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Review of dissertation thesis
submitted to the First Faculty of Medicine of Charles University in Prague

Title: *Ca^{2+} signalling in magnocellular neurones of the rat supraoptic nucleus*
Author: *Ing. Štěpán Kortus*
Supervisor: *RNDr. Martin Zápotocký, Ph.D.*
Consultant: *Dr. Govindan Dayanithi, Ph.D.*

The submitted dissertation thesis is a great introduction to the physiology of magnocellular neurosecretory cells of the hypothalamus. Most importantly, however, the bulk of the thesis contains an excellent description and analysis of several important experiments concerning the dynamics of intracellular concentration of Ca^{2+} in vasopressin-producing and oxytocin-producing cells.

Experiments and analyses presented in the thesis represent a much needed combination of experimental approaches and mathematical modelling. The main topic selected for the thesis is certainly ambitious and it is highly relevant for current research on Ca^{2+} signalling in various cell types.

The thesis is written clearly and concisely on 95 pages, it is divided into five chapters, and includes an extensive reference section. Two appendices add 33 more pages which contain copyright permission statements and also copies of three publications (co-)authored by Štěpán in Appendix B. Figures, schematics, and tables throughout the thesis are consistently of very high quality and convey the intended messages really well.

The first chapter contains a description of important physiological roles of vasopressin and oxytocin, followed by detailed description of physiological properties of magnocellular cells producing and releasing oxytocin and vasopressin. The main part of this initial portion of the thesis then introduces in detail the main topic, i.e. intracellular Ca^{2+} dynamics and associated experimental approaches.

Aims of the thesis are summarized succinctly in the second chapter. While ambitious, the aims represent a logical and naturally evolving sequence of experiments,

ranging from exploring the mechanisms underlying spontaneous Ca^{2+} oscillations and induced changes in intracellular Ca^{2+}, to experiments promising to reveal the physiological role(s) of Ca^{2+} dynamics in magnocellular cells.

The third chapter introduces the main experimental and analytical approaches used in the thesis. The Methods section starts with the description of experimental animals as a source of identified magnocellular cells and experimental setup for calcium imaging. As a side note, I could not find any mention about sampling rate at which the fluorescence signal was recorded. This information might be important, for example, when interpreting the definition of baseline calcium level, which is defined as the mean of twenty samples.
The most relevant part of Methods, however, concerns the description of several experimental protocols that allowed the author to use pharmacological manipulations designed to control individual well-defined Ca^{2+} fluxes, and consequently tease apart their contributions to the overall Ca^{2+} dynamics. This is accompanied by a detailed description of the mathematical method underlying the 'Ca^{2+} flux decomposition.'

Main results of the thesis are described in detail in chapter 4. Among several important results, the author starts by demonstrating that spontaneous Ca^{2+} oscillations do not depend on spiking activity of a given neuron. In a series of elegant experiments, Štěpán then manages to decompose depolarization-induced changes in Ca^{2+} levels, and tease apart sustained depolarization-evoked Ca^{2+} influx and a faster component which depends on the state of intracellular calcium stores. Finally, the author describes the impact of the physiological state (e.g. dehydration, lactation) or related extracellular stimuli (e.g. osmotic change or direct application of vasopressin) on spontaneous Ca^{2+} in magnocellular cells.
The results alone represent important contributions to the field. Moreover, presented experimental and computational approaches can form a foundation for future in-vivo studies aiming at determinng the full physiological function of magnocellular cells and related regulatory systems.

I have very few minor comments on the results and their presentation in the fourth chapter. The thesis is written in excellent English, but several typos seem to have escaped proofreading. For example, 'component is present*ed*' (p.65), 'adaptation (a)rises' (p.68), or 'did not survive*d*' (p.48).
The example skewness values described and discussed on p. 70 do not correspond to the values reported in Fig. 4.17, and the boundary between traces Types II and III should be defined as 0.53 instead of 0.43. Section 5.3 (p. 81) refers to section 0,

whereas it should most likely refer to section 4.1.
At several places the text reports 'significance' of results, where the correct term should be 'statistical significance' to distinguish *statistical significance* from *biological significance.*
Figures 4.18 and 4.20 lost their colors, probably when they were exported and reprinted from the publication in appendix B.II.
Responses refered to as 'green area' (p.65) should be refered to as 'blue area', if I'm reading the protocols and results correctly. Similarly, in Fig 5.1 (p. 84) the terms 'blue' and 'green' in the caption should be swapped.

Chapter 5 then wraps up the thesis with a nice discussion of reported findings, placing them in context, and demonstrating again the importance of these results in the field. The conclusions provide a succinct summary of all results described in the thesis.

The thesis is well-written, well-documented, and very interesting to read. The author designed and performed clear and useful experiments combined with mathematical modelling, and demonstrated his ability to think critically and contribute to the field. I believe the thesis fulfills all requirements and I recommend it for defense in the field of Medical Biophysics.

Finally, I have two questions for the author:

1. All calcium indicators are subject to bleaching and the resulting gradual loss of signal could affect measurements similar to those performed in your experiments. Did you control for or prevent bleaching in your experiments, for example by limiting the duration of measurements? Would it be possible to replace loading dyes with genetically encoded calcium indicators in some future experiments?

2. The important relationship between spiking activity and Ca^{2+} dynamics in magnocellular cells is hinted at throughout the thesis. Could you *speculate* on the nature of the relationship, i.e. is it known how the interplay between spiking and Ca^{2+} oscillations (for example) might determine the release of hormones in magnocellular cells?

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