



BIOLOGY CENTRE CAS

Institute of Entomology

address: Branišovská 1160/31, 370 05 České Budějovice, Czech Republic

IBAN: CZ39 0300 0000 0006 0077 3445 | SWIFT CODE: CEKOCZPP | VAT No.: CZ60077344

phone: +420 387 775 211 | www.entu.cas.cz | e-mail: entu@entu.cas.cz

Posudek na magisterskou diplomovou práci

Bc. Jakuba Opelky

Perception of periodical temperature cycles regulating the circadian clock in larvae of *Drosophila melanogaster*.

General Thoughts:

This Thesis is based on solid amount of work and brings interesting and novel results on anatomical and functional development of neurons involved in sensing thermocycles in the larval stage of the vinegar fly. The text, however, is formally poor and makes an impression that it did not go through any output control at all.

Strengths:

The novelty of findings can be considered the major strength of the Thesis:

1. The depth of information presented in Introduction is impressive. It is clear that the author has made an effort to read and digest the most current literature on neuronal networks involved in the fly circadian clocks.
2. The text of Introduction is accompanied with very nice schematic figures – apparently the author's own work (though the authorship is not acknowledged).
3. The findings of the neuroanatomical localization of the receptor molecule IR25a and its involvement in sensing the thermocycles in fly larvae are highly original.

Weaknesses:

Unfortunately, whole text must be seen as a single big weakness:

1. The first warning for a reader comes already in 'Poděkování' where the names of three acknowledged people are either distorted ('Šumanovi') or shown in grammatically incorrect way ('Brabci', 'Šerýmu').
2. The first two sections (1.1. and 1.1.1.) are fine but the problems start to occur from section 1.2. and are so numerous, that I have decided not to list them one by one and will make just general comments.
3. The overall organization of the text is highly confusing. There are abrupt transitions from one topic to another. Long passages are obviously repeated twice (pages 12-13; 14-16). Many sentences are simply unclear. Numerous typos and spell errors make the reading unpleasant.
4. It is important to explain meaning of all abbreviations on their first occurrence in the text (in fact, having a list of abbreviations would be highly helpful). The very first abbreviation that occurs in the text is 'TRP' – on page 4 (unexplained). Here, the reader is left to guess that TRP is maybe distorted, should be TPR, and that it most likely means 'thermoperiodic response'. Unfortunately, the same abbreviation TRP is also used for Transient receptor potential channel (which first occurs on page 11 – unexplained). Moreover, on page 14 – the abbreviation TPR is finally decoded as a 'thermal preference rhythm' ... the third possible



meaning. The abbreviations TPR and TRP then occur many times in the text, in some places clearly distorted, in other places with unclear meaning.

5. The schematic figures (otherwise very nice) usually come too late and there is no reference to them early in the text. This makes the orientation for a reader difficult.
6. The Introduction makes an impression of a continuous stream of information that fails to proceed from general to specific and fails to stress that the molecule IR25a will be in the focus of experimental part. Why, for instance, the sentence ‘Studies on *D. melanogaster* adults have identified a gene, *IR25a*, that plays crucial role in synchronizing organisms to low temperature cycles’ does not come in the Introduction – it only comes in Discussion (page 42, moreover without any reference).
7. Similarly, the Introduction fails to explain what ELAV is (*elav* gene is laconically mentioned on page 26 for the first time – but unexplained). Later, ELAV is used as an important marker molecule throughout the experimental part, while short and partial explanations of ELAV importance come only on pages 36 and 42.
8. Six figures present the results of experimental part. Not only they are wrongly numbered (Fig. 15 is missing, the text refers to non-existing Fig. 18), but parts remain unexplained in the figure caption (Fig. 12 C, D), or the captions fail to clearly describe the figure content. Fig. 14 apparently presents the key evidence for localization of IR25a in the chordotonal neuron Ich1 – the quality of this figure is, unfortunately, not very high (missing contrast between signal and dark background) and the information presented in parts A, B, C is not logically interconnected. I believe that the author has more than a single micrograph documenting this important finding and could present those in supplements.

Discussion Questions:

1. The Figure 15 (which is wrongly labelled as Figure 16) clearly shows that the null mutation of the *ir25a* gene, as well as the GAL4/UAS-driven apoptosis of all *ir25a*-expressing cells, cause complete loss of PER staining in DN1 neurons. Could not this result mean that the *ir25a* is expressed in the DN1 cells? You seem to exclude this possibility though you have detected clear signals of IR25a in different sensory organs of the larval head (Fig. 12 and 13).

In addition, on page 39 you state that: ‘No significant oscillation in PER expression was found in either LNV nor DN2 neurons (Figure 15 C).’ On the panel C of Fig. 15, however, the oscillation of PER in DN2 neurons, with the phase shift similar to *ir25a* mutation, is more or less obvious. If the oscillation is truly present, would that have any influence on your conclusions?

2. The Figure 16 suggests that the wild-type larvae can anticipate both the transition to cold and warm phase of the thermoperiodic regime. This itself is very important finding as it documents the full functionality of circadian system already in the larval stage. What is your explanation of the fact that *ir25a* null mutation abolishes only the anticipation of cold, but not warm, phase?
3. The Figure 17 has really poor descriptions both in the caption and in the text. Could you spend some time to explain what exactly is on Figure 17 and what it means?

Conclusion:

I would like to postpone my final decision after the oral presentation and defense of Jakub Opelka is done. The Thesis is apparently based on solid analysis of theoretical background and also contains original results of experimental work. The formal presentation of the Thesis, however, is of low quality and it is thus difficult to recognize whether the lack of clarity and organization stems simply from the lack of time (no output control of the text) or from a more serious lack of understanding.

In České Budějovice, 9 May 2023

Vladimír Košťál