2:30-13:30 "The influence of diet on neuronal degeneration and repair". **Andrea Calixto**

13:30:15:00 Lunch Break

15:00-17:00 Hands on

Thursday, September 6th

Chair: Andrés Chávez

9:00-10:00 "On resolution, super resolution and implications in neuroscience" **Dilia Aguirre**

10:00-11:00. "Connexin hemichannels in models of depression". **Juan C. Sáez**

11:00-11:30 Coffee Break

11:30-12.30 "Neuronal Glutamate Transporter EAAT3: a novel target in Obsessive-Compulsive Disorder". **Pablo Moya**

11:30-12:30.

12:30:14:30 Lunch Break

14:30-17:00 Hands on

Friday, September 7th

MODULE 3. THE QUEST FOR NEW TREATMENTS OF NEUROPATHOLOGIES

Chair: Juan C. Sáez

9:00-10:00

10:00-11:00 Mitochondrial function in glial reactivity" **Patricia Cassina**

11:00-11:30 Coffee Break

11:30-12:30 "Detrimental effects of α-synuclein in major functions of astrocytes" **Juan A. Orellana.**

12:30:14:30 Lunch Break

14:30-15:30 "Lysosomal storage diseases". **Laurent Counillon**

15:30 and on Hands on

Saturday, September 8th

Chair: Chiayu Chiu

9:00-10:00. "Participation of glíal cells in the pathogenesis of ALS" **Patricia Cassina.**

10:00-11:00. "Metabolic and functional changes during astrocyte activation" **Sonia L. Albarracín**

11:00-11:30 Coffee Break

11:30-12-12:30 "Mechanism of syndromic deafness mutations in Cx26". **Agustín Martínez**

12:30:14:30 Lunch Break

14:30-15:30 "How to write papers" Juan C. Sáez and Agustín Martínez
15:30 and on Hands on

Monday, September 10th

Chair: Agustin Martínez

9:00-10:00 “Structure/function relationships coded at the molecular architecture of Cx-based channels”. **Tomás Pérez-Acle**

10:00-11:00 “Molecular Simulations applied to ion channels”. **Danilo González**

11:00-11:30 Coffee Break

11:30-12:30 “Structure-Based Virtual Screening”. **Carlos Lagos**

12:30-14:30 Lunch Break

14:30 and on. Discover new bioactive compounds by virtual screening -Carlos Lagos.

Tuesday 11th
Chair: Helmuth Sánchez

9:00-10:00 “““H^+ channels new players in important diseases”. **Carlos González**

10:00-11:00 “Thermal and pain sensation”. **Ramón Latorre**

11:00-11:30 Coffee Break

11:30-12:30 "Thermo-TRP channels and Kv1 channels in damage-triggered peripheral neuropathies" **Rodolfo Madrid**

12:30-14:30 Lunch Break

14:30 Hands on

Wednesday 12

Chair: Juan C. Sáez

9:00-10:00 "The role of TRPM8 channels in basal tearing and dry eye sensation". **Rodolfo Madrid**

10:00-11:00 "*In vivo* heavy ethanol exposure and its impact on neuroinflammation and hemichannel activity" **Juan A. Orellana**

11:00-11:30 Coffee Break

11:30-12:30 "Epigenetic Editing to treat neurodegenerative diseases**". Brigitte van Zundert**

12:30:14:30 Lunch Break

14:30- 15-30 "Intracellular NHEs, Christianson's Syndrome, ADHD and Intellectual disabilities." **Laurent Counillon.**

15:30-17:30 "How to prepare a grant application". **Laurent Counillon and Agustín Martínez**

Thrusday, September 13th

Chair: Helmuth Sánchez

9:00-10:00 "GABAergic synaptic Plasticity in health and disease." **Marco Fuenzalida**

10:00-11:00 "Obesity and hypothalamic neurons" **Eugenia Morselli.**

11:00-11:30 Coffee Break

11:30-12-30 "Cellular and molecular basis of the memory-stress interactions." **Jimmy Stehberg**

12:30:14:30 Lunch Break

14:30 Preparing data for Student presentations

Friday, September 14th

9:00-11:00 Students presentation Part I (Laboratory Results)

11:00-11:30 Coffee Break

11:30-12-30 Students presentation Part II (Laboratory Results)
12:30-14:30 Lunch Break

Free afternoon

Saturday, September 15th

12:30-13:00 Closing ceremony and awards

13:00-15:00 Final Banquet

Hands on Laboratory seminars

August 27th to September 11th

First week: rotation in different laboratories with demonstrative experiments.

Second and third weeks: 1 or 2 students per lab will conduct experiments with techniques that could be complementary to their current work.

1. Kathleen Whitlock. Introduce techniques using the zebra fish model to study development of the nervous system.

Techniques to be learned:

-Imaging" with confocal y Spinning Disc microscope to know their possible uses to visualize alive and fixed nervous tissue.
-Visualization of gene expression using in situ hybridization and inhibition of gene functions using morpholinos.
-Immunocytochemistry in zebra fish embryos.
-How to navigate through the zebra fish genome using tools available on line.
-Tools to comprehend the neurobiology of behavior

2. Helmuth Sánchez / Isaac García. **Electrophysiology of Connexin Hemichannels and Gap junction channels linked to genetic deafness**

Mutation in Cx26 gene is the most common cause of genetic deafness in humans (50% of cases) and can cause syndromic or non-syndromic deafness. In syndromic deafness, like KID syndrome, deafness is associated to severe and life threating skin disorders. Whereas

non-syndromic deafness is mostly associated to lost of function of gap junction channels, syndromic deafness mutations cause gain of function hemichannels.

Techniques to be learned:

-The effect of different mutations in Cx26-based channels that produce both types of deafness conditions.

-Expression and analysis of Cx-based channels in Xenopus oocytes and HeLa cells.

-Using wild type and mutated Cx26 representative of human deafness (e.g., Keratitis Ichthyosis Deafness (KID) syndrome)

-Recording and analyses of macroscopic and single channel currents. Techniques of whole cell voltage clamp, dual whole cell voltage clamp, and two electrode voltage clamp will be used.

3. Oliver Schmachtenberg. **Electrophysiology and calcium imaging in the retina.**

Patch clamp and fluorescence imaging in retinal slices treated under conditions emulating diabetes and diabetic retinopathy.

Diabetic retinopathy is a common consequence of diabetes, and one of the principal causes of vision impairment in the world. This is a neurovascular disease, in which the neuronal component and its role in the initial stages of the pathology are poorly understood. Organotypic retinal explants offer the advantage of allowing the study of the neuroretinal response to diabetic conditions without an involvement of the vascular system, which is dysfunctional in culture. By doing comparative electrophysiological recordings from identified bipolar cell types of the retina under hyper- versus normoglycemic conditions, we expect to identify sensitive early biomarkers of diabetes-induced pathological changes in this part of the CNS, at stage at which the classical hallmarks of diabetic retinopathy, vascular alterations seen in eye fundus imaging, are not yet observed.

Techniques to be learned:

Patch clamp in neuronal tissue.

- NO/calcium imaging.

- Retinal slice preparation.

4. Nicolás Palacios /Agustín Martínez / Jaime Maripillán. **Functional analysis of gap junction channels**.

Electrical Synapses or Gap Junctions are fundamental intercellular communication systems that are critic for the functioning of all organs and cell tissues, including the nervous system. In this activity students will learn how to measure the activity and functional

properties of these intercellular channels in cultured cells by the following methods and activities:

Techniques to be learned:

-Measurement of the intercellular diffusion of fluorescent tracers with different size and charge. Data Analysis.

Determining the gap junctional conductance (Gj) by double whole cell voltage clamp technique.

Data Analysis.

5. Andrés Chavez / Chiayu Chiu. **Classical and new methodologies to study synaptic function and plasticity**

Techniques to be learned:

-Retinal and brain slices
-Electrical recording of excitatory and inhibitory synaptic function and plasticity in the brain and the retina to study single neurons and network activity.
-Students will also learn to use advanced optical methods such as optogenetics and two-photon microscopy, to activate neuronal activity in a cell type specific manner and to monitor Ca^{2+} signals in dendrites and single synapses, respectively.

6. R. Sotomayor. **Brain neurochemistry.**

Evaluating neurotransmitter release using polarimetry.

The students will learn:

-How to calibrate

- Measure

-Interpret results obtained with this technique.

7. Carlos González / Karen Castillo. **Electrophysiological and fluorescent techniques to study ion channels structure-function relationship**

Patch clamp and fluorometry for structure-function relationships studies in ion channels.

The goal standard to probe the functionality of an ion channel is to electrically record the currents flowing through their pores as a consequence of specific stimuli, such as changes in membrane potential, pH, temperature or different kind of ligands. Currents fluctuations reflects the transitions between different states of the channel: closed and open states. Moreover, electrically silent channel states, such as intermediate states or transitions (closed, inactivated and desensitized states) can be tracked by patch clamp fluorometry (PCF), by the site directed labeling of specific residues in the channel. By this method the

state of the channel under different conditions can be examined by the simultaneous measurement of fluorescence and currents, making possible to correlate structural with functional transitions of the channels.

Techniques to be learned:

-Heterologous expression of ion channels in cell lines or Xenopus laevis frog oocytes.

-Electrophysiological recordings of ion channels currents at macroscopic and single channel level (patch clamp, two electrodes voltage-clamp, artificial lipid bilayers). Acquisition software.

-Site-directed labeling of ion channels with environmental sensitive fluorophores, followed by PCF determinations. Acquisition software.

-Data analysis.

8. Carlos Lagos. **Discover new bioactive compounds by virtual screening**

Learning how to run Structure-Based Virtual Screening. The students will learn the procedure using their own computer.

Techniques to be learned:

-Ligand data base preparation and characterization

-Protein structure analysis and binding site definition.

-Combined ligand and structure-based virtual screening.

-Binding mode and interaction energy evaluation.

9. Patricio Orio. **Mathematical Modelling of Neuron Behavior**

The analysis of dynamical systems that arise from the physical and chemical principles underlying the transmission of electrical signals in living organisms, has been a great aid in the understanding of experimental data, and is also a whole research field on its own.

Topics that can be taught:

- Mathematical modeling of neural excitability: from ion channels to networks.
- Principles of dynamical applied to Neuroscience. Stability, bifurcations, and chaos.
- Using mathematical models to understand diseases.
- Inferring connectivity from experimental data.

10. Arlek González-Jamett / Ana María Cárdenas Díaz. **Monitoring the release of transmitters using electrochemical techniques.**

Amperometry is an electrochemical technique with high sensitivity and time resolution that allows monitoring in real time the release of oxidizable molecules. It is widely used to monitor single exocytotic events from neuronal, neuroendocrine and endocrine cells, providing information about the kinetics and quantal size of the transmitter released per individual event upon different experimental conditions.

Techniques to be learned:

-Isolation and culture of adrenal chromaffin cells, a widely used experimental model to visualize catecholamine release.

-Evaluating the impact of differential stimulation on the mode of exocytosis.

-Evaluating the role of the actin cytoskeleton dynamics in the mechanism of exocytosis. Actin organization and remodeling will be manipulated by using pharmacological approaches.

-Analysis and interpretation of the amperometric records.

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