5 November 2012

Activity report

Between September 2,2012 and October 28, 2012 I have performed a training stay in the lab led by Dr. Eric Lingueglia at the Institute of Molecular and Cellular Pharmacology in Sophia Antipolis, France. During my two-month stay with the Dr. Lingueglia's group and under his supervision I have acquired essential knowledge in both theoretical and practical aspects of the real time PCR technique by learning the steps required for the preparation of PCR experiments, as well as performing the actual PCRs.

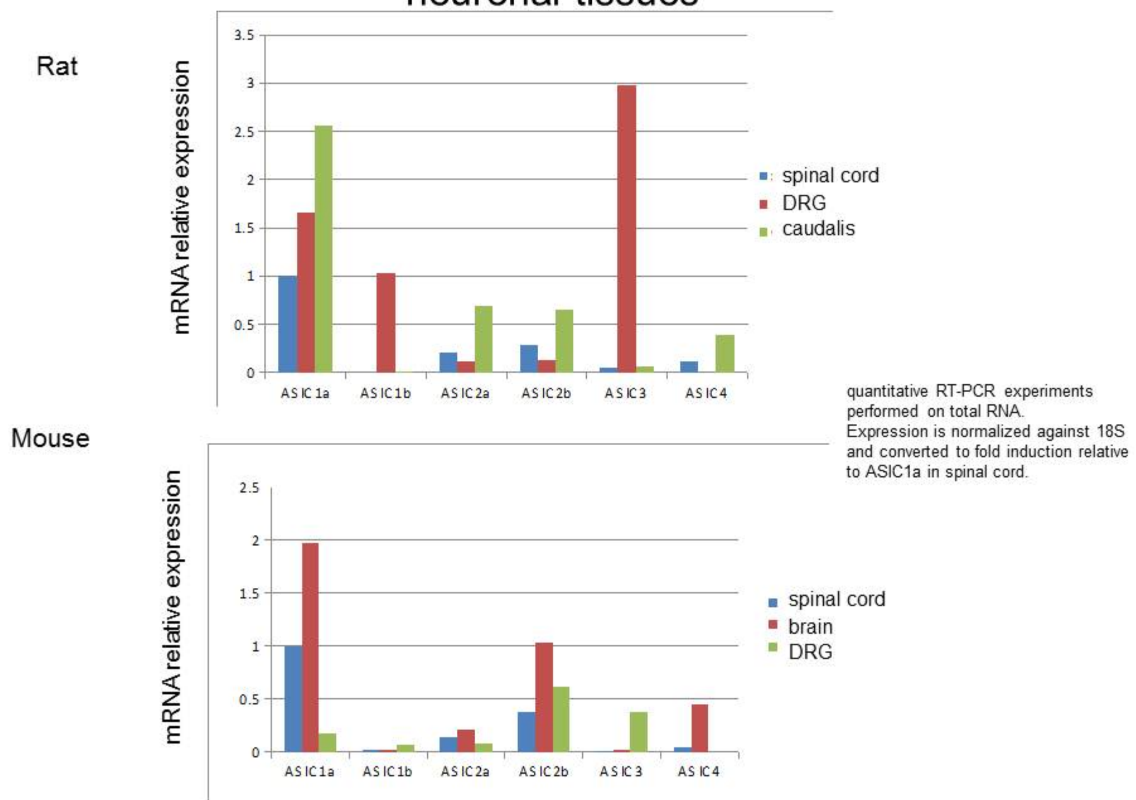
Initially, I have been instructed in the process of primer design, utilizing different bioinformatics tools to ensure the fulfillment of all the criteria necessary for generating efficient primers, such as high target specificity, suitable length of both primer and amplicon, absence of self and cross complementarity etc. These primers were designed for rat and mouse Acid-Sensing Ion Channels (ASICs), the main research focus of Dr. Lingueglia's group, in such a way as to ensure the detection and discrimination of different isoforms and the exclusion of any genomic DNA amplification. This was not the case of the primers already available in Dr. Lingueglia's group.

After the completion of the first phase, the next step was to verify the efficiency of the newly-designed primers by running PCR plates of cDNA serial dilutions and plotting the standard curve for each primer. If the efficiency (curve slope value) and linearity coefficient of the primer fell within accepted limits, it would be kept to be used in the following experiments.

Next, a comparison was made between the new set of primers and older already-validated by not fully optimized (see above) ones by assessing the expression levels of the ASIC channels in different neuronal tissues and comparing the results, in order to verify previous results and provide a complete picture of the channel distribution pattern.

Another objective was to extend the choices with regard to the housekeeping genes that could be used in these experiments. After consulting the literature on commonly used housekeeping genes and their expression stability, the choice was narrowed down to 4 such genes for each species, whose expression was later verified for constancy between different neuronal tissues and within the same tissue in different conditions (physiological and neuropathic pain, respectively).

As a result of this training, my skills and self-assurance in handling this technique have increased substantially, which will allow for its confident implementation in my home lab, revising past experiments and designing new ones while generating reliable data.



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