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FACULTY OF BIOLOGICAL SCIENCES

DEPARTMENT OF ANIMAL DEVELOPMENTAL BIOLOGY
ul. H. Sienkiewicza 21
50-335 Wrocław | Poland
tel. +48 71 375 40 30 | +48 71 375 40 24
zbrz@uwr.edu.pl | www.zbrz.uni.wroc.pl

Review of the Doctoral Thesis of Ms. Debarati Bhattacharya M, SC.

Faculty of Biology ATUT PhD Programme in Biology

title: *The Role of Adenosine Receptor in the Living Processes of Drosophila melanogaster*

Supervisor: Prof. dr hab. Elżbieta Pyza, Department of Cell Biology and Imaging Institute of Zoology and Biomedical Research, Faculty of Biology

Introduction

The history of research on the phenomenon of periodical processes conditioned by the existence of the biological clock dates back to the 17th century, when Carl Linnaeus observed the daily movements of flower petals, the closing and opening of flower goblet. These observations prompted the famous researcher to arrange a flower clock from various species of plants. Although scientists have known that almost all organisms undergo changes called circadian rhythms, for a long time they could not discover, where this biological clock is or what does control it. Winners of Nobel Prize in Medicine in 2017, three researchers (Jeffrey C. Hall, Michael Rosbash and Michael W. Young) – modified fruit fly genes and created insects that stopped falling asleep regularly. The gene that was responsible for this change was named by the scientists *'period'*. More genes and mechanisms operating in individual organs were discovered. Today we know how disruption of the circadian rhythm affects human health. Currently, research on these processes belongs to world research leader.

The doctoral dissertation presented for review concerns on, not fully known, role of adenosine receptor in the life processes of *Drosophila melonagaster*. The doctoral dissertation was carried out under the supervision of Prof. dr hab. Elżbieta Pyza, a world-class specialist in circadian clock of *D. melanogaster*. In the doctoral project, the PhD candidate studied the role of the adenosine receptor in survival processes, physical condition, sleep and locomotor activity of fruit fly. Based on the knowledge that caffeine interacts with adenosine receptors, the PhD student investigated the effects of caffeine on adenosine receptors in the regulation of sleep, aging and behaviour in fruit fly.

Thesis' Review

The PhD thesis begins with an extensive *introduction* to the issues related to the doctoral

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project. The theoretical part of the thesis includes information focuses on issues related to adenosine receptors, their molecular structure, distribution and functions and its one counterpart in *D. melanogaster*. The chapters on the circadian rhythm and clock were very interestingly presented by the PhD student. Author presented the advantages of fruit fly in the study of circadian rhythm due to the simple organization of neuronal networks in the brain. Author also described first discovered 'clock gene', clock neurons and so-called 'Zeitgebers' that synchronize the clock to daily changes in environment. The last chapters of introduction parts focuses on sleep in *D. melanogaster*, type of neurotransmitters in sleep and the role of caffeine in this process. It is evident that the PhD student gained a deep understanding of the theoretical knowledge and the discussed problems.

The next chapter *The aim of the thesis* has been written very precisely and clearly by the author. The PhD student points out the fact that despite the discovery of the adenosine receptor in *Drosophila*, its role in sleep and regulation of other processes in fruit fly is still unknown. The assumptions of the project was to determine the function of the adenosine receptor in fruit fly by the overexpression or silencing of *dAdoR* in several cells, and influence above treatments on flies' survival, fitness, daytime and nighttime sleep, and locomotor activity. The second objective of PhD project was to determine whether lower expression of *dAdoR* can alter the Bruchpilot protein level and circadian pattern. The last objective of the study was an assessment on age-dependent changes in sleep of WT flies after feeding them with caffeine, and caffeine influence on *Drosophila siesta* and nighttime sleep, circadian clock after overexpression/silencing of *dAdoR* in transgenic flies.

In the chapter *Materials and Methods*, the PhD student describes in details the methods used in the project. In her project PhD student used Canton-S wild-type flies and several transgenic strains. In research, she uses the UAS / Gal4. In her studies, PhD student, also carried out survival assay and standard behavioural tests e.g. climbing and locomotor activity. Sleep in *Drosophila* was defined as a 5-min period of inactivity during locomotor test. In another experiment, flies were exposed to different concentrations of caffeine added to the medium. PhD student planned immunocytochemistry and immunoquantification methods. I would like to emphasize that the student showed a good knowledge of statistical methods that allowed for an in-depth analysis of the results. This proves that the PhD student is very well prepared to

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experimental work, and the methods she had used, allowed her to obtain very interesting, novel data.

Chapter, *Results*, were divided into subchapters related to the main objectives, included in *aim of thesis*, of the dissertation.

The most important achievements of the doctoral dissertation are:

- Overexpression or silencing of *dAdoR* in photoreceptors, neurons, and glial cells affects living processes in *D. melanogaster* such as lifespan, fitness, sleep and locomotor activity.
- Survival assay revealed that overexpression *dAdoR* mRNA in photoreceptors decreased the median survival and lifespan of males and females. *dAdoR* overexpression causes early deaths in younger flies whereas *dAdoR* silencing are characterized by a reduced overall lifespan. *dAdoR* overexpression in the retina photoreceptors does not influence on total sleep of flies but their daytime sleep was significantly longer whereas the nighttimes sleep was shorter.
- Overexpression of *dAdoR* in fruit fly neurons showed improved climbing ability of 60-day old males and 30-60-day old females. On the other hand, silencing of *dAdoR* causes decreased climbing ability. *dAdoR* overexpression in neurons causes that flies slept significantly longer (daytime and nighttime sleep increase) to control flies during the light and the dark part of the 24 h cycle.
- In glial cells overexpression of *dAdoR* causes that middle-aged male flies have better climbing ability in comparison to control flies. Silencing of *dAdoR* in glia caused reduction of climbing. Overexpression of *dAdoR* in fruit fly glial cells induced a significant increase in nighttime sleep.
- Studies on the level of presynaptic protein Bruchpilot (BRP), and its daily pattern in the flys's first optic neuropil (lamina) in silencing of *dAdoR* in photoreceptors and glial cells revealed that decrease in fitness is caused by lower level of the BRP protein .
- Studies on the possible effects of caffeine on adenosine receptors in sleep regulation, ageing, and behaviour revealed that caffeine affects WT female flies, this mainly affects the *siesta*.
- Results reveal that caffeine is unable to disrupt the circadian clock in situation when

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dAdoR is overexpressed or silencing.

- It was demonstrated that adenosine receptors are involved in the regulation of *D. melanogaster siesta*.

In the last chapter, *Discussion*, the author summarizes all the results obtained during the implementation of the doctoral project. Well clarified conclusions were precisely discussed by PhD student. The Discussion contains all the issues that the author has included for the purposes of the study, and correctly interpreted on the basis of the available literature.
However, several issues require deeper analysis. Therefore, I have a few questions for the student.

- PhD student showed that *dAdoR* overexpression in fruit fly induced more sleep at night and total sleep whereas in rodents this effect was not observed. How do you explain this situation?
- Author reveal that *dAdoR* silencing in photoreceptors, neurons or glial cells do not change the amount of total sleep, only a small decrease of the nighttime was observed. The lack of effect is explained by the student with a weak signal as a result of the down-regulation of the *dAdoR* gene. Have similar studies been conducted on mammals?
- PhD student has written that studies on caffeine signalling mechanism in fruit fly may be important for clinical trials on adenosine receptor –based therapy. Could you please, develop this issue?
- What is the reason, why caffeine has a stronger effect on males than on females?

Podsumowując, główne cele rozprawy doktorskiej zostały przez Doktorantkę w pełni zrealizowane. Uzyskane wyniki są oryginalne i wartościowe. Autorka rozprawy doktorskiej precyzyjnie wykonała szereg przemyślanych eksperymentów, z których wyciągnęła logiczne wnioski. Uzyskane przez Doktorantkę wyniki z pewnością przyczynią się do lepszego zrozumienia zegara biologicznego w kontekście receptora adenozynowego i jego roli w procesach życiowych *D. melanogaster*.

Rozprawa doktorska Pani mgr Debarati Bhattacharya. pt.: *Rola receptora adenozynowego w procesach życiowych Drosophila melanogaster*, spełnia warunki określone w art. 187 Ustawy z dnia 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce (Dz. U. z

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2018r. poz.1668 z późn. zm.). Na tej podstawie wnoszę do wysokiej Rady Naukowej Nauk Biologicznych Uniwersytetu Jagiellońskiego o dopuszczenie mgr Debarati Bhattacharya do dalszych etapów rozprawy doktorskiej. Jednocześnie zwracam się do Rady Dyscypliny o nadanie Pani magister stopnia naukowego doktora w *dziedzinie nauk ścisłych i przyrodniczych, w dyscyplinie nauki biologiczne*.

Rozprawę doktorską oceniam wysoko. Dysertacja została bardzo dobrze zaplanowana, cele pracy zostały osiągnięte, a studentka wykazała się bardzo dobrym przygotowaniem, zarówno merytorycznym, jak i metodologicznym. Na tej podstawie proszę Radę Naukową o wyróżnienie rozprawy doktorskiej Pani mgr Debarati Bhattacharya.

Prof. dr hab. Małgorzata Daczewska