

Students Name: Bc. Jakub Opelka

Thesis Title: Perception of periodical temperature cycles regulating the circadian clock in larvae of *Drosophila melanogaster*

The Master thesis of Bc. Jakub Opelka explores the role of Ionotropic Receptor 25a (IR25a) in temperature regulation in regards to the circadian clock in *Drosophila melanogaster*.

The thesis is written in English and apart from some small errors, the language is generally good. Although the structure is logical in terms of chapter order; it is very difficult to read in parts. It is clear that an effort was made to research the topic, however there are very few citations, especially in the introduction, even when a direct quote is given ("everything is rhythmic unless proven otherwise." Page 1). There are multiple figures throughout the introduction that aid in the understanding of the mechanisms discussed, however these also lack referencing. If figures are adapted, credit should still be given to the author. Additionally, the introduction is long and has several paragraphs that are repeated (page 10 & page 12-13 (highlighted in pink) and page 10-12 & page 14-16 (highlighted in green)). There also seems to be a lot of focus on adult sensory system despite the majority of the work focusing on larvae.

The experimental design seems well constructed, allowing the candidate to investigate the aims laid out in this thesis. The methodology is clearly explained, although there are some parts lacking, for example the dissection of *Drosophila* brains.

The results, for the most part, are presented well, the confocal images are of a high quality but the graphs (Figures 16 (15), 16 and 17) would benefit from further explanation. For example in the first Figure 16 (15) there are green and red stars present on the graph that are not explained, the significance indicated in the figure legend is not clearly displayed on the graph and it's unclear what is being compared. The confocal images in this figure would benefit from being labeled to match the corresponding graph; as well as a scheme or labeling to orientate the reader to the position in the brain. I would also expect the 'n' of the experiments to be stated in the figure legend. One can assume the blue and yellow on the graphs (Figures 16 (15), 16 and 17) represent day and night phase respectively, but it would be prudent to explain this.

The discussion is relatively short and therefore limited. There is little direct reference to the results section in terms of what figure is being referred to. An attempt has been made to compare the results presented in this thesis to published literature; however, some of the claims made require further explanation. The conclusions addressing the difference between adult and larval *Drosophila* are vague and should be elaborated on.

In conclusion, the entire thesis is severely under referenced. I have some serious concerns about how this thesis was presented; it seems little care was taken to read over the work prior submission.

Questions to candidate:

1. In the introduction the candidate states “of three transcriptional loops, of which the per/tim loop is the most important” page 8, what is meant by ‘the most important’, what is the evidence for this?
2. Please describe the process of the antibody staining, how do the primary and secondary antibody interact, what is the benefit of preabsorbing the PER antibody in per⁰ embryos?
3. The candidate utilizes UAS-rpr, please explain how this induces apoptosis in a cell type specific manner?
4. Can the candidate please explain Figure 16 (15) page 38. Which values are compared to determine significance?
5. Why did the candidate choose to perform behavioral experiments on adult animals, rather than larvae when the focus of your other experiments are on larvae?
6. For the Drosophila activity monitoring experiments why did you select males? Would you expect female animals to behave in the same way as the males you tested?
7. The candidate concludes that the molecular mechanisms of low temperature perception in adult and larval animals differ, what are those proposed mechanisms?

Ellen McMullen

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