Opponent assessment of the dissertation 'Selective regulation of presynaptic receptors by SGIP1', presented by Oleh Durydivka, M.S.

Opponent: Josef Lazar, Ph.D., First Faculty of Medicine, Charles University, Prague

The presented dissertation summarizes the results of work carried out by O. Durydivka, M.S., within the field of cannabinoid receptor signaling and physiology, under the supervision of Asst. Prof. J. Blahoš, Ph.D. The work focused largely on the role of SGIP1 in cannabinoid receptor signaling and physiology. The topic fits well within the scope of the doctoral study program 'Biochemistry and Pathobiochemistry' of the Second Faculty of Medicine of Charles University, as cannabinoid receptors are molecular targets of existing and potential analgesics and other pharmaceutical drugs. Both the amount and quality of the presented work meet the requirements for a Ph.D. dissertation. The dissertation is written in English. In contrast to most dissertations I have evaluated to date, the text (with the exception of the Methods) is not structured hierarchically into many short sections. Rather, it is split into a few larger chapters, in a fashion similar to what one would typically find in a review article in a scientific magazine. Thanks to the author's writing skills and very good command of English, this makes for an engaging read. The Introduction section is particularly well written, and manages to clearly, concisely and comprehensively explain many aspects of cannabinoid molecular signaling, physiology and pharmacology. The text is accompanied by well-chosen, clear illustrations.

The Results section describes work in three interrelated areas of research: observations of effects of a knockout of the SGIP1 gene on chronic pain perception in mouse models; investigations of various splice variants of SGIP1 in the mouse; and studies of molecular pharmacology of hexahydrocannabinol, a minor naturally occurring phytocannabinoid. Behavioral experiments on pain-sensitized Sgip1 knock-out and wild-type mice allowed identifying the cannabinoid receptor 1 (CB1R) as the mediator of the studied chronic pain response. Absence of SGIP1 mildly, but statistically significantly increased sensitivity to painful mechanical stimuli in male mice. Somewhat surprisingly, SGIP1 absence had no effect in female mice. Also surprisingly, the possible reasons for this dimorphism are not investigated further, or even discussed. Although the overall study design seems solid, no control treatment (injection of carrier only) was performed in female animals. This is a flaw that should have been avoided.

Most of the experimental work performed by Mr. Durydivka concerned identifying distinct splice variants of the SGIP1 protein and investigating possible differences in their physiological roles. The work was done in a logical, systematic fashion, and involved a wide range of techniques of molecular biology (RT-PCR, cloning, restriction digestion), as well as protein science (SDS-PAGE/Western blotting, immunoprecipitation, proteolytic cleavage, mass spectrometry). The main rationale for this work, a Western blot showing at least two distinct bands, is shown in Fig 11. Frustratingly, the photograph of the blot is overexposed, making it impossible to judge the homo/heterogeneity of the ~130 kDa band, or the relative abundances of the ~130 and ~110 kDa bands. However, it does appear that the ~130 kDa band is many times stronger than the ~110 kDa band, suggesting that the physiological importance of the SGIP1 variants with lower molecular weight is small. The difference between the expected (94 kDa) and observed (130 kDa) molecular weight remains unexplained and virtually undiscussed. The fact that the observed shift of ~10 kDa upon treatment with phosphatase would require removal of approximately 125(!) phosphate groups is glanced over. The possibility that the apparent molecular weight observed by electrophoresis is an artifact caused by peculiarities of the SGIP1 protein sequence should have been discussed more extensively. Furthermore, it would have been advisable to accurately determine both the molecular weight of SGIP1 and the relative representation of its splice variants by mass spectrometry (MALDI-TOF). Thus, although the dissertation contains a commendable amount of work on investigating SGIP1

splice variants, and the complex results are presented in a clear, well-organized fashion, the rationale for the work is not very strong, and neither are the conclusions.

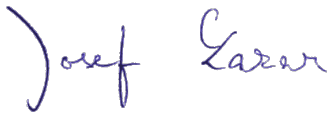
The final part of the presented work concerns investigations of molecular signaling in response to hexahydrocannabinol. The work utilized state-of-the-art approaches (bystander BRET) and well-designed biosensors, and touches on topics that are currently of high interest in the field of GPCR signaling (biased agonism, receptor internalization). The work appears to be done well. A minor criticism would concern the use of three significant digits in the stated values of Log(EC50), which seems excessive.

To summarize, the dissertation describes research effort in multiple aspects of cannabinoid signaling. The work encompassed a wide variety of experimental techniques, and yielded a good amount of results. The results have been published in two first-author publications and in one other publication. The publications are in respectable journals (Gene, IF 3.9; Frontiers in Neuroscience, IF 5.2; J. of Neurochemistry, IF 5.5). The student gained valuable experience in one of the field's most prominent laboratories (M. Bouvier, U. Montreal). The dissertation is well organized, and written in very good English. Therefore, despite my above-mentioned reservations, I fully recommend an award of a doctoral degree.

Questions to be answered during the dissertation defense:

1) Please discuss possible causes of the observed sexual dimorphism in the effects of the Sgip1 deletion.
2) Can you please clearly compare/contrast/discuss the effects of Sgip1 deletion on acute and chronic nociception? The similarities/differences are not clear enough from the text.
3) Can you please show a different version of Fig 4A that allows better assessment of the relative abundances of the 130 kDa and 110 kDa proteins? Was this quantification attempted by other techniques (such as gel filtration chromatography or MALDI-TOF)?
4) Is it possible that the observed multiple bands in Fig 4A are a result of proteolytical cleavage, rather than of differential mRNA splicing? How could you distinguish between the two possible mechanisms?
5) Fig. 7B and 7B' show two distinct intracellular localization patterns of SGIP1 853. Was one of them perhaps more common at higher expression levels and the other one at lower expression levels? Is it possible that the punctal localization is an artifact caused by overexpression?
6) Can you please briefly describe your experience from the Bouvier lab? What skills/know how did you obtain during your stay?

Nov 22, 2023 in Prague



Josef Lazar, Ph.D.