NENS Exchange Grant

- Final Report -

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Project title of the training stay: Viral vector production to define connections, electrophysiology and impact of activity modulation of lesion-induced striatal neuroblasts

Premise

My stay in Prof. Berninger Lab was a great opportunity provided by the NENS Exchange Grant. The aim of this stay was to learn how to produce retroviral vectors for the analysis and modulation of distinct aspects of adult neurogenesis, particularly the integration of newly generated neurons into the preexisting networks, study neuroblasts maturation and functional connectivity and study the effects of altered electrical activity.

Retroviral vector production

I produced viral vectors by transfection of a retrovirus packaging cell line that stably express gag and pol genes derived from Moloney Murine Leukemia Virus (MMLV). The gag region encodes genes which comprise the capsid proteins; the pol region encodes the reverse transcriptase and integrase proteins. I have learned how to grow the 1F8 packaging cells that is a monoclonal cell line derived from HEK293GPG cells. These cells also express the envelope VSVG gene under Tet-off control, that is rapidly induced upon withdrawal of Tetracyclin.

To allow the cells to start producing viral particles, once reached 70-80% confluence, I cultured them in packaging medium without any antibiotics nor Tetracyclin. In parallel I prepared the plasmid prep including the construct pCAG-IRES-DsRed, using the PEI transfection reagent. The following day I performed the transfection maintaining the cells always in fresh packaging medium and wait two days before the first virus harvest. I performed 3 harvests for each dish in total, waiting one day between one harvest and the following. At each time I collected the supernatant from the dishes in a Beckman tube (5 P100 dishes for each tube). After an initial centrifuge followed by a filtering step I spinned each tube using an ultra-centrifuged for 2 hours in order to obtain a viral particle pellet. Finally, I discarded the supernatant, re-suspended the viral pellet in TBS-5 and freezed the aliquots.
To analyze the virus titer, I infected HEK293GPG cells with serial dilutions of the produced virus and after one day of incubation I counted the number of infected cells with the epi-fluorescent stereomicroscope.

Notably, all the required equipment and reagents are available at my home lab institution and I only need the adequate cell line and plasmids in order to readily produce the other retroviral vectors stated in my original project such as the ones expressing, along with the GFP reporter, the activator or inhibitor DREADD hM3Dq or hM4d(Gi).

Testing DsRed-carrying retroviral vectors *in vivo* following excitotoxic lesion of the adult mouse striatum

Preliminary results in the quinolinic acid injury model indicate that lesion-induced striatal neuroblasts have a transient existence, however as they become older these cells attain complex morphologies and at least a fraction of them integrate in the pre-existing circuits. It is well established in multiple models that immature neurons integration into pre-existing circuits is critically dependent on neuroblasts connectivity and electrical activity.

In this view, during my stay in Prof. Berninger Lab I also had the opportunity to directly test *in vivo* the DsRed retroviral vector with the aim of performing for the first time an electrophysiological characterization of newborn striatal neurons activity with patch-clamp recordings. Hence, beside training my previously acquired knowledge on viral vectors stereotaxic injections, I also learned the preparation and maintenance of acute brain slices for electrophysiological assessment of newborn striatal neuron activity. In collaboration with Nicolas Marichal, an expert electrophysiologist working in the Prof. Berninger Lab, we performed patch-clamp recording in whole-cell configuration and found out that two weeks old newborn neurons have basic membrane properties typical of neuroblasts (i.e. similar to those derived from canonical neurogenic niches). In addition, these cells display transient inward sodium currents and accordingly are able to fire single action potentials (Fig. 1).

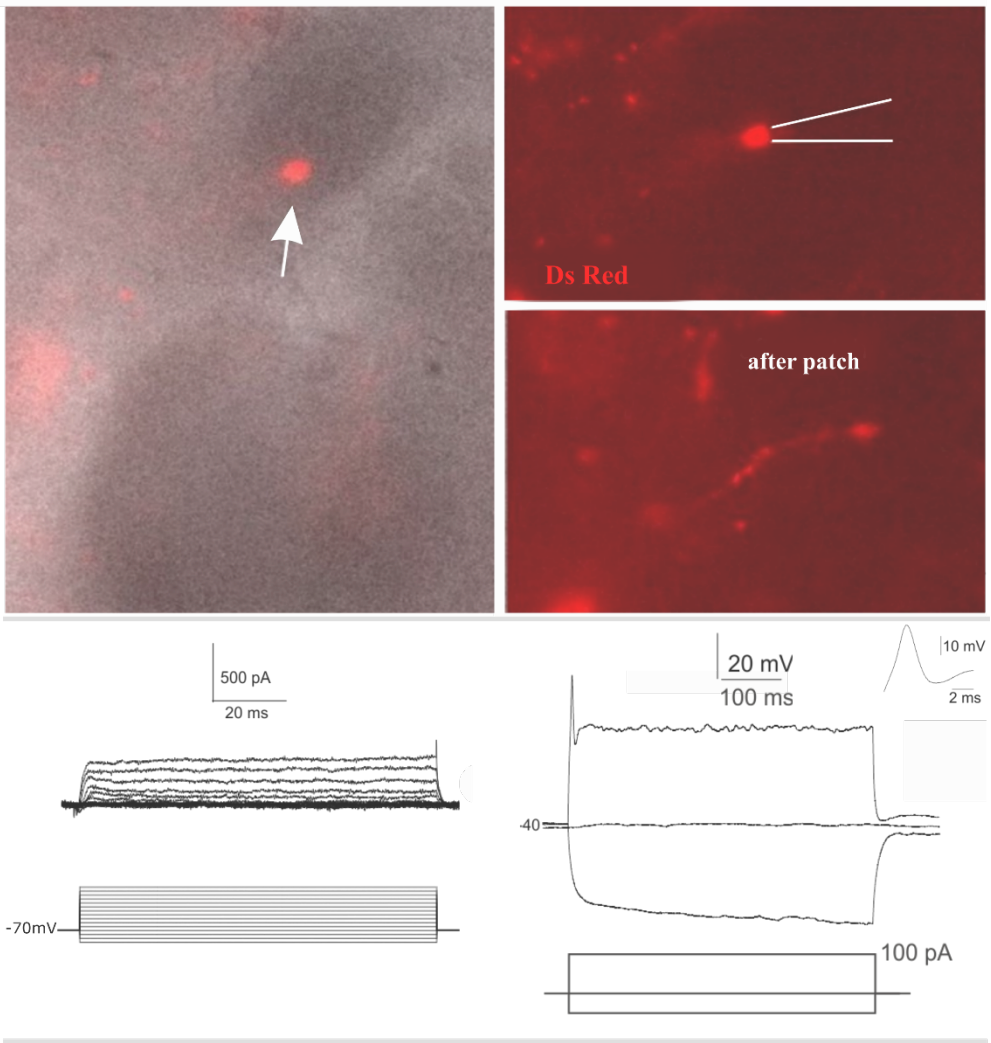

Figure 1. Top panel shows an example of retroviral-traced newborn striatal neurons expressing the DsRed fluorescence reporter during and after patch-clamp recording. Bottom panel shows representative traces obtained in voltage-clamp configuration (bottom left) and current-clamp configuration (bottom right).

Conclusions

During my training stay I had a great supervision from different post-doc and PhD students working in Prof. Berninger lab. I also had the opportunity to be active part of the Prof. Berninger group by participating to Lab meetings, journal clubs and seminars. It was very stimulating to work in this inspiring and heterogenous environment.

Therefore, I would like to thank the Network of European Neuroscience Schools (NENS) for awarding me the stipend for my training stay. I would also like to thank Prof. Benedkit Berninger for receiving me. Special thanks to Andrea Gamir Morralla and Jeronimo Jurado Arjona for teaching me the virus production, and Nicolas Marichal for the electrophysiological analysis.

Overall, the NENS exchange grant gave me one of the most valuable experiences in my scientific career so far. I obtained data and feedback to continue my research project and it was a great chance for me and my laboratory to strengthen our ongoing collaboration.