COST EMF - MED (Action BM1309):
European network for innovative uses of EMFs in biomedical applications

STSM Report:

The effect of dorsal root ganglion electrostimulation on pain-related behaviors and electrophysiological properties of neurons

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Abstract:

Chronic pain is a disease that seriously affects patients' quality of life and burdens the health care systems. The DRG electrostimulation therapy has initial positive results although only few clinical studies are available. Our pilot study indicated that the surgery for implanted stimulators didn't affect the pain related behaviors although the chronic compression of the dorsal root ganglion, due to stainless steel rod implant, changed the parameters of action potentials. In conclusion, our findings will contribute to a better understanding on how the excitability of neurons can be altered by electrostimulation and how pain related behaviors can be decreased.

A. Purpose of the STSM

The purpose of this short term scientific mission was to confirm that the electrical stimulation of the injured dorsal root ganglion from rats can affect the pain related behaviors and excitability of DRG neurons. Our behavior data indicated that the chronic compression of the dorsal root ganglion (CCD) can induce changes, which we further investigated with whole-cell patch-clamp recordings.

B. Work Description

During the period of my short term scientific mission I worked a lot and I did different activities for each of the three proposed objectives:

- Objective 1: to do surgery for spinal nerve ligation (SNL), chronic compression of the dorsal root ganglion (CCD), sham stimulator implant and sham electrode implant
- Objective 2: to test the pain behavior in animals after each type of surgery
- Objective 3: to analyze the parameters of action potentials to characterize the excitability of DRG neurons

At the beginning, we planned the next days of work according with our proposal and started the experiments. The surgeries and behavior tests were made in the specific days to have the animals tested as indicated in Figure 2. For the electrophysiological recordings we made primary cultures of sensitive neurons, isolated from the dorsal root ganglion of adult rats. Also, the patch clamp setup was calibrated and adjusted to our experiments. In the same day of culture and 24h later the neurons were recorded and action potentials analyzed for changes in parameters. Periodical lab meetings were necessary to discuss the experiments and further plan the work. Also, lunch and dinner were other occasions to talk about personal and scientific issues and also to plan future experiments and our international collaboration.

C. Results

In the proposal of my short term scientific mission I described three main objectives. The first objective was to learn the spinal nerve ligation (SNL) surgery and to implant electrodes for stimulation. All the surgeries were performed on adult male Sprague-Dawley rats under isoflurane-anesthesia. We made a small skin incision and exposed the vertebrae after which we removed the L6 transverse process and exposed the L4 and L5 spinal nerves (Figure 1). The rod was inserted near the L5 dorsal root ganglion after which the muscle was sutured and the skin was closed with wound clips.

Figure 1. The surgery for the rod implant. The small incision in the lumbar region (left) is followed by the exposure of the spinal nerves L4 and L5 (center) and then the rod implant (right).

For our second objective, we tested four different behaviors, withdrawal response, von Frey withdrawal threshold, cold withdrawal response and heat threshold. These behaviors were made after spinal nerve ligation (SNL), chronic compression of the dorsal root ganglion (CCD), sham stimulator implant, sham electrode implant and control conditions, before the surgery or 1, 3, 7 days after the surgery. The experimental data is showing that the withdrawal response and cold withdrawal response can elevate after CCD while sham electrode and sham stimulator implant is not changing significantly the measured behaviors (Figure 2).

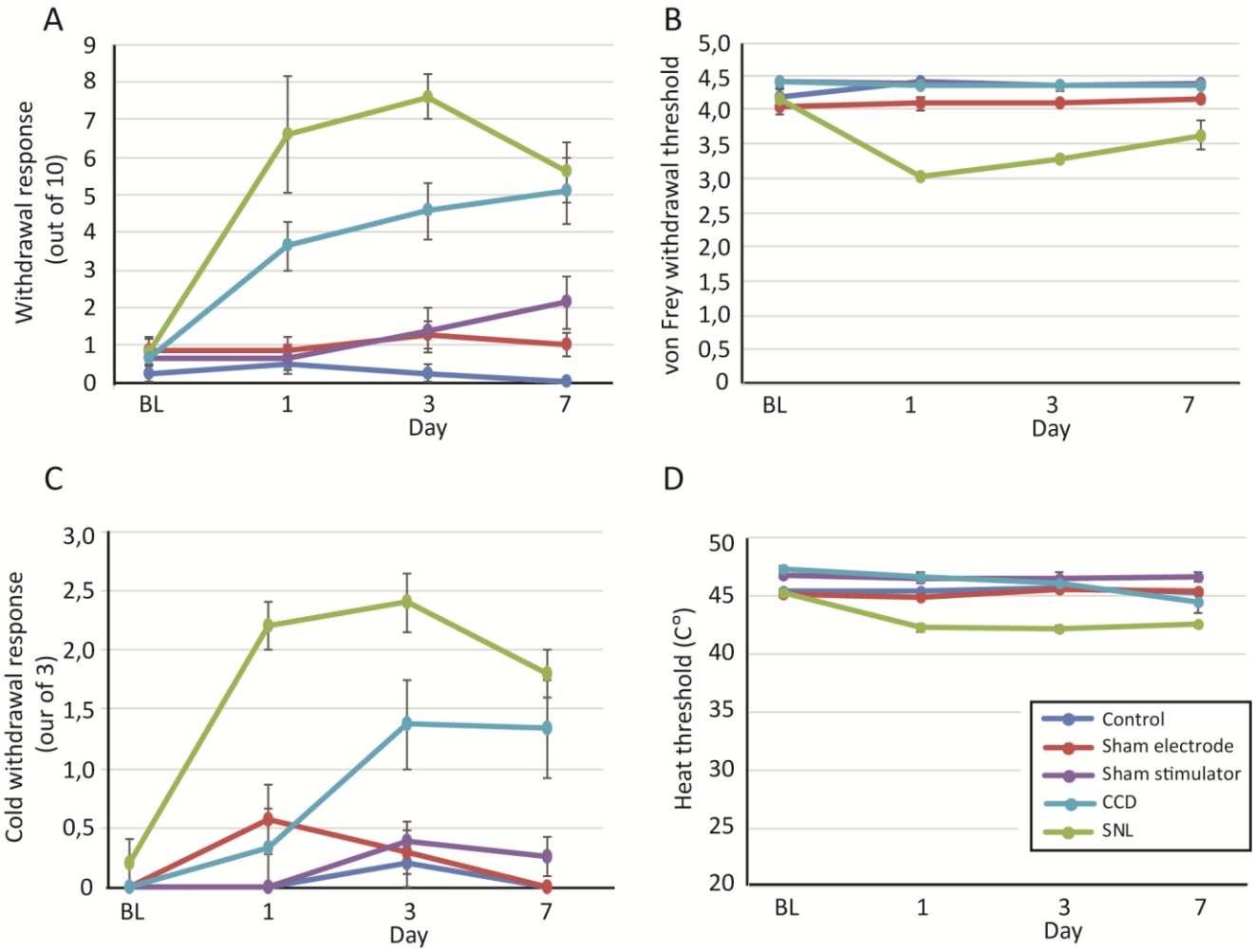
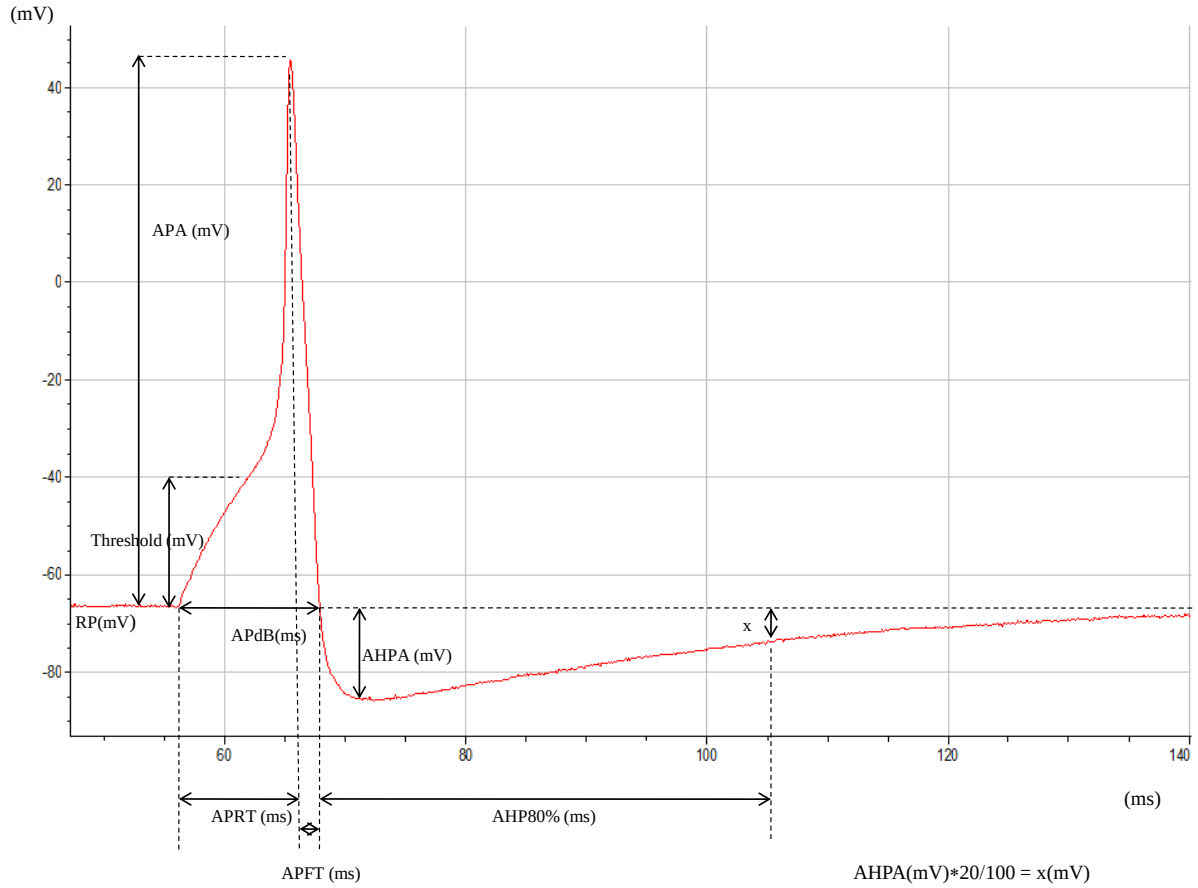


Figure 2. Withdrawal response (A), von Frey withdrawal threshold (B), cold withdrawal response (C) and heat threshold (D) tested in our experimental conditions, after spinal nerve ligation (SNL), chronic compression of the Dorsal Root Ganglion (CCD), sham stimulator implant, sham electrode implant and control.

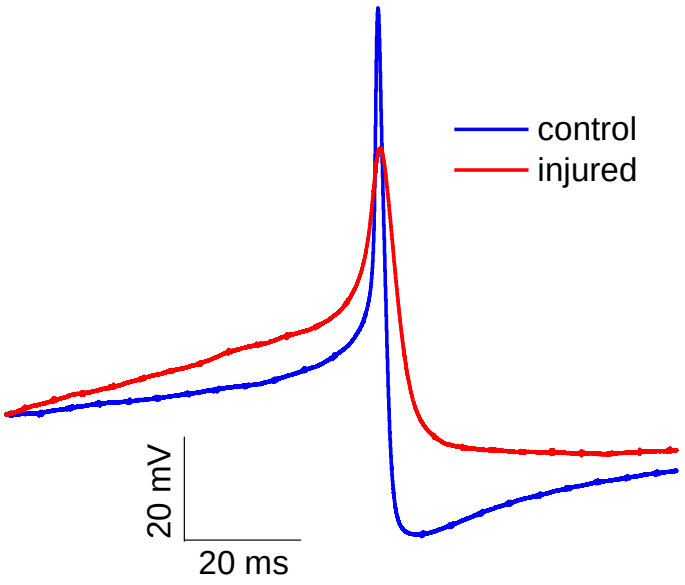
To better characterize the excitability of neurons we can compare different parameters of the action potentials depending on the conditions. After different pain models, injuries or other pathological states it can be observed different changes in the action potentials including the resting membrane potential, rising and falling time, afterhyperpolarization, amplitude and other. In the Figure 3 some parameters of the action potential can be identified.



RP=Resting potential/baseline (mV)
Threshold (mv)
APA=AP amplitude (mV)
AP-dB=AP duration at the base (ms)
AP-RT=AP rising time (ms)
AP-FT=AP falling time (ms)
AHPA=Afterhyperpolarization amplitude (mV)
AHP80 (ms)=time when 80% of afterhyperpolarization amplitude is reached (ms)

Figure 3. A model of action potential illustrating the main phases as listed in the table.

For our last objective, the experiments showed that after the chronic compression of the dorsal root ganglion (CCD) by a rod implant introduced during surgery, we can identify changes in different action potential parameters. In Figure 4, action potentials in our two conditions, control and CCD, are presented and the table below is indicating different parameters identified using Patch Master (Harvard Bioscience, Holliston, Massachusetts, USA).



	Baseline	AP amplitude	time to AP amplitude	repolarization amplitude	time to repolarization amplitude	rise time	up slope	rise time delay	decay time	down slope	decay time delay	decay tau
control	44.404	77.152	76.88	-24.982	91.68	5.1789	8.9383	70.993	2.0107	-23.023	77.757	20.259
injured	30.637	57.665	198.84	-1.7234	267.46	89.518	386.5	106.49	7.0654	-4.897	201.95	20.08

Figure 4. Action potentials in control (blue) and after the rod implant (red). The table indicate different parameters of the above action potentials.

D. Future collaboration with host institution

The future collaboration will be based on joint publications. The work necessary for future publication will be divided between our labs. The plan is to finish acquisition of the data during next 5 months. Future project are not planed at this point since we first want to publish joint papers that will serve as a basis for the future joint grant applications.

E. Expected Publications

We plan three joint publications. The first one will be submitted to Journal of Neuroscience Methods and will describe the construction of DRG stimulators and consequences of its implantation in healthy animals. The second paper will be submitted to Neuromodulation journal and will describe the consequences of DRG stimulation in SNL model. The third paper will deal with electrophysiological characterization of the DRG cells grown on micropilar substrates.

Confirmation by the host institution of the successful execution of the STSM:

We confirm that Alexandru DEFTU has performed the research work as described above.

Contact Person of Host Institution

Prof. dr. sc. Damir SAPUNAR

Signature

Name of researcher

Dr. Alexandru DEFTU

Signature