

NENS Exchange Grant – Final report

Period: 07 November 2022 – 07 February 2023



Anastasia Megalokonomou, BSc, MSc, PhD candidate
Institute of Biosciences & Applications, NCSR "Demokritos"
T. Patriarchou Grigoriou & Neapoleos, 153 10 Athens, Greece
Email: anastasiam13@yahoo.fr
lab: +30 210 6503586
cell: +30 6977127402

Host program: Master in Health Sciences and PhD in Health Sciences

Host Lab: ICVS, Medical school of University of Minho, Portugal

Home program: Graduate Program in Neuroscience – Crete

Home Lab: Institute of Biosciences and Applications, NCSR "Demokritos", Athens, Greece

During my 3-month-long training stay at ICVS, I was able to acquire valuable knowledge and master techniques that will be extremely useful during my Ph.D., while they will also form part of my skill set as a future researcher. Specifically, I was trained in a technical assay for primary neuronal cultures established by the team of my host supervisor Dr. Joana Silva. This assay includes the sacrifice of mice at postnatal day 0-1 (P0-1), isolation of the hippocampus and cortex for neuron collection, plating, and treatment, followed by collection methods for further analysis.

After learning this technical assay, I was able to complete a part of the *in vitro* studies of my Ph.D. project. Based on previous work from Dr. Joana Silva showing the precipitating role of chronic stress and high levels of the main stress hormones, the glucocorticoids, on Tau pathology (Silva JM, et al CDD 2019; Monteiro et al Mol Psychiatry 2021), I tested whether selected Antisense Oligonucleotides (ASOs) against Tau protein can be protective against neurotoxicity induced by the synthetic glucocorticoid dexamethasone (DEX). For this experiment, I was trained and worked in the preparation of primary neurons from wild-type (WT) C57BL/6 mice as well as P301L-Tau Tg mice for 2 weeks, which I treated with ASOs for 72h and DEX for 48h.

During my training stay, I was also trained in molecular and cellular techniques for primary neurons analysis, such as a cell viability assay (MTS), and immunofluorescence (IF), while I also applied a specific IF protocol for double staining of phosphorylated Tau and a synaptic marker (PSD-95), created and optimized by the host lab. After completing the IF staining at the ICVS Institute, I was also trained in super-resolution confocal imaging in order to obtain high-quality fluorescence images, in order to proceed with image analysis.

In addition, as Dr. Joana Silva is highly experienced in animal surgeries, after completing my *in vitro* experiments, I was offered the opportunity to be trained in surgical procedures including anesthesia and analgesia, as well as i.c.v. injections of ASOs using a stereotaxic apparatus. This was essential as it provided me with the appropriate techniques to apply during the *in vivo* experiments of my Ph.D. project that will take place in my home laboratory in Greece. Briefly, during these experiments, I will inject P301L-Tau Tg and WT mice with selected ASOs, and after 6 weeks, I will conduct behavioral experiments to evaluate memory, mood, and locomotion, while the animal brains will be isolated in order to proceed with molecular, cellular, neurostructural, proteomic, and transcriptomic analysis.

My host supervisor, Dr. Joana Silva, was a very valuable mentor, providing me with extensive training on various techniques, advising me on methodological and theoretical issues regarding my

project, but also continuously offering me opportunities to learn and pose questions, inspiring me to become a better scientist. She encouraged me to present my Ph.D. proposal during an ICVS meeting, an occasion that offered me valuable feedback and novel ideas regarding the future development of my Ph.D. studies, by experienced scientists such as the host program coordinator Prof. João Bessa, Assistant Professor at Medical School of University of Minho as well as Dr. Luisa Pinto and Dr. Ana João Rodrigues, ICVS Group leaders who are experts in brain plasticity and connectomics, respectively.

It is important to mention that most of the techniques I learned are not yet used in my home laboratory. Therefore, my newly acquired expertise in these methodologies will allow me to implement them to continue my current Ph.D. experiments, but also to contribute to other experiments of my home Institute team in Greece in a constructive way.

Finally, it is worth mentioning that during my stay in ICVS I had the opportunity to interact with researchers from different countries and cultures (Portuguese, Italian, Brazilian, Greek) and familiarize with novel ways of thinking in both science and life. Portuguese hospitality (both in and out of the lab) was a great gift for me and thus, I am grateful to the **NENS exchange program** that offered me this important opportunity at the beginning of my Ph.D. studies and scientific career.

My photo with the group of Dr. Joana Silva's in the "X-mas lab meeting".

