External Referee's Report on the Ph.D. thesis of Mgr. Olena Butenko: "Expression and functional characterization of transient receptor potential vanilloid-related channel 4 (TRPV4) in hippocampal astrocytes after ischemia/reperfusion"

The Ph.D. thesis by Mgr. Olena Butenko is focused on investigations of the functional role of the transient receptor potential TRPV4 receptor channel in hippocampal astrocytes in an animal model of ischemic injury.

Particularly, the new results demonstrate that TRPV4 channels participate in the increase of astrocytic calcium signalling after cerebral hypoxia/ischemia and characterize the underlying changes in TRPV4 expression and functioning. The new results presented in this work are highly topical in the field and are based on two primary publications in refereed scientific journals (PlosOne, IF 3.73 (2012) and Neurochem Int. IF 3.60 (2010)) and one review article in Physiological Research (2008, IF 1.6).

The Ph.D. thesis is written in English and is composed of an Introduction (29 pages) in which the author presents basic information about astrocytes, their proposed role during cerebral ischemia and basic functional characteristics of the TRPV4 channel. The aims of the work are clearly formulated on page 44 in five specific points, which include the overall characterization of pathological changes in hippocampal CA1 region under a model of ischemic injury with a specific focus on elucidating the functional role of TRPV4 in this process. The Methods section (12 pages) is followed by the Results section (47 pages) which is logically structured into seven subchapters. The Discussion (14 pages) very well and compactly covers the experimental results. The thesis is well and carefully written and the results are illustrated by relevant figures.

I had the opportunity to read the first version of this dissertation as an external opponent in the process of internal doctoral thesis defence. Small inaccuracies and typos were identified and have been fixed by the author in this submitted version. On the other hand, I still remain a bit uncertain what fraction of the currents illustrated in Fig. 54, Page 81, is mediated by TRPV4. I would be curious why the author did not use the TRPV4 inhibitor at the end of each recording? The progressive increase in the current in the recordings shown in the panel A could be attributed to losing the GΩ seal and not to TRPV4-mediated activation (as in Fig. 65, the abrupt changes in the amplitude of the red representative trace). The experiments with the blocker ruthenium red (RR, Page 86, Figure 56, panel B) are not much convincing – the inhibition precedes RR application. In addition, I would expect a certain asymmetry in the inhibitory effects of RR at negative vs. positive potentials. Could the candidate please comment on this?

Beside the impressing amount of new experimental data that were obtained in this work, I value the way both the new results as well as the limitations of the experimental approaches used to answer the experimental questions are discussed based on the help of the most recent literature. This thesis contains a large amount of original material and adds to the understanding of the role of TRPV4 in astrocytes. The candidate has demonstrated a high degree of expertise in a variety of relatively sophisticated electrophysiological, molecular biology and biochemical techniques.

Conclusion:

In my opinion, Mgr. Olena Butenko possesses the requisite qualifications, powers of original thought, and has proved to be capable of efficiently solving new research problems. Parts of the Ph.D. thesis have been already published in reputable impacted scientific journals. The thesis significantly contributes to the advancement of research in its specific area and fulfills the requirements for the degree of Ph.D.. Therefore, I recommend the thesis submitted by Olena Butenko for the award of the degree of Ph.D. by the Committee at the 2nd Faculty of Medicine, Charles University in Prague.

Praha, 25th March 2014

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The themes for debate; questions:

1. In addition to TRPV4, astrocytes have been shown to express also TRPV2, 5 and 6 ? In your experiments, did you notice some functional signs of possible involvement of other TRPV channels?

2. The expression pattern of TRPV4 is relatively broad and the physiological function of this channel is also supposed to be more general than only sensory. Can you please comment a possible role of astrocytes in some known phenotypes of TRPV4 deficient mice in this context?

3. The 4αPDD-evoked currents from astrocytes display a linear current-to-voltage relationship, rather than rectifying one. The author hypothesizes about possible mechanisms on Page 101. Did the author consider to repeat some key experiments with another (and maybe more potent) TRPV4 agonist ?