NENS Training Stay - REPORT

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Host Laboratory: TRP Ion Channels and Pain Group, Department of Cellular Neurophysiology, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague
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TRPM8 belongs to the TRP (Transient Receptor Potential) family of ion channels and it is involved in innocuous and noxious cold transduction in mammals. TRPM8 is highly expressed in afferent nerve endings of dorsal root ganglia neurons, which are depolarized in response to cold. TRPM8 is also activated by chemical stimuli, such as the natural cooling agents menthol, eucalyptol and other derivatives or the synthetic compound icilin (Chuang et al., 2004). Our group in Bucharest has recently demonstrated that camphor directly activates and sensitizes human TRPM8 to cold. However, the chicken homologue is not activated by camphor (Selescu et al., 2013). The aminoacids residues responsible for camphor activation of human TRPM8 have not been identified yet.

The aim of my training stay in the laboratory of Dr. Viktorie Vlachová was to generate TRPM8 point-mutations and chimeras between chicken and human TRPM8 in order to identify the molecular determinants of the camphor sensitivity of this protein. For this purpose my work there focused on learning the technique of PCR-mutagenesis. I received training in modern molecular biology techniques, including designing of specific primers and point-mutagenesis using a kit from Stratagene. During my training stay I generated one point-mutation in chicken TRPM8 and three point-mutations in human TRPM8. These last point-mutations were introduced in previous experimental studies in TRPM8 gene from other species (rat or mouse) and none of them was used in experiments performing stimulation with camphor before. Furthermore, I learned how to generate chimeras, producing one chimera between chicken and human TRPM8. I used the BioEdit 7.2.5.0 (Tom Hall, Ibis Biosciences) programme for alignments between DNA sequences.

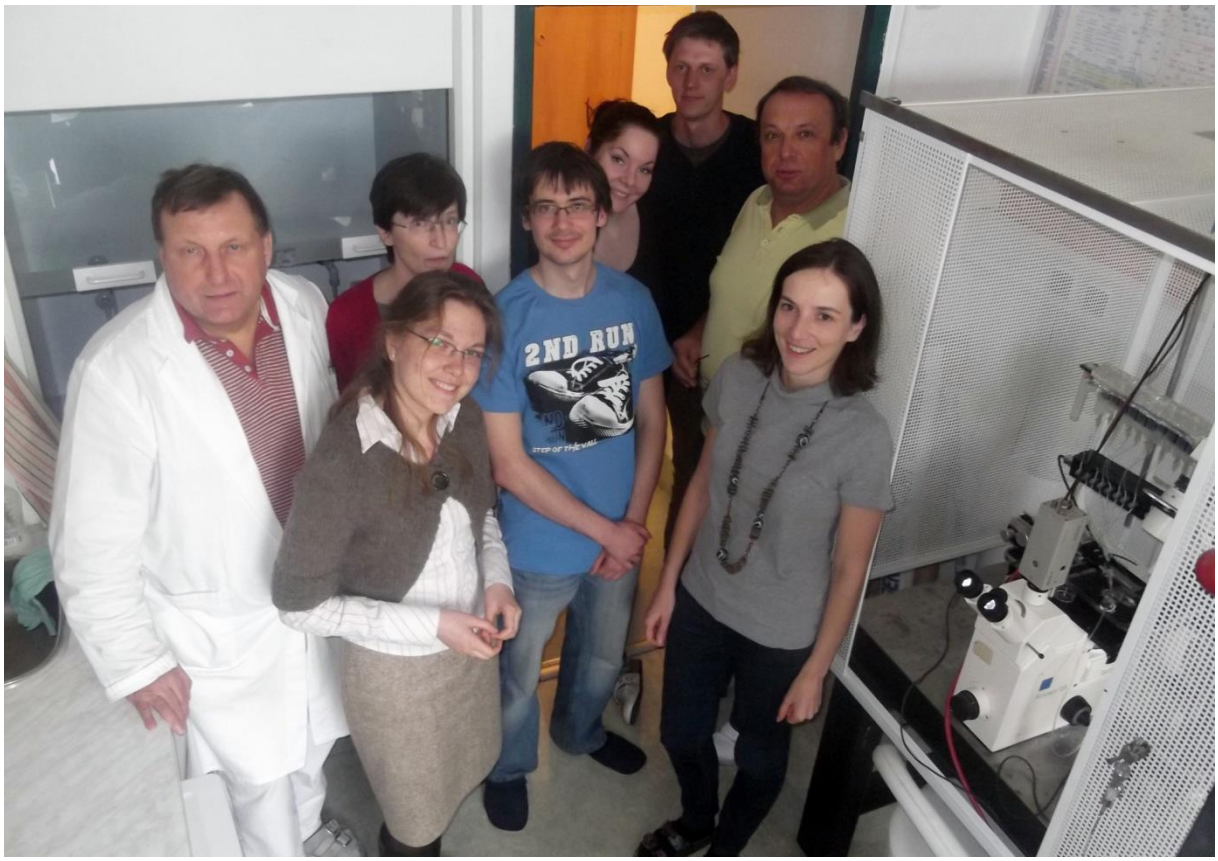
In order to see if the modified residues are important in camphor sensitivity of TRPM8, we performed calcium microfluorimetry measurements in a heterologous system. We prepared transient transfections of TRPM8 mutants, chimera or wild-type in HEK cells using MATra

(Magnet-Assisted Transfection) method (a different transfection method from those used in my home laboratory). I have improved my knowledge of calcium imaging techniques because I received training in ratiometric calcium imaging, using Fura-2 indicator, while in my home lab we use nonratiometric calcium microfluorimetry (with Calcium Green 1 AM). Calcium imaging measurements were performed using Cell R imaging system based on an Olympus IX - 81 inverted microscope from Olympus. Intensity profiles were measured using Image J software from NIH (National Institutes of Health) and data analysis was prepared using Sigma Plot 10.0 software from Systat Software Inc, San Jose, California, USA.

We have preliminary results concerning the activity of camphor on the TRPM8 mutants we generated and further investigation regarding these mutants, including calcium microfluorimetry, but also electrophysiology recordings will be performed in my home laboratory. We also intend to generate other chimeras in order to find out which is the molecular determinant of the sensitivity to camphor of human TRPM8 receptor.

NENS training stay had an important contribution to my professional development because I could see and learn different experimental protocols and equipments that are used in other laboratories studying the neurophysiology of pain and thermotransduction. I acquired new skills, I gained professional experience and independence, I am more able now to choose the appropriate method for a certain purpose, taking into account the advantages and disadvantages of using one or another. NENS training stay was a valuable step for the evolution of my career as a neuroscientist.

My dissertation project involves the investigation of the pharmacological properties of thermoTRP channels, particularly focusing on the natural compound camphor and my work during the training stay allowed me to get close to the aim of my thesis. Receiving a NENS stipend was a great opportunity for me because it allowed me to learn new techniques from experts in the field of molecular biology and electrophysiology of TRP channels, the time spent in my host laboratory being very pleasant and useful. It was an important professional experience for my research career in neurophysiology and also a great life experience in a wonderful historical and cultural place.



From left to right: Ivan Dittert, Mihaela-Olivia Dobrică, Viktorie Vlachová, Viktor Synytaya, Katarina Lichnerová, Štěpán Chvojka, Ladsislav Vyklicky, Lenka Maršáková