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CAEN Award Category 1A (April 2016 Round)

Report

Project title: Live microscopy to decipher the impact of ER-mitochondria connection on Alzheimer's disease neuronal bioenergetics performance.

Awardee: Pamela V. Martino Adami, MSc.

Advisor: Laura Morelli, PhD.

Home laboratory: Laboratory of Amyloidosis and Neurodegeneration, Fundación Instituto Leloir – IIBBA CONICET. Ciudad Autónoma de Buenos Aires, Argentina.

Host laboratory: MitoCare Center for Mitochondrial Imaging Research and Diagnostics, Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University. Philadelphia, Pennsylvania, United States of America.

Host laboratory's advisor: György Hajnóczky, MD, PhD.

Dear Dr. Caroline Rae,

I was awarded with a CAEN Award Category 1A in April 2016 to visit the laboratory of Dr. György Hajnóczky, located in Thomas Jefferson University, Philadelphia, United States of America, from 15th July to 9th September. Dr. Hajnóczky was a very kind host. He provided me all the reagents and equipment needed to pursue my project, made me join the laboratory meetings and gave me the opportunity to discuss the results several times. The members of his team also helped me throughout my stay in Jefferson to get familiar with the use of microscopes for live imaging and I could exchange scientific ideas with them. I am thankful to

Dr. Hajnóczky and his team in this regard. Furthermore, I was able to help the host lab with insights about neuronal cultures, which is a new research model in the laboratory.

The objectives of my stay were (1) to visualize and quantify the physiological endoplasmic reticulum ER-mitochondria contacts and (2) to evaluate the Ca^{2+} transfer from ER to mitochondria in rat hippocampal primary neurons by live imaging. In order to save time and get more out of my short stay, I cryopreserved wild type embryonic neurons (E18) that I isolated in Buenos Aires and sent the cryovials with cells ready-to-use to Philadelphia.

During my first week, I got used to visualization techniques with a cell line (H9c2 cells) which is very useful for imaging due to the size of the cells and the high transfection efficiency. Once I got familiar with the microscope, I thawed the cryopreserved primary neurons and began evaluating ER-mitochondria contacts and Ca^{2+} transfer in neurons with 7 or 14 days *in vitro* (DIV7 or DIV14). In order to address objective (1), I transfected neurons with plasmids coding for “linkers” targeting the outer mitochondrial membrane (OMM) and the ER that heterodimerized and formed a FRET pair upon rapamycin addition, indicating the areas where OMM and ER were naturally close. I used linkers with different lengths to evaluate the contacts with variable distances between OMM and ER. Regarding objective (2), I transfected neurons with a plasmid coding for a Ca^{2+} sensor targeted to the mitochondrial matrix and loaded them with Fura-2AM to monitor cytoplasmic Ca^{2+} levels at the same time. All the plasmids were cloned and provided to me by Dr. Hajnóczky and his team. By comparing the results obtained with primary neurons to those with H9c2 cells, I could observe how different neurons are in terms of ER-mitochondria contacts (different FRET kinetics depending on the length of the linkers) and in Ca^{2+} buffering (neurons rely much more on mitochondria to buffer increased Ca^{2+} levels). These results obtained in MitoCare Center will be contrasted to those I am going to perform in Argentina with the transgenic Alzheimer model I am working with.

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In summary, this short stay gave me the opportunity to get training in live imaging, a technique that will allow me to address the last scientific questions regarding my PhD project with state-of-the-art experiments. This is a very different approach from the one I have been using, which was based mostly on neurochemical techniques. In this way, the possibility of visiting Dr. Hajnóczky's laboratory undoubtedly added value to my project and benefited my home institution since I brought back home tools which will allow us to get more out of the microscopy facility we already have. This kind of activities is essential for my institution located in Argentina, which is a low-income country where there are low budgets and serious difficulties to support science & innovation. I am really thankful to the International Society for Neurochemistry for having considered my application and providing me the opportunity to perform part of my research in a laboratory located in a developed country.

Regards,

Pamela V. Martino Adami, MSc.

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Dr. Hajnóczky (bottom, in the middle) and his postdocs on my last day in MitoCare Center

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