



Přírodovědecká
fakulta
Faculty
of Science

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice



BIOLOGICKÉ
CENTRUM
AV ČR, v. v. i.

STATEMENT OF THE DIPLOMA THESIS SUPERVISOR

Name of the student: Bc. Jakub Opelka

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

Supervisor: Mgr. Hana Sehadová, Ph.D.

Supervisor's affiliation: Institute of Entomology, Biology Center Czech Academy of Sciences and Faculty of Science, University of South Bohemia in České Budějovice, Branišovská 31, 370 05 České Budějovice, Czech Republic

The diploma thesis focuses on the synchronization of the circadian clock of *Drosophila melanogaster* larvae to low temperature cycles. Previous studies in adults have shown that the input pathway transmitting the low temperature cycle signal to the central clock oscillator involves the Ionotropic Receptor 25a (IR25a). An IR25a mutation results in a loss of the ability to be entrained by low temperature cycles. Jakub detected IR25a in 3rd instar larvae using the *IR25aGAL4-UAS-RFP* line and identified IR25a-positive neurons by immunohistochemical colocalization with embryonic lethal abnormal vision protein (ELAV) as a marker for active neurons. To verify the role of IR25a in entrainment of larval clock, he tested the ability of IR25a mutants and larvae with ablated IR25a-expressing neurons to synchronize expression of the central clock protein Period in the larval brain, as well as their ability to generate rhythmic oscillations in respiratory activity under the low temperature cycles. Jakub discovered that mutation of the IR25a gene in larvae causes a phase shift in the expression of the Period protein in the central clock and eliminates its expression particularly in dorsolateral clock neurons. Although IR25a mutants retain the daily cycles of CO₂ production, they lose the ability to anticipate the cold phase of the temperature cycle. Because ablation of IR25a-positive cells caused arrhythmicity in Period protein expression and resulted in the loss of both the anticipation of the cold and warm phases of CO₂ production, we can conclude that IR25a-positive neurons are involved in the entrainment of the larval

circadian clock by low temperature cycles, but IR25a is not the only protein involved in this synchronization. These are very valuable results that provide a closer insight into the mechanism of synchronization of the larval circadian clock by temperature cycles.

During this project, Jakub improved in immunohistochemical detection of antigens followed by laser scanning confocal microscopy and signal intensity analysis performed with the software FIJI. He also mastered respirometry and overcame all the pitfalls associated with measuring CO₂ production in the larval population and analyzing the data obtained. He also performed behavioral tests on adult flies and analyzed corresponding data. He learned the software BioRender, which he used to create many of the diagrams used in the introduction of the thesis.

Jakub showed a high degree of independence in solving the tasks assigned to him and familiarized himself with a large amount of scientific literature. Therefore, I'm sorry that he managed his time poorly and didn't have enough time for the final proofreading of his thesis. The many typos and formal deficiencies detract from the value of the thesis.

Jakub is a thoughtful and ambitious student who tries to bring the tasks assigned to him to a successful conclusion and to look for possible alternatives in case of difficulties. He will continue research on circadian clock in *D. melanogaster* larvae during his PhD studies, which he plans to start soon. He has already arranged a graduate internship under the Erasmus program in our collaborative laboratory of Prof. Ralf Stanewsky at the University of Münster, where he will go in the autumn of this year. He is also actively applying for student grants awarded by the University of South Bohemia in České Budějovice.

In České Budějovice, 15.5.2023

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Mgr. Hana Sehadová, Ph.D.