

ACADEMIC REFERENCE LETTER

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In reference to the dissertation work on the topic "Effects of the subchronic administration of ligands of cannabinoid receptors on learning and memory processes of olfactory bulbectomized rats" for awarding the educational and scientific degree "PhD"/ "Doctor" to Dobrinka Kalinova Doncheva, assistant professor at the Department of Physiology and Pathophysiology, ESS in Physiology, Faculty of Medicine, Medical University, "Prof. Dr. Paraskev Stoyanov", Varna. She is a PhD student in the scientific speciality "Physiology of Animals and Man", professional field 4.3. Biological studies, admitted to the program with the Order № P-109-428 as of 16.07.2018, completed the program with Order № P-109-86 as of 23.02.2022.

This academic reference letter was written according to the Order of the rector of the Medical University, "Prof. Dr. Paraskev Stoyanov", Varna, № P-109-86/ 23.02.2022 and Protocol №1/ 25.02.2022 taken at the preliminary meeting of the Scientific jury.

1. Evaluation of the topicality of the dissertation.

The dissertation work "Effects of the subchronic administration of ligands of cannabinoid receptors on learning and memory processes of olfactory bulbectomized rats" carries out research on one of the important and particularly topical aspects of the effect of the endocannabinoid signaling system (ECS) in the regulation of brain functions and more specifically the role of endocannabinoids (EC) in the processes related to learning and memory. In contrast to the other neurotransmitters, which have been known for a long time and their effects thoroughly studied, EC have been unknown till the beginning of the 90ies of the previous century. It was during the last 2, 3 decades that some scientific evidence has been accumulated on the neurotransmitter and neuromodulatory role of EC and their participation in the regulation of different physiological, learning and memory processes inclusive.

As has been properly emphasized by the doctoral student, the research aimed at revealing the role of the endocannabinoid signaling system (ECS) in the cognitive functions shows contradictory results. The pharmacological ligands (agonists and antagonists) used that act through cannabinoid receptors (CB1 and CB2) are able to either harm or improve different stages of learning and memory formation (acquisition, consolidation, retrieval). That is why the research work presented in this dissertation in relation to the effects of the subchronic administration of ligands of cannabinoid CB receptors on memory deficits in the experimental model of olfactory bulbectomized rats (OBX), supplements the scientific knowledge on this subject and makes this dissertation especially topical with possibilities for further research.

On the basis of the profound analysis of the existing data in the scientific literature carried out by the doctoral student, it is obvious that the changed ECS activity accompanies a number of psychiatric (depression, anxiety, schizophrenia) and neurodegenerative (Alzheimer's and Parkinson's) diseases. It is a well-known fact that during the last decade depressive disorders have become diseases of social importance on a worldwide scale. In this context there still exist a number of unsolved problems in

relation to the connection between the cognitive and depressive symptoms and the differentiation of the specific mechanisms related to the effect of ECS on the cognitive and emotional processes, which happen to be the main idea of the dissertation presented.

2. Evaluation of the results.

The very precisely formulated aims and objectives by the PhD student that fully correspond to the topic of the dissertation are definitely important for the evaluation of the results achieved. The large number of experimental animals - 294 sexually mature white male Wistar rats, confirms the reliability of the results. The pharmacological ligands used HU-210 (CB-agonist) and Rimonabant, SR-141716A (CB1-antagonist) are applied following a scheme confirmed in the experimental pharmacology. This scheme allows for precise information about the effects to be obtained.

The results presented reflect the effects of HU-210 and SR-141716A, Rimonabant in series of 7- and 14-day i.c.v. administration, as well as of Rimonabant (SR-141716A), administered intragastrically in the course of 14 days (before, immediately after and 14 days after olfactory bulbectomy (OBX) when a depressive like disorder has already been developed) on the exploratory behavior and the related motor activity of rats with OBX model of depression and of Sham-operated rats. The PhD student's choice to study the bilateral OBX as a broadly used model of depression is very appropriate since this model allows for a pharmacological testing of new types of depressants to be carried out on the one hand and for the mechanisms of a depressive state, neurodegeneration and the cognitive disorders related to them to be explored on the other hand. Lately OBX is also used as a model of Alzheimer's disease for assessment and evaluation of the therapeutic effect of new substances in normalization of memory deficit.

The results obtained are significant, sufficiently informative and fully correspond to the aims and objectives set. They show that the exploration of the effects of subchronic administration of ligands of cannabinoid receptors of OBX model rats contributes to revealing the role of CB receptors in the development of memory deficits that tend to accompany this model. Comparing the effects of OBX and Sham-operated rats allows for evaluating the physiological importance of the CB receptors for the cognitive processes in the organism. Since the existing data in the literature about the modulatory action of ECS in relation to the mechanisms of learning and memory are rather divergent, the results obtained bring additional information about this subject matter. The results of the research support the data that prove the participation of ECS in the development of memory deficit in OBX model rats. CB-receptor agonist HU-210, induced by subchronic administration, i.c.v., produces an antidepressant-like effect, it normalizes the disorders in the exploratory behavior and the motor activity and improves the learning and memory of OBX model rats while CB1-selective antagonist (SR-141716A) induced by subchronic administration (i.c.v. or intragastrically), tends to aggravate the motor skills disorders of the OBX model rats. Divergent effects have also been demonstrated depending on the route of administration. The importance of the time interval between the application and the response produced by the intragastrically administered CB1-antagonist on the memory deficit in OBX rats, has also been emphasized.

The results have been presented in three publications. The PhD student is the first author in one of them while the other two publications have an impact factor.

3. Evaluation of the contributions.

Dr Doncheva's results presented in her dissertation and the conclusions drawn doubtlessly make an important scientific contribution. Two of her contributions basically confirm previous findings in the field, and five of them are original and are of a potential clinical importance. I accept her contributions and conclusions without any critical remarks.

4. Critical remarks and recommendations.

I think that the subject of this dissertation has been thoroughly explored and I do not have any substantial critical comments or recommendations to make.

5. Conclusion

The dissertation presented "Effects of the subchronic administration of ligands of cannabinoid receptors on learning and memory processes of olfactory bulbectomized rats" written by the PhD student Dr Dobrinka Kalinova Doncheva, contains both scientific and scientifically applicable results which constitute an original contribution to the clarification of the potential participation of the cannabinoid receptors in the mechanisms of memory disorders and the possibilities for pharmacological modulation of their activity while treating depression and neurodegenerative diseases accompanied by a cognitive deficit.

The PhD student has the necessary number of publications on the topic and she has participated in seven scientific forums, two of which were held abroad. I think that the requirements of the Law for the Development of Academic Staff in the Republic of Bulgaria and the Regulations on Academic Staff Development at Medical University, "Prof. Dr. Paraskev Stoyanov", Varna have been met. Therefore I am convinced that this dissertation deserves a positive evaluation and I recommend it to the honorable members of the scientific jury so that they support it with their positive vote for awarding the educational and scientific degree PhD to the doctoral student Dr Dobrinka Kalinova Doncheva, assistant professor at the Department of Physiology and Pathophysiology, ESS /education and science sector/ in Physiology, Faculty of Medicine, Medical University, "Prof. Dr. Paraskev Stoyanov", Varna and a PhD student in the scientific speciality "Physiology of Animals and Man".

21.03.2022
Varna

Reviewer: Associate prof. Dr P. Nikolova, MD, PhD

