



To the President of the Scientific Commission
nominated by Order № ПД-15-27 - 19.03.2021
of the Director of the IEMPAM- BAS

OPINION

For dissertation entitled 'Biochemical bases of Alzheimer disease' of prof. Lyudmil Peniov Kirazov for scientific degree 'Doctor of Sciences' in 'Biochemistry' (01.06.10) in the field of 4.3. Biological Sciences.

Reviewer: Prof. Stefan Vacev Panaiotov, PhD, DSc

Scientific speciality: Microbiology

Institution: NCIPD - Sofia

I declare that I have no conflict of interests within the meaning of art. 4, par. 5 from the Law for Development of the Academic Staff in Bulgaria. I have no common publications with prof. Lyudmil Kirazov.

This OPINION is prepared according to the requirements of the Law for the Development of the Academic Staff in Bulgaria, and according to the internal Regulation for conditions and procedures for obtaining the scientific degree 'Doctor of Sciences' of Institute of Experimental Morphology, Pathology and Anthropology with Museum (IEMPAM-BAS).

I. Analysis of candidate's career profile.

Lyudmil Kirazov studied in Germany and graduated with a MS degree in biochemistry and a PhD. He speaks fluently German and English languages. His career development passes in several institutes of the Bulgarian Academy of Sciences. He was elected Professor in Biochemistry (June 1, 2010) at IEMPAM-BAS. His scientific metric data show upward and gradual development over the years. He passed several specializations in Germany related to productive long-term cooperation. His publications are in prominent international and national journals. His results were presented at national and international scientific forums. His works obtained over 450 citations, Hirsch index = 9 with a total IF 43.32. From 2017 to 2021 he took part as a participant and Chairman of several commissions of the National Science Fund at the Ministry of Education and Science. He has been a member of the Scientific Board of IEMPAM since 2010.

II. General description of the dissertation for 'Doctor of Sciences'

Prof. Kirazov presents:

- Dissertation written on 280 pages, which contains the standard sections. The results and their discussion are presented on 150 pages and occupy the largest part of the dissertation. The description includes 9 tables and 86 figures. The research in the dissertation is focused on Alzheimer's disease (AD), which is a current social, health and economic problem. The dissertation overview is concise and well ordered. The incidence of Alzheimer disease increases with age. The increasing trend of the average age of the population worldwide leads to annual increase of patients number. The dissertation describes the amyloid precursor protein (APP), its processing, amyloid beta peptide and its forms ($A\beta$), τ -protein, as well as the role of these components in healthy individuals and in AD. The potential factors for the development of AD and the applied model systems for the study of the disease are described. Special attention is paid to the complex pathology of AD, which is the main reason for the lack of effective treatment. Data from 700 literature sources were used, which is a reflection of the timeliness of the topic.

The description of the results in the dissertation begins with a comparison of the main methods for protein measurement. The Bradford method was found to give lower values for protein content in fractions containing biological membranes, than the Lowry method. Also, the comparative analyzes show a change in the values depending on the duration of storage of the samples at -20°C . The publication describing the study obtained a significant number of citations > 50 citations in Scopus and 90 in Google Scholar.

Various models have been applied to study the involvement of different transmitter systems and signaling molecules. The involvement of vascular endothelial growth factor (VEGF) in amyloid precursor protein (APP) metabolism has been demonstrated using primary astrocyte cultures from transgenic Tg2576 mice and cultured brain sections from the same animals. Vascular endothelial growth factor has been shown to modulate amyloidogenesis. With the help of native brain sections, i.e. under conditions much closer to those in the brain compared to cell cultures, original studies have been conducted on the role of the neurotransmitter glutamate in the processing of amyloid precursor protein. It has been found that there is a relationship between glutamate concentration and the secretion of amyloid precursor protein. The involvement of different types of glutamate receptors has been studied and it has been shown that their stimulation has a different effect on this process, which is relevant for the development of AD. The secreted amyloid

precursor protein was found to have no feedback to secretion and to undergo additional calcium-dependent degradation. The role of interleukin-1 β has been investigated using a cholinergically differentiated cell line (SH-SY5Y) and has been shown to contribute to cholinergic deficits, most likely by inhibition of transcription factors.

Models with immunolesion of cholinergic neurons in the basal ganglia of the forebrain have been used. In a lesion performed in wild-type mice, it was shown for the first time in vivo that APP metabolism in the cortex is below afferent innervation. It is very convincing to overcome the induced cholinergic hyperactivity by transplanting cells that produce nerve growth factor. The same lesion was administered to transgenic Tg2576 mice, resulting in a much better in-vivo model with advanced amyloid pathology.

To study the metabolism of APP, a model of an isolated synaptosomal fraction has been developed, which provides clearer responses to stimulation with various agents. A detailed and large-scale study of changes in APP expression, at the protein level and at the RNA level, during ontogenesis from embryonic stages to adult rats was performed. Changes in protein levels were observed in homogenate and for the first time in growth cones and synaptosomes. In-situ hybridization of sections from embryos and brains was used for RNA quantitative studies. A comparison of APP levels in the brain and peripheral organs during development was also made. The main role of APP695 in the brain is shown and conclusions are made about its importance in development.

For the first time, the effect of amyloid beta peptide (A β) on the electrical activity of neurons was demonstrated with an original technique, including neuronal cells cultured on a network of microelectrodes. A β affects electrical activity rapidly, concentration-dependently and reversibly. It has been shown that the effect is not due to oxidative stress, but most likely to stimulation of inhibitory receptors. Differences in the effect of different forms of A β (A β 25-35; A β 1-40; A β 1-42) were found. It has also been shown that inhibition is caused by monomeric forms of A β rather than large aggregates. For the first time, a comparative study of the synaptosomal transcript in fractions obtained from young and adult mice was performed. The accumulation of a large number of long intervening non-coding RNAs as well as circular RNAs has been found in adult synaptosomes. Conclusions are made about the significance of this fact. The effect of lead on APP secretion was studied and an original hypothesis about the mechanism of lead toxicity on neuronal cells was proposed.

III. Evaluation of the candidate's scientific works linked to the dissertation work.

Prof. Kirazov applies with the dissertation:

A total of 62 scientific publications, of which:

- 39 in Bulgarian scientific journals,
- 21 in foreign journals,
- 2 in popular science journals,
- 27 in journals with impact factor
- 63 participations in scientific forums, three of the abstracts were published in journals with impact factor
- Participation in 3 national research projects, one of which as Project Leader.

The attached publications are related to the aims and results of the dissertation.

V. Reflection (citations) of the candidate's publications in the national and international literature (publication image).

All publications, citations and participations in research projects reflect the active participation and significant contribution of Prof. Kirazov to the development, implementation and promotion of the dissertation. The number of 243 citations in Scopus and 220 citations outside the Scopus system, Hirsch index - 9 and the total impact factor of 43.32 make the dissertation sufficiently visible among the scientific community in the country and abroad.

VI. Overall, qualitative assessment of candidate's research

Over the years, Prof. Kirazov planned, developed and published results of the dissertation. The results of the dissertation have a significant contribution to the study of Alzheimer's disease. The dissertation conducts research, which has a significant contribution to his personal career development.

VII. Critical remarks and recommendations.

I have no remarks or recommendations to the candidate. All documents are prepared according to the requirements.

VIII. A general assesment of the candidate's compliance with the mandatory rules and the mandatory quantitative criteria and scientific indicators according to the Law for the Development of the Scientific Staff in Bulgaria.

The documents submitted by the candidate cover all criteria according to the Law for the Development of the Scientific Staff in Bulgaria for a dissertation for the scientific degree "Doctor of Sciences". According to the requirements of IEMPAM and the Law for the Development of the Scientific Staff in Bulgaria for obtaining a scientific degree "Doctor of Sciences" Prof. Kirazov collects 652 points, which significantly exceed the threshold of 350 points. The dissertation of Prof. Lyudmil Kirazov meets the mandatory and specific conditions and scientific criteria for the degree of "Doctor of Science".

Conclusion: I confidently give my **positive assessment** and recommend to the Scientific Jury to award with the scientific degree "Doctor of Science" in specialty "Biochemistry" (01.06.10) from Professional field 4.3 "Biological Sciences" prof. Dr. Lyudmil Kirazov.



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