Dissertation Thesis Review

Title: T-type calcium channels in neurological disorders
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The dissertation thesis of PhD student Robin Stringer, MSc. is focused on the T-type calcium channels and their role in neurological disorders. This basic research provided an important inside into the function of the channels, both wild-type and mutated, the latter being of a high clinical relevance for better understanding of the pathogenesis of related diseases.

The dissertation thesis (202 pages in total) is composed of an introductory section describing the physiology and pathophysiology of the T-type calcium channels with a focus on their subtypes relevant to the nervous system (36 pages), aims of the dissertation thesis (3 pages), methods (4 pages), and a commentary (5 pages) on 7 original studies to which the student contributed either as the first author (3 published papers) or a co-author (3 published papers + 1 unpublished manuscript) and which are attached to the dissertation thesis in the form of Appendix. The discussion follows (8 pages) and the key results are concluded (2 pages). The provided information is supported by 406 references.

In the Introduction, the student first provides a short overview of various subtypes of the voltage-gated calcium channels including their expression and roles in various tissues. A detailed overview of the structural and namely functional properties of the T-type calcium channels including their regulation by interacting proteins and post-translational modifications follows. Subsequently, the student deals with channelopathies and neurological disorders related to variants in T-type calcium channel genes and, finally, relevant drug-channel interactions are discussed.

Then, the aims of the thesis are listed, being divided into three main areas: (i.) regulation of expression of the T-type calcium channels, (ii.) channelopathies associated with these channels, and (iii.) drug-channel interactions.

All the used methods are described in a separate subchapter Methods (*e.g.* patch clamp recordings in a cell line expressing the intended T-type calcium channels, computational modelling, molecular docking, and various techniques from the field of molecular biology, such as co-immunoprecipitation, western blot, or transcriptomic analysis). Details can be found in the attached original papers.

In the Results, all the attached papers are commented on. The key findings of each of the studies are described. The studies were focused on: (1.) the role of non-canonical N-glycosylation in the post-translation processing of Cav3.2 channels; (2.) the role of SCAMP2 in T-type calcium channel expression; (3.) several upregulated genes encoding enzymes in diabetic conditions; (4.) T-type calcium channel dysfunctions associated namely with genetic variants related to (i.) ALS, (ii.) severe developmental and epileptic encephalopathy, (iii.) and familial trigeminal neuralgia; (5.) the effect of a group of surfen derivatives on the activity of T-type calcium channels.

Discussion of the results follows, being divided into three parts focused on the previously specified crucial aspects (see Aims) and the dissertation thesis is concluded by a short chapter and Summary.

Summary: The dissertation thesis is written clearly, with a minimum of typos, well structured, and, besides Introduction, Aims, and Methods, contains commented Results and Discussion of 7 attached original papers. The aims of the dissertation thesis were met, the scientific level of the data is high. The acquired results are clinically relevant and contribute to a better understanding of the function of T-type calcium channels in health and disease.

Questions and scientific comments:

1. Why do the T-type calcium channels miss auxiliary subunits in contrast to other voltage-gated calcium channels? Try to hypothesize.
2. The measured currents are represented as current density in pA/pF as usual. Have you checked that there is a significant proportionality between the current magnitude and cell membrane capacitance in your data? It seems that the proportionality is not always present (at least in cardiomyocytes - e.g. Kula *et al.*, Prog Biophys Mol Biol 2020, 157, 24-32; Ismaili *et al.*, Prog Biophys Mol Biol 2020, 157, 33-39) and the use of current density may then misinterpret the data.
3. The patch clamp data were measured at room temperature. Why? Could this affect the results?
4. Appendix – Paper 1 – Are the residues N345 and N1780 unique regarding the impaired trafficking of Cav2.3 channels or any other residues within the channel known to cause a similar dysfunction? Have you considered the use of confocal microscopy to prove the defective trafficking? If not, why?
5. Appendix – Paper 4 – You concluded that the ΔI153 variant showed a dominant negative effect on the WT channel, but the current was reduced only by 35 % (Fig. 4). I am not sure that this conclusion is correct. Please explain.
6. Most of the studies included in this dissertation thesis were performed using human ionic channels heterogeneously expressed in a cell line. Please consider a possible impact of the technique on the results and discuss limitations of the technique (you partly touch this in the Discussion, but try to be more specific). Do you plan to check the impact of some of the tested dysfunctions in patient-specific neurons or even cerebral organoids in the future?
7. What are your future plans with S13?

Formal comments:

1. I would move the paragraph starting with "These channels are essential ..." (page 23, line 6) before the dysfunctions, to page 20.
2. The attached published papers are not well readable. The size of the letters is too small and they are blurred.
3. Typos can be rarely found in the thesis, *e.g.* on page 15 (line 23: "... are now know to be ...") or 25 (line 11: "... in a similar vein ...").

Conclusion: The PhD candidate Robin Stringer, MSc. Has demonstrated creative abilities in the given field of research, the submitted dissertation thesis meets the criteria set for this type of work. Therefore, I recommend and support its successful defense.

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