REVIEW

from Prof.Dr. Tzveteslava Vesselinova Ignatova-Ivanova,
Dean, Faculty Natural Sciences, University of Shoumen, Shoumen, Bulgaria

Ref.:

Dissertation project for granting educational and scientific degree "Doctor" with a PhD-thesis:
"Influence of subchronically applied ligands of cannabinoid receptors on learning and memory processes in rats with olfactory bulbectomy"

Author: Dr. Dobrinka Kalinova Doncheva, assist.prof., Dept. Physiology and pathophysiology, Faculty of Medicine, Medical University of Varna, registered doctorant in animal and human physiology, 4.3. Biological sciences,
Registered with Order P-109-428/16.07.2018, released with Order P-109-86/23.02.2022
Scientific advisor: Assoc.prof.Dr. Margarita Velikova, MD, PhD

Based on the Order of the Rector of the Medical University of Varna, Prof.Dr. Valentin Ignatov, P-109-86 from 23.02.2022, I have been appointed as a member of the Scientific Jury for a procedure of granting educational and scientific degree "Doctor" to Dr. Dobrinka Kalinova Doncheva, author of the dissertation paper "Influence of subchronically applied ligands of cannabinoid receptors on learning and memory processes in rats with olfactory bulbectomy".
After the decision of the Scientific Jury, taken during the preliminary meeting, performed online and via video link, based on the Order of the Rector of Varna Medical University, I was appointed as a reviewer on the submitted for official defense of the dissertation project.

Short personal details of the dissertation author:

Dr. Dobrinka Kalinova Doncheva was born on 13 April 1990.
She graduated Varna Medical University in 2017.
From September 2017 she was appointed as honorary assistant professor of physiology in the Dept. Physiology and pathophysiology, Varna Medical University. From March 2018, after a successful concourse, she was elected and employed as a regular assistant professor in the same Dept. From July 2018 she was registered in a regular doctorate (PhD-student) in the same Dept., with a scientific supervisor Assoc.prof.Dr. Margarita Velikova, MD, PhD.

Analysis of the submitted materials for my review:

The provided to me materials for a review on the dissertation project of Dr. Dobrinka Kalinova Doncheva contain:

-administrative orders for registration and release
-dissertation project
-autoreference
-biographic and professional details for the doctorate PhD-student
-administrative documents from the Dept. Physiology and pathophysiology, as well as from the Rectorate of Varna Medical University
-list and copies of publications and participations in scientific forums

-author's declaration
-certificates
-information card and verification papers for contribution acts
-corresponding protocols and proofs

The PhD-student provides 3 full-text publications, in 1 she is a first leading author, and in 2 she is a co-author
2 of the provided publications are with impact-factor, which adds certain bonus to the dissertation.
Dr. Dobrinka Doncheva has active participation in 7 scientific meetings, where she has presented her scientific and research attempts, 5 of them are at national forums, and 2 are at international forums abroad.
The dissertation project is worked out and presented on 146 standard printing pages, including: introduction, literature review, aim and tasks, material and methods, results and discussion, final analysis and conclusion, bibliography and applications.
The bibliography includes 418 cited publications.
The PhD-thesis has a total of 26 figures, tables, and applications.
The dissertation is worked out and structured by all required national standards, corresponds to the rules and principles for a PhD-thesis, according to the Law in Bulgaria, and is presented in a fully adequate, excellently prepared and documented variant of a proper dissertation.

A very impressive fact, which I noticed from the very beginning of my review on the dissertation and the autoreference, is that the PhD-thesis is worked out and completed by using a serious number of experimental animals, exclusive selection of materials and methods, exact approach to the realization of the performed tasks, which complexly and as a whole proves its correct and true actuality, as well as importance for the national biomedical science, a section of our science and research which strongly needs young and active scientists, like the author of this dissertation.

The direction and topic of the dissertation "Influence of subchronically applied ligands of cannabinoid receptors on learning and memory processes in rats with olfactory bulbectomy" is very well selected, it is associated to investigation of the role, evaluation and influence of the ligands of cannabinoid receptors on memory disorders, induced by olfactory bulbectomy (OBX). After OBX in the animals (in her PhD-thesis the author uses rats), a complex of disorders (behavioural, immune, endocrine, neurocellular, neurochemical, etc.) is developed, and they resemble those changes, characterized for human patients with depressive states, which, in turn, indicates the wish of the author to look for a possible association between animal and man, thus using a very promising research approach in her dissertation with animal model.
This is a very well-targeted scientific experiment, and in addition, especially the well-chosen topic of the research project tends to expressed actual, contemporary, modern and personal involvement of the PhD-student Dr. Dobrinka Doncheva, when working on her dissertation.
My complements to the author!

The preamble and the review analysis of the available literature

are very well presented and are completely adequate to the topic of the dissertation.

Dr. D.Doncheva accents rather correctively that the influence on the activity of the endocannabinoid system (ECS) tends to be a promising therapeutic target in terms of the depressive conditions and some neurodegenerative diseases, such as Alzheimer's disease. Indeed, during the recent decade the depressive disorders tend to be a certain social-important disease not only in our country, but world-wide. There are still numerous unrevealed questions for the link between cognitive and depressive symptoms, as well as determination of specific mechanisms, associated to the influence of the endocannabinoid system on cognitive and emotional processes in the context of depression, thus being the leading idea of the current dissertation attempt.

Dr. D.Doncheva accents on certain facts in her literature review, analyzing the necessity of obtaining many new data for the effects of manipulating of the ECS, and more precisely the role of the cannabinoid receptors, thus making possible the determination of their participation in the mechanisms of development of depression and Alzheimer's disease, but also to find a way to widen the potential therapeutic approach to treat successfully such disorders.

Very impressive is the fact that the PhD-student Dr. Dobrinka Doncheva includes in her literature analysis citations with actual characteristics, thus, a big number of the discussed literature sources are dated after 2007, with a number of most recent ones from 2018, 2019, 2020.

Over 400 bibliography sources, used by the PhD-student, plus their profound analysis, are a direct link to the leading theme and topic of the dissertation, which, in turn, convinces even the spontaneous reader to feel the impulse of the author to study the latest literature sources, to find the necessary associations from other investigations and to have her own opinion on the actuality of the planned parameters of the PhD-thesis. The broad spectrum of the discussed bibliography and its profound horizon in the main theme of the dissertation creates a very remarkable impression.

The link between the literature analysis and the practical part of the dissertation is obviously smooth, consistent and steady, especially based on the conclusions of the author, starting from her analytical analysis of the bibliography, through the aim and tasks of her dissertation, and continuing into materials and methods of her research.

These concrete directions of the PhD-thesis of Dr. D.Doncheva, based on her serious impact on the publications of other authors, lead to the necessity of revealing of the dissertation topic, which, I'll repeat again, is of serious actuality and definite importance for future investigations in cognitive and emotional processes.

Aim and tasks:

The **aim** of the present PhD project is to study the influence of subchronically applied CB1-ligands (agonist and antagonist of cannabinoid receptors) on learning and memory processes in rats with experimental model of olfactory bulbectomy (OBX).

The **tasks** are very well and adequately formulated, answering to the topic and theme of the dissertation, providing complete information. I am impressed by the exactness of the planned project in three main points for realization of the task and aim, when compared to other dissertations, where

the authors show much more unnecessary points of their tasks and aim. In this aspect, the PhD-student Dr. D.Doncheva manages to indicate clearly and precisely what is her aim and how to fulfil it with corresponding tasks.
Congratulations!

Materials and methods:

This is a very well prepared part of the dissertation with all required parameters of a modern PhD-thesis.
The experiments include 294 sex-matured male white rats, Wistar race, thus, the big number of the used experimental animals tends to considerably reliable study of the scientific idea and the achieved results.
The surgical procedures, the applied pharmacologic substances, the verification, the behavioural methods, the learning and memory of the settled experiments with the used animals, the statistic evaluation of the results – everything is worked out smoothly, complexly and with a special attention of the PhD-student, thus proving the quality of the dissertation theme.

Results and discussion:

Both sections of the dissertation are revealed very well and with exact directions, providing the accent of the author's work on the theme, indicating and proving the exactness of the selected topic, the way to achieve precisely the results and their profound analysis.
A serious working load of experimental study is done in several directions: influence of subchronically applied ligands of cannabinoid receptors on investigational behaviour and association with motion activity of rats with OBX-model (Opto Varimex apparatus), influence of subchronically applied ligands of cannabinoid receptors on learning and memory processes of rats with OBX-model, together with the CB-ligand influence on motion activity, as well as the examination and influence on the explorative behavior of the animals, set in a new environment.
The PhD-student Dr. D.Doncheva accents that the habituation to a new environment can be determined as fading with time of the orientation reflex towards repeating indifferent influence. The habituation is considered to be one of most elementary forms of learning, where the decreased level of learning, as a function to multiple exposure within the environment, can be accepted as a memory index.
The PhD-study includes the influence of HU-210 (CB-agonist) and Rimonabant SR-141716A (CB1-antagonist) in series of 7- and 14-day i.c.v.-application or intragastral application of HU-210 and Rimonabant SR-141716A (from 1-st to 5-th minute) on the explorative behavior and associated with it motion activity of Sham- and OBX-rats, as well as the application of Rimonabant from 14-th to 28-th day after bulbectomy, at the background of developed depressive-like state.
The choice of the PhD-student to select the olfactory bulbectomy OBX as a validated model of depression, which is used as a method for investigation of the activity of new substances with antidepressive and cognitive-improving function influence, also to study the mechanisms of the depressive disorders and Alzheimer's disease, is rather positive, because the OBX recently is more and

more famous as a model to investigate Alzheimer's disease, which tends to the development of neurodegenerative changes of the brain and the resulting heavy memory damages.
This is based at the background of a number of literature data regarding the memory disorders in persons using cannabis, the memory-damaged effects of cannabinoid agonists and the improving memory effects of cannabinoid antagonists.
The basic aim of the present study of Dr. D.Doncheva, by using different methods of application (i.c.v. and intragastral), in conditions of subchronic experiment (7 and 14 days), to evaluate the effects of the ligands of cannabinoid CB1-receptors on learning and memory processes of rats with OBX-model, is fulfilled and completed in full volume and I congratulate the dissertant for her utmost efforts to achieve such excellent results with her PhD-project.
There are enough proofs, indicating that the ECS of mammals plays certain role for learning and memory, whereas the investigation of the influence of subchronically applied ligands of cannabinoid receptors in rats with a model of olfactory bulbectomy, contributes to the disclosure of the CB-receptor's role for development of memory deficits, accompanying the experimental model of the dissertant.
I am rather impressed by the accent of Dr. D.Doncheva on the role of her research study: the dissertation work provides additional data to clear out the relation between the cognitive and depressive symptoms, also the influence of ECS on cognitive and emotional processes in diseases, accompanied by a cognitive effect, such as depression, Alzheimer's disease.
The pharmacologic treatment of such contingent of patients is still not sufficient. New investigations, new drugs and new innovative strategies for new medicines are necessary to reveal and prove which specific therapy would be of help.
"Our modest experience to study the role of cannabinoid receptors in learning and memory processes, specifically in memory deficits, is a slight step forward in our knowledge of cognitive functions of the brain" (words of Dr. D.Doncheva).

Conclusions and contributions:

I am very pleased with the statements of the PhD-student in terms of her conclusions and contributions, because this part of her dissertation reflects the overall load of Dr. Doncheva's project and the impression of well-done work is rather realistic.
The **conclusions** are listed in three main points, throwing light on the achieved results of the PhD-study.
1. The modulation of the activity of cannabinoid receptors exerts considerable influence on learning and memory deficits, accompanying the model of olfactory bulbectomy (OBX).
2. The time interval is important for demonstration of the effects of Rimonabant on learning and memory processes in OBX-model.
3. The influence of the ligands of CB-receptors on the rat's behaviour in avoidance tests depends on the experimental background.

The **contributions** are listed in two groups:

-With confirming character:

1.The received data indicate that the cannabinoid receptors participate in learning and memory processes, as well as in development of depressive-like state of rats with OBX-model, 2. It is established that the CB1-antagonist (SR-141716A) shows a tendency to deepen the depressive-like state, and the way of its application is important for performance of the effects on behavioural reactions of the animals.

-With original character:

1. It is found that the ligands of the cannabinoid receptors exert various influence on learning and memory in Sham-operated and OBX-rats, 2. It is established that the activation of cannabinoid receptors has antidepressive effect with OBX-model, 3. The received data for intragastral application and improvement of CB1-antagonist indicate that the learning and memory processes with OBX-model are improved, 4. It is established that the time interval to apply the CB1-antagonist is associated to the behavioural deficits in the OBX-model, 5. The results contribute to the additional explanation of the role of cannabinoid receptors in learning and memory processes, as well as memory deficits accompanying neurologic and psychic diseases.

Autoreference:

The presented autoreference includes 54 standard pages and is very well structured and edited. The autoreference, as provided to me, answers all required by the Law specifications, and is written in a very good scientific language, representing in summary the entire idea of the dissertation.

My role, as a reviewer of the PhD-thesis of Dr. Dobrinka Doncheva, allows me to express my full satisfaction of her tremendous scientific project, and I would like to accent, that with some additional materials, slightly modified methods, new investigation and analysis, this dissertation could, for sure, be directed to a defense of the next scientific degree Doctor of Biological Sciences.

As part of the publication and presentative activity of the PhD-student, I would like to pay a special attention to the fact that the presented by Dr. Dobrinka Doncheva poster with a title „*Effects of Rimonabant on Locomotor and Exploratoty Acytivities in Olfactory Bulbectomized Rats*" has been awarded with a **Supportive First Award** for a young scientist, based on the decision of the international committee SEEC-IMAB during the forum 11-th South-East European Conference on Infections and Cancer, together with 31-st Annual Assembly of International Medical Association Bulgaria, 28-31 October 2021, Plovdiv, Bulgaria.

This Annual Award of SEEC-IMAB has a tradition from 2010 until now, and it is worth mentioning that this united scientific forum is one of the most prestigious for the South-East European region and has a very high rating of international scientific-practical meeting.

I declare this fact, because I myself participate regularly at this unique annual assembly-conference and I am fully aware of the level and quality of the presentations, included in the Scientific Programme.

This Award is a compliment for the PhD-student Dr. Dobrinka Doncheva for her success as a young scientist and participant, who dares present her scientific production in front of a respectful international scientific jury of the united scientific forum of both organizations SEEC and IMAB.

Conclusion:

Based on the provided to me materials for a review on the dissertation of the PhD-student Dr. Dobrinka Kalinova Doncheva, PhD-student at the Dept. Physiology and pathophysiology, Faculty of Medicine, Varna Medical University, hereby, upon my expertise and solemnly declared opinion, I confirm my positive statement on the quality and indisputable necessity of this dissertation ***"Influence of subchronically applied ligands of cannabinoid receptors on learning and memory processes in rats with olfactory bulbectomy",*** for the national medico-biological science.

I dare request the esteemed members of the Scientific Jury to give their positive vote for granting the educational and scientific degree "Doctor" to Dr. D.Doncheva, in the way I shall do.

Reviewer:

Prof.Dr. Tzveteslava Vesselinova Ignatova-Ivanova, 10 March 2022