ISN School of Neurobiology – Mendoza, Argentina, 26th August – 6th September 2019 *"From Molecules to Systems: Modern Neurobiology at a Glance"*

Focus on: "Somatosensory Systems"

Final Report

Mendoza, Argentina, 3rd January 2020

Dear Dr. Babette Fuss Chairperson of the ISN School's Initiative Committee

I, on behalf of the Organizing Committee, would like to thank you and all members of the ISN School's Initiative Committee and the ISN for your invaluable support for our third School of Neurobiology.

This was the third reiteration of the School to take place in Cuyo, the Western region of Argentina. It counted on the participation of students from all regions of Argentina and from 5 other Latin American countries. As in the previous two events (2015 and 2017), this School proved to be an enriching experience for students and faculty members alike. It also resulted in substantial networking and the initiation of fruitful future collaborations.

The School offered a brief summary of a broad range of topical subjects in modern neurobiology. It covered some fundamentals of the cellular and molecular physiology of the nervous system and linked them to frontier and cutting-edge research in the field of the role of the somatosensory system as a major input and integration player in the nervous system. All participants actively engaged in numerous laboratory-based practical activities. Furthermore, the School allowed the participants to create a network of contacts with colleagues and academics from top research institutes in Argentina and Chile.

The funding granted by ISN to this event guaranteed that all participants received travel grants, accommodation, transportation to and from the school's venue, lunch and access to high quality laboratory material and equipment. It also made possible for highly qualified scientists from Argentina and Chile (who presented plenary conferences and lectures) to be flown to Mendoza and provided with suitable accommodations.

It is thus with immense gratitude and a sense of mission accomplished that I submit this report for your consideration. Please note that I am attaching additional supporting documentation and photographs as requested.

Yours sincerely,

Dr. Cristian Acosta IHEM-CONICET Mendoza

1. Activities undertaken

As in 2015 and 2017, the Organizing Committee of the School compiled, updated and edited a laboratory Workbook that contained all the protocols used during the practical sessions. The aim is to provide the students with a record of detailed instructions and useful tips on how to carry out the different techniques practised during the course. This Workbook was handed to the students at the beginning of the School. Please note that a fully-fledged description of each of the activities listed below is provided in the Student's Workbook. We include it separately (in pdf format) as part of the report.

The School activities consisted of:

a. Eighteen plenary lectures (conference format) of 1.5-2 hours each presented by distinguished scientists from Argentina and Chile. Each lecture was divided in 2 parts: an introduction that imparted the basics of each topic in terms of fundamental neurophysiology and a data-based section, where recent advances and experimental evidence was presented. The following is the full schedule of the first week of the School that includes the lectures (in chronological order):

Aug 26	8.00-9.00	Student accreditation				
0	9.00-9.30	Welcoming speech by the Organizing Committee Members. Presentation of the ISN mission,				
		objectives and programs. Explanation of the School's modality.				
	Coffee break					
	9.45-11.30	Lecture: General organization of the nervous system (Dr. S. Patterson)				
	Coffee break					
	11.45-13.00	Application Lecture: Use of advanced biochemical and biophysical approaches in neurosciences (Dr. S. Patterson)				
	Lunch					
	14.30-16.00 Coffee b	Lecture: Cell biology and histology of the nervous system – part 1 (Dr. A. Seltzer) break				
	16.15-17.15 17.30-19.30	Lecture: Cell biology and histology of the nervous system – part 2 (Dr. A. Seltzer) Student Presentations, part I				
Aug 27	9.00-11.00	Lecture: Principles of electrophysiology (Dr. C. Acosta)				
	11.30-13.00	Lecture: Ion channels and the control of neuronal excitability (Dr. C. Acosta)				
	Lunch					
	14.30-17.00	Laboratory: Computer models of neuronal excitability and the patch-clamp technique (Dr. C. Acosta)				
	17.30-19.30	Student's Presentations, part II				
Aug 28	9.00-10.30 Coffee b	Lecture: Sensory physiology: from receptors to nociception (Dr. C. Acosta) break				
	11.00-12.30	Lecture: Glial cells in neuropathic pain (Dr. S. Gonzalez)				
		Lunch				
	14.00-15.30	Lecture: From rodents to humans: how to translate scientific knowledge into solutions for pain suffering patients (Dr. M. Villar)				
	15.45-17.15	Lecture: Pharmacological advances in the treatment of acute and chronic pain (Dr. C. Laino)				
	17.30-18.30	Athenaeum: Open Discussion and Debate session				

Aug 29	9.00-11.00	Synaptic transmission and neuronal plasticity (Dr. S. Patterson)			
	Coffee l	preak			
	11.30-13.00	Lecture: Pannexins: a new system of synaptic amplification in the peripheral nervous system (Dr. L. Constandil Cordova)			
	Lunch	b)stem (D1. E. Constantan Cordova)			
	15 00 17 00	Lecture: Microglial modulation of multipation and demuslipation: its affects on sensory			
	13.00-17.00	function (Dr. J. Pasquini)			
	Coffee l	preak			
	17.15-18.30	Athenaeum: Open Discussion and Debate session			
Aug 30	9.00-11.00	Lecture: The brain talks to the ear (Dr. E. Katz)			
0	Coffee l	preak			
	11.30-12.30	Application Lecture: Discussion and interpretation of scientific works on auditory physiology (Dr. E. Katz)			
	Lunch				
	14.00-15.30	Lecture: Mechanisms of synaptic plasticity in relevant addiction brain areas following psychostimulant drugs: its impact on somatosensory function (Dr. G. Paglini)			
	Coffee break				
	16.00-16.45	Athenaeum: Open Discussion and Debate session			
	17.15-19.00	Workshop/Lecture: The use of human stereotaxis to treat chronic pain (Dr. F. Cremaschi)			

b. The School included 4 special debate/discussion sessions (athenaeums) of 1-1.5 hours each, where the students and the faculty exchanged points of view, discussed standing questions in the field of their own research and proposed future perspectives. These activities were especially relevant to our students given their lack of previous training in how to handle, organize, discuss and present a body of data, a task of crucial importance when writing up their own research papers. It also encouraged them to think critically and explore the advantages and limitations of a number of relevant techniques and animal models, encompassing currently used genetic models, optogenetics, advanced imaging and proteomics.

c. Two special sessions of 2 hours each at the beginning of the School, where the students used brief power points to present (in English) their thesis projects, with emphasis on the questions they are trying to address, the relevance of their research and their perspectives. Each presentation was followed by 5 minutes of questions and answers, a useful and highly informative exercise. This exercise requires the ability to communicate essential aspects of their work in a short time with conceptual clarity. It is our experience that this demanding exercise is very revealing of individual abilities and deficiencies, giving us time to encourage and train them appropriately. The Faculty board offered tips and recommendations to all presenters on how they could improve their communicational skills.

d. Week 2 consisted of five full days of laboratory sessions (of 8-9 hours each) per group to carry out different experiments, ranging from purely molecular/cellular (i.e. triple immunofluorescence staining, RT-PCR, Western blotting, etc.) to the whole animal (behavioral assessments of various kinds). The 18 selected students were divided into 3 groups of 6 students each under the supervision of a Senior Researcher with the assistance of a Junior Researcher, according to the following detail:

Laboratory 1: Perinatal Damage and Early Development of the CNS (Dr. A. Seltzer/Dr. S. Valdez/Biol. J. Asencio)

Practical: a) Microscopic observation of nervous tissue. Specific staining (Cresyl-violet and others). Uses of bright field, Nomarski and fluorescence microscopy. Introduction to Brain Altlases. **b)** Use of immunohistochemical tools to evaluate neuronal damage and repair mechanisms. **c)** Behavioral experiments to assess motor coordination and nervous system maturation in pups and juvenile rats from the SHR strain versus Wistar rats and Elevated Plus Maze test to assess anxiety-like behavior in adult Wistar rats. **d)** Western

blotting of sample proteins from different brain regions in SHR vs Wistar rats. e) Data collection and statistical analysis.

Laboratory 2: Injury and Repair in the Nervous System (Dr S. Patterson)

Practical: a) Microscopic observation of nervous tissue. Anatomy and physiology of CNS and PNS. Use of specific staining (Hemotoxilin-eosin, Sudan III) to visualize Wallerian degeneration and inflammatory infiltration. Uses of bright field, Nomarski and fluorescence microscopy. b) Use of immunohistochemical tools to evaluate neuronal damage and repair mechanisms using specific markers. c) Behavioral experiments to assess pain and changes in locomotor activity. d) Western blotting of sample proteins from different tissues in rats that underwent peripheral nerve damage vs sham operated rats. e) Data collection and statistical analysis.

Laboratory 3: Neurobiology of Pain (Dr. C. Acosta/D. Messina)

Practical: **a)** Model of cutaneous inflammation induced by Complete Freund's Adjuvant. Behavioural testing: spontaneous pain, mechanical and thermal hyperalgesia, mechanical allodynia. **b)** Intracardiac perfusion and dissection of dorsal root ganglia. Use of the cryostat to serially cut section of the DRG. **c)** Analysis by double and triple immunofluorescence, semi-quantitative PCR and Western blotting of potassium channels expression. **d)** Data collection and statistical analysis.

The participants gain direct hands-on experience in several commonly used techniques in the field of Neurobiology, including cell cultures, Western blotting, immunohistochemistry, advanced microscopy, intracardiac perfusion and behavioral assessment in rodents, as well as the generation of animal models of hypoxia/ischemia, cutaneous inflammation and neurotoxicity induced by glutamate. The students generated and analyzed the data, wrote a short report on their findings in paper-like format and on the final day of the School made a 60 minutes presentation of their experiences and results.

Please note that in all these activities the students gained hands on experience and were given introductory lectures including video material and recommendations for the correct and successful implementation of the various techniques they were trained in.

The students were also offered a series of optional short training sessions in other techniques not covered in the workbook. The sessions on offer were:

- 1. Use of the cryostat to generate sections for immunohistochemistry.
- 2. Surgical methods to generate models of chronic pain (axonotmesis and neuropraxia).
- 3. Design of primers for RT-PCR.
- 4. Dissection of the whole Nervous System of the rat (demonstration).
- 5. Intracardiac perfusion.
- 6. Generation of the model of perinatal Hypoxia-ischemia.

e. A social event, which consisted of a traditional argentine barbecue (much enjoyed by all participants!) was organized in the grounds of the Mendoza Scientific and Technological Centre (CCT, the acronym in Spanish). Afterwards, there was a football match with the active participation of the students and part of the faculty. This activity took place on Sunday 1st September and was a fantastic opportunity for the students to interact with the Faculty and among themselves in a very relaxed and informal environment. A group picture of this event is included as part of the report.

2. Breakdown of disbursement of funds

ITEM/CATEGORY	AMOUNT IN USD*	
Faculty travel expenses (Air		
fares)	912.92	
Faculty Hotel Accommodation		
(single rooms)	913.72	
Student's Accommodation 13		
nights including breakfast	4418.60	
Student's Insurance 14 days	481.39	
Student's & Faculty Lunches (10		
days)	1800.00	
Student's travel grants (air fares)	3471.35	
Faculty Expenses	650.00	
Handouts, printed material,		
xeroxed copies, etc.	346.79	
Use of microscopy suite, IT		
services	500.00	
Workbook, credentials, posters	234.93	
School's coffee breaks	349.77	
Student's local transportation	250.00	
Laboratory reagents (antibodies,		
disposables, buffers, kits)	4354.00	
Social event Barbecue, venue)	214.11	
Total expenditure	18897.58	
Granted by ISN (80% from total		
minus local bank tax)	19398.00	

* Please note that the AR\$ to USD conversion was made at the exchange rate of the day the ISN funds were deposited in our local Bank (1 USD = 54 AR\$). We must highlight, however, that owing to political and economic difficulties (that we are still suffering) the exchange rate jumped to 1 USD = 63 AR\$. As the ISN funds get converted into local currency upon arrival at the Bank, the impact of this devaluation is that we had slightly less money available (in terms of USD). We are waiting to receive the final installment from ISN. This money will be essential to cover the cost of replacing all the reagents and some of the antibodies used by the 3 labs where the students performed their lab practicals. It would also cover the cost of the animals and their maintenance, as well as providing funds towards buying some much needed small equipment. Please, bear in mind that as of December 26^{th} , all purchases in USD made from Argentina will be subject to an additional tax of 30% according to the latest law passed by our Congress dictating a state of Economic Emergency.

3. People who received ISN funding

Due probably to the success of the First and Second Schools, this third edition saw a good number of people interested in taking part. In total, we received over 50 expressions of interest and 32 full applications from candidates from 8 countries (Argentina, Chile, Cuba, Colombia, Costa Rica, Mexico, Cuba, Peru and Venezuela). We implemented a careful selection process that took into account the following criteria:

1. Favor those applicants whom may benefit the most from the School, including those coming from

scientific environments where Neurosciences are less developed or belong to isolated research groups;

- 2. Give preference to students at the start of their PhD training;
- 3. Ensure gender equality, especially for women;
- 4. Guarantee representation from multi-cultural and trans-national backgrounds;
- 5. Give extra credit to those students with better academic achievements;
- 6. Prioritize those students with the best reference letters.

Note that the students were asked to provide a list of 3 referees capable of commenting on their qualifications to take part in the School. To these, the Committee sent a list of specific questions regarding the applicants motivations and lab performance; their social skills and known interpersonal difficulties and also to comment on their overall appreciation of the candidate. The referees' comments were confidential.

This is the **List of Students** selected to take part in the School "From Molecules to Systems: Modern Neurobiology at a Glance", IHEM-FCM, Mendoza, Argentina, 2019

Student	ID or Passport	Nationality	Institution	Gender
1- ALTAMIRANO, Fernando	31848726	Argentinian	UNSL	М
2-BONACCORSO, María	33886660	Argentinian	INBIOMED	F
3-BORTOLOTTO, Emanuel	35611153	Argentinian	UNLP	М
4-CHAMPARINI, Leandro	39301388	Argentinian	IFEC	М
5-GOMEZ CUAUTLE, Dante	G-28075150	Mexican	UBA	М
6-GONZALES CARAZAS, Maryanne	118144933	Peruvian	NR	F
7-HIDALGO LANUSSA; Oscar	AT913722	Colombian	PUJ	М
8-LUNA, Sebastian	34592998	Argentinian	UNR	М
9-McCARTHY, Clara	36571742	Argentinian	IMBICE UNLP	F
10-MESSINA, Diego	32354584	Argentinian	IHEM	М
11-OLAVE OLAVE, Felipe	18694857-2	Chilean	UChile	М
12-PEROTTI, Mayra	36239795	Argentinian	CIQUIBIC	F
13-RÍOS, Maximiliano	36983892	Argentinian	CIQUIBIC	М
14-SARCHI, Paula	29636935	Argentinian	IBCN UBA	F
15-SIGWALD, Eric	35545750	Argentinian	INIMEC	Μ

16-SOSA, Camila	35473201	Argentinian	INIMEC	F
17- VILLEGAS CHAVES, Emilce	94953080	Costa Rican	UNLR	F

Total: 17 students

<u>Classed by Origin:</u> From Mendoza: 2 From other parts of Argentina: 10 From other countries: 5

<u>Classed by Gender:</u> Females: 7 Males: 10

Notes: please, note that we had selected 1 student from Cuba (Ms. Hany Pazos-Espìnosa, Passport number K482264) whom had many difficulties obtaining the required Argentinian visa. Usually, the selection process and notification to the students is completed well in advance of the start date of the School, but this year, due to delays to obtain the funding, the whole process was also delayed. For Cubans wanting to visit Argentina, the errand takes no less than 4-6 weeks. Thus, Hany could not attend the School. We also had a Venezuelan citizen (Mr. Lenin Aponcio, passport E-95487830, presently living in Argentina) who attended the first week of the School only.

We open the first week of the School to all public, so we had a number of additional attendees from the scientific, teaching and student community in Mendoza.

The following Faculty members also received support from ISN funding:

Dr. Juana Pasquini (Faculty of Biochemistry and Pharmacy, University of Buenos Aires) Dr. Eleonora Katz (INGEBI-CONICET, University of Buenos Aires) Dr. Fabián Cremaschi (Faculty of Medicine, Cuyo National University) Dr. Carlos Laino (Institute of Biotechnology, CENIIT, La Rioja National University) Dr. Marcelo Villar (Institute of Translational Medicine, Austral University) Dr. Susana González (IBYME-CONICET, University of Buenos Aires) Dr. Gabriela Paglini (INIMEC-CONICET, Cordoba National University) Dr. Luis Constandil Cordoba (Dept. of Chemistry and Biology, University of Chile) Vet. Julieta Scelta (IHEM-CONICET, Cuyo National University)

Compared to our original proposal, we had two changes in the Faculty: Dr. Belen Elgoyhen suffered a wrist fracture and underwent surgery just 2 weeks before the start of the School. She was replaced by one of her long-time collaborators and one of the most prominent and highly cited researchers in the country, Prof. Dr. Eleonora Katz. Also, Dr. Brumovsky had to attend a World Meeting on Cannabis on the same week the School started. In his place, we had the fantastic opportunity of having Prof. Dr. Marcelo Villar, a highly skilled, experienced and much respected researcher in the field of pain. His lecture on the practicalities of translational medicine was particularly enlightening and much praised and enjoyed by the students, and attracted a large audience of people not participating in the course.

Note: CONICET is the Spanish acronym for the National Research Council of Argentina.

4. Illustrative photographs

Please refer to the attached file containing a full set of images taken during the course. There are group photographs suitable for publication in the ISN website.

5. Evaluation

There is an instance of internal and external evaluation on the performance of the School conducted jointly by the Organizing Committee and PROBIOL (the University PhD program). As part of this ongoing evaluation process the students were sent an invitation to answer a short online-based anonymous survey. In this instance, 15 students choose to answer the survey.

Ten of the students considered that, taking into account the academic aspects and the logistics, the School was excellent (67%). Four thought it was very good (27%) and one said it was good (6%). None considered it to be either regular or bad. We asked the students to make comments on their evaluation of the School. All of them praised the level and depth of the lectures and the compromise and dedication of the Faculty. Most remarked on how useful the contents were to their day to day activities and commented very favorably on the organization of the School.

The students assigned an average score of 92 out of 100 to the Faculty; the practical and laboratory based activities received an average score of 83 out of 100. These we consider to be very good scores indeed, albeit we will aim at improving the quality and diversity of the practical activities for the next edition of the School.

We also asked the students to identify what aspects of the School require the most improvement. This turned out to be the Final Written Exam that 57% considered to be too long and too difficult. Some students also suggested that the duration and long hours of the School could be improved. We acknowledge that the time demands imposed on the students by a very intensive schedule/training can be taxing, particularly as this sort of activity is seldom available in Latin America, and thus the attendees lack the necessary preparation and stamina to meet the requirements. As per the examination, we found that its mere existence compels the students to devote at least some time every day to the revision of the contents of the lectures and practical classes. We will look into the level and complexity of the exam and make adjustments for the next reiteration of the School. Equally, we fully intend to design a new schedule of activities that contemplates more free time that the students can devote to study.

The valuable input we gather in this and previous editions of the School always results in improvements in the next edition of the School.

6. Activities to promote the ISN and its mission

We are committed to promote and disseminate the mission and goals of the ISN to our students but also to the Faculty that take part of the School. The first activity consisted of a powerpoint presentation that outlines the guiding principles and organization of the ISN. We emphasize the key role of the School's Initiative support in making it possible for our School to take place. Furthermore, we made a concerted effort throughout the duration of the School to encourage the students to become members of the Society, not only because they obtain substantial benefits but also because it is a way to assure the ability of the Society to continue its fundamental role in promoting Neurochemistry and Neurosciences in the global arena, and particularly in those regions where the resources and opportunities are limited.

We feel very strongly that it is our duty to promote the activities of the ISN – even after the formal activities have ended we keep sending reminders and invitations for the students to join ISN, sponsoring them. Despite

all ours efforts, only a relatively low number finally formalize the membership. We believe that there are two fundamental reasons for this. Firstly, these are students in the early stages of their doctoral work, who have not yet developed an understanding of the role of personal dissemination and networking in their professional development, nor are they yet committed to a future in Neurochemistry. This will resolve itself eventually. Secondly, the students (at least in Argentina) receive very small stipends (around 480 USD per months) and the cost of life is very expensive. Thus, most of them shy away from joining organization where they are expected to make even a small monetary contribution.