



Progress report CAEN Category 1C Return Home Grant 2014-2015

Awardee: Dr. Sara Sanz-Blasco

Project Title: CHARACTERIZATION OF DIRECT AND INDIRECT STRIATAL PATHWAYS IN L-DOPA INDUCED DYSKINESIAS

Dear ISN CAEN members,

It is my pleasure to write this report about my scientific activities since I was honored with an ISN CAEN Return Home Grant in June 2014. I arrived to Argentina in October of 2013 and joined the Laboratory of Experimental Parkinsonism of the National Pharmacological Research Institute (ININFA, University of Buenos Aires and National Council of Scientific Research CONICET) as a Postdoctoral Associate under the supervision of Dr. Oscar Gershanik, who has been leading the lab for the last 30 years and is the current President of the International Parkinson and Movement Disorders Society. I immediately started working with my colleagues in a very interesting project focused on the molecular mechanism of Levodopa (L-DOPA) induced dyskinesias in a mouse model of Parkinson's disease (PD), which is currently funded by the Michael J Fox Foundation for Parkinson's Research - Target Validation Grants (2014-2016). Moving to Argentina and starting working in Parkinson's disease was a little bit of a challenge for me since my previous work, first during my doctoral studies in Spain and later during my first postdoctoral at Sanford Burnham Institute (La Jolla, California), was mainly focused in the physiological mechanisms of toxicity in Alzheimer's disease, so it took me some time to adapt myself to new methodologies and theoretical concepts.

In our laboratory we study how the prolonged use of L-DOPA modifies the brain in rodent models of PD. The administration of L-DOPA is the most effective symptomatic pharmacological therapy for PD. Despite its benefits, most patients develop side effects known as L-DOPA induced dyskinesias (LID). To control LID in PD therapy it is necessary to better understand the multiple cellular and molecular changes that take place during LID. Some protein and gene changes have been reported within the dyskinetic striatum, but the mechanisms in which they are involved are not fully understood. My proposed research project aims to characterize some of the molecules implied in L-DOPA induced dyskinesias (LID) in the two different striatal pathways, direct (D1) and indirect (D2).

It has been previously reported by our group that Pleiotrophin and its receptor RPTP ζ/β are upregulated as a consequence of dopaminergic cell loss and L-DOPA treatment. RPTP ζ/β interacts with PSD95 at the postsynaptic density complex and regulates the protein kinase Fyn, a key molecule involved in synaptic plasticity and cytoskeleton stability. We found an increase in the number of Pleiotrophin(+) neurons, and high levels of phosphorylated Fyn in the striatum of dyskinetic rats (Figure 1).

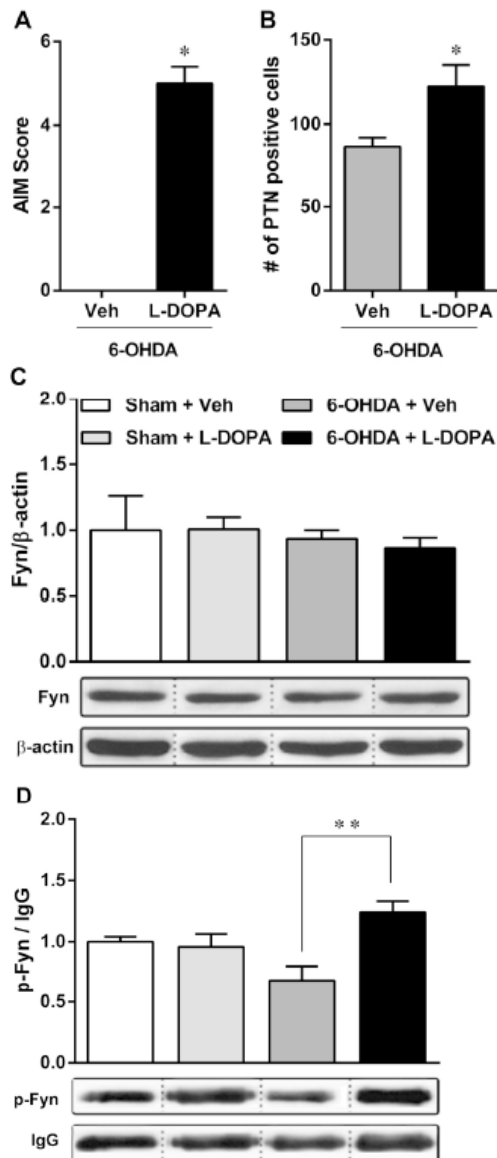


Figure 1. AIM score, PTN cell counting and Fyn protein amounts and phosphorylated status. A) AIM score of 6-OHDA lesioned rats treated either with vehicle (Veh) or L-DOPA. Only 6-OHDA+L-DOPA treated group developed dyskinesia. B) PTN cell counting in the dorsolateral striatum. Data are mean \pm SEM ($n = 7$ rats /group). * $p < 0.05$ vs. 6-OHDA+Veh group, by Student's *t*-test. C) Amounts of total Fyn determined by western blot from ipsilateral striatal homogenates. D) Analysis of phosphorylated Fyn (p-Fyn) in the striatum of dyskinetic rats compared to non-dyskinetic animals. Data are mean \pm SEM ($n = 6$ to 8 striata/group). ** $p < 0.01$, determined by one-way ANOVA ($F = 6.133$) and Tukey's test.

According to these interesting results, we decided to perform behavioral tests and determine abnormal involuntary movements (AIMs) in a model of LID in Fyn knockout (Fyn-KO) mice compared to wild type (WT) mice. Dopaminergic denervation was confirmed by immunodetection of nigral and striatal tyrosine hydroxylase (Figure 2). Fyn-KO mice showed a significant reduction in the development of LID in relation to WT animals (Figure 3).

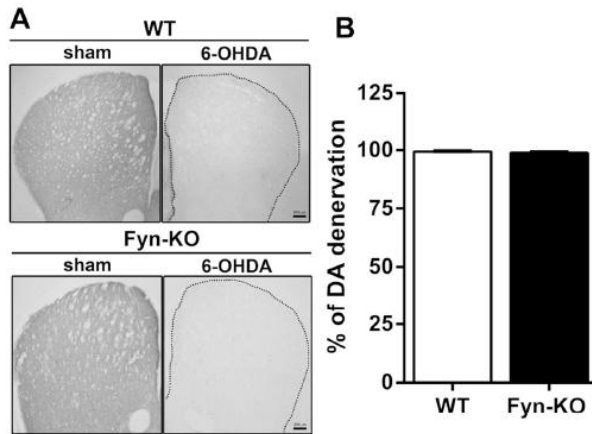


Figure 2. Tyrosine hydroxylase (TH) immunodetection. A) Illustrative photomicrographs of TH striatal immunostaining in sham-operated or 6-OHDA-lesioned Fyn-KO and WT mice. Scale bar = 200 μ m. B) Quantification of TH immunoreactivity expressed as percentage of dopaminergic denervation relative to sham-operated mice. Data are mean \pm SEM ($n = 11$ mice/group), compared by Student's *t*-test.

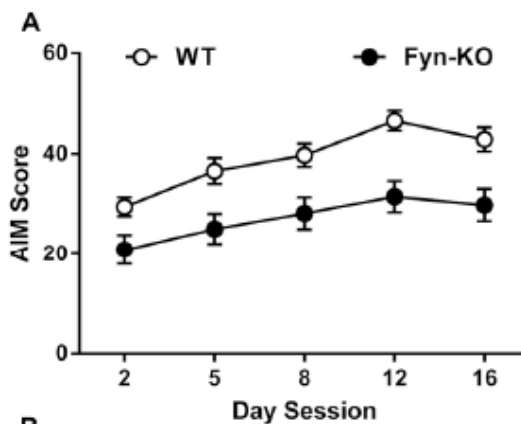


Figure 3. Evaluation of AIMs in Fyn-KO vs. WT mice. A) Sum of values for axial dystonia, orolingual and limb dyskinesia per day throughout treatment. Data are mean \pm SEM ($n = 30$ for Fyn-KO and $n = 28$ for WT littermates). Two-way ANOVA with repeated measures showed no interaction effect (time \times genotype) [$F(4,252) = 1.239$; $p > 0.05$], a significant effect of genotype [$F(1,63) = 14.27$; *** $p < 0,001$] and time [$F(4,252) = 25.09$; *** $p < 0,001$].

In addition, the levels of molecular markers involved in LID, as FosB and pErk, were determined by Immunohistochemistry and/or Western blot, and the results confirmed the previous findings. Our data suggest that Fyn might be involved in the development of LID, and suggests this kinase as a potential target to control LID.

Once we established the role of Fyn in the development of dyskinesias we started studying the expression levels and phosphorylation state of other molecules related with Fyn as NR2A

and NR2B subunits, DARPP-32 and CaMKII, and how they are involved in both striatal pathways. We have induced dyskinesia in a group of mice which express a red fluorescent marker under the control of a D1 receptor promoter (D1-tdTomato). It is our intention to do the same with mice which express an EGFP marker under the control of a D2 promoter (D2-EGFP), but unfortunately we lost this colony some months ago and we are presently trying to restart it. As D1 receptors have been largely associated with LID, our favored hypothesis is that changes in Fyn phosphorylation will take place mainly in this subpopulation of neurons. To evaluate if some of the studied molecules participate in the development and/or consolidation of LID, we will knock down their expression by RNA interference in a temporal/spatial conditional strategy using lentiviral vectors. We will design short hairpin RNA sequences Three to five shRNAs will be cloned into the LV and tested in primary cultures, by co-transduction of shRNA-LVs and a LV expressing CRE. The expression will be quantified by qPCR and western blot to determine the most efficient shRNA-LV. This LV will be injected into the striata of transgenic mice expressing CRE recombinase either in D1 or D2 expressing neurons.

Financial Costs - CAEN award:

Surgery/LID:

6-OHDA	\$325
L-DOPA	\$500
Saracatinib (Fyn inhibitor)	\$1000

Immunohistochemistry:

Antibodies	\$2000
(CaMKII, NR2A, NR2B, PhospoNR2B, TH, secondaries)	

qPCR:

RNA tissue extraction kit	\$500
microplates (10x)	\$200
qPCR kit (50x)	\$1000

Data Analysis:

Computer MacBook Pro 13-inch	\$1600
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Academic Training:

Registration fee 19th International Congress of the PD and MD Society	\$600
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shRNA cloning & molecular biology:

EndoFree Plasmid Buffer Set	\$844
RNeasy Lipid Tissue Mini Kit	\$492

TOTAL Expenses: \$ 9061

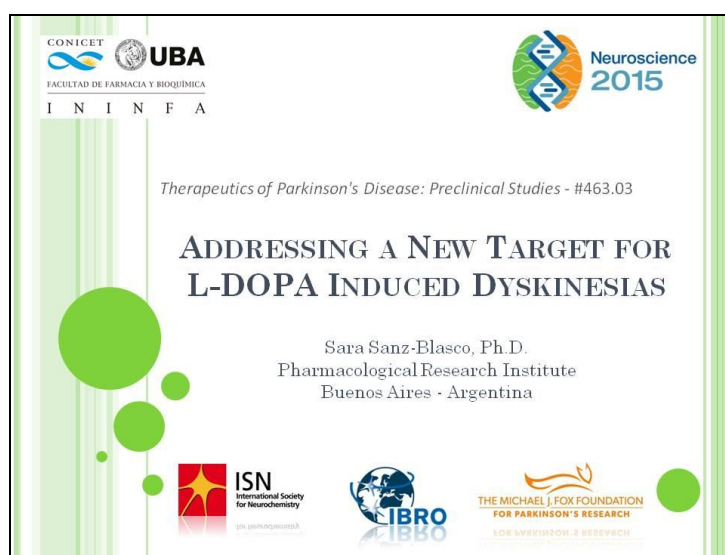
(Remaining: \$400)

Recently we have submitted a manuscript to Annals of Neurology with the results of these two years of work, with me as a first author (see picture of Acknowledgments and Author Contribution part below)

ACKNOWLEDGMENTS: This work was partially supported by EMBO short term fellowship (JEF), IBRO Return Home Fellowship (JEF), ISN CAEN Return Home Grant (SSB), ANPCYT PICT 2011-Argentina (OSG) and Michael J Fox Foundation for Parkinson's Research (JEF & OSG).

AUTHOR CONTRIBUTION: SSB, MDS, MPB, MEA and JEF designed, performed research and analyzed and discussed data. AD, SC, MAB, IT & GG performed research and discussed data, DPH and OSG designed research and discussed data. All authors have participated in discussion of the results, contributed to and approved the final manuscript.

In addition, I got a travel grant to present a poster explaining part of this work at the 19th International Congress of Parkinson's Disease and Movement Disorders in San Diego, California (June 2015), and a few months later I was awarded with the IBRO travel grant to attend the Society for Neuroscience 45th annual meeting in Chicago (October 2015) where I had the opportunity of presenting my work orally in the Nanosymposium entitled: "Therapeutics of Parkinson's disease: preclinical studies" (see photo of first slide of the presentation below).



In November 2014 I was designated Assistant Researcher by the National Council of Scientific Research (CONICET) at my Department, position that I presently hold. In December 2015 I was invited by the Institute of Biology and Molecular Genetics (IBGM) in Valladolid, Spain, to give a talk about these results and my project at their weekly research workshops. Finally, I am preparing a review manuscript about the neurochemistry of dyskinesia to be submitted to the Journal of Neurochemistry in the next few months.

Overall, I think this time has been a very productive stage in my career, in large part thanks to the International Society for Neurochemistry support.

Sincerely,

A handwritten signature in black ink, appearing to read 'Sara Sanz-Blasco', with a horizontal line underneath.

Sara Sanz-Blasco, Ph.D.

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