Evaluation of the dissertation work of Bohdan Kysilov, MSc. entitled
THE STUDY OF FUNCTIONAL AND PHARMACOLOGICAL PROPERTIES OF GLUTAMATE RECEPTORS

The dissertation of Bohdan Kysilov, MSc. dealing with the study of the properties of glutamate receptors was prepared on the basis of experimental work carried out in the Institute of Physiology of the Czech Academy of Sciences under the supervision of Prof. Ladislav Vyklický, M.D., DrSc. The dissertation is based on the results published in three prestigious international journals (Br. J. Pharmacol., J. Neurosci. and Front. Neurosci.); the articles are attached to the disseration *in extenso*. In addition to these papers, Mr. Kysilov is also a co-author of three other papers, two of which have been published in renowned impact factor journals.

Ligand-gated ionotropic glutamate receptors, particularlly NMDA receptors, play a key role in normal brain function, and alteration of their properties as a result of mutations may be associated with the development of various neuropsychiatric disorders, including schizophrenia, epilepsy, or autistm-spectrum disorders. A better understanding of the properties of NMDA receptors and modulation of their function by neuroactive steroids may be useful for potentially more effective therapy of these disorders. The chosen topic of the dissertation is very timely and in line with the long-term focus of experimental work in the Department of Cellular Neurophysiology of the Institute of Physiology of the Czech Academy of Sciences.

The work is structured in the usual way and divided into several main chapters and subchapters. The Introductory section (21 pages) reviews current information on NMDA receptors and their potential use in neuropsychiatric disorders. There are relevant data about NMDA receptor antagonists and modulators, and a separate chapter is devoted to neuroactive steroids. The aims of the dissertation are clearly stated and the methodological approaches (11 pages) are described in sufficient detail. The Result section represents the most extensive part of the work (36 pages) and the description of the results is well organized into several subsections. The results obtained are critically discussed in the Discussion section (12 pages) in the context of the relevant literature and then summarized in the Conclusions.

It can be stated that the objectives of the work were achieved. The author gained a number of new interesting information about the functional and pharmacological properties of NMDA receptors with *de novo* mutations in the hGluN2B subunit that may affect receptor expression, affinity for glutamate or glycine, and possibly desensitization and ion channel opening probability. It has been possible to identify the sites of action of pregnenolone sulfate at NMDA receptors and the mechanism by which PE-S positively modulates the function of these receptors. Last but not least, the author succeeeded in describing the effects of selected neuroactive steroids at NMDA receptors and in establishing requirements for the structure of these molecules with respect to the mechanism of their action.

In conclusion, Mr. Kysilov´s dissertation shows that the author has an excellent orientation in the field of NMDA receptors and neurosteroids and demonstrates his skills in the practical performance of experiments based on advanced molecular biology and electrophysiological techniques. The clearly presented dissertation is of high professional quality and meets all the requirements for this type of work. Therefore, I recommend that Mr. Kysilov be awarded the title Ph.D. upon successful defence of the dissertation.

Questions for discussion:

1. What is known about the trafficking of NMDA receptors within cells and the mechanism of their insertion into the plasma membrane? How accurately were you able to assess (by immunofluorescnce microscopy) the surface expression of wild-type and mutant NMDA receptors? How did you normalize the fluorescence intensity?
2. What were the experimental conditions for using γCDX in intracellular solution to deplete intracellular PE-S (20-oxo-pregn-5-en-3β-yl-sulfate)? How efficient was the depletion of PE-S using this method? Did you verify the efficiency of the method?
3. Do neuroactive steroids have any possible undesirable side effects? What cell structures and processes may be affected by neuroactive steroids?

Prague 22.2.2023

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