The travel grant was used for a research visit to the laboratory of Professor Ignacio Lizasoain and Professor Maria Moro (department: Unidad de Investigación Neurovascular) at the Universidad Complutense Madrid. It took place from the 1st March 2009 to the 14th March of 2009

Purpose of the visit

It is our aim to investigate the question whether adult neural stem cells divide with an intrinsic asymmetry and to characterize the proteins that are involved in this process. In a first series of experiments we want to determine the subcellular localization of our candidate genes in mitotic neural stem cells in the subventricular zone (SVZ) of mouse brains. Under homeostatic conditions the adult neural stem cells only produce new neurons for the olfactory bulb, therefore they only show a relative low level of activity. Consequently at any time point only a few cells are in mitosis. But, to investigate the mechanisms of asymmetric cell division in detail it would be necessary to increase the rate of mitosis and probably also to synchronise mitosis in these adult neural stem cells. This can be achieved by infusion of the drug AraC (Cytosine Arabinoside) into the SVZ (Doetsch et al., 1999). Firstly AraC kills all cells that are mitotically active at the moment of infusion leaving just dormant adult stem cells in the SVZ. However, shortly after stopping the infusion these dormant stem cells become active and undergo mitosis. Therefore this technique allows to image mitosis in a lot of adult neural stem cells in their *in vivo* environment.

During the two weeks of my visit to the lab of Professor Ignacio Lizasoain and Professor Maria Moro I wanted to learn the technique for the infusion of AraC with a mini-osmotic pump into the lateral ventricle of the rodent brain. This technique is very well established in their lab at the *Universidad Complutense* in Madrid (Morales et al., 2008; Barros et al., 2008).

Barros C. A., Ekuni R., Moro M. A., Pereira F. M., Pereira M. A., Milani H. (2008) The cognitive and histopathological effects of chronic 4-vessel occlusion in rats depend on the set of vessels occluded and the age of the animals. Behav Brain Res., (Epub ahead of print)

Doetsch F., Caillé I., Lim D. A., García-Verdugo J. M., Alvarez-Buylla A. (1999) Subventricular Zone Astrocytes Are Neural Stem Cells in the Adult Mammalian Brain. Cell, 97: 703-716

Morales J. R., Ballesteros I., Deniz J. M., Hurtado O., Vivancos J., Nombela F., Lizasoain I., Castrillo A., Moro M. A. (2008) Activation of liver X receptors promotes neuroprotection and reduces brain inflammation in experimental stroke. Circulation, 118: 1450-1459

Description of the work carried out during the visit

For the implantation of the mini-osmotic pumps it is necessary to learn how to perform a surgery at the open rodent brain with minimal harm to the animal and how to use a stereotactical apparatus. So the first thing to do was to implant several cannulas for single injections to learn the surgery procedure on the one hand and to optimize the implantation coordinates by injecting of coomassie blue into the lateral ventricle on the other hand.

After verification of the right coordinates we started with the implantation of the mini-osmotic pumps (Alzet; model 2001). Before the implantation these pumps have to be prepared after a certain protocol (http://www.alzet.com/products/filling.php) to ensure the correct pumping velocity. This includes the sterile filling of the pumps without including air-bubbles and the overnight-'priming' of the pumps during which the pumps soak up fluid and come to the correct operation temperature of 37˚C.

The pumps then are implanted into a fur-pocket in the animal's neck or back and connected by a sterile tube to a cannula, that is placed at the coordinates of the lateral ventricle with a stereotactical apparatus (Fig. 1 and 2).

During my visit at Maria Moros lab five pumps were implanted under different conditions. The first pump was filled with 0.9% sterile NaCl-solution and was lasted in the animal for 24

hrs before killing the animal and the injection of coomassie blue to verify the injection coordinates. Two more pumps were filled with 0.9% saline solution and were maintained in the animal for 72 hrs before killing the animal and injecting the dye. Two pumps were

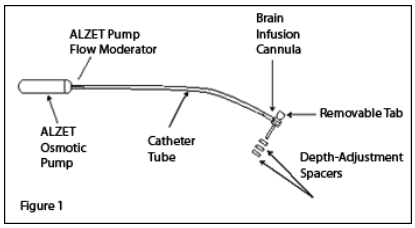
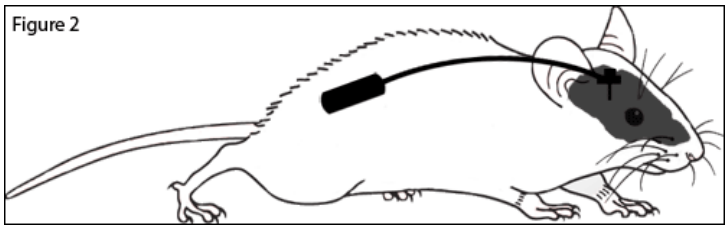


Figure 1



implanted in animals and maintained there for 48 hrs. One of them was filled with the neuroprotective agent CDP-choline (250mg/ml) and the other one was filled with 0.9% NaCl solution as a control. After killing the animals, the brains were used for cDNA production and for western blot analysis, which was performed after I left the lab, so that no results can be described here.

Description of the main results

The aim of the visit was to learn how to implant mini-osmotic pumps from Alzet into the adult rodent brain lateral ventricle. In the two weeks of work in the lab of Prof. Maria Moro I learned how to perform this technique and in the future I will be able to perform this surgical procedure on my own.

Future collaborations with host group

Both sides have proposed future collaborations, but no topic for collaboration has been specified, yet.

Projected publications resulting from this grant

The learned technique will play an important role in future work of our lab. Therefore some publications are expected to be derived from this grant.