



"2012 - Año de Homenaje al Dr. D. Manuel Belgrano"

*Ministerio de Ciencia, Tecnología e Innovación Productiva
Consejo Nacional de Investigaciones Científicas y Técnicas*

Buenos Aires, October 28th, 2015

Final Report: **Category C - Return Home Award**

Awarded to: **Silvina Laura Diaz**

Affiliation: **Instituto de Biología Celular y Neurociencias Prof. E. De Robertis, Universidad de Buenos Aires & CONICET.**

Dear CAEN Return Home Committee,

I was awarded the CAEN Return Home in February 2014 to set up my new laboratory, since I had just obtained a post at the National Council of Science & Technology (CONICET) to return to my country, Argentina, after a 6 year postdoctoral period in France.

The local conditions made it difficult to follow the timetable proposed for the grant and thus, I asked the CAEN Committee to postpone the deadline for the final report. You accepted to give me some extra months and make some changes in case there are problems or other priorities. Given that some conditions did not change very much, namely bureaucracy with importation & purchases abroad, the main objectives were kept, but certain activities proposed in the original project were modified. Some preliminary results are presented below, but most of the data produced is currently under analysis. Also, a table with the expenses corresponding to grant awarded is presented at the end of this report.

Final report

Convergent mechanisms modulating adult neurogenesis

Our laboratory is focused on the study of the role of trophic factors and neurotransmitters systems modulating adult hippocampal neurogenesis. Our final aim is to shed light for the comprehension of the mechanisms governing this process and detect potential new targets for more precise neurogenesis-based therapies.

Even though increases in BDNF have been classically associated with increased neurogenesis, some contradictory results observed by us and others, suggest that others aspects apart from the mere increase of BDNF (like differential clivage of proBDNF), are relevant for the neurogenic response to antidepressants. Therefore a deep dissection of neurotrophin biology was proposed in this project.

The specific aims proposed were as follows:

Objective 1: To determine the BDNF isoform involved in DG induced-neurogenesis in adult mice.

Objective 2: To identify the BDNF transcript/s involved in the response to proneurogenic treatments.

Experiments performed

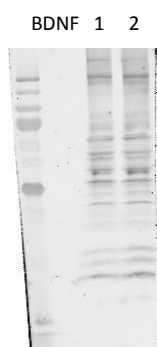
Objective 1:

To determine the expression of BDNF isoforms, experimental conditions were set up. Given the poor specificity of commercial antibodies directed to BDNF, we tested different antibodies and conditions. We also studied the expression of the BDNF receptors, as well as some proteins potentially involved in the clivage of proBDNF. Although these last analysis were not proposed in the original project, we decided to carry out them to better understand the role of BDNF on the adult hippocampal neurogenic process.

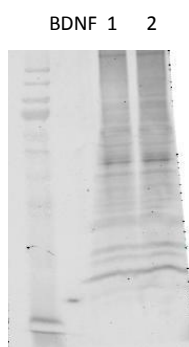
We have originally planned to perform this study in wild type mice chronically treated with antidepressants, in which cell proliferation and survival is increased due to increased serotonin levels. To go deeper on the participation of this neurotrophin and its interaction with the serotonergic system, we also performed the study in hiposerotonergic mice and in a group of transgenic mice knock-out for the 5-HT_{2B} receptor that do not respond to the neurogenic effect of serotonergic antidepressants.

Western blots experiments were carried out to analyze the expression of several proteins in the hippocampus of adult mice. The following proteins were analyzed: proBDNF, mBDNF, TrkB receptor, PhosphoTrkB receptor, p75 receptor, MMP-9 (matrix metalloprotease) and tPA (tissue plasminogen activator). Quantification is currently being carried out but some preliminary observations are presented:

- m- and pro-BDNF levels are poorly detected with the antibodies employed. Also, several non-specific bands are obtained with all of them, as can be seen in the photos bellow, corresponding to 2 samples of hippocampus of wild-type mice (1 & 2) and pure BDNF (50 ng)



Rabbit anti-proBDNF 1:500 (Abcam)

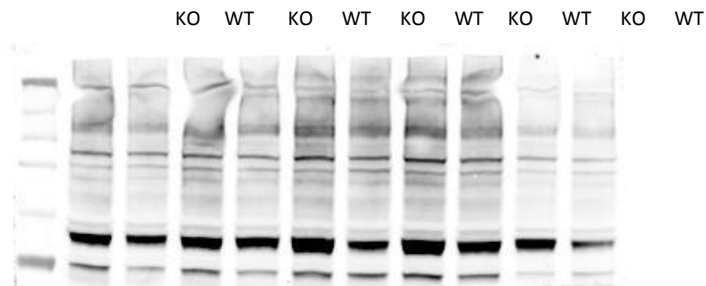


Sheep anti-mBDNF 1:500 (Abcam)



Rabbit anti-BDNF 1:1000 (Sta. Cruz)

- TrkB receptors were more activated in mice knock-out for the serotonin receptor 5-HT_{2B} (KO) than in wild-type (WT) mice, as revealed in the photo below, by the stronger labeling observed for the Phospho TrkB antibody in the KO mice.



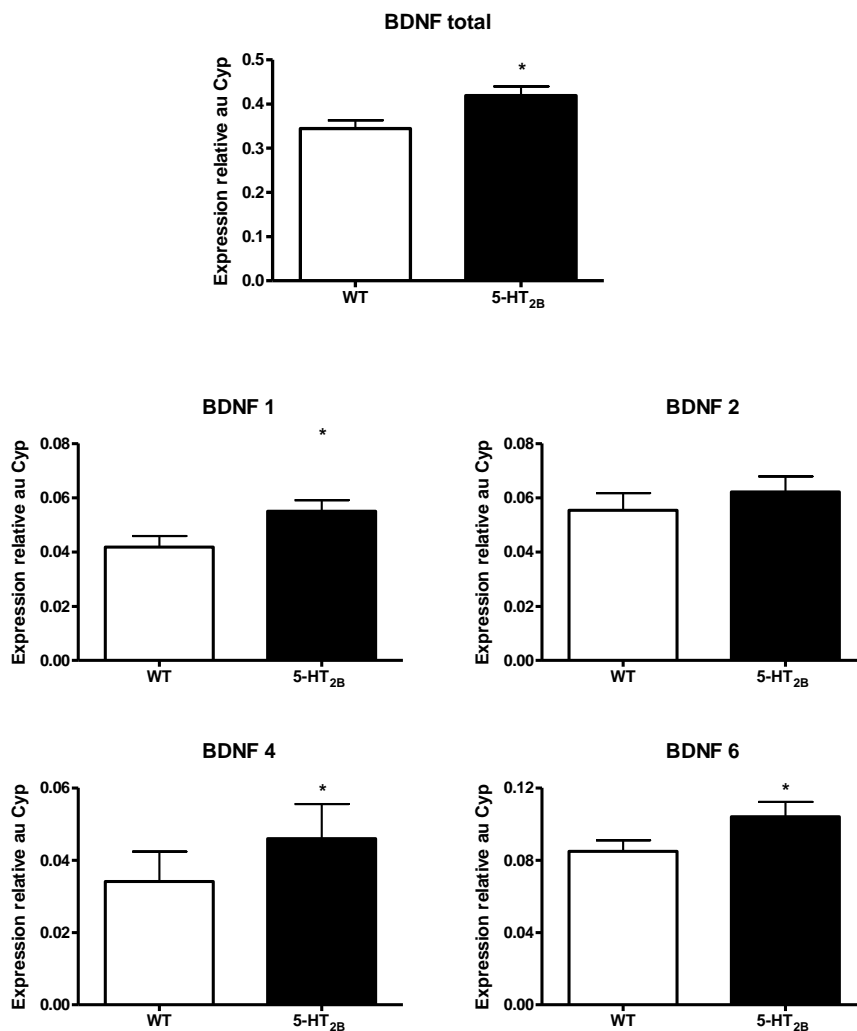
Rabbit anti-Phospho (Y515) TrkB 2ug/ml (Abcam)

The analysis of western blot experiments is expected to be finished by the end of the year.

Objective 2:

In a first step, expression of the different BDNF transcripts in the hippocampus of adult naïve mice was evaluated by qPCR. Among the eight different transcripts, only transcripts 1, 2, 4 & 6 were detected in our experimental conditions. We also measured the expression of these transcripts in mice knock-out for the 5-HT_{2B} receptors and found significant differences in the levels of expression between KO and WT mice in basal conditions. We have then chronically treated mice with serotonergic antidepressants. These results are currently under analysis. We expect that these experiments will reveal the BDNF transcript/s potentially involved in the neurogenic effects of SSRIs.

The graphs below, show the expression of total BDNF and four of its different transcripts in the hippocampus of WT and mice knock-out for the 5-HT_{2B} receptor. BDNF levels are expressed as values relative to Cyp expression.



I would like to add that I was recently awarded the EJM Best Publication Award 2015 for an article published in the European Journal of Neurosciences in 2013. I was asked to give a talk in the last FENS Featured Regional Meeting, in Thessaloniki, Greece, where I presented the results of that paper and my ongoing research, which is partly supported by the CAEN Home Return grant. Therefore, below, there is a photo with my sincere acknowledgment to the ISN.



FENS Featured Regional Meeting, Thessaloniki, Greece, October 9th 2015.

Below, it is detailed how the grant was spent.

Items	Date	\$ ARS	Euro	US\$
Library items	15/02/2014	30		3,4
Library items	15/02/2014	40		4,6
Library items	01/03/2014	971,5		111,7
Library items	01/03/2014	465,1		53,5
Library items	25/03/2014	125		14,4
Library items	26/05/2014	15		1,7
Air Conditioning	28/02/2014	4099,99		471,3
Postal charges	10/03/2014	1121,7		128,9
Postal charges	07/04/2014	663		76,2
Photocopies	08/04/2014	49,5		5,7
Prints B/N	23/04/2014	121,53		14,0
Computer memory 2B	25/04/2014	435		50,0
Mouse for computer	16/04/2014	99		11,4
AFSTAL Adhesion	25/04/2014	1460		167,8
Heater	02/07/2014	1299		149,3
Inhalatory anesthesia apparatus	10/02/2015	39199,87		4505,7
Anti tPA ab (Santa Cruz)	26/06/2015		225,28	248,3
Rb polyclonal to pro BDNF (Abcam)	26/06/2015		405	446,4
Sheep polyclonal to BDNF (Abcam)	26/06/2015		425	468,4
Rbpolyclonal to PhospoTrkB (Abcam)	26/06/2015		410	451,9
Rb x Rat to MMP-9 (Merck-Millipore)	26/06/2015		407,02	448,6
Int. transport of tissue (World Courier)	30/06/2015	12436,6		1323
PC portable ASUS UX305FADQ193T	28/09/2015		799	879,6
Total expenses				10035,8



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Should you need the receipts of the expenses detailed above, please, do not hesitate to ask me and I will send you the scanned copies of them.

I take advantage of this opportunity to warmly thank again the CAEN Return Home Committee for the grant awarded, which was fundamental to set up my own laboratory and allow me to follow my scientific career in my country Argentina.

Yours sincerely,

A handwritten signature in black ink, appearing to read "Silvana Diaz".

Dra. Silvana Diaz
Inv. Adjunta CONICET