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Review of the PhD Dissertation entitled "The Role of Adenosine Receptor in the Living Processes of *Drosophila melanogaster*"

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The subject of this PhD dissertation is in general the study a role of adenosinergic system in the living functions of the in vivo insect model using *Drosophila melanogaster.*

Organization of the dissertation.

The dissertation is composed in typical form of monography subdivided into following chapters: 1. Introduction; 2. Materials and Methods; 3. Results; 4. Discussion; 5. Conclusion; 6. Summary; 7. References; 8. Statement and 9. Acknowledgement. The abstract in English and Polish is also attached as well as list of Abbreviations, list of Figure Legends (25) and list of table Legends (15). References (355) are presented in alphabetical order.

The background and state of the art.

Adenosine is a very important neurmodulator in the CNS of mammalian brain and differs from classical neurotransmitters by not having specific neuronal pathways and mechanism of storage in cellular vesicles. However, adenosine is synthetized and metabolised by specific enzymes, it is also released in response to certain stimuli and reuptaken into cells by its own transporters. Several well characterized G-protein coupled receptors are demonstrated in the brain as well as in peripheral organs and number of tissues. This system is cooperating with other neurotransmitter pathways, serves as important modulatory system and is involved in body functions. The study undertaken by Author of this work is characterization more deeply the role of adenosine in the simpler animal model of *Drosophila melanogaster* which may give insight into adenosine action in the living body of higher systems.

Introduction.

In the Introduction Author described in detail adenosine synthesis, its metabolism with specific enzymes, the structure and signaling of 4 types of adenosine receptors found in mammalian brain and in the peripheral tissues. Receptors distribution and their involvement in various cellular functions are well characterised. Author also shortly mentioned the therapeutic applications of adenosine receptor ligands such as their role in cognition, neurodegeneration, epilepsy, immune

responses, cell stress. Here, the important role of adenosine A1AR subtype in the periphery is not described in a more detailed way; however in the Table 1.1 Author summarises main functions of all receptor types.

Chapter 1.6 and the next of Introduction are strictly devoted to receptors, neurotransmitter systems and living functions of fruit flies such as circadian clock and sleep regulation. This part of Introduction concerning the main subject of the study should be extracted as independent section. Similarly, "The Aim of the Thesis" should be placed as the separate paragraph.

Introduction is very detailed, contains encyclopaedic, gargantual knowledge on circadian clock and sleep in *Drosophila melanogaster*. Author gives description of neurotransmitter systems in Drosophila, recently discovered adenosine receptor and its putative role in insect living functions including sleep regulation. The circadian clock neurons and schematic representation of clock cells presented in graphical form helps to understand its complexity. The same refers to molecular mechanism of the circadian clock including feedback loops, function, signaling and regulation by light. In my opinion it is to detailed and material collected by Author may be published as independent monographic article.
The section devoted caffeine in sleep is good background for next chapter **"The aim of the Thesis"**.

The main objectives of PhD thesis formulated shortly are:

1. To examine the role of *dAdoR* in flies' survival, sleep and locomotion
2. To find out how changes in expression of *dAdoR* alters functioning of the presynaptic protein Bruchpilot related with pattern of circadian clock
3. To determine influence of caffeine as antagonist of adenosine receptors on sleep and circadian clock.

Methods.
In the methods chapter Author presents equipment used, list of chemicals and media, fly strains, conditions of flies maintenance, measurement of flies activity and sleep, immunohistochemistry assay and statistical analysis.

Results.
The results are presented in 14 figures and 8 tables. Author shows impact of overexpression or silencing of *dAdoR* on flies survival, fitness and sleep pattern.

Results show that overexpression of *dAdoR* affects flies survival, mostly decreasing it in 50 days old flies. Interestingly, strongest effect was observed when *dAdoR* was overexpressed in neurons but not in photoreceptor cells. However, *dAdoR* overexpression in glial cells affected more potently older flies. The silencing of *dAdoR* in photoreceptors and glial cells mildly protected flies from early death.

Overexpression of *dAdoR* in neurons improved climbing ability of old males and females, while its silencing had opposite effect. Overexpression of *dAdoR* in glia improved climbing of younger males but not females. Silencing of *dAdoR* in glia caused decline of climbing in older males and females.

Adenosine receptor signaling was also involved in regulation of sleep in *Drosophila melanogaster*.

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Overexpression of *dAdoR* in retina photoreceptors increased daytime sleep, but decreased nighttime sleep. Overexpression of *dAdoR* in neurons and glial cells increased day- and nighttime sleep. Interestingly, the circadian clock was not distorted by the overexpression of *dAdoR* in all types on cells.

The next objective of the study was to check the effect of *dAdoR* on synaptic protein BRP involved in circadian plasticity. Confocal image of BRP protein in Drosophila visual system showed that its level changes during the day (the lowest level was seen in the middle of the day). Silencing of the *dAdoR* in the photoreceptors resulted in lower fluorescence in the middle of the day which may affect behaviour. The higher expression of BRP at the beginning of the day and night seems to correlate with increased locomotion. The pattern of daily changes in BRP level in flies with silenced *dAdoR* in glial cells varied during the day and at the beginning of the night.

Feeding of flies with caffeine affected nighttime sleep and siesta. There were sex difference in response to caffeine feeding. Caffeine decreased male total sleep, daytime and nighttime sleep, but at higher concentrations it increased total and daytime sleep but decreased nighttime sleep. In females, caffeine at low concentration (0.1 mg/ml) decreased total sleep and nighttime sleep, at higher concentration 0.5 mg/ml increased total and daytime sleep but decreased nighttime sleep. At the highest concentration (1 mg/ml) caffeine increased daytime sleep but decreased nighttime sleep. Age dependent changes were also observed in caffeine effect on total sleep. Interestingly, females were more sensitive to caffeine-induced age-dependent changes in total, daytime and nighttime sleep.
Interesting observation was inability of caffeine to influence s*iesta* in flies with *dAdoR* overexpression in dopaminergic neurons. Exposure of flies to caffeine with silencing *dAdoR* in dopaminergic neurons caused a decrease in total sleep and *siesta*.

Discussion.
Discussion is mainly descriptive and summarizing data. The most interesting findings are:

1. Enhanced adenosine signaling was harmful to young flies, but if they survive initial stage then can live longer.
2. Flies with silenced *dAdoR* were protected against early death, but later showed higher mortality.
3. Adenosine signaling was important for fitness (silencing *dAdoR* in neurons and glia decreased fitness).
4. Overexpression *dAdoR* increasesd total sleep but decreased *siesta;* important role in sleep regulation plays neuron-glia crosstalk.
5. Presynaptic protein BRP related with *dAdoR* signaling was crucial for fitness and locomotion in *Drosophila.*
6. Caffeine affected *Drosophila* sleep (total and *siesta*) depending on concentration and gender. When *dAdoR* was overexpressed in all neurons, caffeine decreased total sleep, *siesta* and nighttime sleep. In transgenic flies with overexpression of *dAdoR* in *pdf*-neurons no effect on sleep was observed, while decrease in total and nighttime sleep was observed in *tim*-neurons and dopaminergic neurons.
7. Author explains the changes in sleep by caffeine through mechanism of formation of A2A-D2 receptor dimers in dopaminergic neurons and their negative interaction. According to this explanation, shorter sleep after silencing *dAdoR* can occur when caffeine blocks A2AR and increases activity of D2R. In turn, overexpression of *dAdoR* may result in decrease of D2

receptor activity and no change in sleep. The explanation of this data through negative interaction of A2AR-D2R known in mammalian brain cannot be directly transferred to *Drosophila* visual system. A2AR-D2R complexes in rodent or human brain are formed in GABAergic striato-pallidal neurons **but not in dopaminergic neurons**. It is not known whether these receptor complexes exist in *Drosophila*. What's more, adenosine receptor in *Drosophila* is not the same as in mammalian brain in spite that it has some similarity to A2A and A2B mammalian receptors.

8. Changes produced by caffeine in regulation of sleep did not cause distortion of the circadian clock.

In main conclusion Author suggests that discovery of adenosine receptor in *Drosophila* may have translational value and may help to understand the sleep regulation in man. However, invertebrate adenosine receptor is not the same as mammalian A2A or A2B receptors and its cell expression is not fully confirmed. Moreover, caffeine used in this study as a pharmacological tool, is antagonist not only of A2A but also A1 adenosine receptors, which are more abundant in mammalian brain as well as peripheral tissues and many of caffeine effects depend on both receptors blockade.

Summing up, in my opinion this PhD dissertation presents very interesting data and many problems which appeared during investigation need further studies. I recommend this dissertation to the **Biological Sciences Council of Jagiellonian University** for further steps of public defence and conclude that PhD thesis meets conditions set out in article 187 of the Higher Education and Sciences Law Act of July 20, 2018 (Dz. U. 2018 r. poz. 1668 z późn. zm.).

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W podsumowaniu stwierdzam, że rozprawa doktorska kandydatki spełnia warunki określone w artykule 187 Ustawy z dnia 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce (Dz. U. z 2018 r. poz. 1668 z późn. zm.) i rekomenduję **Radzie Dyscypliny Nauki Biologiczne Uniwersytetu Jagiellońskiego** o dopuszczenie mgr Debarati Bhattacharya do dalszych etapów postępowania o nadanie stopnia doktora *w dziedzinie nauk ścisłych i przyrodniczych w dyscyplinie nauki biologiczne*.

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