Final Report for CAEN Award
Category 1A: Visit by the applicant to another lab.

Applicant: Laura E. Montroull

Home Laboratory: Dr. Daniel H. Mascó. Institute of Biological and Technological Research. CONICET-UNC. Argentina.

Host Laboratory: Dr. Wilma Friedman. Deparment of Biological Sciences. Rutgers University. Newark, NJ. USA.

Award period: 4 months (between August 20 and December 20, 2012).

Outcome and benefit of the award:

The purpose of the visit was to study how the regulation of Neurotrophins (NT) determines the availability of ligands and their binding to the NT receptors leading finally to survival or neuronal death using organotypic hippocampal cultures. Dr. Friedman has been studying the role of NT in regulating survival and death of brain neurons for over 25 years thus she has a great experience in this area. In particular, she is interested in how the precursor of Nerve Growth Factor (proNGF) induces neuronal death in an *in vitro* and *in vivo* model of epilepsy (Status Epilepticus). On the other hand, our lab in Argentina has been working for many years in a project which investigates the effect of Brain-Derived Neurotrophic Factor and its precursor (proBDNF) in the mechanisms of neuronal death in an *in vivo* model of Status Epilepticus.

Brain slices maintain many aspects of *in vivo* biology, including functional local synaptic circuitry with preserved brain architecture, while allowing good experimental access and precise control of the extracellular environment, making them ideal platforms for dissection of molecular pathways underlying neuronal dysfunction. Learning this technique improved my research in our laboratory back in Argentina, allowing new challenges in our model of study. Also, during my stay I had the opportunity to exchange ideas and work with a group of very talented scientists. Likewise, this visit favored further collaborations between our laboratories, and fostered the visits of future researchers.

I am highly grateful to the ISN-CAEN for this opportunity. This experience definitely impacted into my academic career in a positive way and had a great contribution to my intellectual and personal growth: I had to communicate my ideas effectively in other language, discuss experiments and literature, write a project, generate new hypotheses and resolve technical issues.

Summary of results:

We carried out *in vitro* experiments using hippocampal organotypic culture witch were treated with Kainic Acid (KA) to induce neuronal hyperexcitability (similar to SE *in vivo*) and the modifications in BDNF system were evaluated. During the performance of the experiments, it was difficult to maintain the cultures in good condition thus; the results shown below (Figure 1-4) are those where the survival of the culture could be maintained throughout the treatment.

Hippocampal slices (300 µm) from postnatal day 7 rat pups were maintained at 37°C in 95% O_2 and 5% CO_2 for 7 d. Afterward, the media was changed to serum free media (SFM) (containing 1:1 F12/ME with 6 mg/ml D-glucose, 100 mg/ml transferrin, 25mg/ml insulin, 20 nM progesterone, 60 µM putrescine, 30 nM sodium selenide) for 24 h. Cultures Slices were treated with MK-801 for 30min to block NMDA receptors and excitotoxicity, and then with 5µM kainic acid (KA). After 1 h, KA was washed out and slices were returned to

fresh SFM containing 10 µM MK-801. Propidium iodide (PI) nucleic acid stain (5µg/ml) was added to the slice cultures as an indicator of cell death. Slices were lysed for Western blot analysis at 24 h and 48h. Control slices were prepared similarly but were treated with vehicle instead of KA (Fig.1). For analysis of BDNF and proBDNF secretion, media samples were concentrated using chloroform:methanol protocol.

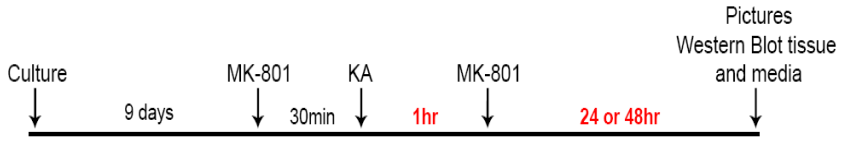

Fig 1. Experimental Design. Organotypic cultures of rat hippocampal neurons were prepared from P7-8 Wistar rats pups. Cultures were cultured for 9 days *in vitro* (DIV). On the 9th DIV, KA was added to the culture medium for 1h. Time points analyzed were 24 and 48 hours after KA.

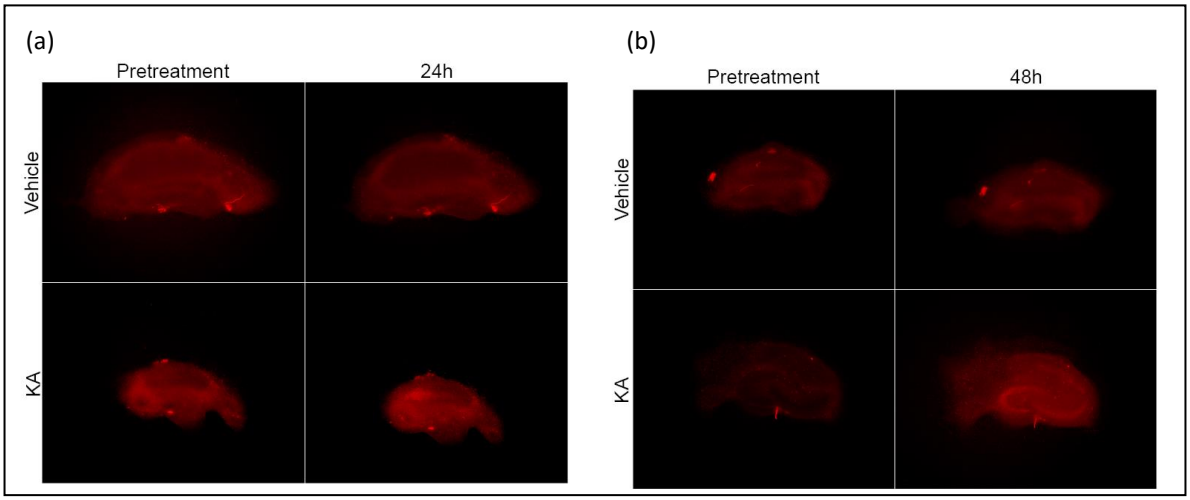

Fig 2. Micrographs of hippocampal cultures stained with Propidium Iodure showing representative slices from each treatment. (a) Slices from P7 rat pups were cultured for 7 d with 50% media changes every 3 d. On day 7, slices were placed into SFM containing 5µg/ml PI. After 1d, pretreatment photographs of each slice were taken (left column). Slices were treated then with 10µl of MK-801 and 1.5 µl of vehicle or 5 µM KA (final concentration). After 1 h, media was changed to fresh SFM with MK-801. Photographs were taken again 24 h following return to SFM. Only slices that received KA treatment demonstrated PI uptake 24 h after kainic acid treatment, particularly in the CA1. **(b)** Photographs were taken again 48 h following return to SFM. Only slices that received KA treatment demonstrated PI uptake 24 h after kainic acid treatment, particularly in the CA1, CA3 and hilus.

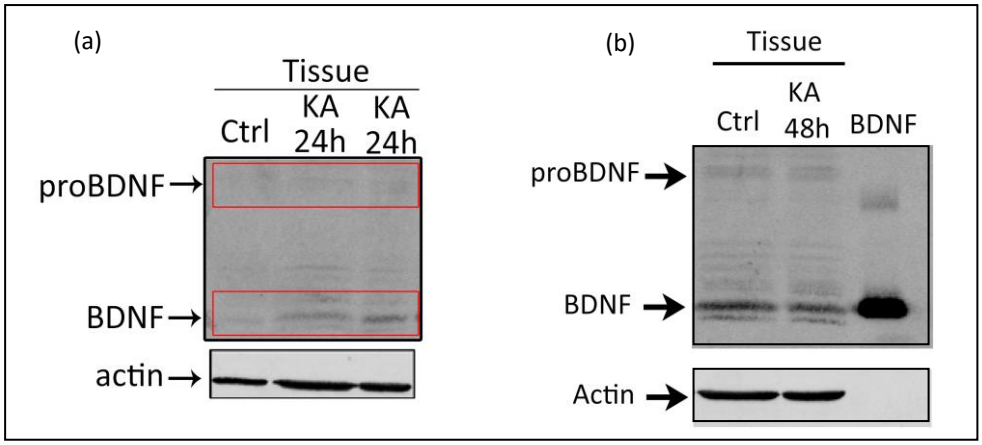

Fig 3. BDNF and proBDNF expression after KA treatment. Following KA treatment, slice culture tissue was harvested at 24 h and 48 h, lysed in buffer containing protease inhibitors, and subjected to Western blot analysis to examine levels of BDNF at 24 h **(a)** and 48 h **(b)** after KA compared with untreated control. By 24 h after KA treatment, BDNF and proBDNF levels increased compared with basal levels while 48 h later, the levels of both proteins were equals to control cultures.

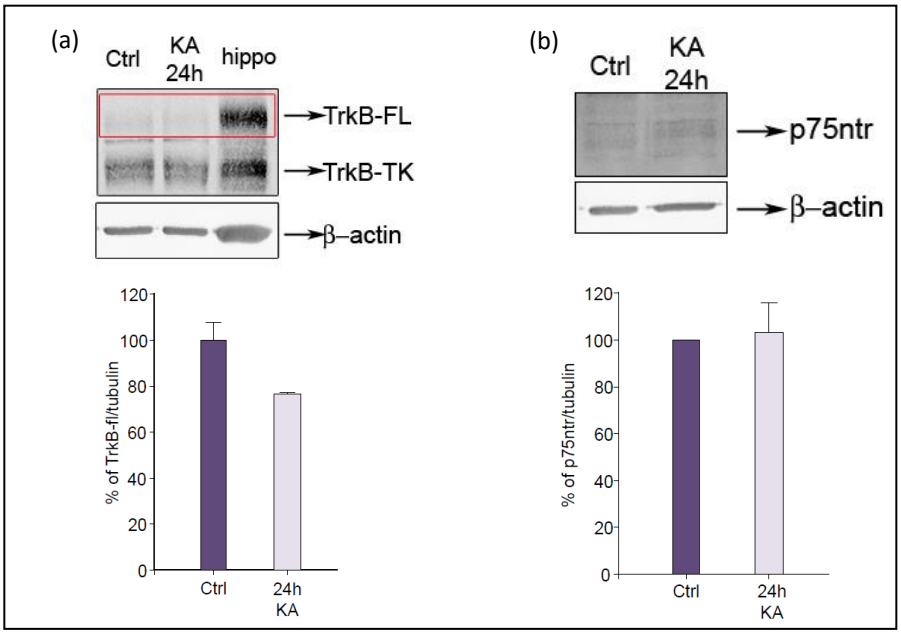

Fig 4. TrkB and p75ntr expresión after 24h KA treatment. Following KA treatment, slice culture tissue was harvested at 24 h, lysed in buffer containing protease inhibitors, and subjected to Western blot analysis to examine levels of TrkB **(a)** and p75ntr **(b)** compared with untreated control. By 24 h after KA treatment, TrkB levels decreased while p75ntr increased compared with basal levels. Error bars represent SEM.

Other academic activities during the short term project:

Discussed my PhD research work with Dr. Friedman's lab members.

Attended the regular weekly discussions and seminars of the Deparment of Biological Sciences.

Dr. Friedman Lab. (*Left to right*): Mr. John Yarotsky; Dr. Marta Volosin; Dr. Wilma Friedman; Dr. Shayri Greenwood; Me; Tech. Dipti Kelkar and Dr. Juan Pablo Zanin.