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**PEER REVIEW**

From prof. Hristo Stefanov Gagov, PhD  
Faculty of Biology, Sofia University, „St Kliment Ohridski”

Of PhD thesis of Zlatina Petrova Nenčovska entitled:  
„INVESTIGATION OF THE EFFECTS OF MELATONIN HORMON ON  
BEHAVIORAL AND BIOCHEMICAL CHANGES ACCOMPANYING  
EPILEPTOGENESIS IN A CAINATE MODEL OF TEMPORAL EPILEPSY ”

For achieving of educational and scientific degree „Philosophy Doctor”

Field of higher education 7. "Health care and sport",

Professional field 7.1 "Medicine",

Scientific speciality "Pharmacology“

The PhD thesis of Zlatina Petrova Nenčovska was made under the supervising of prof. Yana Chekalarova, PhD in the Institute of neurobiology, BAS, Sofia. It is based on 5 research papers. All they are written in English. Three of them are published in high ranking scientific journals (2 in *Epilepsy and Behavior*, IF=2.655 for 2013 and 2014 r. in the fields *Behavior Neuroscience* and *Pharmacology* and one in *Pharmacology Biochemistry and Behavior*, IF=2.82 for 2013 r. in the field *Behavior Neuroscience*). The sum of the points of these publications according to the methodology in the professional field is 37, i.e. it is above the required 30 points. In two of the papers Zlatina Nenčovska is first author, in two – second and in one – third author. The participations of Zlatina Nenčovska in international conferences with materials of this thesis are 5. She has also 5 participations with posters or oral presentation in 5 other conferences.

Biography data. In 2011 Zlatina Nenčovska finished as a MD student in „Animal and Human Physiology“, Faculty of Biology, Sofia University. From March 2010 to December 2011 she worked as a specialist in the Institute of neurobiology, BAS and since December 2011 has been

working as an assistant professor in the same research institution. She uses English and German languages. She was enrolled as PhD student on 05.12.2013 r. and was deducted on 19.04.2016 r.

The relevance of the topic and the appropriateness of the goals and objectives set. Epilepsy is among the most common neurological diseases affecting a huge number of people, many of them young (today, up to 1% of the population or over 50 million people worldwide, in Bulgaria - about 70,000). The impaired excitability of different brain structures causes the retention and excitation processes in different areas of the brain to pull in the direction of the second, i.e. brain structures systematically express a lower threshold of activation. As a result, an epileptogenic outbreak emerges that is able to excite itself and to increase the excitability of other parts of the brain. The investigated model of kainate-induced temporal lobe epilepsy is particularly relevant for applied research, given that about 30% of registered patients generate seizures due to abnormal excitability in the temporal lobe. In addition, kainate-induced epileptic status in the rat is characterized by acute, latent, and chronic phases, which provides additional opportunities for detailed monitoring of the molecular mechanisms of the disease. The use of spontaneously hypertensive rats (SHR), as well as any other disease model, provides an additional opportunity to study comorbid conditions and to optimize their pharmacological treatment.

Interest in studies of the functions of melatonin, which is a hormone of the pineal gland with pronounced antioxidant properties, as well as tissue hormone in the skin, intestines, etc., is generally high. It has been mentioned in more than 16,000 publications in PubMed since 2000, with a significant proportion of them having effects on the brain. In this dissertation, the importance of prolonged treatment with melatonin (10 mg/kg) on spontaneous seizure activity, comorbid depression, and morphological changes in the limbic system (mainly in the hippocampus) in normotensive (Wistar) and spontaneously hypertensive rats (SHR) was investigated on kainate-induced epileptic status.

The dissertation consists of 132 pages (including 15 pages of literature, with line spacing 1), is written and structured very precisely. The work contains all the necessary sections.

The literary review covers 26 pages. It is thorough and well written. A total of 256 literature sources, all in English, have been cited. The quality of the cited publications is adequate to the topic of the dissertation, the goals and tasks set, the results obtained and their discussion.

The review introduces us to known data in two main areas:

(a) epilepsy: mechanisms, animal models, plastic changes in the hippocampus, influence of comorbid depression, oxidative stress and comorbid hypertension on a kainate model of epilepsy in normotensive and hypertensive rats;

(b) melatonin: synthesis, localization and metabolism, hormonal effects of melatonin on various physiological processes, and in particular on the regulation of circadian rhythms and states of alertness and sleep, on types of melatonin receptors and their role in depression and epilepsy. The pathogenesis of melatonin deficiency is also addressed.

The aim is formulated briefly and clearly. It entails five tasks related to the frequency of seizures, behavioral responses, changes in serotonin levels and certain markers of oxidative stress, and to examine histological changes in some brain structures after kainate-induced epilepsy.

Materials and methods.

This dissertation presents data obtained through a variety of methods. The following have been applied for the study of: depressive state / behavior by forced swimming or preference for sweet solutions; hippocampus-dependent spatial memory; levels of 5-hydroxytryptamine were investigated by HPLC; histological examination of neuronal loss in brain structures of hippocampus, gyrus central gyrus, and pyriform cortex; the levels of lipid peroxidation and superoxide dismutase activity in cytosolic and mitochondrial fractions have been accounted for by biochemical methods; Western blot - for the levels of heat shock protein 72 (HSP72). The statistical processing used is relevant.

The results are many and strongly support the conclusions drawn. They are represented in 28 figures.

The discussion is well written, commenting on all the important results and well supporting the conclusions and the contributions.

The conclusions well summarize the data obtained. The conclusions and contributions are formulated briefly and clearly. They contain new information on the effect of melatonin on spontaneous seizure activity, behavioral responses, and morphological changes in the limbic system in kainate-induced temporal lobe epilepsy of normotensive and hypertensive rat lines, and its antioxidant effect. Similar behavioral, biochemical and morphological parameters of intact and epileptic SHR rats have also been identified, which are the basis for their use in the study of comorbid hypertension and epilepsy. Only in Contribution 4 I find ambiguity in the statement that: "melatonin exhibits breed-dependent differences ... suggesting a role for the melatonin system in a model of comorbid depression and epilepsy." In my opinion, there are two contributions here, and it would be better to separate them as follows: a) "For the first time, melatonin has been shown to exhibit different breed-dependent effects on spontaneous seizure activity, behavioral responses, and morphology of structures of the limbic system." (Contribution 4) and (b) "For the first time, melatonin has been shown to have an antidepressant effect in a model of temporal epilepsy in normotensive rats (Wistar) by the restoring of serotonin level in the hippocampus, suggesting a role for the melatonin system in this model of comorbid depression and epilepsy." (Contributing 5). In conclusion, I am convinced that the results of all these interesting studies have a great potential for practical use.

The auto-abstract consists of 62 pages and additional 9 pages in English, which summarize its contents. It accurately reflects the main points and achievements of this PhD thesis, presents the most important results obtained and their discussion, as well as contains the 7 conclusions, 5 contributions, the list of 5 publications on the topic of the thesis and the contributions of Zlatina

Nenchovska in international and national scientific forums and trainings. Finally, it contains data on the various honors of Zlatina Nenchovska - two awards for young scientist with donor Acad. Damian Damyanov for 2013 and 2014, one-year scholarship from the World Federation of Scientists with headquarters in Geneva (for the period 2013-14) and on supported participation in a workshop in neuropsychopharmacology in Nice, France. There are no attached data noted citations of these articles. The information provided showed that by August 15, 2019, they were cited dozens of times (39 citations appear in the Scopus system only). This information should have been available because it testifies both to the relevance of the topic being developed and to the authoritative position of the team and the qualities of the doctoral student herself as a researcher in this very dynamic and competitive field of research.

Critical comments and recommendations. I have no critical remarks or recommendations on how the research was conducted. However, I notice some details that can be corrected:

- add some missing abbreviations such as PKB, IL, HIOMT, TE, RFK, PAC to *Used abbreviations*.

In addition, PAB = RNS, i.e. two abbreviations (in Cyrillic and Latin) are used, as well as two designations - WKY rat and Wistar rat, for the same thing;

- words in this generally well written thesis are often fused, making reading difficult;

- "Hippocampal SHN" - to become "Hypothalamic SHN" - 29 p.

- "intercellular cyclic adenosine monophosphate (cAMP)" - to become "intracellular cyclic adenosine monophosphate (cAMP)" - page 29, second paragraph, below;

- "Neuroprotict" – to become "Neuroprotective" – p. 110, l. 11.

These small technical gaps do not detract from my appreciation for this very interesting, well-executed, written and illustrated work, the results of which have been presented to the scientific public in several excellent publications.

All this convinces me that Zlatina Nenčovska is a promising young scientist who, during her thesis work, has acquired a solid and complex competence that will be important for her future research work in the field of pharmacology.

In conclusion, I believe that the goal and tasks set have been successfully completed. In terms of structure and content, the submitted project of dissertation work fully meets the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its Implementation for the PhD degree. On the basis of the above, I strongly recommend that the members of the scientific jury award assistant professor Zlatina Petrova Nenčovska the educational and scientific degree "Philosophy Doctor".

Signature:

Sofia, August 20, 2019

(Prof. Dr. Hristo Gagov)