ISN School of Neurobiology – Mendoza, Argentina, 28th August – 8th September 2017

"From Molecules to Systems: Modern Neurobiology at a Glance"

Focus on: "Injury and Repair in the Nervous System"

Final Report

Mendoza, Argentina, 25th September 2017

Dear Dr. Dimitra Mangoura Head of the ISN School's Initiative International Society for Neurochemistry

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 to our School of Neurobiology.

This was the second edition of the School to take place in our Cuyo region. It counted with the participation of students from all regions of Argentina and from 4 other South American countries. As in 2015, this School proved to be an enriching experience for students and faculty members alike. It also resulted in 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 Nervous System Injury and Repair Mechanisms. It allowed the participants to actively engage in laboratory-based practical activities as well as 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 (whom imparted plenary conferences and lectures) to be flown to Mendoza and offered suitable accommodations.

It is then 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

Dr. Cristian Acosta IHEM-CONICET Mendoza

1. Activities undertaken

Please note that a fully-fledged and detailed description of each of the activities listed below is provided in the Student's Workbook. This workbook was handed to the students at the beginning of the School and it is included separately (in pdf format) as part of the present report.

The School activities consisted of:

a. Sixteen plenary lectures (conference format) of 1.5-2 hours each imparted 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 a list of the lectures in chronological order:

1. Injury and repair in the nervous system (Dr. S. Patterson)

2. Hypothalamic involvement in neonatal hypoxia: a neuroendocrine crosstalk (Dr. S. Valdez)

3. Neurotrophic factors in the development and regeneration of the nervous system (Dr. F. Ledda)

4. Acid Sensing Ion Channels (ASIC) in physiological and pathological conditions in the CNS (Dr. O. Uchitel)

5. Cellular and molecular mechanisms involved in inflammatory pain associated with nerve injury (Dr. E. Utreras Puratich)

6. Bases and applications of advanced microscopy in neurosciences (Dr. P. Kunda)

7. Brain injury and behavior (Dr. P. Gargiulo)

8. Prenatal hypomyelination and cognitive impairment (Dr. J. Pasquini)

9. Neuroprotection in Perinatal Asphyxia (Dr. F. Capani)

10. Sex steroid hormones and its role in amyotrophic lateral sclerosis, a neurodegenerative disease (Dr. M. C. González Deniselle)

11. Modern applications of human stereotaxic procedures (Dr. F. Cremaschi)

12. Use of advanced biochemical and biophysical approaches in neurosciences (Dr. S. Patterson/Dr. P. Kunda)

13. Extracellular vesicles in human disease - friends or foes? (Dr. A. Quest)

14. Use of animal models in modern neurobiology (Vet. J. Scelta)

15. Stem Cell-based Approaches for the Treatment of Neurological Diseases (Dr. M. Ezquer)

16. Stem Cell Therapy: a new approach for the treatment of alcohol use disorder (Dr. F. Ezquer)

b. The School included 8 special debate/discussion sessions of 1 hour each, where the students and the faculty exchanged points of view, standing questions in the field of their own research and future perspectives. These activities were especially relevant to our students given their lack of previous training on 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 currently used genetic models, optogenetics, advanced imaging and proteomics.

c. Six full days of laboratory sessions (of 8-9 hours each) per group to discuss, plan and execute a small research project. 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:

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

Theory: Histology of the peripheral and central nervous system. Cell types and phenotypic markers. Development of the brain cortex. Development of the cerebellum.

Practical: a) Microscopic observation of nervous tissue. Specific staining (Cresyl-violet and others). Uses of bright field, Nomarskii and fluorescence microscopy. Introduction to the Altlas of the Brain. Stereotaxis. Localization and identification of cerebral regions. **b)** Induction of uni and bilateral brain lesions. Evaluation of injured areas. Fixation and dissection of brain regions. Protein extraction from brain tissue and examination by western blotting. **c)** Use of immunohistochemical tools to evaluate neuronal damage and repair mechanisms. **d)** Data collection, statistical analysis. Write up of final laboratory report and oral presentation of results.

Module 2: Glutamatergic receptors and excitotoxicity (Dr. M. Sosa/Dr. M. Troncoso)

Theory: Dynamic of the intracellular structures involved in traffic and degradation of particles and molecules. The endo-lysosomal apparatus in neuronal plasticity and neurotransmission. Its physiological relevance and relationship to pathologies of the nervous system.

Practical: a) Evaluation of cytotoxicity in striatal neurons. Use of cell lines to study Huntington's disease. Effect of glutamatergic agonists on the integrity of the endolysosomal apparatus. b) Assays of cell viability by MTT. Protein extraction and expression patterns of glutamatergic receptor and lysosomal protein determined by western blot analysis. c) Immunocytochemistry and confocal microscopy to determine sub-cellular localization of proteins associated with neuronal excitotoxicity. d) Data collection and statistical analysis. Write up of final laboratory report and oral presentation of results.

Module 3: Potassium channels and pain (Dr. C. Acosta/Dr. S. Benitez)

Theory: Bases of neuronal excitability: action potentials and their propagation, properties and regulation of ion channels under normal and pathological conditions. Principles of patch-clamp recording and its variants. Modelling of neuronal excitability. Channelopathies. Sensory physiology: pain and nociception.

Practical: **a)** Model of cutaneous inflammation induced by CFA. 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. Write up of final laboratory report and oral presentation of results.

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, intra-cardiac 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 acquired training on.

d. The School also included a special (and informal) module devoted to the presentation by the students of their present topic of research. This exercise demanded ability to communicate essential aspects of their work in a short time plus clarity of concepts. The Faculty board offered tips and recommendations to all presenters on how they could improve their communicational skills.

e. A social event, which consisted of a traditional argentine barbecue (much enjoyed by all participants!) organized in the Sports Centre of the Cuyo National University. There was also a football match with the active participation of the students and faculty. This whole activity took place on Sunday 3rd 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

AMOUNT IN AR\$	AMOUNT IN USD
34955.75	2056.22
20500.00	1205.88
88030.80	5178.28
3900.00	229.41
29061.00	1709.47
69242.46	4073.08
12000.00	705.88
4150.00	244.11
8500.00	500.00
11702.00	688.35
5040.00	296.47
4200.00	247.05
35200.00	2070.59
51459.10	3027.00
5000.00	294.11
<mark>383940.30</mark>	22525.90
243219.27	14380.00
140769.35	8140.00
	AMOUNT IN AR\$ 34955.75 20500.00 88030.80 3900.00 29061.00 69242.46 12000.00 4150.00 8500.00 11702.00 5040.00 4200.00 35200.00 51459.10 5000.00 383940.30 243219.27 140769.35

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 = 17 AR\$). We are waiting to receive the final installment from ISN (equivalent to USD 3,600.00 or 20% of USD 18,000.00 awarded in total). This money will cover the cost of replacing all the reagents, kits and disposables used by the 3 labs where the students performed their lab practices.

3. People who received ISN funding

Due probably to the success of the First School of Neurobiology, this second edition saw a substantial increment in the number of people interested in taking part. In total, we received 106 expressions of interest and 56 full applications from candidates from 8 countries (Argentina, Brazil, Chile, Colombia, Ecuador, Mexico, Peru and Venezuela). There was 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.

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, 2017

SURNAME AND NAME	NATIONALITY	ID OR PASSPORT	INSTITUTION
FUNES, ALEJANDRINA	ARGENTINIAN	34.300.120	UN Rosario – Sta Fé
PAZ, RODRIGO MANUEL	ARGENTINIAN	36.200.750	UBA – Buenos Aires
ESTAY VISCARRA, SEBASTIÁN	CHILEAN	17.392.587-5	U Valparaíso - Chile
MAZZITELLI FUENTES, LAURA	ARGENTINIAN	36.079.946	U Comahue - Neuquén
SCHUMACHER, ROCÍO	ARGENTINIAN	35.708.236	UN Litoral – Entre Ríos
VASQUEZ MATSUDA, VICTOR	PERUVIAN	6172472	UFSP – Sao Pablo
APARICIO, GABRIELA INÉS	ARGENTINIAN	35.324.027	UBA – Buenos Aires
FALOMIR LOCKHART, EUGENIA	ARGENTINIAN	35.399.835	UNLP – La Plata
ZAPPA VILLAR, MARÍA FLORENCIA	ARGENTINIAN	33.333.732	UNLP – La Plata
FROST, PAULA DA SILVA	BRAZILIAN	FH101847	UFRJ – Río de Janeiro
UNDA VELAZCO, SANTIAGO RENÉ	ECUATORIAN	95.111.760	UNLR – La Rioja
RODRIGUEZ VARELA, SOLEDAD	ARGENTINIAN	34.608.568	UBA – Buenos Aires
BENEDETTO, MARÍA MERCEDES	ARGENTINIAN	32.566.079	UNC - Córdoba
MUSTAFA, EMILIO ROMÁN	ARGENTINIAN	35.219.446	UNLP – La Plata
FERRERO RESTELLI, FACUNDO	ARGENTINIAN	34.500.938	UBA – Buenos Aires
DIAZ VALDIVIA, ALEJANDRA	CHILEAN	17.838.089-3	U Valparaíso - Chile
ARDILES GALVEZ, NICOLAS	CHILEAN	16.322.630-8	U Valparaíso - Chile
HAROS, VALENTINA	CHILEAN	15.829.958-5	U Valparaíso - Chile

Total: 18 students

<u>Classed by Origin:</u> From Mendoza : 0 From other parts of Argentina: 11 From other countries: 7

<u>Classed by Gender:</u> Females: 11 Males: 7

The following Faculty members also received support from ISN funding:

Dr. Juana Pasquini (Farmacia y Bioquímica, UBA) Dr. Osvaldo Uchitel (IFIBYNE-UBA-CONICET) Dr. Elías Utreras Puratich (Universidad de Chile, Chile) Dr. Francisco Capani (ININCA-UBA-CONICET) Dr. Marcelo Ezquer (Universidad del Desarrollo, Chile) Dr. Patricia Kunda (INICSA-CONICET-UN de Córdoba) Dr. Fabián Cremaschi (FCM, UN de Cuyo) Dr. Fernando Ezquer (Universidad del Desarrollo, Chile) Dr. Pascual Gargiulo (FCM-UNCuyo) Dr. Fernanda Ledda (IBCN-UBA-CONICET) Dr. Susana Valdez (IMBECU-FCEN-CONICET) Dr. Claudia González Deniselle (IBYME-UBA-CONICET) Vet. Julieta Scelta (IHEM-CONICET) Dr. Andrew Quest (Universidad de Chile, Chile)

4. Illustrative photographs

Please, refer to the attached file containing a full set of illustrative images. 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 all participants of the School (including students, academics and Faculty members) have been sent a short survey. The results of this survey will become available at some point within the next two months. The valuable input we gather from the opinion survey will result in improvements in the next edition of the School, that we plan will take place in the second semester of 2019.