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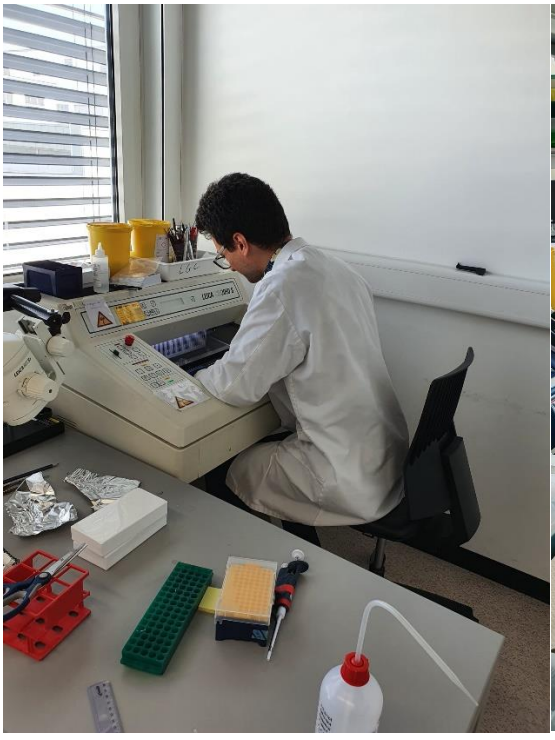
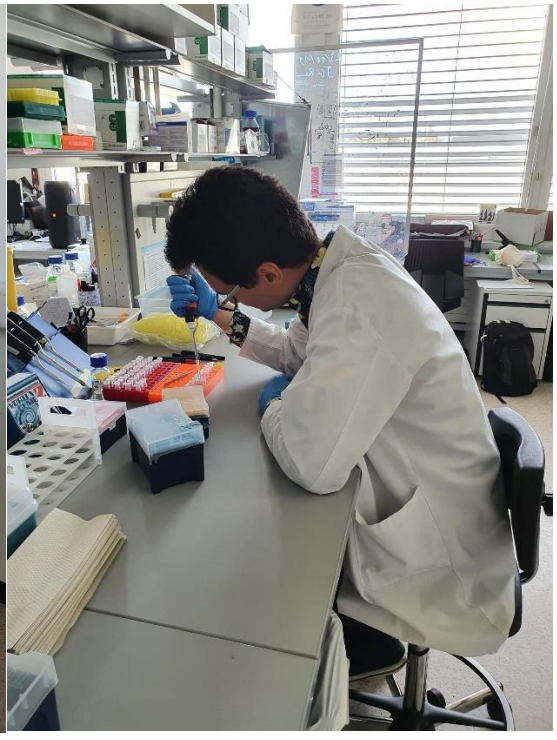
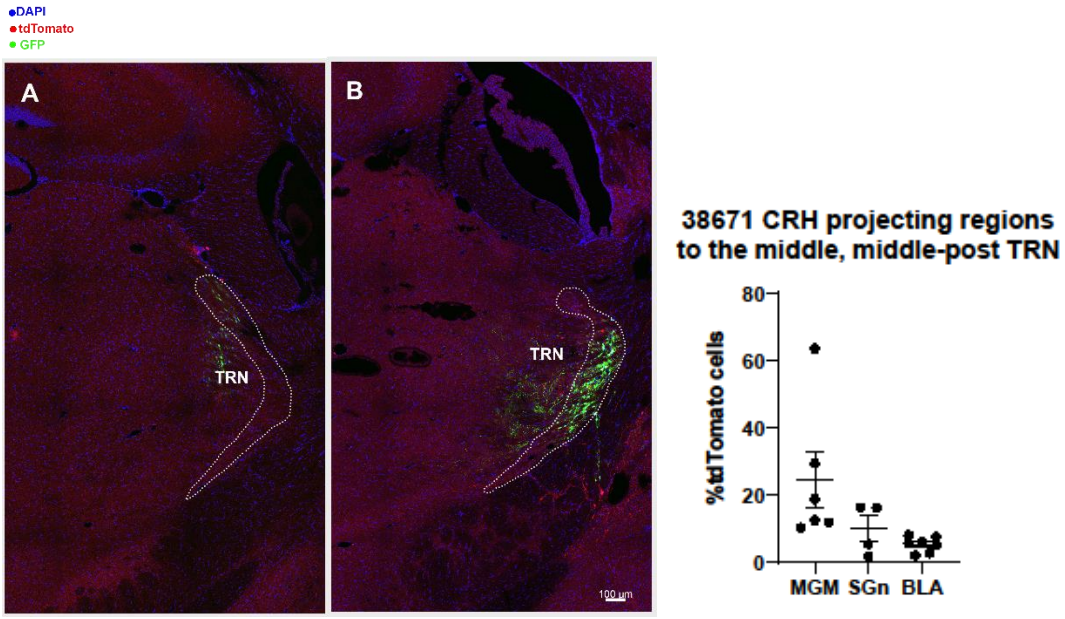
Host lab: Laboratory of Behavioral Genetics led by Carmen Sandi, École polytechnique fédérale de Lausanne
Period of stay: February-April 2022

During my stay at EPFL I got the opportunity to learn plenty of wet-lab techniques as well as a plethora of theoretical information related to neuroscience and also presentation design and data analysis. These two months of training were possible thanks to a NENS exchange grant.

I learned how to use a cryostat and how to mount coronal brain slices on glass slides. As part of this, I learned a great deal about the neuroanatomy of the mouse brain and how to read a brain atlas, plus the whole process from the live animal to the frozen brain. I also did a DAPI stain for the slices before I mounted them. After I processed all the brains, I received training from the BioImaging & Optics Platform (BIOP) of EPFL in order to learn how to use a confocal microscope. The training was split into two parts, first a theoretical session during which I found out how a confocal microscope works and how to optimize different parameters such as pixel size, resolution, laser intensity for obtaining the best possible images. All of these were needed because I had to quantify the images so I needed to have as little artefacts as possible. During the second training I brought some of my samples for the aforementioned optimization and my trainer helped me pick the best setup to balance image quality and acquisition time. The images were acquired with a 20x objective and consisted of three channels, one for DAPI, one for tdTomato and one for the GFP fluorescence. After I imaged all the brains, I had to quantify the relevant regions in them. The brains were extracted from CRH_IRES_Cre transgenic mice. Six of them were injected with two 1:1 viral vectors for a retrograde labelling of neurons that send projections that release corticotropin releasing hormone (CRH) in the thalamic reticular nucleus (TRN). Three of them were injected in the medial geniculate nucleus with a viral vector for an anterograde labelling.

The quantification consisted of several steps. First, I learned how to use ABBA, an ImageJ macro developed by the BIOP. ABBA is a tool that allows you to map the images of the brain slices to an atlas, and then export these annotations into QuPath. This was important because the delimitations made it possible to analyze different areas independently, without having to draw by hand the regions of interest. After all the relevant slices were aligned quantification of the candidate projecting regions was done. For this to be accomplished I first established the total number of cells in relevant regions via the DAPI stain. Afterwards, cells were determined as being CRH positive or not based on the tdTomato signal. Lastly, for the quantification of axons that release CRH in the TRN I had to do a positive pixel count, again based on the tdTomato signal. I didn't have any prior experience with Qupath, but the BIOP team was very helpful in teaching me the ins and outs of this software and now I'm sure I would be able to analyze other samples on my own, a thing that I'm planning to do back in Romania.



Confirmation of the TRN injections and quantification of the candidate projecting regions

Apart from the main project, I followed around other people in the lab that were conducting experiments. I learned how to do electrophysiology on brain slices, and I've also seen how the biological material is prepared for it, from the mouse sacrifice, to the slice fixing at the microscope. I learned how to extract plasma from human blood samples, and I did it several times in order to help the people on the human side of the laboratory. I've also seen how mice behavior experiments are analyzed with EthoVision and how the data is exported and interpreted. Additionally, I got the opportunity to receive training in mice handling and an overview of the animal facility. There, I also got the chance to see set-ups for several classic behavior experiments, like open field, elevated plus maze and fear conditioning chambers. I also observed how surgeries are conducted, mainly the implants of optic fiber and EEG electrodes in mice brains.

Being a temporary student at EPFL meant that I also had access to the Brain-Mind Institute seminars. It was amazing for me to be able to hear almost every week about the work of people that are achieving the cutting edge discoveries in neuroscience.

I was also lucky to arrive just in time for an outdoors lab activity, which consisted in snowshoeing in the mountains. Not only did I got to experience the Swiss mountains, but also meet the very diverse

members of Carmen Sandi's lab. It was really compelling to find out that even though we come from such different places, our interests and hobbies are similar.

This experience opened my eyes about the value of collaborations and meeting new people in science. I learned a lot of useful information in these two months and met very interesting people. I can only imagine how a longer stay would pan out and I hope to find other scholarships that would facilitate that. EPFL is one of the leading institutions in neuroscience and I was fortunate to be able to peer into how things function at this level. I'm sure that the knowledge and the relationships that I acquired here would have a great impact on my future career as a scientist and I'm looking forward to the next exchange experience.