Peer Review File

Article information: https://dx.doi.org/10.21037/jgo-24-50

Review Comments

The paper titled "Inhibition of GABAergic neurons in the paraventricular nucleus of the hypothalamus precipitates visceral pain induced by pancreatic cancer in mice" is interesting. GABAergic neurons located in PVN play a crucial role in precipitating visceral pain induced by pancreatic cancer in mice, thereby offering novel insights for identifying effective targets to treat pancreatic cancer-related visceral pain. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) In addition to the electrophysiological and chemogenetics approaches used in this study, it is recommended to add detection methods such as optogenetics and fiber photometry.

Reply: We appreciate your suggestion. In this study, electrophysiology was employed to uncover a reduced firing rate in PVN GABAergic neurons, and we further investigated the role of these neurons in regulating visceral pain associated with pancreatic cancer through targeted destruction and activation experiments. The results unequivocally demonstrate the pivotal involvement of PVN GABAergic neurons in the modulation of pancreatic cancer visceral pain. Although optogenetics and fiber photometry are valuable techniques in neuroscience research, their implementation necessitates invasive brain fiber implantation, which would exacerbate the distress experienced by mice already enduring compromised health due to pancreatic cancer visceral pain. Therefore, taking all factors into consideration, we refrained from utilizing these two methods.

2) The number of model animals in this study was not mentioned. Is it sufficient to complete all experiments? Please explain the use of animals in the experiment.

Reply: Thank you for your comment. We have indicated the number of animals in each figure legend.

3) How can our research provide new insights into the neural circuit mechanisms of chronic visceral pain? Suggest adding relevant discussions.

Reply: Thank you for your advice. In the discussion section, we have previously described studies suggesting that chronic visceral pain is primarily mediated by the activation of corticotropin-releasing hormone (CRH) neurons. GABAergic neurons serve as the principal inhibitory neurons in this context. However, it remains unclear whether GABAergic neurons can alleviate visceral pain by suppressing CRH neuron activation. This study validates this

notion and enhances our understanding of PVN' involvement in regulating chronic visceral pain. Please see the discussion section, Page 11, Line 267-270, Line 275-282.

4) In the introduction of the manuscript, it is necessary to clearly indicate the knowledge gaps and limitations of prior study and the clinical significance of this study.

Reply: Thank you for your comment. Previous studies have not explored the central regulatory mechanisms of visceral pain in pancreatic cancer, which has led to unsatisfactory treatment outcomes for this condition. Therefore, this study aims to investigate the central regulatory mechanisms of visceral pain in pancreatic cancer, potentially uncovering key central mechanisms that regulate such pain and providing new insights for clinical research. Please see introduction section, Page 4, Line 65-68, Line 90-92..

5) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “The recycling of AMPA receptors/GABAa receptors is related to neuronal excitation/inhibition imbalance and may be regulated by KIF5A, PMID: 36388788”. It is recommended to quote the article.

Reply: Thank you for your comment. We have added relevant content and quote the article as you advised. Please see introduction section, Page 4, Line 78.

6) What molecular changes affect the integration function and response of neurons? Suggest adding relevant content.

Reply: Thank you for your comment. We have added relevant content as follows: Therefore, we suggest that in mice with visceral pain, GABA released by GABAergic neurons is reduced, which leads to increased excitability of CRH neurons and mediates pancreatic cancer visceral pain. By activating GABA neurons, the release of GABA is increased to alleviate pancreatic cancer visceral pain. Please see Discussion section, Page 11, Line 278-282.