NENs Report

Student: Diana Nascimento from Faculty of Medicine University of Oporto

Training stay duration: 6th November to 30th December

Host lab: Institute of pharmacology, University of Heidelberg, Germany

The objective of the training at Professor Rohini Kuner's laboratory, at the University of Heidelberg, was to learn, from skilled researchers, intraganglionic injections in mice. This technique will allow me to do in vivo viral-mediated gene delivery to silence ATF-3 expression (the gene I study) in the DRGs of Monoarthritic rats (model of articular inflammatory pain for a long time under investigation in our lab). This technique is not currently used in my home laboratory (or in any Portuguese lab as far as we concern) and thus there was the necessity to learn it abroad. The knowledge of this methodology will allow the overcoming of some limitations of other common approaches, contributing to a greater reliability of our data. Intraganglionic injections of shRNA viral particles is a more feasible approach to induce gene silencing in DRGs, as it overcomes limitations related with an eventual low migration of the particles from the spinal cord (when using an intrathecal injection approach), and therefore the low transfection/infection of primary afferent neurons, resulting in poorly pronounced and sometimes unreliable effects.

During my training period at the host institution I was able to gain experience and autonomy in this procedure that consists in a specific surgery which lasts around 3 hours and is done under microscope visualization.

To learn the surgical approach I only injected a dye solution (Fast Green), in order to evaluate if the ganglion was being hit with success. However, the final goal (in my home lab) is to inject, unilaterally in L5 DRGs of adult rats, viral particles containing ATF-3-shRNA, in order to silence this gene. These animals will also be subjected to a battery of nociceptive behavioral tests, prior to sacrifice, to evaluate the effects of ATF-3 knock-down in pain modulation. Also particles expressing a control construct containing Green Fluorescence Protein (EGFP) will be injected in control animals to evaluate the efficacy of transfection/infection of primary afferent neurons by these particles. Concerning my PhD project, the implementation of this procedure in my

home institution is crucial as this technique will be used in two of the four tasks planned for my thesis, as referred above.

The know-how of this technique is essential for the accomplishment of my PhD thesis, but it is also of great relevance in other future works involving the studies of mechanisms at the DRG level. This will not only be an asset for my laboratory group but also for all the home institution that will surely benefit from this experience.

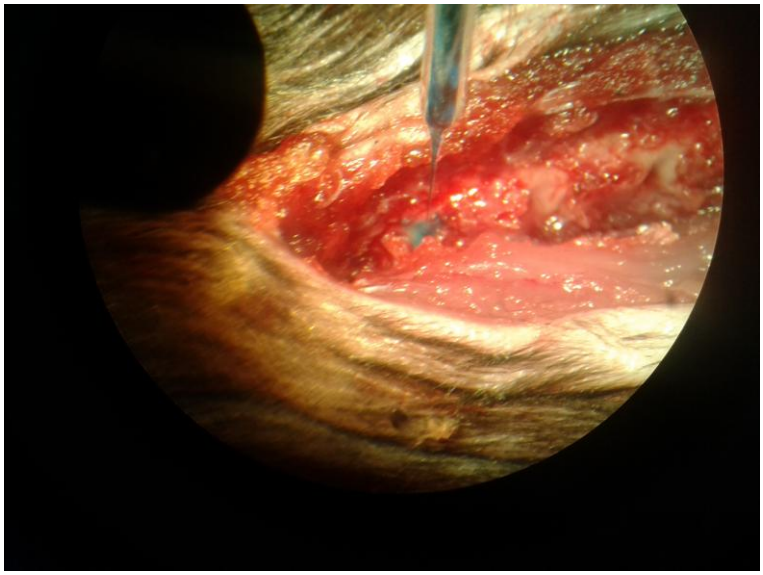


Fig. 1 – L5 ganglion exposed and stained with Fast Green

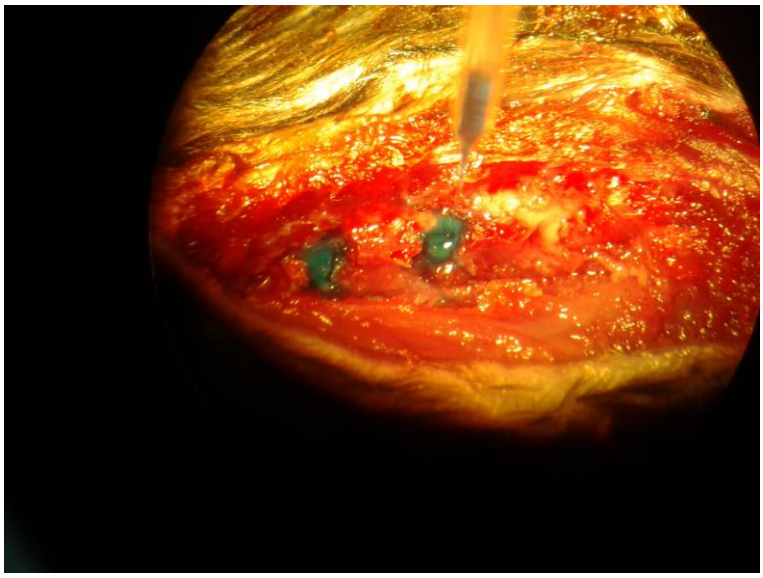
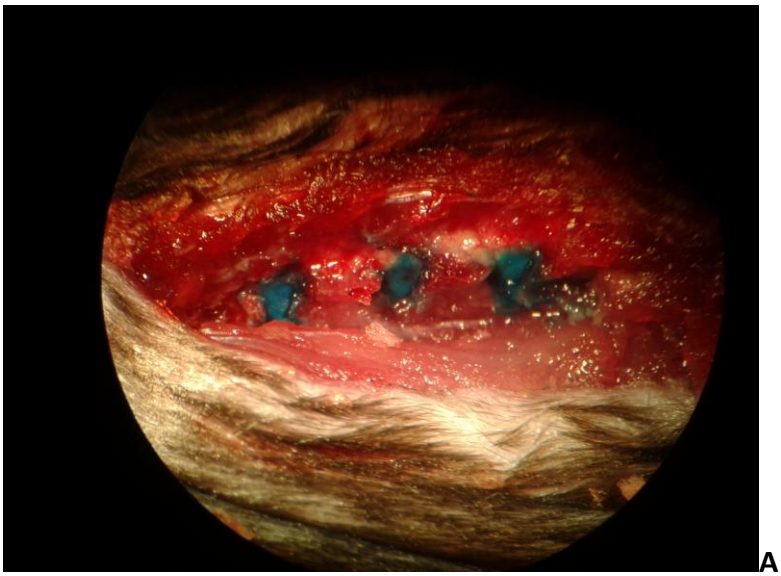
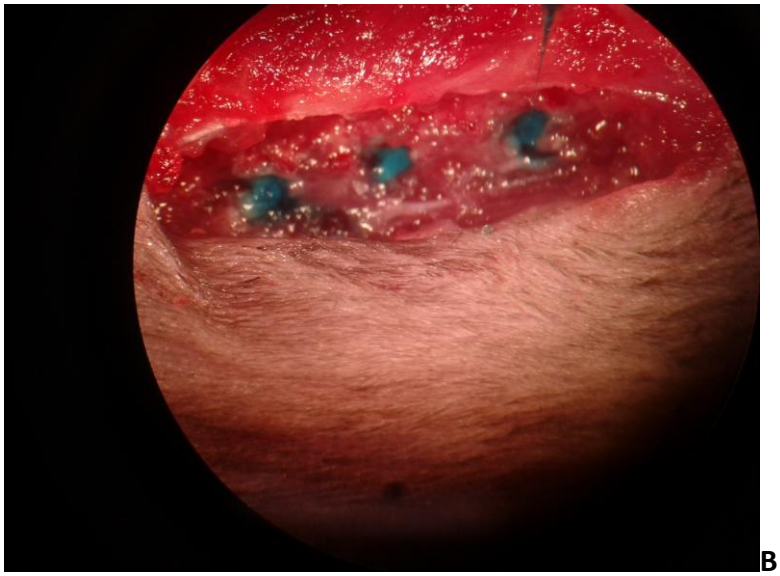


Fig. 2 – L5 and L4 ganglion exposed and stained with Fast Green



A



B

Fig. 3A and 3B– L5, L4 and L3 ganglion exposed and stained with Fast Green

Nadine Gehrig (techinician), Diana Nascimento (me), Vijayan Gangadharan (phD student responsible for my training)