BIOLOGY CENTRE CAS
address: Branišovská 1160/31, 370 05 České Budějovice, Czech Republic
IBAN: CZ39 0300 0000 0006 0077 3445 | SWIFT CODE: CEKOCZPP | VAT No.: CZ60077344
phone: +420 387 771 111 (telephone exchange) | www.bc.cas.cz | e-mail: bc@bc.cas.cz

Alexey Bondar, Ph.D.
Senior researcher
Institute of Entomology
Biology Centre of the Czech Academy of Sciences
Branisovska 1160/31, 370 05 Ceske Budejovice
and
University of South Bohemia in Ceske Budejovice
Branisovska 1760, 370 05 Ceske Budejovice
Czech Republic

In Ceske Budejovice 14.11.2023

Opponents Review: Ph.D. thesis

"Selective regulation of presynaptic receptors by SGIP1"
from Oleh Durydivka, MSc, Charles University, Second Faculty of Medicine

Cannabinoid receptors play a major role in the regulation of multiple physiological functions in the human body with pronounced effects on human behavior. Moreover, these receptors are targets for multiple compounds used in treatments of psychiatric conditions and chronic pain as well as for recreational purposes. Regulation of action of cannabinoid receptors is therefore of utmost scientific and medical interest and importance. The present thesis submitted by Oleh Durydivka significantly contributes to the understanding of cannabinoid receptor signaling pathway and sheds light on poorly understood role of SGIP1 in the regulation of this pathway.

The thesis is based on three principal questions: 1) what is the role of SGIP1 in chronic nociception? 2) how many SGIP1 isoforms are functionally expressed in the mouse brain? 3) how does HHC cannabinoid agonist compare in its action and effects with well-known THC and WIN ligands?

To address these questions Oleh mastered multiple experimental techniques including handling and testing nociception in live animals, RNA isolation, creation of DNA-based constructs, confocal and TIRF microscopy, genomic analysis. Oleh found out that SGIP1 played a role in nociception in mice with surprising sex-based differences. Extensive studies of SGIP1 isoforms allowed Oleh to find and characterize its 15 isoforms in mouse brain with 1 having distorted localization. Finally, a comparative study of cannabinoid receptor ligands allowed Oleh to identify HHC as a less potent alternative to THC, which nevertheless should be strictly regulated.

Oleh carried out a comprehensive research work and his conclusions have a solid base. I am impressed with the diversity of SGIP1-related questions that Oleh managed to address in his present research. I have a few questions to Oleh regarding his thesis which, however, do not deter my enthusiasm for it:

1. How would you explain sex-based SGIP1-related differences in nociception in mice?
2. Can SGIP1, in principle, serve as a drug target for treatment of chronic pain?
3. Does SGIP1 also exhibit postsynaptic localization?
4. Does SGIP1 interact with cannabinoid receptor 2 and regulate its functional activity?
5. Why did you choose GRK3 for your studies? Are other GRKs involved in SGIP1 regulation?
6. What features can define the selective regulation by SGIP1 of the cannabinoid receptor 1 but no other Gi-coupled receptors?
7. Can SGIP1 directly interact with clathrin-coated pits, particularly AP2 protein, through its APA domain?
8. How did you select the region of SGIP1 for eYFP tagging to maintain functional activity of isoforms?

Oleh managed to provide answers to all three principal questions that he posed and had these answers verified by publishing them in reputable peer-reviewed journals. Diversity of studied aspects of SGIP signaling shows that Oleh is a determined and creative researcher. Oleh's thesis is supported by three published research papers, including two first author papers. Overall, I am convinced that Oleh Durydivka performed excellent and extensive research and produced a well-written and comprehensive Ph.D. thesis. **I therefore highly recommend the thesis for the defense and awarding the Ph.D. degree to Oleh Durydivka, MSc.**

Sincerely,
Alexey Bondar