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Final NENS-report

I spent one month, from January 31st to March 1st, in the laboratory of Laura López Mascaraque at the Cajal Institute, in Madrid. The aim of my stay was to take advantage of the novel Star-Track technique they recently developed to analyze cell lineages through the stochastic combinatorial expression of 6 nuclear and 6 cytoplasmic fluorescent proteins, coded by a mixture of 12 plasmids under the regulation of cell-specific promoters (i.e. GFAP to label astrocyte progenitors). The expression of these proteins, after in utero co-electroporation with the PiggyBac transposase under the control of the ubiquitous CMV promoter, produces inheritable marks that permit the long-term lineage of progenitors to be traced in vivo with high accuracy. Namely, this technique, allowing to follow the fate of single progenitors in vivo, represents a useful approach to clarify the mechanisms of specification of the different astroglial phenotypes present in the cerebellum, which are still poorly clarified and in which I am interested.

During my stay, Star-Track plasmids under the GFAP promoter were in utero electroporated in the IVth ventricle of mouse embryos at early developmental stages, i.e. E12 and E13, to target the cerebellar ventricular zone (VN), and label primary astroglial progenitors. Four pregnant mice per week were gently provided by the host animal facility and this allowed to perform several electroporations in different experimental conditions, hopefully permitting to set up the more suitable protocol to reach the cerebellum. Pregnant mice were anesthetized with isofluorane and received a subcutaneous injection of the antibiotic enrofloxacine and of the anti-inflammatory/analgesic meloxicam. The uterine horns were then exposed by a midline incision through the skin and the abdominal wall. At E12, 2 µL of the plasmid mixture was injected with the aid of the high-frequency ultrasound imaging, generating images that could be used in real time to guide the fine glass capillary into the embryonic region of interest (fig. a and b). At E13, instead, injections were performed without the ultrasounds and the IVth ventricle was located through trans-illumination with cold light alone. After all the embryos were injected, the head of each was placed between tweezer-type electrodes connected to an electroporator and electric pulses of different voltages according to known procedures (Garcia-Marques and Lopez-Mascaraque, Cereb Cortex, 2012; Kita et al., PLoS ONE, 2013) and to the embryonic stage (i.e. 30 or 40V at E12 and 33 or 50V at E13) were applied five times for 50 ms, followed by 950ms of interval. At the end of the procedure, the uterine horns were returned into the abdominal cavity, the wound was sutured and the pregnant mice recovered on a heating blanket. The injected embryos were allowed to survive until P0, to test the method efficacy and make preliminary analyses (fig. c), or P30, to perform the analysis of adult clone composition. As regards the first ones, the brains were fixed in Madrid and, after cutting, the analyses were started under prof. Lopez-Mascaraque's supervision. The brains of the latter, instead, will be all sent to Torino, where

the examination of both the cohorts of experimental animals will be completed in the next months.
I was also given the opportunity to hold my own seminar and I could attend the conferences and the journal clubs organized at the Cajal institute.
The NENS exchange grant offered me the great chance to interface to a very rich scientific environment and allowed me to acquire skills and knowledge of which I will for sure take profit for my future career. Overall, I think that this useful experience will positively affect both my personal and professional growth.

(**a**) and (**b**) Ultrasound-guided injection of Star Track mixture in the embryonic IVth ventricle. (**c**) Confocal image at P0 of clones deriving from embryonic progenitors labelled with GFAP-Star Track plasmids at E12. (**d**) Prof. Laura López Mascaraque's lab and me.