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Report on the doctoral thesis of Mgr. Daniel Šmít entitled
Analysis of dynamical interactions of axon shafts and their biophysical modeling

This PhD thesis at the interface between physics and biology investigates the dynamics and the underlying biophysical mechanisms of axon fasciculation. Because axon fasciculation plays a key role in the development of neural networks, the topic of the thesis is of high scientific interest and significance. Previous research focused on investigating the role of growth cone (GC) dynamics on axon fasciculation. In this work, it is uncovered that additional mechanisms independent of GC dynamics and involving direct interactions between individual axon shafts play an important role in regulating axon fasciculation. It is found that the interplay between axon-axon adhesion and mechanical tension in axons leads to zippering or unzippering of axons or axon bundles, which allows for changes in the structure of the neural network and for network remodeling. So far, the novel findings of this thesis have led to a major publication with Daniel Šmít as first author in the high impact journal eLife. This provides a strong validation for the high quality and impact of the work. In addition, an open source software tool to analyze BFP experiments has been developed and published in BMC Biophysics and has been made publicly available to the scientific community via the Github platform.

This thesis is a very good example for modern systems biology where the fruitful interplay between biology, physics, mathematics and computer simulations leads to understanding and quantitative description of complex biological systems. I am impressed by the multitude of sophisticated experimental, physical, mathematical and numerical techniques that have been combined in this cooperative project between the Trembleau and Zapotocky lab to study the regulation of axon fasciculation: generation of a well-defined axonal network by culturing an explant of embryonic mice olfactory sensory neurons, pharmacological and mechanical manipulations of the network, recording of time lapse videomicroscopy and electron-microscopy, force measurements with BFP technique, development of analytic and software tools to analyze data, development of a biophysical model of axon zippering dynamics based on the physics of viscoelastic fluids and solids, mathematical analysis of static and dynamic model properties, statistical analysis of network properties, biophysical parameter extraction and numerical simulations. Although Daniel Šmít did not perform culturing, videomicroscopy, electron microscopy, and pharmacological manipulations, he participated in micromanipulations and BFP experiments, and performed data analysis, modeling, mathematical and numerical analysis.

I much enjoyed reading the thesis manuscript: The physical appearance is impeccable, is very well written, the level of English is flawless, and the content is didactic and clearly structured. It has an extensive bibliography which indicates a profound knowledge of the scientific literature. The thesis manuscript starts with the abstract that is also given in Czech and French language. The introduction explains biological and biophysical aspects of the work, motivation, hypothesis and goals. However, since it has almost 80 pages, it is not well suited to provide a quick and crisp overview. Chapter 2 describes the experimental techniques and the mathematical and numerical methodologies to analyze the various types of experimental data. The heart of the thesis manuscript is Chapter 3. It uses the experimental data to develop and validate the biophysical zippering model for axon fasciculation based on the competition between adhesion and tension. It also provides mathematical analysis and dynamical simulations. The clarity of this chapter could be further enhanced by providing results in a more condensed and less descriptive manner, e.g. using tables that display model parameters and their values. In addition, the inclusion of figures with an overlay between model predictions and observed fasciculation dynamics would have been interesting. Finally, I think that the qualitative two-dimensional foams paragraph would better fit into the discussion rather than the results chapter. Chapter 4 provides a thorough and critical discussion of the results presented in Chapter 3, a comparison with the scientific literature and perspectives for future work. Finally, Chapter 5 completes the thesis with a brief conclusion.

In summary, after careful reading of the thesis manuscript, I conclude that this dissertation makes a significant contribution to the understanding of the role of axon shaft and mechanical tension in axon fasciculation. The thesis exhibits all the expectations for an independent scientific work and without hesitation I recommend awarding the academic doctor degree (PhD) to Mgr. Daniel Šmít.

Questions for the defense:

- Generalization: How specific are the results to outgrowing olfactory neurons? Can they be generalized to other neuron types and other substrates?
- Regulation: Is there a biological regulation of the zippering process via signaling pathways that depend on axon-substrate or axon-axon interactions? How would this change the purely physical model?
- Axon-Substrate interaction: How exactly is the axon-substrate interaction handled in the dynamic simulations? Can it be simply reduced to friction forces proportional to velocity? And what is the evidence for Assumption 2 on page 167 that axons remain straight? Straightening requires to reposition the whole axon against axon-substrate adhesion forces. Why would that be so much faster compared to the axon-axon adhesion dynamics?

I am looking forward to attending the defense.

Sincerely,

Jürgen Reingruber

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