Вх. № 517/09.09.2019г.

REVIEW

prof. Iren Petkova Belcheva, MD, PhD, D.Sc.

Regarding: The thesis of Zlatina Petrova Nenchovska titled: "Study of the effects of the hormone melatonin on the behavioral and biochemical changes accompanying epileptogenesis in a kainate model of temporal epilepsy" presented for the award of educational and scientific degree PhD **in the professional field 7.1. Medicine, scientific specialty "Pharmacology (incl. pharmacokinetics and chemotherapy)"**.

scientific supervisor: Prof. Yana Jana Tchekalarova

By Order № 610A/08. 08. 2019 г. of the Director of INB - BAS, I was appointed as a member of the Scientific Jury for the defense of Zlatina Nenchowska's PhD thesis in the 7th area "Health and Sport", Professional Field 7.1 "Medicine" in the scientific specialty "Pharmacology (including pharmacokinetics and chemotherapy)".

With the decision of the Scientific Jury (Protocol No. 1/13.08.2019) I was appointed as a reviewer of the dissertation work of Assistant Professor Zlatina Petrova Nenchovska, PhD student in the Department of Behavioral Neurobiology, Institute of Neurobiology, BAS.

This review has been prepared in accordance to the Law of the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of the Academic Staff Development Law and the Regulations for the Conditions and the Procedure for Acquisition of Academic Degrees and Academic Position Occupation in INB-BAS.

I declare that I have no co-publications with the PhD student.

Brief biographical data

PhD student Zlatina Nenchovska was born on May 28, 1986. She graduated in 2005 high school "Aleko Konstantinov" in Pravets, profiling with foreign language. The following 5 years she studied at the Faculty of Biology, Sofia University "St. Kliment Ohridski " and in 2010 receives a Master's Degree in Animal and Human Physiology. After graduation Nenchovska has been appointed as a specialist biologist at the Institute of Neurobiology, BAS. Since 2011 she has been an Assistant

Professor at the department of Behavioral Neurobiology, INB-BAS. In 2013 (order N 568/05.12.2013 / Nenchovska was enrolled as a PhD student in the PhD program "Pharmacology (including pharmacokinetics and chemotherapy)" in the Department of Behavioral Neurobiology, INB-BAS and in 2016 she was released with the right of PhD thesis defense.

Zlatina Nenchovska speaks German and English.

Before I say my opinion for the dissertation, I would like to emphasize, that the Nenchovka has took into account the remarks and the guidelines which I made earlier at her internal defense.

General characteristics of the thesis. The PhD thesis is written on 132 pages and includes the following sections: Contents, Abbreviations used and Introduction - 11 pages; Review - 26 pages; Purpose and tasks - 1 page; Materials and methods - 12 pages; Results - 47 pages; Discussion - 12 pages; Conclusions - 1 page; Contributions - 1 page; List of publications included in the dissertation - 1 page; List of participations and scientific reports included in the thesis - 4 pages; Awards- 1 page; References - 15 pages, including 256 authors in latin.

The work is well constructed, well written and illustrated with schemes, tables and figures. The literature used reflects the focus and specificity of the literature on the topic of the dissertation. The experimental part is illustrated with 30 figures representing statistically processed data from the conducted experiments.

Topicality of the subject and expediency of the set goals and tasks

The dissertation presented is dedicated to an important problem related to studying the effect of melatonin on the behavioral and biochemical changes accompanying epileptogenesis in a kainate model of temporal epilepsy. Epilepsy is the fourth most common neurological diseases, the main symptom of which is recurrent, unprovoked seizures that cause lasting neurochemical, morphological and behavioral changes - cognitive impairment, depression and emotional disorders. Epileptic seizure is actually brain over-excitability due to the over-discharge of a particular population of neurons in a given brain region.

In recent years, the neurochemical mechanisms and signaling pathways involved in the pathophysiology of epilepsy have been intensively studied, as well as the influence of the genetic factors that determine the predisposition to epilepsy. The topicality of the problem is also determined by the fact that melatonin, as a regulator of various physiological processes, is notably important for both physiology and pathophysiology as well as medical practice.

Studies in this area are still isolated, which is a prerequisite for clarifying the efficacy of the hormone melatonin on the circadian rhythm of epileptiform activity, the role of hypertension in the development of epileptogenesis, and the involvement of melatonin in the pathophysiology of epilepsy and essential hypertension.

The Nenchovska's thesis are focused on the effects of lomg-term treatment with melatonin on epileptogenesis, associated with the development of spontaneous seizures, comorbid depression, biochemical and morphological changes in the limbic system in two breed of rats- normotensive Wistar rats and spontaneously hypertensive rats (SHR).

Knowledge of the problem

In the literature review, assistant Nenchovska has presented in clear and concise form both historical data and the most important literature data presenting the level of contemporary knowledge of epilepsy - classification, mechanisms of the convulsive reactions, pharmacological approaches for treatment.

In the separate dissertation chapters were discussed the experimental models of temporal epilepsy, the features of different rat breeds in the model of temporal epilepsy.
The melatonin, its synthesis, localization and metabolism, its receptors and their role in epilepsy, depression and circadian rhythms have been discussed also in detail.

In the literature review, Nenchovska summarized the present state of the problem in a very good way.
At the end of the literature review a very good impression makes the chapter "State of the problem" where more important uncleared questions are indicated.

The literature review is a good achievement for the PhD student and deserves a positive evaluation.

It should be noted that the material included in the literature review is presented in good Bulgarian language, which is characteristic of the subsequent presentation of the author.

Aim and tasks of the dissertation

The aim and the tasks of the dissertation are clearly and concretely indicated.

The aim of the dissertation is to investigate the role of melatonin on the frequency of spontaneous motor seizures and the concomitant behavioral, biochemical and morphological changes in limbic structures after kainic acid status epilepticus in an experimental model of temporal epilepsy in normotensive (Wistar) and spontaneously hypertensive (SHR) rats.

In order to accomplish these goals, Nenchovska has set 5 main tasks, which are clearly formulated and allow targeted experimental studies.

From the "**Materials and Methods**" section, it is clear that Nenchovska correctly has chosen methodological approaches to solve the aim.

The experiments were performed on two breeds of male rats - normotensive Wistar and spontaneously hypertensive rats. An experimental kainate model of temporal epilepsy was used in the dissertation. The seizures have been accounted after the epileptic status induced by kainic acid. Adequate and informative *in vivo* and *in vitro* methods have been used.

EEG activity has been recorded by stereotaxically implanted electrodes (brain cortex and hippocampus) on the fifth day post-status epilepticus.

Behavioral methods have been applied to study the effects of melatonin - open field test (for locomotor activity), elevated plus maze (for anxiety), Sucrose preference and Forced swim tests (for

depressive-like symptoms) and Radial arm maze test (for memory). All behavior tests were recorded using an infrared sensitive CCD camera and a video tracking system (SMART PanLab software).

Have been also used informative histomorphological, biochemical (determination of lipid peroxidation and cytosolic superoxide dismutase) methods.

For the Statistical analysis has been used one- , two- or three-way ANOVA. Post hoc comparisons were conducted by Bonferroni t-test or Mann–Whitney U-test.

Experimental results

Many experimental data were obtained and analyzed. The studies were conducted in a logical sequence. The data obtained using adequate approaches are convincing. The results from the different experiments are well and clearly presented in 30 figures, most of them (27) are double (A and B), 1 table, 1 scheme, authentic EEG recordings, 12 color photographs from histological examination of the hippocampus, pyriform cortex and basolateral nucleus of the amygdala of control and experimental animals in both rat breeds - normotensive Wistar and spontaneously hypertensive. This creates excellent transparency and documentation of the obtained scientific facts, which is characteristic of all scientific work. To analyze her own results, Nenchovska used appropriate statistical methods, which creates a basis for discussing the data obtained and the conclusions.
The work presented is quite sufficient in volume.
Overall, I give a positive assessment of the experimental investigations.

Discussion

In the **discussion**, the findings are summarized in a clear and concise form. Thus, systematically as the study has been conducted, the discussion of the obtained data have been constructed. Nenchovska uses her literature knowledge efficiently, to discuss competently and critically her own results. In the course of the discussion, she skillfully compared and contrasted her data with those obtained by other authors, as trying to find a logical explanation for the found differences.

The discussion presents the PhD student as an erudite scientist.

As a result of the precisely done work and the correct discussion of the results, **7 conclusions** have been formulated.

On the basis of the conclusions, a reference is made to the scientific contributions. The contributions are **five** and reflect the achievements in science. I am agreed with the formulated contributions, which are not only of theoretical importance but would also be of interest in medical practice.

Reference

The reference in PhD thesis includes 256 titles in English, cited in the chapters. Both classical and recent newly authors have been cited. This speaks of an excellent and thoroughgoing theoretical training of Nenchovska.

Abstract

The abstract in volume of 71 pages (in Bulgarian and English) has been compiled in accordance with the requirements of the rules and represent the dissertation successfully and sufficiently.

Personal impressions

As a head of Department of Behavioral Neurobiology INB, BAS, I have known Zlatina Nenchovska since her employment at the Institute. My personal impressions are excellent. She is a very good and precise experimenter, able to thoroughly interpret the data obtained, her ability to work in a team,

Scientific indicators

In her dissertation, Nenchovska has included a total of 5 publications, 3 of which have a high IF (over 2.6) in 1 of them she is the first author. With results of the dissertation, Nenchovska has participated in 6 international (on 4 is the first author), 2 national with international participation (on 2 is the first author) and 3 national scientific forums (on 3 is the first author).

Nenchovska fully meets the minimum requirements for the educational and scientific degree "Doctor" in according to the Law of the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of the

Academic Staff Development Law and the Regulations of Institute of Neurobiology-BAS (37 points with a minimum of 30).

Awards

Nenchovska has 2 awards for prominent young scientist, one-year fellowship for a young scientist from the World Federation of Scientists in Geneva and a Funded Participation in a Workshop from the European College of Neuropsychopharmacology in France.

I evaluate the PhD thesis of assistant Nenchovska as her personal work, without data on plagiarism, fully responding to the requirements and recommended scientometric criteria for awarding the educational and scientific degree "Doctor".

In conclusion, my assessment of the PhD thesis of Zlatina Petrova Nenchovska titled: "Study of the effects of the hormone melatonin on the behavioral and biochemical changes accompanying epileptogenesis in a kainate model of temporal epilepsy" is fully positive and with this review I express my deep conviction that the dissertation corresponds to the requirements for the award of the PhD degree in accordance to the Law of the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of the Academic Staff Development Law and the Regulations for the Conditions and the Procedure for Acquisition of Academic Degrees and Academic Position Occupation in INB-BAS.

On the bases of the provided data, I will vote positively to award the educational and scientific degree "Doctor" to Zlatina Petrova Nenchovska. I would like to recommend to the honorable members of the scientific jury to vote positively and to award Zlatina Petrova Nenchovska the educational and scientific degree "DOCTOR" in the 7th area "Health and Sport", Professional Field 7.1 "Medicine" in the scientific specialty "Pharmacology (including pharmacokinetics and chemotherapy)".

10 September, 2019
Sofia

Reviewer:
/prof. Iren Petkova Belcheva, MD, PhD, D.Sc. /