NENS Exchange Grant – Final Report

Student: Loredana Cumpănă
Period of training stay: 1st August – 3rd October 2018
Home Institution: Master in Neurobiology, University of Bucharest, Bucharest, Romania
Host Institution: Graduate Program in Neuroscience, University of Copenhagen, Copenhagen, Denmark
Host Supervisor: Dr. Mihai Moldovan
Project Title: Acute TRPV4 pharmacologic modulation of motor axon excitability during intermittent burst activity

- **Background**

At the University of Bucharest, as part of my Master thesis we have shown that Schwann cells functionally express TRPV4 (Transient Receptor Potential Vanilloid 4) *in vitro*. During the two months of the NENS internship at the University of Copenhagen, under the guidance of Dr. Mihai Moldovan, we have investigated the functional role of TRPV4 in Schwann cells *in vivo.* Our hypothesis was that TRPV4 is involved in ionic homeostasis, therefore we decided to test if TRPV4 pharmacology has an effect on alpha motor axonal excitability in an activity dependent manner.

- **Project**

Two strains of mice were used, C57BL Wild Type and PMP22 transgenic mice that model the motor neuropathy Charcot-Marie-Tooth type 1A with hypomyelinated peripheral axons phenotype. Electrophysiological recordings were obtained from stimulating the tibial nerve and recording the evoked Compound Muscle Action Potential (CMAP) from the plantar muscles with platinum needle electrodes inserted percutaneously. Multiple axonal excitability measures were recorded using the software QtracS by "threshold-tracking" 40% of the maximum CMAP.

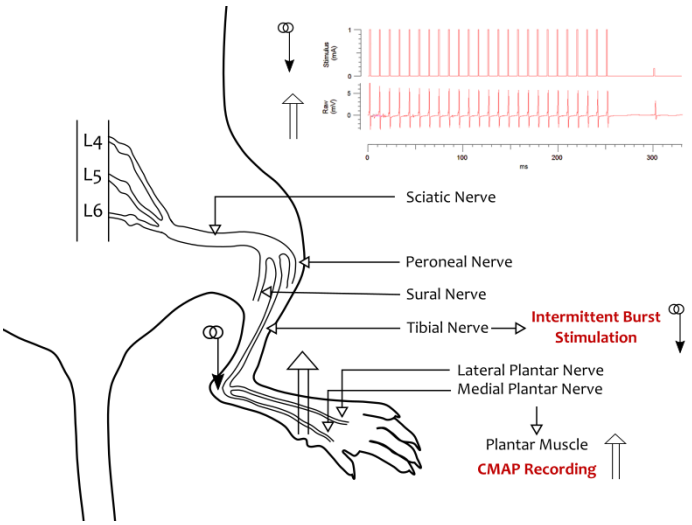

Fig. 1 – Intermittent Burst Stimulation Protocol. In order to induce a strenous activity the tibial nerve was stimulated with intermittent bursts of 100 Hz for one hour. The TRPV4 agonist, antagonist or vehicle (2% DMSO in water) were subcutaneously injected at 10 min into the protocol. Axonal excitability changes were compared before and after the burst stimulation.

In order to investigate TRPV4 pharmacology in an activity dependent manner we've developed an Intermittent Burst Stimulation protocol (Fig. 1) that stimulates the tibial nerve with intermittent bursts of 100 Hz without depleting the neuro-muscular junction of acetylcholine. Axonal excitability was compared before and after one hour of Intermittent Burst Stimulation under three conditions by subcutaneously administering the TRPV4 agonist (GSK1016790A), antagonist (HC067047) or vehicle (1% DMSO in water).

Our results (Fig. 2) showed that TRPV4 pharmacology does have an effect on axonal excitability in an activity dependent manner. Interestingly, the effects were different in wild type and PMP22tg mice, suggesting they are due to Schwann cell TRPV4 modulation.

- **Overview**

The hypothesis for the project is supported by the results, which suggest TRPV4 is involved in an activity-dependent homeostasis that manifests differently in mice with hypomyelinated axons. On 19th of October, I participated with a poster presentation with these results at the National Neuroscience Society of Romania's 9th Annual Conference. We will continue investigating this hypothesis in my home lab, on C57BL WT and PMP22tg mice sent from Dr. Moldovan's lab. This pilot study will also form the basis for a collaboration between our labs.

During my two months stay at Copenhagen University, I learned to use *in vivo* electrophysiology techniques to measure motor axon excitability by "threshold-tracking". Creating and optimizing the Intermittent Burst Stimulation protocol with Dr. Moldovan helped me get acquainted with the underlying software language of Qtrac as well. I also learned how to apply behavioral measures to assess motor function or touch/temperature sensitivity and about the "Bostock" mathematical model for axonal excitability. I attended seminars held by the Graduate Program of Neuroscience once every two weeks and experiments of Dr. Student Amaia de Diego, where I learned about stereotaxic placement, optogenetics and decision-making behavioral paradigm.

It was a great experience that helped me not only improve my technical skills, but also my thinking process in regard to experimental designs. I'm thankful for Dr. Moldovan's diligent guidance, the insightful discussions with Prof. Krarup and the NENS Committee for granting this opportunity that shaped my professional development.

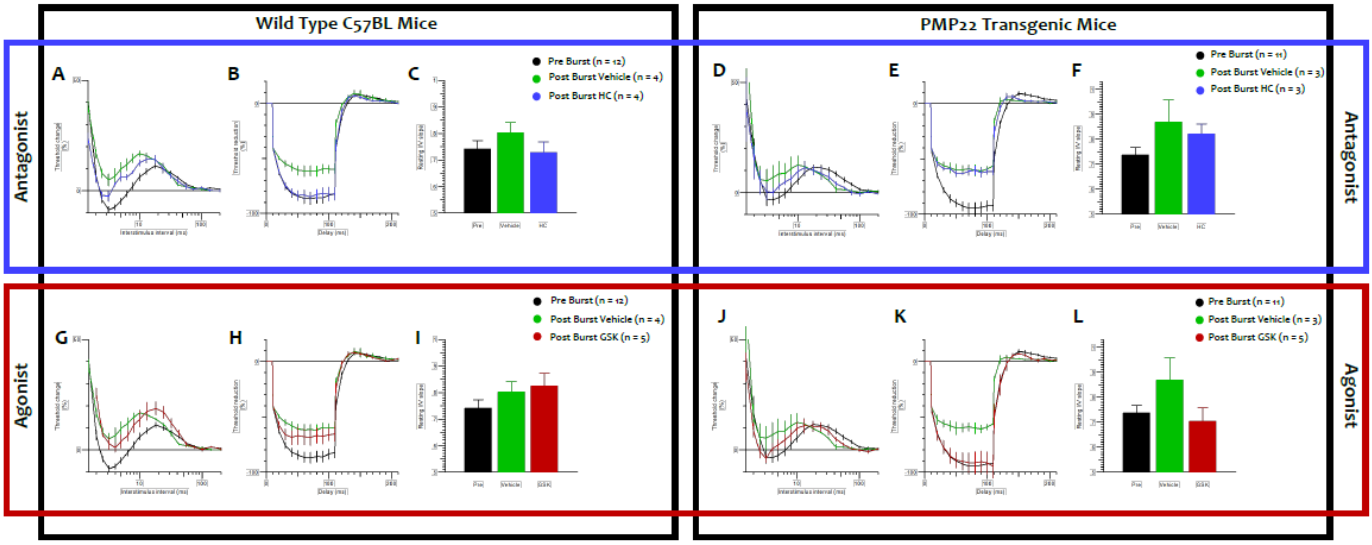

Fig. 2 – Comparing the acute effects of TRPV4 pharmacology on axonal excitability during intermittent burst activity. In WT, the TRPV4 antagonist greatly reduced the effects of the burst stimulation compared to vehicle (B, C), while in PMP22tg mice the same was observed with the agonist (K, L). The agonist in WT (H,I) and antagonist in PMP22tg mice (E,F) does not have an effect on axonal excitability. The recovery cycle (A,D,G,J) in all conditions is a parameter that is not affected by TRPV4 modulation.