The assessment of the doctoral thesis written by **MSc. Monica Pontearso**, titled **„Modulation of nociceptive signaling on a spinal cord level under normal and pathological conditions“**, completed at the Institute of Physiology of Czech Academy of Sciences as a part of the doctoral study program Animal Physiology, Faculty of Science, Charles University in Prague.

This doctoral thesis is focused on the study of the modulation of nociceptive signaling, with a particular emphasis on the spinal cord level, under pathological pain conditions induced by peripheral neuropathy or inflammation. The thesis aims to reveal the modulatory mechanisms of synaptic transmission by inflammatory cytokine MIF, the endocannabinoid anandamide, and the regulatory protein of cannabinoid receptor 1, SGIP1. The study of spinal nociceptive mechanisms induced by peripheral neuropathy is extended by neuro-immune interaction at the site of injury of the peripheral nerve, where infiltration of immune cells plays a substantial role. This experimental work is important in advancing our understanding of the fundamental nociceptive mechanisms and addressing the pressing social implications of various painful conditions for which satisfactory therapeutic approaches have not yet been identified.

This experimental study provides novel insights into the mechanisms underlying pain associated with peripheral nerve injury and neuroinflammation. The presented research yielded several significant findings. Systemic treatment of mice with the MIF inhibitor, ISO-1, attenuated mechanical and thermal hyperalgesia induced by sciatic nerve injury in males but not in females. This ISO-1 treatment partially restored the neuropathy-induced imbalance between excitatory and inhibitory synaptic transmission in males at the spinal cord level. Additionally, it decreased macrophage infiltration at the site of nerve injury and dorsal root ganglion and regulated signs of neuroinflammation. Furthermore, a recent publication in a prestigious first-quartile peer-reviewed international journal demonstrated the impact of peripheral inflammation on the dual role of anandamide in modulating synaptic transmission at the first nociceptive synapses of the ascending pathway. At these synapses, the enhanced inhibition of spinal nociceptive transmission during stimulation of cannabinoid receptors was observed in SGIP1 knock-out mice.

The topic of this thesis is certainly current and interesting, and its findings may contribute to the clinical practice of human medicine in the future.

I have several comments regarding individual sections and the overall dissertation manuscript.

- Its formal processing is standard, and contains all the essential components.
- The text contains quite a few typing errors and English language deserves native speaker’s inspection, especially for future publication purposes.
- From a clinician’s point of view, some statements in the general part of Introduction are unnecessarily categorical and imperative, almost showing gaps in understanding the clinical aspects of the problematics. (For example, page 1: regulatory mechanisms of the pain pathway do not maintain physiological properties of the body after an injury, it is the neurohumoral stress response, …the difference between acute and chronic pain is not based on the extent of the

stimulus, … as regards somatosensory pain pathways, the spinothalamic tract does not process motivational and affective components of the pain sensation, these components arise after integration of the cortical perception with limbic system,… etc.) Furthermore, opioids are far from being the most effective in the treatment of neuropathic pain, representing only the second line medication, after antidepressants, anticonvulsants and locally used analgetic substances.

- The part concerning general immune mechanisms is somewhat confusing. (For example, chapter 5 is called “Immune cells”, whereas chapter 6 “Cells involved in the immune response”.) The humoral part of the immune system is not mentioned at all. The same information is often repeated over again in different places of the text, what further adds to the disarray.
- The second part of Introduction, concerning the subject of author’s research, is written in incomparably higher quality. Reduction of the quantity of theoretical information, particularly in the general part including human physiology, and focusing on the topic of the thesis instead, would certainly improve the quality of the text and add to its clarity. It would also help the author to show and present in which specific area she is an expert.
- The main 3 aims of the study are stated in the relevant sections. The first and the second one perfectly clearly, including experimental goals. The third aim is thematically closely related to the second part. As for the first experimental part, its highlight is the use of several different experimental methods to confirm the findings.
- In Methods section, I miss the description of the design of at least the most important experiments.
- The Results are well analyzed and clearly presented, giving straightforward answers to questions the author was asking. Especially the first part of the study investigating neuroinflammation is very well designed - the discrepancy in findings in one set of experiments is nicely explained by another method, used in the following set of experiments. As regards the PCR experiments, instead of all the unnecessary text in the Introduction, there should be at least a basic information about the proteins involved in regulation of MIF activity the author is investigating (only CD 74 is mentioned), since not all the readers are familiar with this issue. The second part, concerning the modulatory role of anandamide in nociception under inflammatory conditions using patch clamp recording, is also very well and rationally designed. I appreciate the sequence of the individual sets of experiments, gradually clarifying the mechanisms of interest, to reach the experimental goal. The results are presented in a clear, easily understandable way, and their publication in impacted journal speaks for their high quality. The third part, by far the most concise, is thematically related to the second and could represent its last set of experiments. It would make better sense to present the native recordings of mEPSC under control and inflammatory conditions at the very beginning of the second part of the study.

- Discussion chapter is trying to explain obtained results and confront them with previous findings. Again, as a clinician, I would be very reserved to claim that "the most of existing medications used in neuropathic pain treatment are characterized by loss of efficacy", let alone a non-expert. Detailed language revision would help this chapter. Some statements are difficult to understand, trying to figure out what the author means. (For example, page 74: "in models of CCI, MIF inhibition did not cause hypersensitivity", or page 78: "sex differences include different timing in the management of pain states"). Collaboration between neural and immune cells is mentioned repeatedly at the beginning of each single section, and lot of other information is also repeated over again. The explanation of MIF and CD 74 discrepancy in PCR experiments is quite vague.
- The discussion of AEA experiments is much better. A comment about TRPV1 receptor antagonist + AEA experiments is missing completely.

Taken together, during her Ph.D. studies, Miss Monica Pontearso did learn to work as an independent scientist responsibly. She managed to set exact experimental goals and design relevant experiments to reach them. She learnt to use as many as 4 different, highly sophisticated, neurophysiological methods and number of other modern technologies. The results of her work are certainly a contribution to basic science, and maybe in the future, to the clinical practice as well. Her journal publications with impact confirm that she mastered her job.

In conclusion, the submitted dissertation of Monica Pontearso MSc. meets all the requirements for a dissertation in the scope and quality of the obtained professional results and therefore I recommend the thesis for defense and after successful defense to award the degree of Ph.D.

Questions to be answered:

1. How would you explain the time delay of ISO-1 effect on both types of hyperalgesia?
2. Can you compare both animal models you used, with focus on the type of pain state they induce, and its pathophysiology? What are the similarities and what are the differences?
3. What do you know about the interactions between cannabinoid and opioid receptors?

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