REVIEW OF THESIS

by Prof. Dr. Dimitar Kadiysky, MD,Ph.D, DSci

Institute of Experimental Morphology, Pathology and Anthropology with Museum (IEMPAM) at Bulgarian Academy of Sciences (BAS), Sofia,

of dissertation thesis for awarding the academic scientific degree Doctor of Sciences (01.06.10 Biochemistry, Professional field 4.3. Biological Sciences).

Author: Prof. Ludmil Penuv Kirazov, Ph.D.

Topic of the thesis:

BIOCHEMICAL BASIS OF ALZHEIMER'S DISEASE

1. General presentation of the author, the procedure and the materials.

The set of materials presented to me on paper and electronic media by Prof. Ludmil Kirazov includes: 1. Application form for admission to defense 2. Professional CV on European model 3. Copy of a document for the acquired educational and scientific degree "Doctor" 4. Dissertation work 5. Project for abstract (in one paper copy and 8 electronic copies) 6. List of publications related to the topic of the dissertation 7. Copy of the publications included in the dissertation 8. List of scientific events where are presented results from the dissertation 9. List of citations of the publications included in the dissertation 10. Reference for the scientific contributions 11. Reference for fulfillment of the minimum requirements of IEMPAM for obtaining the scientific degree “Doctor of Sciences”. There are all the required documents for conducting a defense of a doctoral thesis for obtaining the scientific degree "Doctor of Sciences".

The reference for fulfillment of the minimum national requirements shows that the author's scientometric indicators, beyond the included volume in his previous registrations in *NACID*, are completely sufficient according to the requirements of *Academic Staff Development Act* and the *Regulations* for opening a procedure for defense of dissertation-thesis for awarding the academic degree "Doctor of Sciences".

Prof. Ludmil Kirazov graduated in 1981 with a degree in Biochemistry from the University of Leipzig, Germany, and in 1982 defended his dissertation in the field of neurobiochemistry at the Paul Flexig Institute for Brain Research, Leipzig. From 1984 to 1988 he was a doctoral student in neurobiochemistry and defended his doctoral dissertation at the Paul Flexig Institute for Brain Research, Leipzig.

After this period, Prof. Kirazov consistently realized his academic and career growth from ordinary biologist, through research associate, to associate professor and professor in the field of neurobiology and biochemistry respectively. In the period 1994-1998 Prof. Kirazov was a post-doctoral student at the Paul Flexig Institute for Brain Research in Germany. In Bulgaria, the research activity of Prof. Ludmil Kirazov finds its place in the Central Laboratory of Regeneration, the Institute of Cell Biology and Morphology, the Institute of Experimental

Morphology and Anthropology and the Institute of Experimental Morphology, Pathology and Anthropology with Museum (IEMPAM) at BAS. At the moment Prof. Kirazov continues his research activity as an experienced researcher in the Department of Experimental Morphology of IEMPAM in the field of neurobiology, biochemistry and neuromorphology, again in collaboration with neurobiologists from Germany.

In general, the author's activity is concentrated in organizing and conducting research aimed at revealing intimate biochemical mechanisms directly or indirectly related to the etiology and pathogenesis of Alzheimer's disease, as well as in developing and promoting modern research methods for the benefit of biomedical research. .

Prof. Kirazov is a holder of grants, scholarships and numerous collaborations with participations in international projects, mainly with prominent neurobiologists in major research centers in Germany.

Prof. Kirazov's thesis is presented on 279 pages and is illustrated with 86 figures and 9 tables, including diagrams, graphics and photomicrographs. 697 literature sources are cited in these thesis.

2. Relevance of the topic of the thesis.

The prolongation of human life expectancy in recent centuries and the corresponding increase in the number of senile populations in the developed part of the world is a new process in the history of the human species. Contrary to expectations, this process intensifies some negative health consequences for the individual based on the growth of a wide range of age-specific pathological changes, which inevitably occur in this case. The research included in the thesis is focused on Alzheimer's disease (AD), which is a current social, including economic problem and its prevalence is increasing as a result of the above mentioned reasons. Clarifying the pathogenesis of Alzheimer's disease and possibly preventing its occurrence is a promising strategy for achieving better health in old age for society. Unfortunately, the unclear etiology and unspecified features of irreversible chronic neurodegenerative brain damage in Alzheimer's disease significantly thwart efforts to prevent it and to create potential therapeutic approaches. All this emphasizes the exceptional relevance to date of the content of the presented work.

3. Literary awareness of the author.

The literature review includes a complete historical reference of the disease and defines the structure of the degenerative processes in the central nervous system (CNS) in Alzheimer's disease known from the point of view of medicine and morphology. The review emphasizes the importance of amyloid precursor protein, the role of secretory precursor protein, and amyloid β peptide in the body. Their physiological and neurotoxic role is presented in the conditions of the functioning of the CNS. From neurobiological, and incl. a morphological point of view, Prof. Kirazov summarizes the processes of cerebrovascular deposits of amyloid β peptide, the role of microglia and the loss of synapses in the course of Alzheimer's disease. The author correlates the changes of certain biochemical parameters with the clinical data in the stages of Alzheimer's disease. Special attention is paid in the literature review to the existence of genetic causes of

Alzheimer's disease, as well as to the role of inflammatory processes and oxidative stress. An overview of the attempts for experimental modeling of Alzheimer's disease in order to create successful therapeutic approaches.

By reviewing existing theories and hypotheses in the literature, Prof. Ludmil Kirazov examines the causes of biochemical restructuring in the brain, leading to neurodegenerative changes, the formation of plaque composition and the resulting deformations in brain morphology, leading to mortality.

In the review part of the paper a separate section is devoted to the transformation of a complex of biochemically active components of the CNS, leading to the classical neurodegenerative view of brain morphology. The structure of the nucleus of senile plaques, as a major pathognomonic phenomenon for Alzheimer's disease, is mainly a concentration of aggregated amyloid β peptide (Aβ). This peptide is the important suspected thing in the destruction and damage of neurons observed in Alzheimer's disease, and it was later understood that amyloid β peptide (Aβ) is part of a large precursor protein - amyloid precursor protein (APP). This understanding in modern neurobiology has opened a huge field for research on the biochemical basis of Alzheimer's disease, on the basis of which are the numerous research approaches of Prof. Kirazov, reflected in the scientific work.

4. Construction and scope of the thesis. Scientific approaches, presentation of results.

The aim of the thesis is quite specific **- to establish basic biochemical approaches for scientific screening of the etiology and pathogenesis of Alzheimer's disease**. The clear formulation of the goal on the basis of the extensive review of the problem in the review part is a pledge for the creation of a completed work with a fundamental and at the same time scientifically applied character, essential for the respective branch of medicine.

To achieve this goal, Prof. Kirazov has included in his dissertation the results of a large volume of modern and classical biochemical, histological, immunohistochemical, biotechnological, morphological, statistical and many other new modern methods, such as *in situ* hybridization. Cell culture, including of neuronal populations, was used as the basic technique for most of the experiments. The results are presented concisely and clearly.

The logic of sequence of ordering the results in the dissertation work strictly follows the trend of scientific research stated in the title and in terms of composition. Prof. Kirazov has achieved a smooth and understandable arrangement of the material. On the contrary, the title of a scientific paper is an exact derivative of the content.

At the beginning, a key result of a sensitivity comparison of the main methods for determining protein is presented. This widely used in science methods have been used repeatedly to evaluate the results of specific biochemical approaches in the author's research. The finding that the *Bradford method* gives lower results for the protein content in fractions containing biological membranes compared to the *Lowry method* was taken into account in the evaluation of all further results included in the dissertation. The unexpected fact established by Prof. Kirazov and team for the existence of fluctuations in the data on protein content depending on the duration of

storage of samples at -20 ° C is important for the final results and this was taken into account by the author in a number of experiments. Bringing to the forefront in the dissertation of this result is a real preliminary request for accurate screening of the biochemical reliability of everything that the author presents to us below. The importance of establishing this scientific fact is evidenced by its assessment among the international scientific community - it has been cited more than 50 times in Scopus and 90 in Google Scholar.

An important part of the work is the presentation of results for different transmitter systems and signaling molecules, and here the author introduces various experimental models. For example, to demonstrate the involvement of vascular endothelial growth factor (VEGF) in amyloid precursor protein (APP) metabolism, experiments were performed on primary astrocyte cultures from transgenic Tg2576 mice and cultured brain sections from the same animals. As a result, VEGF has been shown to modulate amyloidogenesis.

In the model with native brain sections - in conditions much closer to those in vivo in the brain compared to cell culture, original studies were performed on the role of the neurotransmitter glutamate in the processing of amyloid precursor protein. The author's results show that glutamate affects the concentration-dependent secretion of amyloid precursor protein. Evidence in these studies that stimulation of different types of glutamate receptors has a different effect on the process is of great importance for establishing the intimate mechanisms of the pathogenesis of Alzheimer's disease. In this experimental setting, it was also found that the secreted amyloid precursor protein has no feedback to the secretion and undergoes additional calcium-dependent degradation.

The role of interleukin-1β has been investigated using a cholinergically differentiated cell line (SH-SY5Y) and has been shown to contribute to cholinergic deficiencies, most likely by inhibition of transcription factors.

In another experimental setting, immunolesia of cholinergic neurons in the basal ganglia of the forebrain was modeled. In the lesion performed in mice, it was shown for the first time *in vivo* that the metabolism of amyloid precursor protein (APP) in the cortex is below afferent innervation. The demonstration of overcoming the induced cholinergic hypoactivity by grafting nerve growth factor-producing cells is very convincing. When immunolesia is administered to transgenic Tg2576 mice, optimal in vivo modeling of advanced amyloid pathology is achieved.

To study the metabolism of amyloid precursor protein, a model for the study of an isolated synaptosomal fraction has been promoted and validated, which provides clearer responses to stimulation with various agents.

In general, the dissertation is dominated by the results of a detailed and large-scale study of changes in amyloid precursor protein expression during ontogenesis from embryonic stages to adult rats, both at the protein and RNA levels. Changes in protein levels were observed in homogenate and for the first time in growth cones and synaptosomes. *In situ* hybridization of embryo and brain sections was used for RNA level studies during the same stages of development. A comparison of the content of amyloid precursor protein in the brain and

peripheral organs during development was made. The main role of the amyloid precursor protein APP695 in the brain is shown and conclusions are made about its importance in development.

With extremely original and accurate technology - culturing neurons on a network of microelectrodes - for the first time the effect of amyloid beta peptide (Aβ) on the electrical activity of neurons was directly demonstrated. The amyloid beta peptide Aβ affects the 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 the amyloid beta peptide Aβ (Aβ25-35; Aβ1-40; Aβ1-42) were found. Inhibition has also been shown to be caused by monomeric forms of the amyloid beta peptide (Aβ) rather than large aggregates.

The results of an original comparative study of the synaptosomal transcript in fractions obtained from young and adult mice were considered in terms of their importance for the biochemistry of Alzheimer's disease. Thus, in the synaptosomes of adult animals, the accumulation of a large number of strands of long intervening non-coding RNAs as well as circular RNAs was found.

The analysis concerning the effect of lead on the secretion of the amyloid precursor protein APP allowed the author to propose an original hypothesis about the mechanism of lead toxicity on neuronal cells.

Finally, it is worth noting that the discussion of the numerous scientific data in the presented dissertation goes hand in hand with the presentation of the results, which greatly facilitates its consideration.

5. Conclusions, contributions, publications and impact on the scientific community

Twelve strictly formed conclusions related to the results presented in the separate chapters of the dissertation summarize significant neurobiological, biochemical and methodological findings, the result of the research work of Prof. Kirazov for a long time.

The report on the contributions of the dissertation shows 10 original scientific achievements and two others separately, defined by the author as scientific-applied contributions, but in fact they represent important original methodological results, highly valued in the scientific community.

The overall research activity of the author related to the topic of the dissertation is reflected in 45 scientific papers in various forums and is the subject of presentation and discussion in 31 scientific papers of Prof. Kirazov, published in important publications such as *Brain Res., Eur. J. Neurosci., Int. J. Dev. Neurosci., Neuroscienc Neurobiol., Aging,* et al. The total impact factor of the lied to dissertation publications is 30.23.

The citations found by Prof. Kirazov abroad (in publications, dissertations, books and patents) of works related to his dissertation are 425. The citations of the dissertation publications reported in Scopus are 257.

6. Critical remarks and recommendations.

I have not noticed any incompleteness in terms of structure, facts and results. The volume, structure and content of a dissertation in all cases are largely a function of the judgment and decision of the author himself. Some of the terminological discrepancies noticed by me in the initial dissertation version, due to an as yet unidentified Bulgarian translation of nosological units, names and terms from foreign languages, after a personal conversation with the author most of these discrepancies are corrected in the text. It should be borne in mind, however, that this problem often arises in the specialized scientific literature.

7. The presented abstract (in a volume of 95 pages) fully reflects the results and is extremely well illustrated with graphics, diagrams, color and black and white micrographs. It gives an accurate picture of the content of the dissertation. Its volume and design meet the accepted requirements according to the regulations and *Academic Staff Development Act*.

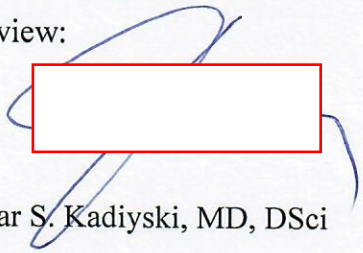
8. Conclusion.

The presented dissertation provides modern data specifying the subtle biochemical mechanisms of neurodegenerative processes in brain pathology, addressing both current scientific and medical problems. The author has invested his in-depth theoretical knowledge and professional skills in the field of neurobiology during his research work in his academic and career growth. Results have been obtained that have helped to compile a qualitative scientific paper highlighting the basic biochemical features of the etiology and pathogenesis of Alzheimer's disease. The scientific and scientific-applied results synthesized in the conclusions of the dissertation represent an original contribution to science. The work has been developed competently, and the maximum use of research opportunities in leading European universities and institutes also contributes to the quality of the work.

In connection with the above, I confidently give my positive assessment of the work and invite the esteemed scientific jury to vote positively regarding the qualities of the previous dissertation.

To the author - Prof. Lyudmil Kirazov – I propose to be awarded the scientific degree Doctor of Sciences in the specialty Biochemistry – (01.06.10), professional field 4.3. - Biological Sciences, because the material presented to me, summarized as a thesis, is fully responsible for awarding this type of academic degree.

Prepared the review:



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