NENS Exchange Grant Report

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Period of my stay: 01/09/2022 - 30/11/2022

Acknowledge:

It has been a great pleasure to be granted with NENS Exchange Grant. Thanks to this stipend I had the chance to learn new techniques that were not possible in my home lab, such as patch clamp, RNAscope and reprogramming using viral injections. From the first day, all Professor Benedikt Berninger's lab hosted me like one more of their team and I could participate in their lab meetings, round tables and journal clubs. I would like to thank especially Professor Berninger for giving me the opportunity to be in his lab and Ana Beltran for the technical training. This three-month training has given me new knowledge for my research that I will bring back to my lab and I will teach to my lab colleagues.

I would like to encourage everyone to apply for the NENS exchange grant because thanks to their support, I had an invaluable life experience in the beautiful city of London learning new techniques, improving my communication skills and making new friends.

Report:

The scientific formation during my thesis includes a training stay in the laboratory of Professor Benedikt Berninger in Guy´s Campus at King´s College London (United Kingdom). During these three months, we performed lineage reprogramming to assess the gene expression using RNAscope and the functional properties using patch-clamp.

First of all, Benedikt's lab is focused on reprogramming glial cells into neurons. I observed Ana Beltran, a PhD Student, performing **virus injection** (Figure 1A). For this purpose, P5 mice were anaesthetized with a "sleep solution" and kept in a chamber at 33°C. An incision was made in the skin in the region of interest and using a needle the skull was scratched very carefully to expose the brain. The glass capillary with the retrovirus was down until the head, which was set up like "0". Glass capillary was introduced in the cortex 0.55µm. The mixture of viruses that allows the reprogramming of the cells was injected (1ul per animal). The glass capillary was removed and the incision was closed with surgical glue. An "awake" solution was administrated and pups were placed in a 33°C chamber to ease their recovery. The observation of retrovirus injections has been very useful to my lab to adapt the StarTrack (clonal method develop in my lab to tag single progenitors and their progeny) to retrovirus or lentivirus to can labelled cells in adult mice.

Later, I learn to reveal the expression of some genes using **RNAscope** (Figure 1B). This technique consists of RNA in situ hybridization (ISH) that allows the visualization of single RNA molecules in individual cells. In this way, we can do an approximative quantification of the RNA and localise their expression in the specific cells. For this purpose, after drying the brain slides at 60°C for 60min, the tissue was dehydrated with 50%, 70% and 100% EtOH. Later, to inactivate the peroxidases, hydrogen peroxide was used. Then, target RNA were exposed using a target retrieval solution at 90°C, followed by protease III treatment. Probes against specific genes were used and amplified. Finally, the signal was revealed using horseradish peroxidase (HRP) and different opal fluorophores. The tissue was mounted and let dry.

Afterwards, images of the samples were taken in Zeiss confocal at 40x (Figure 1C).

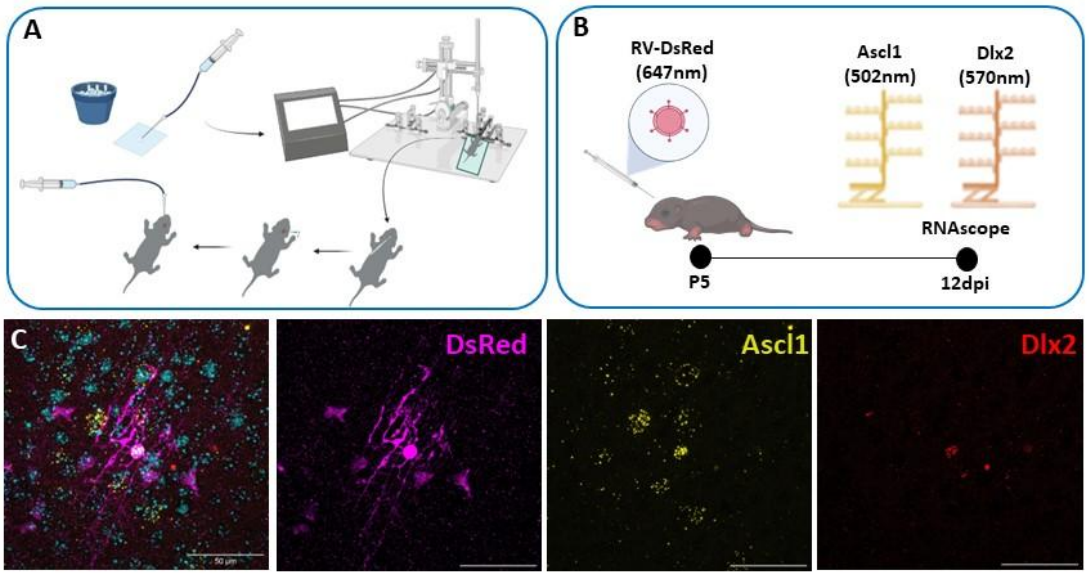

Figure 1. Gene expression in reprogrammed cells. A) Virus injection in mice pups. B)Timeline of virus injection and RNAscope analysis. C) Image of Ascl1 and Dlx2 gene expression using RNAscope in cells injected with a retrovirus (DrRed).

In my thesis, I am interested in deciphering some aspects of Neural Progenitors heterogeneity. In Benedikt's lab, I could combine the use of our genetic tool StarTrack and RNA scope to find or discard the expression of genes in the progeny of some progenitors (Figure 2). In the future, the knowledge acquired using RNA scope will help me to unravel the presence and quantity of different genes to know more about progenitor diversity.

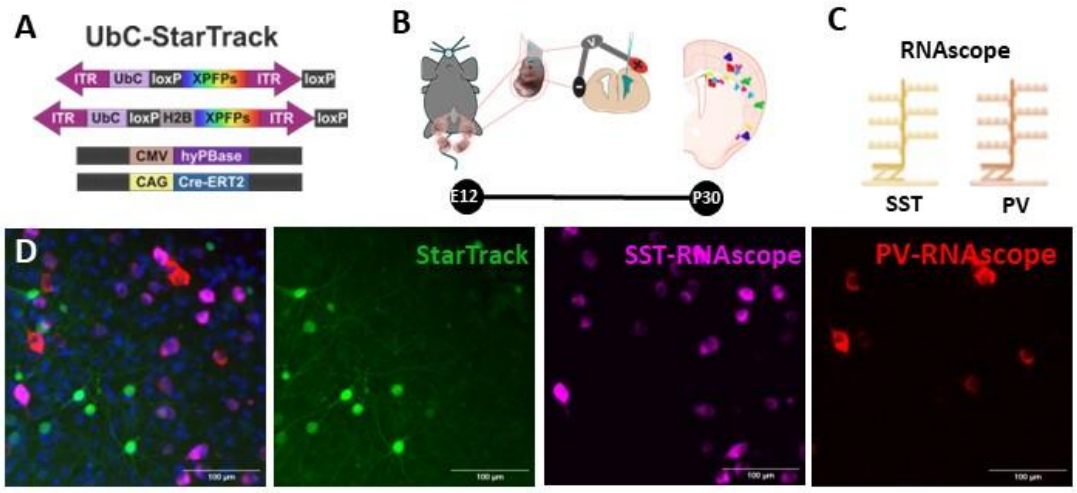

Figure 2. Gene expression in StarTrack Cells. A) StarTrack constructs. B) In-utero electroporation to inject StarTrack plasmids at E12 and analysis at P30. C) RNAscope strategy

to analyse gene expression. D) Image of SST and PV gene expression using RNAscope in StarTrack (EGFP).

About **Patch-clamp**, Nicolas Marichal Negrin taught me the complete process. First of all, 2 weeks after the virus infection to reprogramme glial cells into interneurons, mice brains were cut into 300µm slices (a proper width for the tissue survival and observation in the microscope) and using a cold ASCF medium. Tissue was recovered in ASCF medium at 34°C for 1:30h. Glass capillary was made using a puller to obtain a resistance of 10-15MOhm. Slices were placed in a chamber in the setup and a proper medium was flux. The intracellular solution was placed in the glass capillary using 0.22µm. Capilar was approximated to our cell of interest using negative pressure. Then, the seal with the cell membrane was done changing the pressure using a syringe. Once the pressure was in the order of GigaOhm, the membrane was opened and cell activity was recorded. All procedures were developed in PFA-free conditions and the medium was maintained with 5%CO2/O2. In my laboratory, we have a setup but didn't know how to use it. The acquisition of electrophysiological skills during the stay will allow me to perform the functional assessment of the progeny of neural progenitor cells.

Additionally, I could enrich my scientific knowledge by attending their lab meetings, round tables and journal clubs weekly, as well as seminars that were organized in the Department. Furthermore, I participated in the Stem Cell Network Meeting.

The picture with the host lab.