NENS Training Stay Report - Enrica Paradiso

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Getting a NENS Grant Stipend gave me the great opportunity to learn in vivo optogenetic, one of the cutting edge techniques that allows precise temporal control of cells during behaviour, in the laboratory of Prof. Andreas Lüthi at the Friedrich Miescher Institute for Biomedical Research.

The aim of my project was to test the hypothesis that Vasoactive Intestinal Polypeptide-expressing interneurons (VIP$^+$-INs) in the mouse basolateral amygdala (BLA) are involved in fear memory acquisition, and that through direct inhibition of other types of interneurons they in turn disinhibit principal cells. This study represents an important part of my project that so far mainly focused on the long-range connectivity of VIP$^+$-INs in the BLA.

Previous published work from the Lüthi's group showed that Parvalbumin- (PV$^+$-INs) and Somatostatin-expressing interneurons (SOM$^+$-INs) in the BLA are inhibited upon presentation of an unconditioned stimulus (US; footshock) during a fear conditioning paradigm, a simple form of emotional learning that it is known to recruit the BLA. Moreover, in vivo optogenetic activation during presentation of the US was able to reduce the fear memory, measured as freezing response. On the other hand, in vivo optogenetic inhibition of these neurons reversed this behavioural output leading to an increase in the freezing response.

For my project we decided to test the involvement of VIP$^+$-INs using in vivo optogenetic inhibition during a specific fear conditioning paradigm. As a first step in my training, I learned how to build the optical connectors which have to be subsequently implanted in the mouse brain. These connectors consist of a stripped and cleaved optical fibre (200 µm core diameter, 0.48 NA) inserted into a metal holder and protruding 5 mm (custom cut to be implanted in the BLA), kept in place with the use of a superglue combined with prosthetic repair material that hardens fast. It is important to polish the fibre on the side of the metal connector to allow a better transmission of the light. I also learned how to build the optic fibre that connects the laser output with the mouse. It consists of a long (4-5 m) unstripped optical fibre (covered with black paint, to avoid light dispersion) terminating on one side with a connector designed to fit into the laser bench output, and on the other side with a metal hose that perfectly fits the connector implanted in the mouse brain. Both extremities of the fibre have to be polished and tested for power transmission. For both the connectors and fibre an output of 80% is generally adequate.

In order to modulate the VIP$^+$-INs we took advantage of a cre-dependent adeno associated vector expressing the outward proton pump ArchT coupled to a GFP reporter. This opsin generates a hyperpolarizing current in mammalian neurons in response to a 500-600 nm light and is an effective optogenetic silencer of neuronal

activity. In these experiments it is important to have appropriate controls, therefore we used a cre-dependent adeno associated vector carrying only GFP. The stereotactic injections consisted of bilateral injections in the BLA (AP -1.5 mm, ML +/- 3.4 mm, DV -4.4 mm) of 100 nl of the AAV vectors in 8 week-old Vip-cre male mice. Although I was already able to perform stereotactic injections, here I learned the implantation of the connectors. It is crucial to place the connectors in the right position above the BLA (DV -4.00 mm) so that the laser light can reliably reach the infected cells. Moreover, the connectors have to be glued very well to the skull with ultragel glue and prosthetic repair material, and have to be covered with a mixture of dental cement and black paint to create a protection from the environmental light.

As part of the training I learned also how to design an optogenetic protocol using an automatized software (Plexon), how to handle mice properly in order to be able to connect them during the behavioural sessions to the optic fibres, and how to properly handle lasers. Choosing the appropriate laser power is an integral part of the experiment. We decided for a laser power output of 15 mV, based on previously published work that used ArchT as an opsin.

Lastly, I implemented a new fear conditioning paradigm tailored to test my working hypothesis. Mice underwent behavioural testing 4 to 5 weeks after surgeries. After habituation to the retrieval context, 2 different CSs (30s of 27 pips of 200 msec, 6 kHz and 12 kHz) and 4 times 4.5s presentation of a yellow laser light (to control for non-specific effects of laser presentation), mice were exposed in the fear conditioning context to one CS (12kHz or 6 KHz) coupled only once to a US (0.65 mA). During the behavioural paradigm, we shined light for 4.5s (laser light 590 nm wave length) during the co-presentation of the US. At retrieval, 24h after the fear conditioning protocol, blocks of 4 CS were presented and the freezing response to the CSs was assessed in both groups (ArchT and GFP).

Preliminary results show that modulation of VIP$^+$-INs during fear acquisition (US presentation) show a tendency towards a reduction of freezing behaviour at retrieval day compared to GFP-injected mice controls.

Through this experience has I was able to obtain important preliminary results that are critical for my PhD project. My home lab is currently establishing a facility for in vivo optogenetic, therefore, and I will be able to replicate the experiment performed in Basel and expand my preliminary results. Moreover, I can transfer the knowledge acquired in Basel to my home laboratory.

Being at the FMI for this period, I not only learned this very sophisticated technique for behavioural control, but also how to troubleshoot when things do not work. I was intensively supervised for all the steps of the training and I was able to get useful insights for the progression of my PhD project. Moreover, I had the possibility to be surrounded by a vibrant neuroscience community made of many international PhD students and post-docs.

Therefore, I would like to thank the FENS Committee of Higher Education and Training for having given me this great opportunity.