Report on ISN/CAEN Category 1A Research Grant, 2016

Awardee: Felipe Campos Ribeiro

Project title: Targeting decreased mRNA translation initiation in Alzheimer's disease

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Home Supervisor: Dr. Sergio Teixeira Ferreira

Host Laboratory: Sonenberg lab, Biochemical Institute, McGill University, Canada

Host Supervisor: Dr. Nahum Sonenberg

Duration of visit: February 2017 to November 2017

Introduction:

Neuronal mRNA translation (also known as protein synthesis) is a process that has a major function in synaptic physiology, plasticity and cognition and there is already evidence of decreased general translation on Alzheimer`s disease (AD) patients and also in AD animal models. Here, we used Aβ oligomers (AβOs)-injected mice as a model of AD to investigate if manipulating protein synthesis regulators could rescue AD phenotype in mice.

Aβ is a neurotoxin found on AD brain that has a tendency to aggregate into higher order species. AβOs was found as one of the most toxic forms of this peptide and several studies found that it can model many different aspects of the disease and it correlates well with cognitive deficits in patients.

In order to study protein synthesis on AD we used a mice that has only one allele of the translational repressor 4E-BP2 (thus enhancing translation), injected AβOs intracerebroventricularly (i.c.v.) and after 7 days analyzed protein synthesis and memory.

Results:

To evaluate if the 4E-BP2 +/- mice could rescue AβOs effects on translation, we injected Wt and 4E-BP2 mice with AβOs or vehicle and after 7 days we made hippocampus slices with a vibratome, treated the tissue with puromycin and them prepared the tissue for Western blotting analysis. This technique is called SuNSET and it uses puromycin in low concentrations that is uptaken by the translational machinery as a tyrosine analog and we probe the newly synthesized protein with the anti-puromycin antibody.

Our preliminary results indicate that AβOs reduced protein synthesis on Wt mice, but on the 4E-BP2 +/- mice, protein synthesis` levels are as the control mice (Fig. 1).

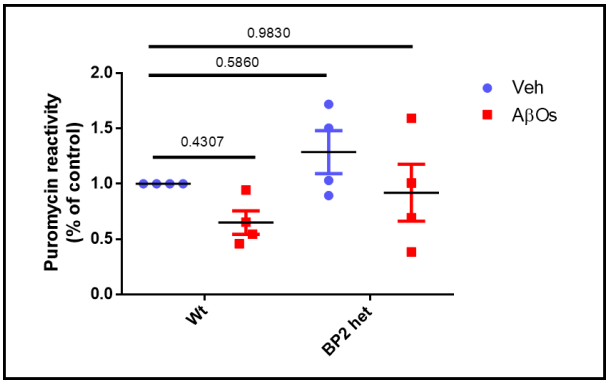


Figure 1: 4E-BP2 attenuation rescues protein synthesis levels on AβOs-infused mice. 3 months-old C57 Bl6 and 4E-BP2 +/- mice were i.c.v. injected with AβOs or vehicle and 7 days after the injection, the hippocampus were extracted, sliced and treated for 45 min with puromycin for labeling of newly synthesized proteins. Our preliminary results indicates that 4E-BP2 reduction rescues protein synthesis levels on AβOs-injected mice.

In another batch of mice, we injected the AβOs in the same way and evaluated memory with the fear conditioning task. On the 6th day after injection, we trained the mice giving a shock in a specific context and on the next day we tested with the mice retained the memory, by assessing their freezing behavior.

We show here that AβOs induced impairment on memory in the Wt mice, the 4E-BP2 +/- alone showed deficits on fear memory, but the AβOs-injected mice performed as the control (Fig. 2). This results suggests that an optimal level of protein synthesis is necessary for memory function.

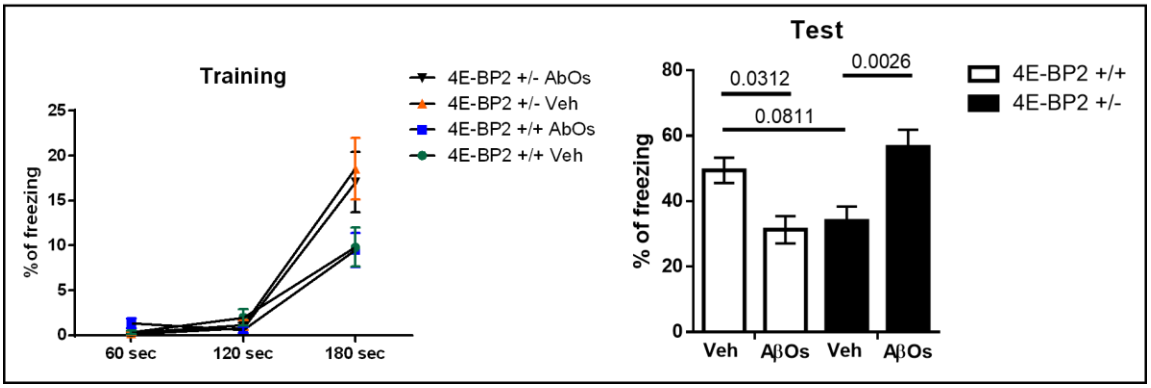


Figure 2: 4E-BP2 attenuation rescues memory loss on AβOs-infused mice. 3 months-old C57 Bl6 and 4E-BP2 +/- mice were i.c.v. injected with AβOs or vehicle and 7 days after the injection, tested on the fear conditioning task. The mice didn`t show statistical difference on freezing in the training session. In the test session, AβOs-injected Wt mice showed decreased freezing, while the 4E-BP +/- mice performed as the control.

Financial report during 10 months of stay in Montreal:

Visa expenses: $300 USD

Air ticket: $1 000 USD

Accommodation: $5 000 USD

Food: $3 000 USD

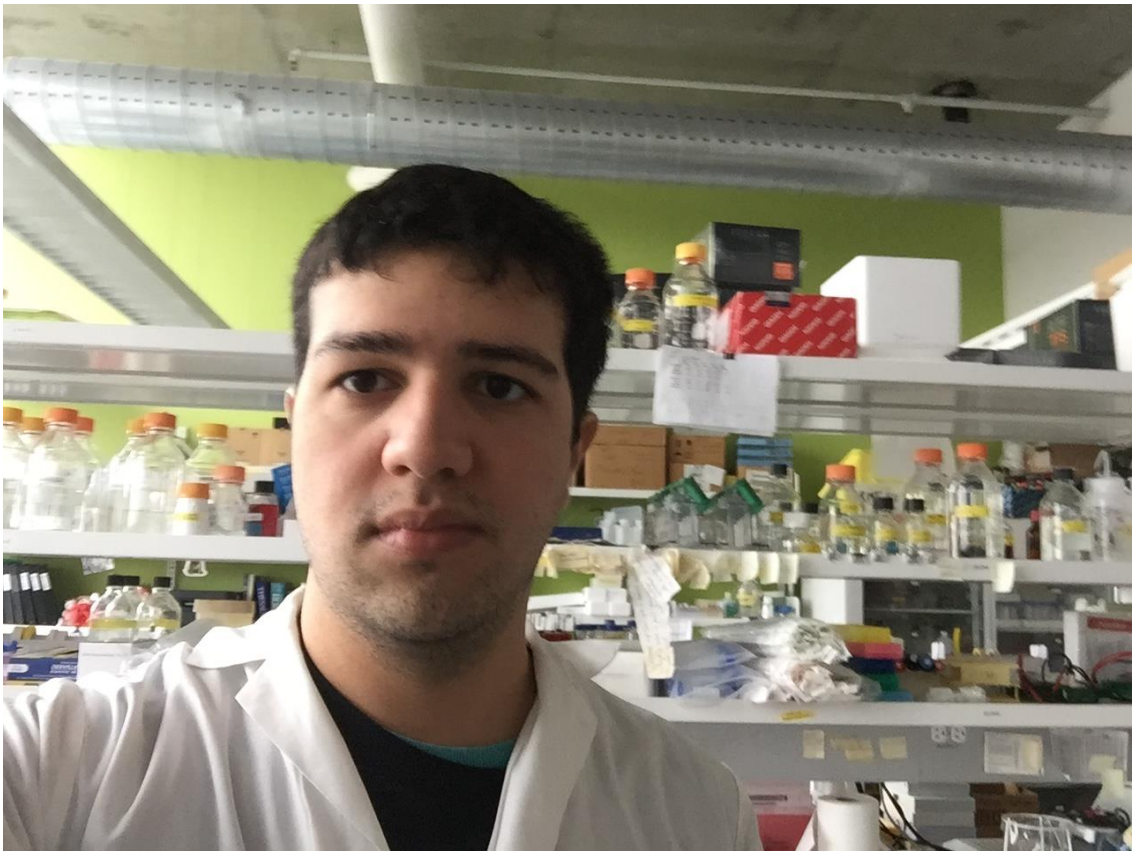
Transportation: $700 USD

Total expenses: $9 000 USD

From the total amount of the expenses $4000 USD were thankfully obtained by the ISN CAEN support and the remaining amount were used from another grant that I received from IUBMB with the amount of $4000 USD and also by personal funds.

I`ve used the ISN CAEN grant to buy the air ticket and for the first 4 months of my stay.

I`d like to greatly thank the ISN and the CAEN committee for the financial support that was indispensable for the work.



Lab photo at the Sonenberg lab

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