Institute
of Experimental
Medicine, CAS

Opponent's review of the dissertation of Akash Shivling Mali, MSc "Exploring the role of opioid signaling in modulation of microglial function"

The submitted dissertation is written in the classical form based on the two publications where the candidate is first author. Both publications have a solid impact factor; in addition, Mali at al. 2022 was published in Elsevier publishing group. The contribution of the candidate on the publication is adequate and contain the technical work as well as data evaluation and publication writing.
The thesis has 112 pages, and it is written in English.

The study was focused on the role of opioids in modulation of the microglial functions The study of opioid agonists, including DAMGO, DADLE, and U-50488, revealed that they could influence microglial polarization. They suppress M1 polarization (proinflammatory state) and promote M2 polarization (anti-inflammatory state). This is indicated by changes in phagocytic activity, nitric oxide (NO) production, and the expression of proinflammatory and anti-inflammatory cytokines. The study also explores the impact of opioids on mitochondrial function and energy metabolism in microglia. It found that opioids have cytoprotective effects by reducing reactive oxygen species (ROS) production, increasing NADPH synthesis, and enhancing glucose uptake. These effects are associated with an upregulation of the Nrf2/HO-1 pathway. The results suggest that the activation of opioid signalling pathways can be harnessed to regulate microglial behaviour, promote anti-inflammatory and antioxidant effects, and inhibit inflammatory stimuli. This could have implications for the development of therapeutic approaches to address neurodegenerative diseases, where dysfunctional microglia are implicated.

The literary review is written meticulously and comprehensibly. I would suggest incorporating more schemes and images, as they often convey more than words, e. g when explaining molecular pathways and its role in the different pathologies. The role of microglia in neurodegenerative diseases is well described, but for the research presented here the information is rather marginal.

The Materials and Methods chapter is very well written and contains all the necessary information about the research procedures. The project usually uses well-established methods or commercial assays. The main hypothesis is well postulated, and the methods are well designed to address the specific objectives of the project.

The Results chapter is organized according to the methods presented in the previous chapter. The individual experiments are well planned, and the results well presented, and a sufficient number of biological replicates were performed for each experiment. The data are adequately presented, and the statistical analysis is well chosen for such an experimental design.

In the Discussion section, the results of the experiments are one by one presented and discussed in detail with the literature. The results of the project are explained in detail, but the overall

Vídeňská 1083
142 20 Prague, CZ
+420 241 062 230
+420 241 062 780
www.iem.cas.cz
iem@iem.cas.cz

conclusion and summary of the project as a whole is not sufficient, and I hope that the individual results will be more connected as a one story in the public defense.

From the comments and questions, I noted while reading, I choose:
Comments:

1. Many abbreviations, especially from the Introduction are not explained or listed in the list of abbreviations (MFG-E8, DAP12, CCR7 etc.).
2. Some images are of lower quality, e.g. the aspect ratio is not preserved in Figures 15, 18 and 19.
3. The majority of references in the discussion part are more than 5 yeras old.

Questions:

1. The candidate brought up the historical perception of the central nervous system (CNS) as immune-privileged due to its absence of conventional lymphatic drainage. Given the discoveries made in the past decade regarding the "glymphatic system," how should we discussed the results presented here in the context of the CNS's pathologies ant he glymphatic system?
2. As the candidate also emphasized, the M1 and M2 classification simplifies the immune response, given that the response to pathological events is a dynamic process especially *in vivo*. How might this impact your *in vitro* results?
3. On which levels the opioid ligands modulate anti inflammatory reactions, e.g.on the protein level, gene expression levels etc. ?
4. What are the main objective of the using a scratch assay and what are the limitations of this technique?

The current dissertation includes data that have been previously presented in publications but are now discussed and contextualized within the dissertation. While the thesis would benefit from more improved handling of images and text, the student exhibits the necessary qualifications for independent scientific research. Therefore, I recommend this thesis for defense.

Sincerely,

Lucia Machová Urdzíková MD.PhD.
lucia.machova @iem.cas.cz
Institute of Experimental Medicine AS CR
Vídeňská 1083
142 20 Prague 4, Czech Republic
email: lucia.machova@iem.cas.cz
Tel.: +420 241 062 619