Instituto de Investigación Médica Mercedes y Martín Ferreyra
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ISN-CAEN

CATEGORY 1C Return Home Grant - 12 month progress report

Agustin Anastasia PhD.

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Cordoba (Argentina) – June 2nd, 2016

Dear Dr. Caroline Rae,

I am glad to report about my first year back in my home country Argentina where I am setting my laboratory. I have worked as a postdoctoral fellow at the Weill Cornell Medical College (New York, USA) under the direction of Dr. Barbara L. Hempstead for 5 years (2010-2015). Besides Dr. Hempstead mentorship, I worked closely in collaboration with Dr. Francis S. Lee (Weill Cornell Medical College) and Dr. Moses V. Chao (New York University, New York, USA). During my postdoc I studied a common single nucleotide polymorphism (SNP) in brain-derived neurotrophic factor (BDNF), which is associated with enhanced risk for depression, anxiety and memory impairment in humans. My research has contributed to clarify the mechanisms by which this SNP alters human behavior, and to find targets for future therapeutics (Anastasia et al., 2013 Nature Communications). During this training period I gained expertise on structural biology, biochemistry, cell biology, behavioral neuroscience, as well as broadened my knowledge on neurobiology of disease. On April 2015 I was appointed as an Assistant Investigator, which is a full-time research position from the Argentinean National Research Council (CONICET). With this position I am setting my laboratory in the Instituto de Investigacion Medica Mercedes y Martin Ferreyra (INIMEC-CONICET-UNC) which is a CONICET and National University of Cordoba University Institution, located in Cordoba, Argentina. I am also teaching cell biology for the Medical Doctor's program at the Instituto Universitario de Ciencias Biomedicas of Cordoba (IUCBC).

During this first year I have established collaborations, recruited and started training students, and set my laboratory which is now producing results. I have started a collaboration with Dr. Alfredo Caceres who is a world renowned cell biologist expert on cytoskeleton, membrane trafficking and neuronal polarity. With Dr. Caceres we are studying how the "pathogenic" BDNF Met prodomain alters neuronal structure to affect human behavior. Specifically, we will determine how the Met prodomain affects the cytoskeleton in growth cones and in dendritic arbors. I also maintain collaborations with my postdoctoral mentors Dr. Barbara L. Hempstead and Dr. Francis S. Lee. I have recruited 3 students (2 undergraduate and 1 graduate student) which are learning techniques and already conducting some experiments independently in my laboratory. Moreover, I have set to work essential techniques to reach the aims of the project. For example, we have optimized the generation and maintenance of mesencephalic cultures enriched in dopaminergic neurons (from C57Bl6 mice), and the generation of lentiviral vectors.

The Instituto Ferreyra (INIMEC-CONICET-UNC) has common equipment and facilities which allow me to perform experimentation. Moreover, Dr. Alfredo Caceres, who is my local mentor, opened his laboratory to allow me use bench-top equipment while I buy my own.

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Scientific Progress

Brain-derived Neurotrophic Factor (BDNF) is the most highly expressed and studied neurotrophic factor in the mammalian brain. BDNF is synthetized as a precursor proBDNF that can be processed to release mature BDNF and a prodomain. We recently published that the BDNF prodomain is a neuronal secreted ligand in the hippocampus, and that a very frequent human single nucleotide polymorphism (SNP) which induces a Val66Met substitution in the prodomain, confers bioactivity to this protein. Moreover, we found that this bioactivity is mediated through interaction of the Met prodomain with the sortilin-related VS10 domain containing receptor 2 (SorCS2) and the p75 neurotrophin receptor ($p75^{NTR}$) (Anastasia et al, Nature Communications, 2013).

In collaboration with Dr. Francis Lee's laboratory (Weill Cornell Medical College) we have recently found that the Met prodomain alters the structure of mature neurons in culture. We observed that this ligand changes the dendritic spines morphology from mushroom shape (considered to be mature and functional spines holding active synapsis) to thin/filopodial spines which are immature and probably dysfunctional. We have found that the Met prodomain administration alters key actin cytoskeleton regulators which begins to shed light on the mechanism. We propose that the Met prodomain effects on dendritic spine morphology could be affecting synaptic transmission and consequently altering circuitry and behavior in the human Met carriers (manuscript in preparation to be submitted soon).

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons of the substantia nigra and the concomitant loss of dopamine in the target striatum. It has been reported that the Val66Met polymorphism is associated with increased incidence of PD in certain populations but the pathogenic mechanism through which the Met allele contributes to the disease occurrence remains to be elucidated. Therefore, the general aim of my laboratory is to study the regulation and role of the BDNF prodomain (both Val and Met variants) in the development, maintenance and degeneration of the nigrostriatal system.

To test the hypothesis that the BDNF prodomain function as an active ligand in the nigrostriatal dopaminergic system, in my laboratory we first study if young and adult mice express the BDNF prodomain and its known receptors SorCS2 and $p75^{NTR}$ in the substantia nigra and striatum. We found that all of them are expressed in these brain regions. Moreover, we have recently generated preliminary data indicating that the Met prodomain induces superior cervical ganglia (SCGs) dopaminergic neurons death, implying that this ligand can affect other neurons besides the hippocampal ones. We have set up the protocol to culture primary mesencephalic neurons which are enriched in dopaminergic neurons. This primary cultures are being used to test the impact of the Val or Met prodomains on dopaminergic neuron survival and morphology.

Manuscripts published

I have recently published as the first and co-corresponding author a paper that describes the oligomerization status of the $p75^{NTR}$ receptor and proposes a novel method of activation of this receptor. This data is critical to understand how the BDNF prodomain triggers signaling by activating a complex of two unrelated receptors as SorCS2 and $p75^{NTR}$. Some of the experiments for this publication were finished in my laboratory at the Instituto Ferreyra (INIMEC-

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CONICET-UNC). The ISN-CAEN is acknowledged in this paper as some of the funds were allocated to these experiments. Paper details:
"Detection of $p75^{NTR}$ Trimers: Implications for Receptor Stoichiometry and Activation"
Anastasia A*, Barker PA, Chao MV, Hempstead BL*. *co-corresponding authors.
The Journal of Neuroscience. 2015 Aug 26;35(34):11911-20.

Poster presented in the first year of funding

1- Agustin Anastasia, Olav Olsen, Marc Tessier-Lavigne, Phillip A. Barker, Moses V. Chao, Barbara L. Hempstead Detection of p75NTR trimers: implications for receptor stoichiometry and function. Neurotrophic Factor Gordon Research Conferences. May 31 – June 5, 2015. Salve Regina University, Newport, RI, USA.

Selected oral presentations during the first year of funding

1-18th TWAS ROLAC Young Scientist Conference. November 3-4 2015. Rio de Janeiro, Brazil.
2-XXX Meeting of the Sociedad Argentina de Investigación en Neurociencias (SAN) - Young Investigator Symposium. October 1 2015. Mar del Plata, Argentina.

Short stay in Weill Cornell Medical College (New York, USA)

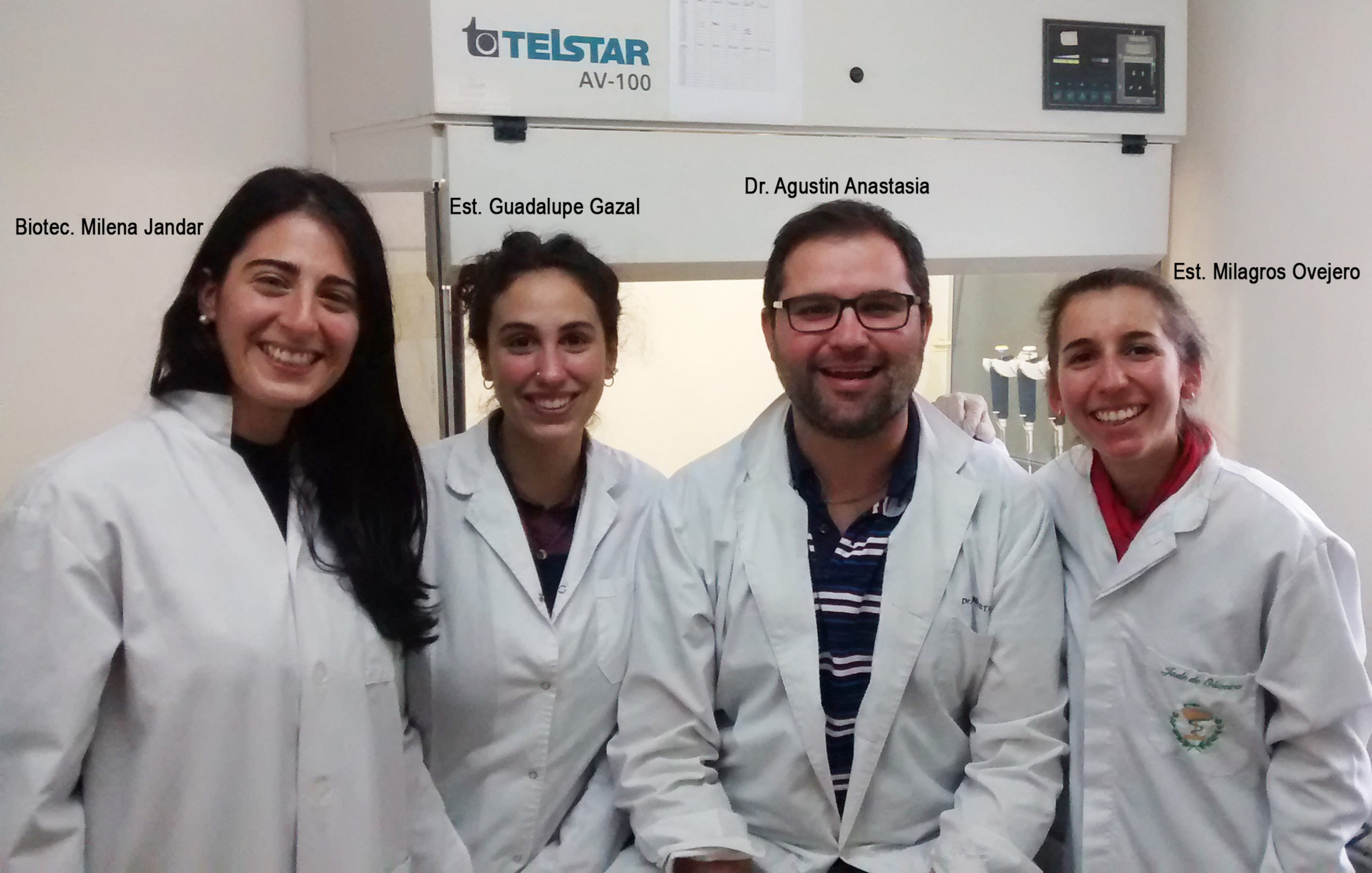
In 2015 I spent 2 weeks in Dr. Barbara L. Hempstead laboratory conducting experiments for my current project. The reason for the short stay is that I don't have yet in my Institute BDNF knock-in mice which are critical for my project. Therefore, while waiting for the paperwork to import those mice to Argentina, I went to Weill Cornell Medical College and cultured ventral mesencephalic neurons from knock-in $BDNF^{Val/Val}$ and $BDNF^{Met/Met}$ mice to study their neurites and dendritic arbor development. Soon I will have those mice in my Institution (a kind gift of Dr. Francis S. Lee) to continue with the experiments.

Acknowledgement to the ISN-CAEN

I am very grateful for the support from the ISN-CAEN. The ISN-CAEN return home grant allowed me to finish a paper (recently published, see above), helped me to set my laboratory and office, and to generate results which were/are utilized to apply for national and international funding. The ISN-CAEN will be acknowledge in future publications and meeting presentations (poster/oral).

Financial report

Item	USD
Lab supplies (reagents and small equipment)	700
Mice maintenance and time pregnant mice for primary culture	400
Computer / western blot scanning system	1800
Office supplies	540
Travel expenses to the Neurotrophic Factor Gordon Conference	1500
Short stay at Weill Cornell Medical College	1750
Knock-in mice importation: courier + paperwork/documents	1100
Amount left	210
Total	**8000**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