Outcome and benefits

The support I received from CAEN for a research stay in the Cajal Institute (López-Mascaraque Laboratory) in Spain allowed me to become independent to apply the StarTrack[5] technique to a research project that will continue in my home country, Argentina, in collaboration with other local groups. The project focuses in the characterization of the maturation process of astrocytic networks. Astrocytes are glial cells that form selective and dynamic networks that start to be conceived in terms of the interplay between neuronal and glial networks[1]. At the first relay of the olfactory pathway, olfactory sensory neurons innervate the superficial layer of the olfactory bulb establishing a sensory map, such that sensory neurons with the same odor specificity converge on a few of around 2000 neuropils (glomeruli)[2]. This convergence is refined during postnatal development and depends on sensory experience[4]. In the glomerular layer of the olfactory bulb, astrocytes form gap junction-connected networks mainly circumscribed to individual glomeruli[3]. The size of these networks is modulated by neuronal activity[3], however, whether postnatal refinement of astrocyte networks occur in parallel with olfactory map refinement is unknown. In addition, clonally-related astrocytes, defined by sibling cells originated from a single cell, are anatomically located in association with the same glomerulus[5]. The aim of this project is to test the following hypotheses: 1. That astrocytes belonging to the same mature functional network share the same origin. 2. That the clonal composition of astrocyte networks is refined during postnatal development through experience-dependent mechanisms.

In order to test the postulated hypotheses, we proposed the study of the clonal composition of astrocyte glomerular networks using the genetic tracing strategy, StarTrack[6], which attributes a specific and unique color-code to single neural precursors, allowing all their progeny to be tracked in order to characterize functional networks using electrophysiology and dye-coupling.

During my stay at López-Mascaraque's Lab I learned and gained experience in several procedures and analyses related to the use of StarTrack technology. The procedure involves performing *in utero* surgery in young (E13–14) mouse embryos involving intracerebroventricular injection of the StarTrack plasmid mix and

electroporation of brain embryos. The plasmid mix contains 12 plasmids which carry different fluorescent protein genes (YFP, GFP, m-Cherry, mT-Sapphire, mKO and m-Cerulean) downstream the GFAP protomoter, and a plasmid coding a ubiquitous transposase (protein that inserts the fluorescent proteins construction in the cellular genome). In 6 of the fluorescent protein plasmids, the coding sequence is fusedto an H2B sequence, tagging the protein to the cellular nucleus, whereas the other 6 plasmids allow citoplasmic expression of fluorophores. When the plasmid mix is electroporated, all types of cells are subjected to electroporation and integration of the fluorescent protein coding region, but only GFAP expressing cells have actual expression of fluorophores. After electroporated mice are born, intracardiac paraformaldehyde perfusion of neonate mice (post-natal day 0-7) is performed, and the brain is dissected for later sectioning using a vibratome. After searching electroporated areas of the olfactory bulb, by using an epifluorescence microscope, photos of selected sections are taken by using confocal microscopy. The StarTrack technique provides each electroporated cell and its progeny (clones) with a different combination of 6 fluorescent proteins (color code) in two possible localizations (nucleus of citoplasm). For clonal analysis of combinatorial expression of fluorescent proteins, a specifically developed macro algorithm in ImageJ software is used.

During the 8 weeks of my stay, I performed 15 surgeries and *in utero* electroporations of the StarTrack plasmid mixture with a strategic position of injection pipette and electrodes to target the olfactory bulb. I also isolated 12 GFAP-directed fluorophore plasmids and the transposase plasmid from bacteria cultures, using maxiprep protocols, to be able to develop this technique in at least two interested laboratories in Argentina (System Neuroscience Group, IFIBIO-Houssay Institute and Molecular Neuropathology laboratory, directed by Dr. Javier A. Ramos in IBCN Institute).

As a result of my training, I was able to obtain evidence of olfactory bulb astrocytic electroporation in neonatal mice (Fig. 1). All plasmids were electroporated, as we found examples of the 6 fluorophores in both cellular locations. In addition, different types of astrocytes were observed, astrocytes located in the pial surface organized as

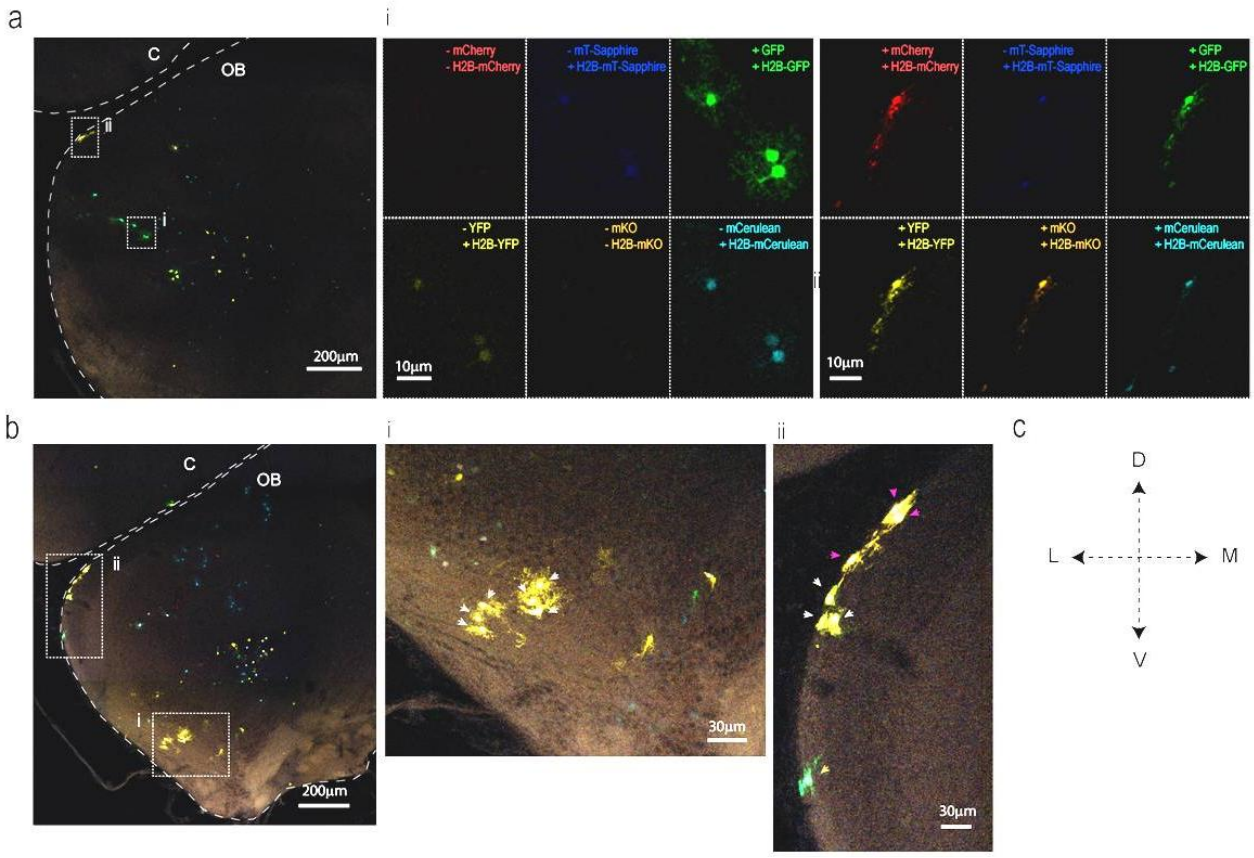

Figure 1. A. Representative image of an olfactory bulb section of a P5 mouse subjected to *in utero* electroporation at E13(C: Cortex, OB: Olfactory bulb). **i,ii.** Representative cells are shown with specific combinations of fluorophore expression and localization that defines a color code for each cell. **B.** Representative image of an olfactory bulb section of a P5 mice subjected to electroporation at E13 (C: Cortex, OB: Olfactory bulb). **i.** Protoplasmic astrocytic clones are shown. **ii.** Different pial astrocytic clones are marked with colored arrows.

small clonal clusters (Figure 1Bii), and others with protoplasmic morphology (Figure 1Ai and Figure 1Bi), recognized by a spherical projection of fine branched processes[5].

In sum, I was able to obtain the preparation necessary for clonal analysis and posterior experiments. As part of my project, electrophysiological recordings were to be performed in electroporated astrocytes of olfactory bulb slices. For this purpose, during the 2 months I worked in López-Mascaraque's lab, I used my skills in this technique to set up an *in vitro* electrophysiology recording station that is now ready to be used.

During my stay I had multiple opportunities to discuss my current work and the results I generated in my graduate research so far, showing evidence of maturation of

the molecular subtract for olfactory glia network connectivity along the post-natal refinement. I attended several institutional seminars presented by local and foreign researchers. These experiences enabled me to meet other researchers and graduate students at the Cajal Institute. As part of the lab meeting schedule I presented my work in two opportunities, one as part of a group of brief presentations during the visit of Dr. Peter Mombaerts to the lab (Fig. 2), and the other as part of the weekly lab seminars, were I gave a full presentation of my current work and future perspectives.

Statement of financial support

The financial support from CAEN-ISN was essential to have this worthy experience and make fruitful connections with other labs and researchers. I estimate a total cost of US$1500 for lodging for 2 months and an additional U$S300 for local transportation. Most of the funds were spent in flight fares, totaling US$1500. After my return I gave an institutional seminar of about 30 minutes reporting the activities during my stay abroad to an audience of around 10 researchers and graduate and undergraduate 20 students. I am convinced that many students will be motivated to apply for future CAEN calls based on what they could learn from my experience.

Figure 2. Lab members at MB02, Cajal Institute, Madrid, Spain. From left to right: Lucila Brocardo (awardee), Laura López-Mascaraque (host lab director), Mario Sánchez Villalón, Ana Cristina Ojalvo Sanz, María Figueras Oñate, Sonsoles Barriola, Ana Bribian.

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