

Attitude of Reviewer

of Prof. Rositsa Zamfirova, PhD

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Concerning a thesis aimed at public defence for awarding the PhD degree in higher education field 4. Natural sciences, professional field 7.1. Medicine. Scientific specialty "Pharmacology (incl. Pharmacokinetics and Chemotherapy)" (code 03.01.24)

entitled "Effects of hormone Melatonin on behaviour and biochemical changes accompanying epileptogenesis in kainite model of temporal epilepsies".

by the post-graduate student ZLATINA PETROVA NENCHOVSKA, with Director of studies Associate Professor Yana Chekalarova, Ph.D.

The presented work includes 132 pages, consisting of Introduction (3 pages), Review (24 pages), Aims and tasks (1 page), Materials and methods (13 pages). The Results are described in 48 pages, the Discussion - in 11 pages, the Conclusions and Contributions covers 2 pages. The cited references are 256.

The review is detailed, well-balanced and intelligently presented. It outlines the types of epilepsy, and shows the reasons, that could provoke its generation (genetic, morphological changes, traumas, metabolic disorders, etc.) It emphasized that the reasons remain unknown very often. The chapter summarizes the current knowledge of epileptogenesis and the main characteristics of the focal and generalized seizures types, as well as the common drug therapy. A substantial role in choosing the topic for the research is the appreciated need for a new type of therapy, which not only affects the symptoms, but suppresses epileptogenesis. Hence, an extensive research on the pathophysiology of epilepsy is necessary. Z. Nenchovska draws attention to the fact that, after SE, a differently long latency period without seizures is observed. During the latent phase, a plastic changes in the brain are made, related to loss of inhibitory GABA-ergic and expansion of excitatory neurons. In turn, this causes behavioural disorders (concomitant depression, attention deficit disorder and hyperactivity disorder), inflammatory and immune responses, apoptosis and oxidative stress. A separate chapter outlines the available literature on the hormone melatonin and its physiological role, including its known effects on epilepsy and depressive states.

The reason to use spontaneously hypertensive rats (SHR) in the study, as a model of attention-deficit hypertonia, is well grounded by literature and clinical data. The aim is to elucidate the role of comorbid hypertension in epileptogenesis. Some facts, described in the literature, provide the basis for such research: 1) Hypertension is associated with increased release of free radicals, which could be a factor in epileptogenesis development; 2) A reversal of circadian rhythm of blood pressure has been found in SHR, observed also in people with epilepsy; 3) Increased expression of myocardial antioxidant enzymes has been reported with SHR; 4) In chronic epilepsy models, SHRs have a lower seizure threshold; 5) SHRs are an widely used model for studying disorders in neurological diseases (incl. epilepsy), which are associated with vascular dementia and accompanying behavioural abnormalities as a result of hypertension development.

Based on the data in the literature, **the aim** of the PhD thesis is to investigate the effect of melatonin on epileptogenesis, as well as on the accompanying biochemical, morphological and behavioural

changes in a model of KA-induced post-SE of TLE in normotensive Wistar and SHR rats. The resulting five tasks include the study on the changes in circadian rhythm and frequency of spontaneous recurrent seizures, the 24-hour variations in behavioural responses, the morphological changes in some brain structures after epileptogenesis, and changes in 5-HT levels in the frontal cortex and hippocampus and markers of the oxidative stress caused by the SE. It has to be noted that a large number of experimental models and methods, both classical (open field, forced swimming, cross maze) and modern (biochemical and histological), were used, according to the requirements of the separate tasks. The video and EEG monitoring of spontaneous recurrent seizures is a prerequisite for obtaining accurate and reliable results.

The results are well described and illustrated with 30 figures and one table. The figures present in parallel the data for the Wistar and SHR rats, which facilitates the understanding of the material. Along with behavioural changes, the effects of melatonin on 5-HT levels in the hippocampus, on SE-induced morphological changes, as well as on OS markers, have been demonstrated. Statistical methods are carefully selected according to the experiment. The use of two-factor and three-factor analysis makes it possible to statistically demonstrate whether or not there is a relationship between epilepsy and more than one variable factors. In the **discussion**, Mrs. Z. Nenčovska relates her own data to literature, medical practice and previous team-research. She skilfully combined the results of other authors with her own studies to explain the differences in the effects of melatonin on Wistar and SHR rats. The study gives additional knowledge on the behavioural changes accompanying epilepsy and the effects of melatonin on them - depression, anxiety, motor activity, and memory. The author explains the unequal effect, induced by melatonin on anxiety and depression, to a difference in 5-HT levels in the hippocampus and different activity of adrenergic system in the two animal strains. Interestingly, in the present study, melatonin exerts a significant neuroprotective effect in structures associated with spatial memory (C1 of hippocampus and Pyr). The finding is in agreement with that found in models of other neurodegenerative diseases (such as Parkinson, Alzheimer's, cerebral ischemia) and also with in vitro studies on hippocampal neurons. **The conclusions** correctly summarize the results obtained in the study. I fully accept the five presented contributions. They formulate the most important original data, obtained in the study. In the auto-review, the most significant and beneficial results, the publications and the poster presentations on scientific forums, are included. There is also a summary of the work in English.

CONCLUSION

The thesis is focused on the study of effects of the hormone melatonin on the behavioural and biochemical changes accompanying epileptogenesis in a kainate model of TLE. The work is an extension of the topic elaborated by the laboratory team on TLE. Wistar and SHR rats were used to study the role of hypertension in the development of epileptogenesis. Informative behavioural, biochemical and histological methods were used and the results were statistically processed with appropriate methods. EEG is documented and continuous video surveillance is provided. The results are accurately described and well-illustrated. The review of the literature, as well as the discussion, show Mrs. Z. Nenčovska's broad knowledge on the field in which she works and her ability to convincingly and competently analyse the experimental results. The data obtained and the conclusions drawn are a step in clarifying the pathogenesis of TLE and its features in accompanying hypertension.

The results of the study were published in 3 papers, all with IF above 2.6. In one of them, Z. Nenčovska is the leading author. Other 2 articles were published in national journals without IF. During the work on the thesis, Z. Nenčovska presented her current results as posters at four international forums and two national conferences. Her research activity has been awarded four times, including an one-year

fellowship for a young scientist from the World Federation of Scientists, with Geneva headquarters (2013-2014) and a participation in the Workshop on Neuropsychopharmacology for Young Scientists in Europe, France, Nice, March 2010, funded by the European College of neuropsychopharmacology.

The work fully covers the requirements and fits the ZRASRB, and its application rules, as well as the Regulation for the acquisition of the educational and scientific degree "Doctor" .

The Thesis and its presentation by the PhD student convinced me to support beyond doubt the award of the educational and scientific degree "Doctor" in the scientific specialty "Pharmacology" to the Assistant Zlatina Nenčovska.

30.08.2019
Sofia

Reviewer: 
Prof. Dr. R. Zamfirova