

## STANDPOINT

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Subject: Dissertation work aimed at public defense for the award of the PhD degree in higher education 4. Natural sciences, professional field 7.1. Medicine, scientific specialty "Pharmacology (including pharmacokinetics and chemotherapy)" by assistant professor Zlatina Petrova Nenchovska on "Study of the effects of the hormone melatonin on behavioral and biochemical changes accompanying epileptogenesis in a kainate model of temporal epilepsy" with scientific supervisor prof. Yana Chekalarova, PhD.

Assistant professor Zlatina Nenchovska holds the Bachelor's Degree in Biology and Master's in Animal and Human Physiology from Sofia University "St. Kliment Ohridski", respectively in 2009 and 2011. Since 2010 she started working at the Institute of Neurobiology of the Bulgarian Academy of Sciences. The PhD student is fluent in both English and German. She has very good computer skills, including basic office techniques and statistical programs.

The topic of the dissertation is devoted to the current problem related to the influence of behavioral and biochemical changes in a model of temporal epilepsy induced by the administration of kainic acid. Epilepsy is one of the most common chronic neurological diseases. Despite the large number of antiepileptic drugs currently available and the development of a new generation of anticonvulsants (eg Topiramate, Levetiracetam, Gabapentin, Rufinamide, Eslicarbazepine, Perampanel), seizure control is not achieved in 30% of patients with monotherapy. The results of both in vitro and in vivo studies show the antiepileptic activity of melatonin mediated by an antioxidant effect, an increase in GABA concentration, an affinity for GABA receptors, and a decrease in the excitatory effect of N-methyl-D-aspartate.

The dissertation is written on 132 pages, properly structured and well illustrated with 30 figures, 1 table, and 1 scheme.

The literature review is competently written and includes 10 sections. The written review shows that the PhD student is well aware of the problem presented in the dissertation related to the mechanisms of epileptogenesis, experimental models of epilepsy, and the functions of melatonin as hormone and drug. A significant bibliographical reference has been made, citing 256 literature sources. The considerable biographical information shows that PhD student is



thoroughly acquainted with the problems related to the dissertation work, including the latest studies in this field.

The purpose of the dissertation is precisely and clearly formulated - to study the role of melatonin on the frequency of spontaneous epileptiform activity and behavioral, biochemical and histological changes accompanying epileptogenesis, in an experimental model of temporal epilepsy caused by intraperitoneal administration of kainic acid in two breeds of rats - normotensive (Wistar) and spontaneously hypertensive. To accomplish this goal, five tasks have been identified that are fully consistent with the goal.

The experiments in this study were conducted on sexually mature male rats - normotensive (Wistar) and spontaneously hypertensive, according to the regulations for working with experimental animals in Bulgaria and the rules of the Ethics Committee at IBN, BAS. The experimental groups of animals are properly formed according to the tasks of the dissertation. The methodological approach is modern and sufficient to fulfill the set goals and tasks. Adequate experimental methods were used in the study: implantation of electrodes for EEG registration, model for inducing epileptic status by administration of kainic acid, EEG and video recordings, behavioral tests, histological examination, high performance liquid chromatography, biochemical and statistical methods.

The obtained own data are statistically processed, analyzed and clearly illustrated. The administration of melatonin for 8 weeks on rats with epileptic status induced by kainic acid was found to have protective effects on some of the adverse effects of behavioral epileptogenesis, including impulsive behavior, comorbid depression, and diminished spatial memory, of serotonin in the hippocampus and damage to neurons in the limbic structures. Melatonin exerts an anti-epileptogenic effect in hypertensive rats, reducing the frequency of spontaneous seizure attacks and after discontinuing treatment. Melatonin exhibits antidepressant activity in normotensive rats with a model of epilepsy, restoring the concentration of 5-hydroxytryptamine in the hippocampus to control levels.

The discussion shows the ability of assistant professor Zlatina Nenchevska to analyze the results obtained. In the course of the discussion, PhD student made a precise analysis of the results of the study, comparing its experimental results with those obtained by other authors. The seven conclusions drawn are real and fully consistent with the experimental results obtained. Listed are 5 contributions of a fundamental and applied nature.

In connection with dissertation, assistant professor Zlatina Nenchevska has attached a list of 5 publications, three of which have been published in IF journals (2 articles in *Epilepsy & Behavior*, 1 article in *Pharmacology Biochemistry & Behavior*). In 2 of publications she is the



first author. The publications presented reflect the most important results of the dissertation. The results of the dissertation of assistant professor Zlatina Nenchovska have been reported in 10 scientific forums. Five reports have been presented at international forums and 5 at national scientific forums.

I believe that the dissertation is a personal work of the PhD student. The results obtained and the contributions formulated are a personal credit.

The abstract of the dissertation is prepared in accordance with the requirements of the Rules of the INB - BAS for the application of ZRARB. The attached abstract reflects adequately the main content of the dissertation and the results obtained. It contains 71 pages, including summary in English (pages 63-71).

I have no critical comments on the dissertation presented.

## CONCLUSION

The dissertation contains scientific and applied results, which make an original contribution to science and meet all the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the implementation of the ZRASRB and the Rules of the BNB - BAS.

The dissertation shows that the PhD Zlatina Nenchovska holds profound theoretical knowledge and professional skills in the specialty of pharmacology, demonstrating qualities and skills for independent research.

Because of the above, I am convinced of my positive assessment of the research, presented by the dissertation reviewed above, dissertation abstract, achieved results and contributions, and propose to the venerable scientific jury to award the educational and scientific degree "PhD" of assistant professor Zlatina Petrova Nenchovska in doctoral program in Pharmacology (including pharmacokinetics and chemotherapy).

04.09.2019

Reviewer: ..........

Assoc. prof. Rumen Pavlov Nikolov, PhD