November 16th, 2017
Main Report

Awardee: Mauricio Martins Oliveira, PhD student
Biological Chemistry program, Federal University of Rio de Janeiro
Supervisor: Dr. Sergio T. Ferreira
Institute of Medical Biochemistry Leopoldo de Meis, Federal University of Rio de Janeiro
Host Supervisor: Dr. Eric Klann
Host Institution: New York University – New York (NY)

Dear Drs. Prinetti and Rae,

I was awarded a 2017 ISN/CAEN Travel Award 1A for a scientific visit to another laboratory. For this sake, I was kindly hosted Dr. Eric Klann's laboratory, at the New York University (NYU), New York (NY). I was very pleased to join his group between May and September. Dr. Klann was a very kind and very supportive host, just as the Klann's lab members, which were very supportive and helpful to solve any questions or problems. Every training and courses necessary to work in the university were quickly scheduled by the NYU staff, which facilitated me to start my experiments as quick as possible. For this, I am very grateful to all the university staff, as well as all members of Klann's lab.

I should here disclose that, when I first submitted the ISN/CAEN 1A request, the project proposal was different from the experiments here described. I should acknowledge that this was in common acceptance between me and both host and supervisor. I would like to reinforce that the project here developed had very exciting outcomes, which are currently incorporated in a manuscript under preparation for submission. I would like, thus, to apologize for any inconvenient that might be found by the ISN committee for this change of plans. The experiments here comprised include behavioral analysis of a transgenic mouse model of Alzheimer's disease, protein synthesis rate analysis in cultured neurons, and immunoprecipitation experiments to analyze protein synthesis-related complex formation during cellular stress situations. For

a more detailed version of this data, please refer to the scientific report attached to this report.

The visit to Dr. Klann's lab provided me with the possibility to interact and exchange scientific ideas with talented and engaged scientists. This experience deeply enhanced scientific notions I had. Moreover, the vibrant scientific atmosphere was evident, and pushed me forward towards pursuing a successful scientific career.

During my visited, I could get in touch with different techniques, that are not usually performed in our laboratory in Brazil. These techniques comprise both electrophysiological measurements (recording of LTP measurements in hippocampal slices), state-of-art biochemical approaches to study protein synthesis (Bio-Orthogonal Noncanonical Aminoacid Tagging [BONCAT] and Fluorescent Non-canonical Aminoacid Tagging [FUNCAT]), advanced co-immunoprecipitation techniques and behavioral experiments (classical Morris Water Maze and contextual fear conditioning). I had the opportunity to learn the theory of each technique I was in touch with, capacitating me to spread this knowledge not only amongst other researcher in our group, but also amongst scientists in our institution.

Dr. Sergio Ferreira's laboratory has increasing interest in the protein synthesis dysfunction role in the progression of Alzheimer's disease (AD, Lourenco et al., 2013). The experiments made during this visit helped to elucidate precise mechanisms underlying protein translation initiation impairment in this disease, and will hopefully add to other great scientific contributions made throughout last years, staging protein synthesis as one major feature of AD.

The results here described comprise answers to two major questions related to protein synthesis impairment in AD: (1) How is translation initiation impaired in AD models?; and (2) Is it possible to prevent AD-related cognitive impairment by counteracting protein synthesis downregulation? To solve these questions, we used a recently described drug, ISRIB, that bypasses protein synthesis regulatory mechanisms to enhance translation initiation (Sidrauski et al., 2015; Sekine et al., 2015). We described a new translation regulation mechanism involved in cellular stress (and possibly in AD), as well as we saw that counteracting translation initiation impairment is sufficient to prevent cognitive decline in the transgenic murine model of AD APP/PS1.

Finally, as part of my training at NYU, I received high-standard training in animal ethics, care and handling that included interactive on-line courses and presential lectures. These courses improved my knowledge over animal research and use.

In summary, this was a very productive visit, as I had a unique chance to greatly enhance the impact of current science I am developing. Moreover, my period at NYU improved my scientific notions, as I was in touch with some of the greatest scientific minds of my research area. I believe that a scientist should answer specific questions in a very complete way, ranging from cellular/molecular approaches to behavioral/physiology assessment. This is what ISN/CAEN Travel Award 1A helped me pursue. I am, therefore, thankful to ISN/CAEN for allowing me such unique experience, supporting my career development through this award.

Sincerely,

Maurício Martins Oliveira

Mauricio M. Oliveira, PhD Candidate
Institute of Medical Biochemistry Leopoldo de Meis
Federal University of Rio de Janeiro
Brazil

Enclosed picture with host supervisor

Scientific Report

Alzheimer's disease (AD) is the most common form of dementia in the world, and is mostly characterized by memory loss. Although a number of hallmarks were already described in AD brains, the best correlate for cognitive decline is synapse loss (Masliah et al., 1990; Terry et al., 1991). Neurotoxic events that lead to memory loss are mostly associated to small soluble neurotoxins called amyloid-β oligomers (AβO; Ferreira et al., 2015). These molecules are proved capable of binding to neuronal synaptic terminals and induce aberrant signal transduction, impacting memory formation. Amongst signaling pathways that are affected by AβOs, protein synthesis impairment emerges as one central feature of the disease, as this process is long staged as essential for long-term memory formation (Flexner, 1962), and was already shown to be correlated to synapse loss in AD (Lourenco et al., 2013). Our group and others shown that one leading cause of protein synthesis defect is the increased phosphorylation of the eukaryotic initiation factor 2 alpha (eIFα-P) by stress kinases, which ultimately result in protein synthesis impairment (Lourenco et al., 2013; Ma et al., 2013). eIF2α, once phosphorylated, blocks the eIF2B-dependent exchange of GDP for GTP in the eIF2γ subunit of eIF2, therefore impeding the translation initiation complex formation (reviewed by Trinh and Klann, 2013). Thus, counteracting protein synthesis arresting emerges as an attracting approach to jeopardize disease progression.

Reducing levels of eIF2α-P in AD brain was shown to be a challenging task, due to 4 different stress kinases that can mediate its increase. Therefore, an approach that bypasses these kinases activity, by acting directly over eIF2α-P activity, could represent one novel, untested therapeutic approach. A recent pharmacological advent developed by Dr. Peter Walter's group, from UCSF, called ISRIB, was shown to act directly over eIF2α-P effects on translation initiation impairment (Sidrauski et al., 2013; Sidrauski et al., 2015; Sekine et al., 2015). Furthermore, this drug was already shown to have preventive effects in the progression of neurodegenerative diseases (Halliday et al., 2015; Chou et al., 2017). We thus hypothesized that ISRIB could rescue memory decline in AD models by recovering brain protein synthesis to basal state.

During my visit to Dr. Klann's laboratory, I sought to investigate two major questions: (1) What are the precise mechanisms involved in eIF2α-P-dependent protein

synthesis impairment in stressful conditions?; (2) Does ISRIB counteract protein synthesis impairment and cognitive decline in AD models?

To answer the first question, we exposed HEK293 cells to Thapsigargin and/or ISRIB, and evaluated the integrity of both eIF2 complex and the interaction between eIF2 and eIF2B through co-immunoprecipitation. *Our results indicate that Thapsigargin induce a disruption of eFI2 complex, as well as its interaction with eIF2B. Intriguingly, although ISRIB could not rescue eIF2α-P levels, it prevented both interaction disruptions, offering an explanation on how does ISRIB counteract translation blockage upon stressful situations (Figure 1)*.

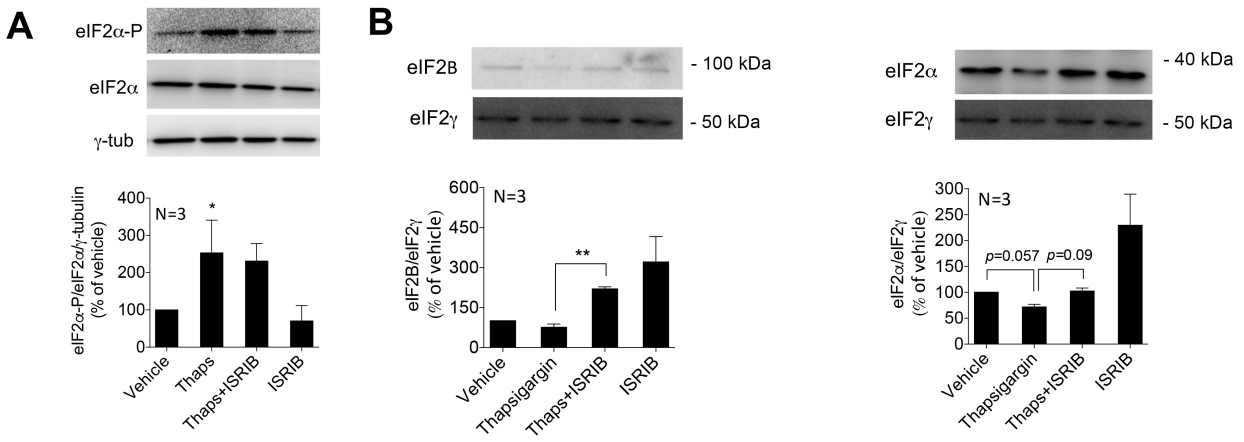

Figure 1. ISRIB prevents stress-induced translation initiation complex disruption. (A) ISRIB does not prevent thapsigargin-dependent eIF2α-P enhancement in HEK293 cells. (B) ISRIB prevents and enhances the interaction between eIF2B and its substrate, eIF2γ, in HEK293 cells. (C) ISRIB blocks thapsigargin-induced eIF2α-eIF2γ interaction disruption in HEK293 cells. Two-Way ANOVA, *=p<0.05, **=p<0.01.

To solve the second question, we administered intraperitoneally 0.25 mg/kg ISRIB daily, during a month, in a transgenic mouse model of AD, the APPSwe/PS1ΔE9. To test whether ISRIB could cause any aberrant locomotor/anxiety phenotype, we tested these mice in the open-field arena two weeks after the first ISRIB injection. To assess cognitive impairment, we performed a Morris Water Maze 3 weeks after the first injection, and Contextual Fear Conditioning 2 days after the Morris Water Maze. *Our results, although*

preliminary, indicate that chronic administration of ISRIB rescues cognitive decline in APP/PS1, without any clear aberrant locomotor side effect (Figure 2). Further experiments will be necessary to conclude these promising results.

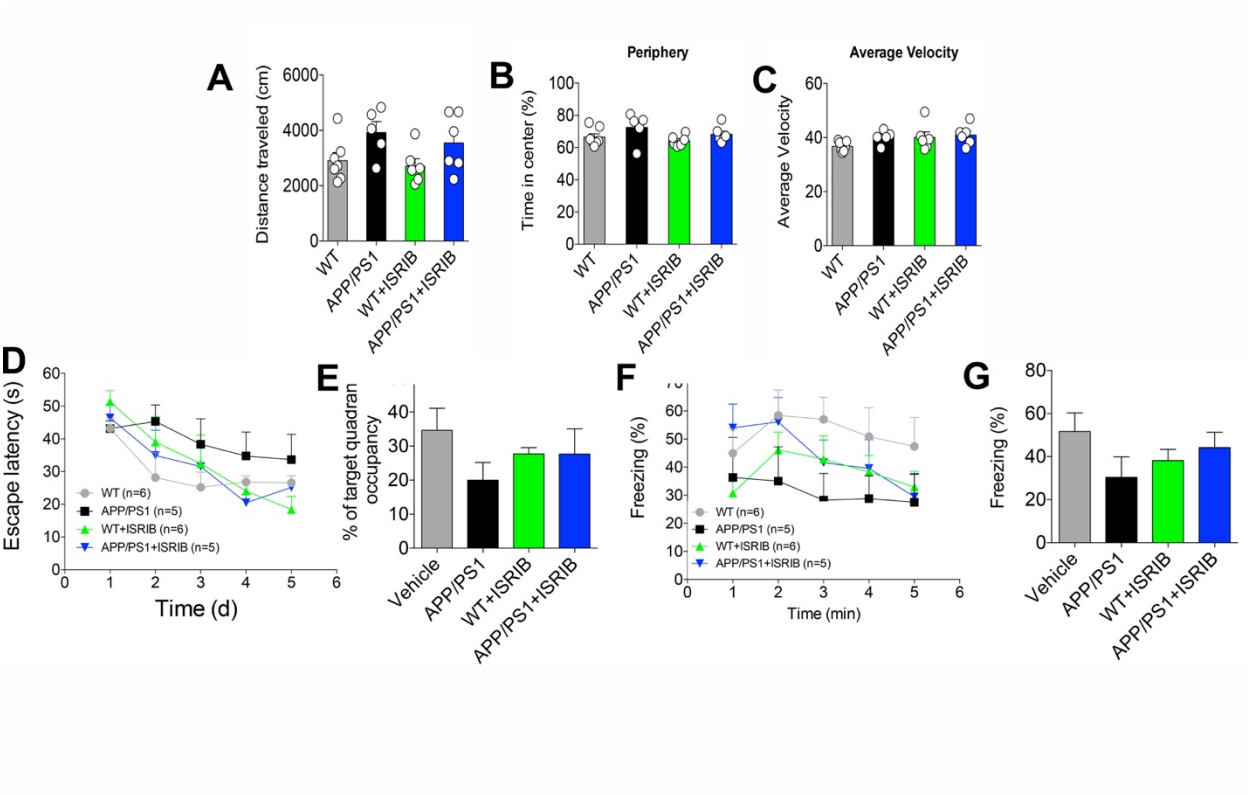

Figure 2. ISRIB rescues cognitive deficits in APP/PS1 mice. (A-C) Open Field Arena measurements of distance traveled (A), time spent in periphery (B) and average velocity (C) of mice. (D) Escape latency during each day of trial of the Morris Water Maze. (E) Time spent in target quadrant in Morris Water Maze's probe day. (F) Contextual Fear Conditioning (CFC), analyzing freezing behavior minute by minute. (G) Percentage of total time spent by mice doing freezing behavior.

Altogether, the findings obtained during my visit to Dr. Klann's lab strongly suggest that ISRIB might rescue memory impairment in a transgenic AD murine model, as well as suggest a new mechanism by which ISRIB could bypass translation initiation impairment, by greatly enhancing both eIF2 complex formation and eIF2-eIF2B physical interaction. These results are in agreement with previous results we obtained in this project, which overall suggests that bypassing eIF2α-P-dependent translation initiation impairment

might represent a suitable therapeutic approach to combat AD-related cognitive decline. Results here described were already incorporated in a manuscript, which is currently in preparation for submission.

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Travel assurance

Conectados tudo é possível

CopaAirlines

A STAR ALLIANCE MEMBER

Panama, 19 de Setembro de 2017

COPA AIRLINES – Compania Panameña de Aviación
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Apartado 0816-06819, Panamá, R. P.

DECLARAÇÃO DE VIAGEM

Copa Airlines declara para os devidos fins, que tem em seus registros a emissão do bilhete **230-1228517051** em nome de Mauricio Oliveira emitido em **26 de Abril de 2017** o qual consta como utilizado nos seguintes trechos:

CM 215 de 20 de Maio/2017 – Rio de Janeiro – Panamá - Tarifa E
CM 804 de 20 de Maio/2017 – Panamá – New York –Tarifa E
CM 803 de 10 de Setembro/2017 – New York – Panamá – Tarifa E
CM 873 de 10 de Setembro/2017 – Panamá – Rio de Janeiro – Tarifa E

Sendo o que se nos apresenta para o momento, subscrevemo-nos,

Atenciosamente

Jonathan Peña
Call Center | Coordinator
Copa Airlines

Flight Details

Código de Reserva:**PR6NHU** Fecha: **26/04/2017**

Itinerário de voo:

Ida:

RESERVA CONFIRMADA, Classe Turística

VOO	CM 215 - Copa	20/05/2017
SAÍDA:	Rio de Janeiro, BR (Rio Int.)	20/05/2017 11:30
CHEGADA:	Cidade do Panamá, PA (General Omar Torrijos Herrara Intl. Airprt)	20/05/2017 16:53

LOCALIZADOR COMPANHIA AÉREA:CM/GTP4RR
DURAÇÃO: 07:23

BAGAGEM PERMITIDA: 2 Pedaços
PARAGENS TÉCNICAS: -
OPERADO POR: Copa, CM

RESERVA CONFIRMADA, Classe Turística

VOO	CM 804 - Copa	20/05/2017
SAÍDA:	Cidade do Panamá, PA (General Omar Torrijos Herrara Intl. Airprt)	20/05/2017 18:27
CHEGADA:	Nova Iorque, US (John F. Kennedy Intl.)	21/05/2017 00:35

LOCALIZADOR COMPANHIA AÉREA:CM/GTP4RR
DURAÇÃO: 05:08

BAGAGEM PERMITIDA: 2 Pedaços
PARAGENS TÉCNICAS: -
OPERADO POR: Copa, CM

Volta:

RESERVA CONFIRMADA, Classe Turística

VOO	CM 803 - Copa	10/09/2017
SAÍDA:	Nova Iorque, US (John F. Kennedy Intl.)	10/09/2017 09:45
CHEGADA:	Cidade do Panamá, PA (General Omar Torrijos Herrara Intl. Airprt)	10/09/2017 13:57

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DURAÇÃO:05:12

BAGAGEM PERMITIDA: 2 Pedaços
PARAGENS TÉCNICAS: -
OPERADO POR: Copa, CM

RESERVA CONFIRMADA, Classe Turística

VOO	CM 873 - Copa	10/09/2017
SAÍDA:	Cidade do Panamá, PA (General Omar Torrijos Herrara Intl. Airprt)	10/09/2017 15:19
CHEGADA:	Rio de Janeiro, BR (Rio Int.)	11/09/2017 00:25

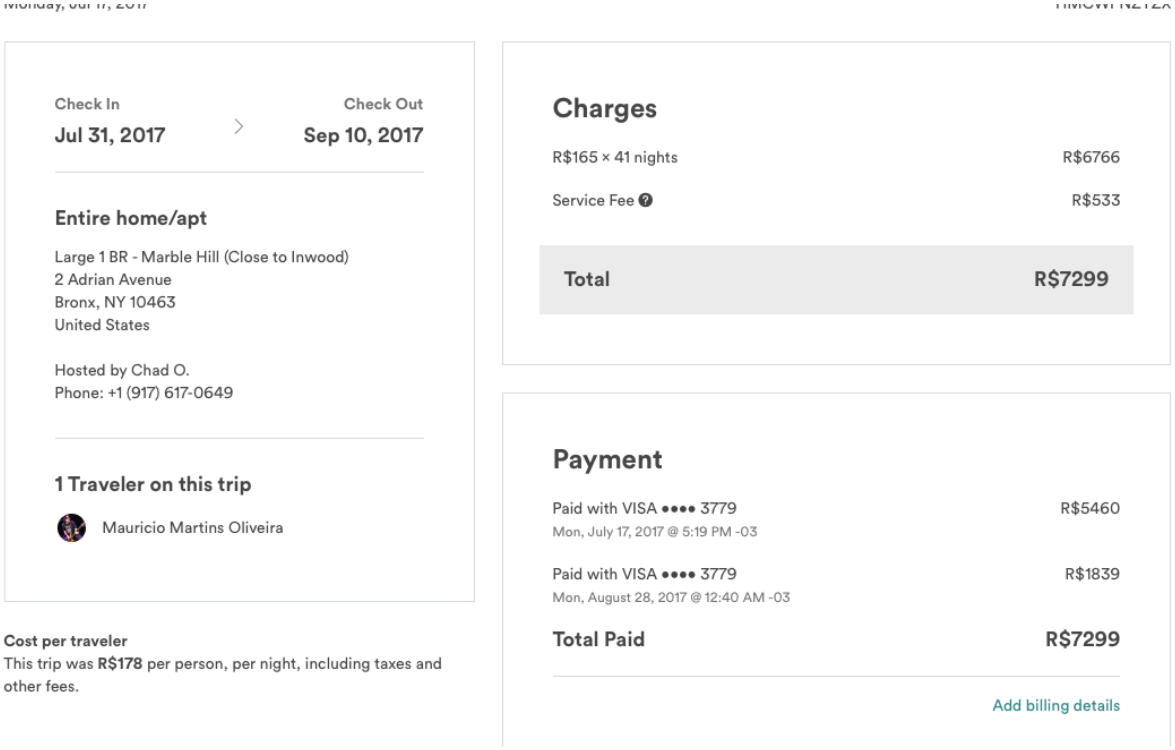
LOCALIZADOR COMPANHIA AÉREA:CM/GTP4RR
DURAÇÃO:07:06

BAGAGEM PERMITIDA: 2 Pedaços
PARAGENS TÉCNICAS: -
OPERADO POR: Copa, CM

Bilhete/s Eletrônico/s

BILHETE: **2301228517051 - Oliveira Mauricio**

Housing



Check In
Jul 31, 2017

Check Out
Sep 10, 2017

Entire home/apt

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Bronx, NY 10463
United States

Hosted by Chad O.
Phone: +1 (917) 617-0649

1 Traveler on this trip

Mauricio Martins Oliveira

Cost per traveler
This trip was **R$178** per person, per night, including taxes and other fees.

Charges

R$165 × 41 nights	R$6766
Service Fee	R$533
Total	**R$7299**

Payment

Paid with VISA •••• 3779 Mon, July 17, 2017 @ 5:19 PM -03	R$5460
Paid with VISA •••• 3779 Mon, August 28, 2017 @ 12:40 AM -03	R$1839
Total Paid	**R$7299**

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R$7299 = USD 2224,71

Finantial report

To cover partial expenses of my visit to Dr. Klann's laboratory, I was kindly awarded by ISN/CAEN with USD 2.000,00, which were primarily employed in housing payment. The award stacked up to a Brazilian fellowship awarded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), which granted me USD 11.900,00.