COMENIUS UNIVERSITY IN BRATISLAVA
FACULTY OF NATURAL SCIENCES
Department of Animal Physiology and Ethology
Ilkovičova 6, 842 15 Bratislava 4

Opponent Review of Doctoral Dissertation of Mgr. Petra Honzlová

Title: Role of interaction between circadian and immune systems in development of obesity and type 2 diabetes mellitus

The doctoral dissertation of Mgr. Petra Honzlová is focused on the study of the mutual interactions between three important physiological systems: metabolism, immunity and the circadian clock. The thesis is based on three research projects and the presented results have already been included in three original papers published in high-quality scientific journals. The candidate is the first author of all these papers, demonstrating her substantial contribution. The thesis contains a number of interesting and original findings in the field of chronobiology, such that misaligned feeding regimen differentially affected the circadian clocks of the exocrine and endocrine pancreas and that the properties of the circadian clock in macrophages and microglia change dynamically with their polarization.

The thesis is written at a very good and professional level. The concept is clear and presented in a logic way. Regarding the formal part, I only have one comment on the style of citing literature in the text, as it is not consistent (sometimes the initials of the authors' first names are used). To achieve the aims of the dissertation, the author used a range of sophisticated methodological approaches and model systems, which were appropriately combined. I was also impressed by the creation of mathematical simulation to confirm the measured data.

The results are organised into three main chapters, parallel to the objectives. They are easy to follow and nicely visualised by the comprehensive figures. A minor objection is the missing statistics from cosinor analysis, particularly in the first chapter of the results. Otherwise, since all the data have already been published and subjected to a rigorous peer-review process, I have no reason to comment critically on them.

Questions

1. Your data showed that misaligned feeding-induced loss of circadian rhythmicity in the exocrine pancreas can be caused by conflicting insulin and corticosterone signalling. Did you consider or measure also other metabolic hormones that could play a role?
2. Interestingly, individual treatment of macrophages with LPS and IFNγ resulted in different effects on the rhythmic parameters of the circadian clock than the combination of LPS+IFNγ. Do you have any explanation for this? Could the treatments differentially also affect proinflammatory phenotype of macrophages?
3. You demonstrated differences in the daily profile of core clock genes between polarized M1 and M2 BMDM, but it could be interesting to show the daily profile of clock genes also in nontreated M0 macrophages. Have you measured this group? Would you expect any differences?

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4. Rosiglitazone-induced PPARγ activation affected PER2 bioluminescence rhythms in M2 polarized macrophages. Can this effect also correlate with their immune function? In addition, since the clinical use of rosiglitazone has been restricted in several countries because of its potential cardiovascular risk, do you think that other PPARγ agonists may affect the macrophage clocks in the same way?
5. Do different daily profiles of clock genes in polarized microglia reflect pro- respectively anti-inflammatory properties of individual clock components?

In conclusion, the proposed scientific objectives have been met and the submitted doctoral dissertation provides a number of original scientific findings. The candidate has demonstrated her ability to creatively orient in a given field of research and to master challenging laboratory techniques. I therefore recommend the grade "passed" and, following a successful defence, the award of the academic degree Philosophie doctor (PhD).

In Bratislava, 31.5.2024

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