NENS Stipend for Training Stay

Report

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Period of training stay: 1st of March, 2017 to 1st of June, 2017 (3 months)

Contact information of home institution supervisor: Prof. Gerald Zernig, Medical University of Innsbruck, Experimental Psychiatry unit, Innrain 66a, 6020 Innsbruck, Austria, gerald.zernig@i-med.ac.at

Contact information of host institution supervisor: Prof. Andreas Frick, Neurocentre Magendie, INSERM U1215146 rue Léo Saignat, 33077 Bordeaux Cedex, France, andreas.frick@inserm.fr

Project title of the training stay: Production and purification of pseudotyped rabies virus for mono-trans-synaptic tracing

My training stay in Prof. Andreas Frick group was a great opportunity provided by the NENS Grant Stipend and it was a very successful training since the goals of my training were achieved: I have learned the production and purification of the pseudotyped rabies virus. The laboratory of Prof. Andreas Frick in the Neurocentre Magendie provided me the necessary guidance, knowledge, techniques and equipment.

My PhD project is entitled "Neural networks in cocaine vs social interaction preference: Investigating the possible differential involvement of presynaptic partners of accumbens D1- and D2-medium spiny neurons". We are using the rabies virus approach to trace the first order presynaptic partners of DA-D1- and D2-expressing medium spiny neurons in the nucleus accumbens. This is for us of relevance in order to identify which networks may be preferentially affected by cocaine vs social interaction. For the mono-trans-synaptic retrograde tracing approach, a helper virus (AAV-DIO-RG) and a mutant rabies virus EnvA-SADΔG-EGFP are stereotaxically injected into the nucleus accumbens of Drd1a-cre::TVA-LacZ mice.

Therefore, it is a great advantage to know how to produce and purify the rabies virus, obtaining a high titer, in order to proceed with my PhD project. Besides, this training has also provided me the knowledge for the set up of this technique in our campus. We are currently buying all the required material, according to Andreas Frick lab's advices, to start the production of this virus in my home institution very soon.

During this stay I learned how to grow the different cell types necessary for the virus production: B7GG (for growing G coated SADΔG), BHK-EnvARGCD2 (for growing EnvA coated SADΔG), BHK-WT (for titering G coated SADΔG), and HEK293-TVA (for titering EnvA coated SADΔG) (Figure 1).

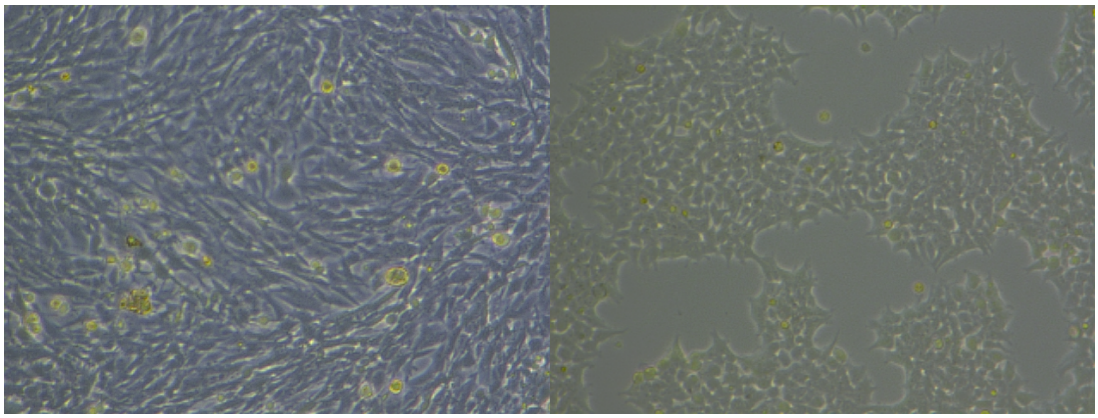


Figure 1. Examples of B7GG cells (left panel) and HEK293-TVA cells (right panel) at different confluences.

Production and titering of rabies viruses

The starter stock of SAD-ΔG-GFP is initially amplified by growing the virus in a complementing cell line (B7GG cells) that provides the rabies glycoprotein. To make EnvA-pseudotyped rabies virus it is necessary to perform another round of amplification followed by pseudotyping in BHK-EnvARGCD2 cells, a cell line that expresses the avian sarcoma and leukosis virus (ASLV) envelope protein EnvA, fused to the cytoplasmic domain of rabies glycoprotein.

The different cell lines are seeded to reach 80% confluency and then infected with the virus, splited and incubated until cells have a 80% confluency. The infection in the cells is confirmed using the epifluorescent microscope. Then the medium is collected 4-6 times, an ultracentrifugation is done and the virus (pellet) is eluted. The virus is aliquoted and freezed at -80º. To analyse the virus titer, the cells (BHK cells for G-coated viruses, 293T-TVA800 cells for EnvA-coated cells) are infected with the virus in a serial of dilutions and after a period of incubation the infected cells are counted with the epifluorescent stereomicroscope and the calculations are made to determine the final virus titer. The virus is ready to be injected (stereotaxically) into the mouse brain.

Additionally, I also had the opportunity to perform stereotaxic injections in mice, using different viral vectors and more importantly; injecting the rabies virus we have produced during my training. In figure 2, an example of presynaptic neurons expressing the rabies virus (RABV-EnvA-mCherry) can be seen. I also had the opportunity to learn and practice how to analyse the obtained data, which is extremely useful for my own PhD project.

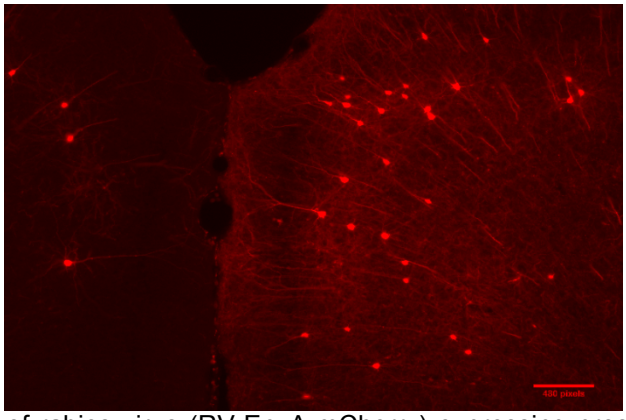
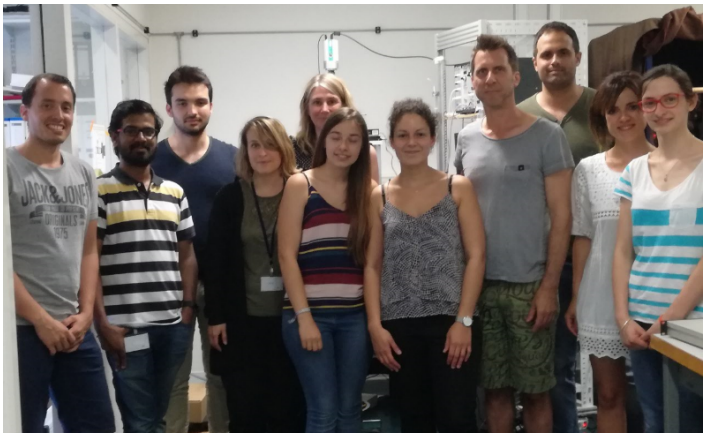


Figure 2. Example of rabies virus (RV-EnvA-mCherry) expressing presynaptic neurons in the anterior cingulate cortex and motor cortex of the mouse brain.

I had a great supervision during my training and I also had the opportunity to be part of the neuroscience community at the Neurocentre Magendie, meeting many other PhD students and post-docs and also participating in the Institute's symposium. It was very stimulating to be in this inspiring and diverse 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. Andreas Frick (host institute) for receiving me. Special thanks to Katy Le Corf for teaching the rabies virus production, and all the members of Prof. Andreas Frick's lab for making my stay a remarkable one.



Andreas Frick' team and I