# NENS Exchange Grant – report

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by

Alexandru-Florian DEFTU

050095 Splaiul Independentei Nr. 91-95, Department of Anatomy, Animal Physiology and Biophysics, Faculty of Biology, University of Bucharest, Romania

Phone: +40766620443

E-mail: bio.florian@yahoo.com


Home lab under the supervision of Prof. Violeta RISTOIU, Research Center for Neurobiology and Molecular Biology, Department of Anatomy, Animal Physiology and Biophysics, Faculty of Biology, University of Bucharest, Romania.

Host lab under the supervision of Prof. Bert BRONE, Biomed Center, Department of Medicine and Biomedical Sciences, Faculty of Medicine and Life Sciences, University of Hasselt, Belgium

During my master and PhD-period I worked intensely in the research field of pain in the peripheral nervous system and in particular on the influence of chemokine CXCL1 and CXCL2 in cultured neurons from dorsal root ganglia (DRG), using electrophysiological recordings. CXCR2, the receptor of the chemokines, has a large distribution in the peripheral and central nervous system, including the hippocampus, an important area in pain sensibility. Apart of the chemokine influence on neurons from peripheral or central areas of the nervous system, new data suggest an important contribution of microglia activation in the orchestra of chemokine signaling. Therefore, the characterization of microglia activity due to chemokine modulation during pain sensibility will bring new targets of interest in this field. A dynamic group focused on microglia activity in the nervous system is the one from Prof.Jean-

Michel Rigo and Prof. Bert Brone lab, a group which helped at the foundation of the research on microglia cells and their importance in the nervous system. They have in house expertise in calcium imaging and electrophysiological properties of microglia both in culture and brain slice and are the best candidates from which I can learn patch-clamping microglia in brain slice. Taken together the background of this research group will for sure have a big benefit in my personal research field and future as a young scientist. In the end, this short-term project I plan to do, I hope will also generate new ideas and maybe new opportunities of collaboration between our lab and the host lab.

The pathogenesis of pain involves the interaction between neurons and glial cells mediated by inflammatory chemokines. CXCL2 is a chemokine that exerts its functions through CXCR2 that has a wide distribution inside the nervous system including the CA1-hippocampal formation, important area in pain modulation. Gene expression along with neurogenesis is altered in the hippocampus during persistent pain. Moreover, inhibition of microglia activation protects hippocampus neurogenesis and their depletion induces a decrease in neural precursor cell activity. Our main goal is to investigate changes that appear in microglia activity from the CA1 and dentate gyrus from the hippocampal region of 5-8 weeks old CX3CR1-eGFP mice due to 4h incubation with 3.6 nM CXCL2, which can serve to clarify its effects on the central pain structures.

Our result show that a hyperpolarization step of -130 mV the current densities expressed by GFP-microglia from the dentate gyrus decreases to -5.64 ±3.06 pA/pF, n = 6 after 4 h incubation in the presence of 3.6 nM CXCL2 compared with control conditions -3.43 ±0.5 pA/pF, n = 11, P > 0.05. At a depolarization step of + 40 mV the current density expressed by microglia cells after 4 h incubation with 3.6 nM CXCL2 increased at 5.62 ±0.75 pA/pF, n = 6 compared with control 4.88 ±1.06 pA/pF, n = 11, P > 0.05 (Figure 1).

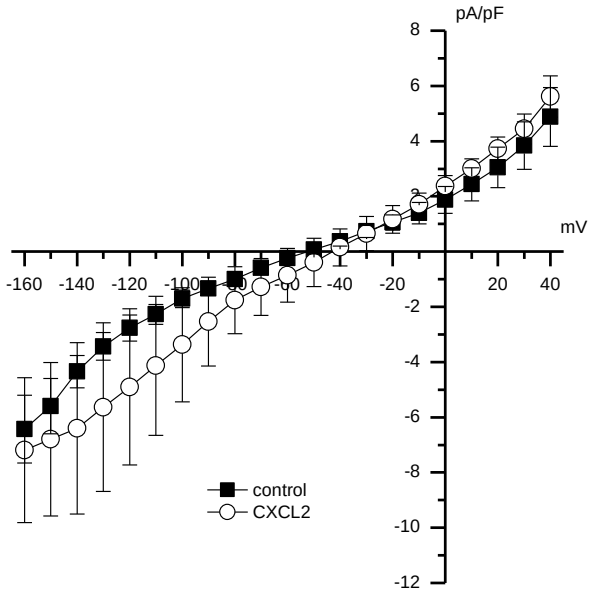


*Figure 1. The IV curve of current density in GFP-labeled microglia from the dentate gyrus.*

Patch-clamp, as a branch of electrophysiology, can be used for single ion channel recording or for whole-cell recordings. By using the whole-cell patch-clamp technique you can either record cells in culture or in slice. The advantage of patching in slice is the rapid isolation of the area of interest and the maintenance of local network from which your cell belongs. In this application I proposed to learn new techniques that I can implement in my home lab. My main concern is to learn how to make a hippocampus brain-slice from mice and how to maintain the slice so that the cells are viable and well preserved in network. Following brain slice, I would like to learn how to approach cells which are in slice and to resolve what impediments I would encounter while making whole-cell patch-clamp in microglia from brain slice. I think that this method will bring new possibilities in my personal research field and also in my home lab in which I will implement this technique after my return and hope to keep a vivid collaboration with the group of Prof. Bert BRONE.

The NENS Exchange Grant gave me as a PhD student the occasion for two month to start a collaboration with the lab from Hasselt, Belgium. The grant was very useful to learn new techniques as electrophysiology and to see the research at advanced levels made in other labs compared with Eastern Europe countries. Through this exchange I have started this new collaboration that I hope it will last for a long time and hope to have a visit also from my host mentor in my country. The techniques which lacked from my lab were very useful to learn, enlarging even more my personal research field, offering new opportunities in my career and I have started to implement them with success in my home lab.