



Instituto de Investigación Médica Mercedes y Martín Ferreyra
INIMEC - CONICET - Universidad Nacional de Córdoba

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Progress report CAEN Category 1C Return Home Grant. August 2014 Round

Project Title: **Characterization of actin cytoskeleton dynamics during axonal degeneration and investigating its potential role in axonal fragmentation.**

Responsable: **Nicolás Unsain, PhD**

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To whom it may concern,

It is a pleasure for me to write this report, for the support provided by ISN for returning to my home country to develop my scientific career has been very meaningful and had an appreciable impact on this difficult early career stage. After receiving my PhD in Biological Sciences in my home University (Universidad Nacional de Córdoba, 2009) under the supervision of Daniel Mascó PhD, I moved to Montreal to perform by postdoctoral studies in the laboratory of Dr. Phil A Barker (Montreal Neurological Institute, McGill University). Those five years were very productive, and thanks for the unconditional support from Dr Barker to develop my future career I set up the stage to earn a Researcher position in Argentina. The financial support received from ISN was also very important for building my own lab and to encourage making the big move. I also have to mention the support from the National Research Council (CONICET) for the funds to move me and my family back to Córdoba. My workplace is now the Instituto de Investigación Médica Mercedes y Martín Ferreyra (INIMEC-CONICET, Universidad Nacional de Córdoba), and I am incorporated in the Neurobiology group of Dr. Alfredo Cáceres, who kindly supports good part of my experimental work and provides significant mentoring.

My first 6 months in Argentina I started my activities as a postdoctoral fellow, under the supervision of Dr Alfredo Cáceres, in his world recognized Neurobiology Laboratory. He was kind to allow me to establish my independent lines of research and at the same time providing insights, mentoring and lab facilities and reagents to that end. I am profoundly grateful for his support and that received by all the lab members, and most especially to Dr Mariano Bisbal, another young investigator.

In March 2015 I was finally appointed Assistant Researcher of the National Research Council (CONICET). As a young investigator I am still in the laboratory of Dr Caceres, with the facilities and mentorship above mentioned.

Current research

During my postdoctoral studies in McGill University, I focused on axonal degeneration, with special attention to the role of caspases and its regulators. My research goals today merge the expertise I acquired in Montreal with the expertise of the laboratory of Dr Caceres on neuronal cytoskeleton regulation and dynamics. Hence, I have been studying the dynamics and changes suffered by the actin cytoskeleton during different modalities of axonal degeneration. I have already presented some of that research in National meetings and we hope to have a manuscript finished in the next months. In it, we show evidence that the actin cytoskeleton is affected early during the course of different modalities of axonal degeneration and that its changes occur well before axonal fragmentation. We believe the data will be of much interest our colleagues studying axonal degeneration.

Research project in collaboration with Dr. Stefani and Dr. Caceres

I am currently part of a multi-group team (recently funded by one of the biggest national grants) for studying basic neurobiology questions using state-of-the-art super-resolution microscopy. The laboratory of nano-photonics lead by Dr Fernando Stefani in Buenos Aires is the one developing these technologies in the country, and we provide to his group with experiments to analyze. This collaboration is very fruitful, especially with respect to the recently discovered periodical organization of axonal subcortical network, which we are now able to observe using STED microscopy and we are also evaluating how these structures change as a function of degeneration. It is a privilege to be part of this exciting inter-disciplinary group and the interaction is already giving its fruits. Also, because this grant is one of the biggest the Argentinean Agency provides to research, is very helpful for the growth of my research.

Collaboration with Dr. Grasselli

I am also currently sharing a grant with Dr. Mariano Grasselli (Universidad Nacional de Quilmes). I contacted Mariano in the beginning of my return, with the goal of designing a culture method based on culture inserts that would allow for the differential treatment of large volumes of axonal material, which is something not possible with any commercial device at the moment. The idea turned into an approved grant proposal by the FAN ("Argentinean Foundation of Nanotechnology") so that is another financial source my lab is being built with. The final goal of this project is to develop a prototype that will allow for the differential isolation and treatment of axons, with the possibility to transform it in a nano-technology-based, commercial enterprise. The laboratory of Dr Grasselli provides the know-how and expertise in biochemistry and nanotechnologies, and my lab provides the cell biology part of the project.

Advocacy

As part of my duties as a National Researcher of CONICET, I devote some time to advocacy, giving public talks and visiting schools to show kids and the lay public some of the work neuroscientists do in their labs. I am grateful for the Sociedad Argentina de Investigación en Neurociencias (SAN) and CONICET for providing the financial needs to cover those projects. In sum, I have given 2 public talks and visited 5 elementary schools and a book show. I have also organized and coordinated two consecutive Brain Awareness Week initiatives (2015 and 2016, in course) that in sum we have visited 40 classes of 4th grade of the elementary school in the city of Córdoba.

Assistance to meetings

Part of the ISN grant for my establishment was used for attending to regional scientific meetings. This has been fundamental for my insertion in the neuroscience community and to start fruitful collaborations with research groups in the region. In sum, I have attended to 2 of the Sociedad Argentina de Investigación en Neurociencias annual meetings, to the annual meeting of the Sociedad de Bioquímica y Biología Molecular of Uruguay and to annual meeting of the Chilean Society for Cell Biology. These have triggered fruitful ideas and contacts with colleagues with whom collaborate in the future.

Short stay in Montreal

In 2015 I decided to make a 2 month lab stay in the laboratory of Dr Barker, in Montreal, during July and August of 2015. I mostly made original and independent work with Barker's support using a newly-acquired Super Resolution STORM microscope (Vutara). The experiments are part of my independent line of research to which Dr Barker is a kind collaborator. With the data collected there and more in my laboratory in Argentina we are now preparing a manuscript, where the role of actin and actin rings in degeneration is explored.

At the end of this letter you will find a summary of my financial report and a detailed report of the activities performed during the funded period.

I am very grateful for the opportunity given by ISN with this grant. I am now also a proud Full Member of the society and in doing so I hope to help making this world-wide society an excellent place to foster the new generation of great neuroscience.

I am also grateful for all the other funding programs that ISN continue to support, like the ones supporting excellent symposia and workshops in the region. My published material includes an acknowledgement note to ISN and the CAEN award. I hope some more will be published soon.



Yours sincerely, Nicolás Unsain

Financial report

Item	U\$D
Laboratory Reagents (cell culture reagents and media, antibodies, etc.)	3,165
Mouse and rat time pregnancies	1,500
Service and maintenance of automatic pipettes	450
Surgical instruments	650
Travel costs for Chilean Society for Cell Biology, 28th Annual Meeting - 2014	500
Travel costs for national meetings (SAN Annual Meetings, 2014 and 2015)	500
Office supply (Desktop computer, external hard drivers, office consumables)	1,600
DNA sequencing	200
Laboratory stay in Montreal, living expenses for 2 months	1,435
TOTAL	10,000

Activities and publications during the funded period.

Work experience and positions

2015-present Researcher, CONICET. At Instituto de Investigación Médica Mercedes y Martín Ferreyra – INIMEC/CONICET – UNC

2014-2015 Postdoctoral fellow, Instituto de Investigación Médica Mercedes y Martín Ferreyra – CONICET – UNC. Works in the laboratory of Dr Alfredo Cáceres while await its assignment as independent Investigator to lead his own lab. Studies the function of modulators of cytoskeleton dynamics in axonal degeneration.

Publications

2016 Gibon J, Unsain N, Gamache K, Thomas RA, De Leon A, Johnstone AD, Nader K, Séguéla P, Barker PA. The X-linked Inhibitor of Apoptosis regulates long-term depression and learning rate. *FASEB Journal*. 2016. *In Press*. **IF-2014: 5.043**.

2015 **Unsain N**, Barker PA. New Views on the Misconstrued: Executioner Caspases and Their Diverse Non-apoptotic Roles. *Neuron*. 2015 Nov 4;88(3):461-74. doi: 10.1016/j.neuron.2015.08.029. Review. **IF-2014: 15.054**.

2015 Cueva Vargas JL, Osswald IK, **Unsain N**, Arousseau MR, Barker PA, Bowie D, Di Polo A. Soluble Tumor Necrosis Factor Alpha Promotes Retinal Ganglion Cell Death in Glaucoma via Calcium-Permeable AMPA Receptor Activation. *J Neurosci*. 2015 Sep 2;35(35):12088-102. doi: 10.1523/JNEUROSCI.1273-15.2015. **IF-2014: 6.344**.

2015 Gibon J, Buckley SM, **Unsain N**, Kaartinen V, Séguéla P, Barker PA. proBDNF and p75NTR Control Excitability and Persistent Firing of Cortical Pyramidal Neurons. *J Neurosci*. 2015 Jul 1;35(26):9741-53. doi: 10.1523/JNEUROSCI.4655-14.2015. **IF-2014: 6.344**.

2014 **Unsain N**, Higgins JM, Parker KN and Barker PA. (2014) *Production and isolation of axons from sensory neurons for biochemical analyses using porous filters*. *J Vis Exp*. 2014 Jul 8;(89). doi: 10.3791/51795. **IF-2014: 1.325**.

Manuscripts prepared to be submitted

- 1 **Unsain N1**, Johnstone AD2, DiPolo A3, Barker PA2. Caspase-3 and Calpain Activation during injury-induced axonal degeneration participate in the degenerative process but are not inhibited during NAD⁺-mediated protection. 1 Laboratorio de Neurobiología, Instituto de Investigación Médica Mercedes y Martín Ferreyra, INIMEC-CONICET, Universidad Nacional de Córdoba; 2 Montreal Neurological Institute, McGill University. 3 University of Montreal. Por ser enviado a Journal of Neuroscience.
- 2 **Unsain N1**, Dorval G2, Sheen J2, and Barker PA2. Generation and characterization of mouse lines carrying floxed and knock-out alleles of the p75^{ntr} homolog NRH2/NRADD. 1 Laboratorio de Neurobiología, Instituto de Investigación Médica Mercedes y Martín Ferreyra, INIMEC-CONICET, Universidad Nacional de Córdoba; 2 Montreal Neurological Institute, McGill University. Por ser enviado a Plos One.

Research Grants and Advocacy Grants

- 2015 Advocacy grant. Brain Awareness Week 2016 Grants, Sociedad Argentina de Investigación en Neurociencias (SAN), 2015, to organize and conduct advocacy activities in the city of Cordoba during BAW-2016.
- 2015 Research grant. ANPCyT, PICT-Class V. MinCyT-Argentina. 2015. 4 year grant to conduct research in the field of neuronal development using nanoscopy (super-resolution microscopy).
- 2015 Technology development grant. Start-up Funding. Fundacion Argentina de Nanotecnología. 2015. To develop a specialized neuronal culture system using nanotechnology tools. For one year.
- 2015 Research grant. ANPCyT, PICT-Return Home Grant. MinCyT-Argentina. 2015. 3 year grant to conduct research in the field of neuronal degeneration.
- 2014 Advocacy grant. Brain Awareness Week 2015 Grants, Sociedad Argentina de Investigación en Neurociencias (SAN), 2014, to organize and conduct advocacy activities in the city of Cordoba during BAW-2015.
- 2014 Research grant. ISN-CAEN: Return Home Grant. International Society for Neurochemistry (ISN), 2014. To start-up by own laboratory as a young investigator. 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

Abstracts in Scientific Meetings

- 2015 D Revillo, E Sigwald, L Freitas, A Guzman, I Zalosnik, P Avalos, F Mir, A Vivinetto, E Cotella, V Rozes, A Anastasia, P Helguera, M Bisbal y **N UNSAIN**. Póster: 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. Sociedad Argentina de Investigación en Neurociencias (SAN) Anual Meeting. Mar del Plata, Buenos Aires, Argentina. Septiembre de 2015.

2015 Barabas F, Remedi M, Bisbal M, Barker PA, Stefani FD, Cáceres AO, **UNSAIN N**. Póster: Characterizing cytoskeleton changes during axonal degeneration. Sociedad Argentina de Investigación en Neurociencias (SAN) Anual Meeting. Mar del Plata, Buenos Aires, Argentina. Septiembre de 2015.

2015 **UNSAIN N** Oral communication: Se hizo la luz, ¿y ahora qué?: Experiencias didácticas en análisis de imágenes de un biólogo cualquiera. IX Jornadas de la Sociedad de Bioquímica y Biología Molecular (SBBM-SUB). Montevideo, Uruguay. Octubre de 2015

2014 JL Cueva Vargas, J Nemargut, I Osswald, M Arousseau, **N UNSAIN**, PA Barker, D Bowie and A Di Polo (2014) Glia-derived tumor necrosis factor-alpha promotes retinal ganglion cell death through overexpression of philanthotoxin-sensitive calcium permeable AMPA receptors. Investigative Ophthalmology and Visual Science. 55 (5), 2416

2014 **UNSAIN N**, JOHNSTONE A AND BARKER PA. Caspase-3 and Calpains become active durings (and play a role in) axonal degeneration but are not inhibited during NAD+-mediated protection. Póster. Chilean Society for Cell Biology, 28th Annual Meeting. 26-30/10/2014. Puerto Varas, Chile

2014 **UNSAIN N**, JOHNSTONE A AND BARKER PA. Caspase-3 and Calpains become active durings (and play a role in) axonal degeneration but are not inhibited during NAD+-mediated protection. Presentación oral, Young Investigator Symposium, 29th Annual Meeting, Sociedad Argentina de Investigación en Neurociencias (SAN). 29/09/2014 - 03/10/2014. Huerta Grande, Córdoba, Argentina.

2014 JOHNSTONE A, **UNSAIN N** AND BARKER PA. *Reactive oxygen species and NOX complex signaling are required for developmental axonal degeneration*. Gordon Research Conference on

Post-Graduate Courses

2014 *Coaching* for groups leadres in sciences. 12 hours. By MSc Claudia Caballero and MSc Orietta Sferco, CEOSS.

Fellowships and Awards

2014-2015 **Posdoctoral Fellowship** from CONICET, Argentina. Works in the laboratory of Dr Alfredo Cáceres. Instituto de Investigación Médica Mercedes y Martín Ferreyra – CONICET – UNC.

Organization of Scientific and Advocacy Meetings and Events

- 2015 Workshop Gabriela Mistral School, Córdoba. Invited by CCT-Córdoba-CONICET, City Science Week. June.
- 2015 Workshop "Neuroscience of the Senses" CONICET Boot at the 41st International Book Show of Buenos Aires. With Dr Mariano Bisbal. Abril de 2015.
- 2015 Organizes and coordinates a 2-week event for Brain Awareness Week 2015 in Córdoba city, entitled "Neuroscience of the Senses". Visiting 4th grade kids from 10 primary schools. May.
- 2014 Open public talk for secondary school students from Instituto Mark Twain. Title: Axonal Degeneration. Inst. de Inv. Médica Mercedes y Martín Ferreyra, 17 de Noviembre de 2014.
- 2014 Public Conference "Conociendo el cerebro a través de las enfermedades neurodegenerativas" en el Ciclo de Conferencias Culturales 2014, organizada por la Academia Nacional de Ciencias de Córdoba. Miércoles 19 de noviembre de 2014 en el Salón de Actos de la Academia Nacional de Ciencias de la Ciudad de Córdoba.

Teaching

- 2015 Assistant Professor in Cell Biology, School of Medicine. Instituto Universitario de Cs. Biomédicas de Córdoba (IUCBC).