

Round-1

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade A (Priority publishing)

Conclusion: Major revision

Specific Comments to Authors: This interesting study set out to investigate the effects of acetyl-L-carnitine (ALC) on secondary hypersensitivity and anxiety-like behavioral changes in caerulein (CAE)-induced recurrent acute pancreatitis. Morphological changes indicative of microglial activation in pain and anxiety-related brain regions were quantified after 6 weeks of CAE induced recurrent acute pancreatitis. Efficacy of ALC treatment to alleviate these symptoms in the last 3 weeks of CAE pancreatitis was determined. Mice with pancreatitis had significantly reduced mechanical withdrawal thresholds and heat response latencies, indicating ongoing pain. Treatment with ALC attenuated hypersensitivity.

Major points

1. It would be much better to start the results section with characterization of pancreatitis severity in the various groups (Figure 4 is not enough). Please show histological pictures (with quantification). Do you have characteristic features of chronic pancreatitis? Would it be possible to demonstrate the extent of fibrosis as well? The prolonged CAE-induced pancreatitis model has been used for several decades in mice and is well described in detail in the literature. We have added a description of the histological evidence of the reported histological changes in the pancreas to the introduction.

2. The title needs to be more specific and should reflect the main findings of the study. The title has been changed. **“Effect of ALC on hypersensitivity in acute recurrent caerulein-induced pancreatitis and microglial activation along the brain’s pain neuraxis ”** more clearly describes the main findings of the study.

3. The aim in the abstract is too general (pathological changes) and does not refer to the brain. This has been changed.

4. The introduction is a bit redundant (e.g. the antioxidant and free radical scavenger activity of ALC is mentioned in two sentences). This has been corrected.

5. Methods. On page 7 it would be better to indicate that daily doses of 2x100 mg/kg ALC was administered to mice. It is unclear to me why this dose was chosen. On page 9, the method of euthanasia should be stated. The daily dose of 2x100 mg/kg ALC was chosen based on previous publications as described in the Methods section *ALC treatment* methods (see references #24 and 52). The euthanasia method is now clearly described.

6. On page 10, the first part of the results is really a repetition of the methods, it should be deleted. The first part of the Results section has been removed.

7. I think that it is pointless to provide results to the decimals when the values are in the hundreds or thousands (see main text and suppl. table 1). This has been changed.

8. Figure 1 is not essential and could be moved to the supplementary part. Figure 1 has been moved to the supplemental data section. The description of this figure is now in the methods section.

9. Figs. 5-6 and 7-8 could perhaps be combined in two figures. Each figure corresponds to a different brain region. It is less confusing and keeps the attention of the reader to keep them separate. Therefore, the figures have not been combined which would render the reference brain slices too small to be readable.

10. On page 19, conclusions, there is nothing stated on the effect of ALC which is odd. This has been edited.

11. There are far too many references (more than double) in this paper. There is no limit to the number of references that can be cited in this journal. While the reference list is lengthy, the present manuscript includes many different research areas (CAE-induced pancreatitis, behavioral and anatomical changes during pancreatitis, chronic pain, brain microglia, pain and anxiety brain circuitry) that require discussing. Combining these very diverse topics justifies the number of references used in this article and complies with the journal's guidelines.

12. In general, the size of figures is too small. In figure 4, the n=3/timepoint could be moved to the legends. The figures are now inserted into the text as pictures and the PowerPoint files are uploaded separately as individual files

13. n numbers and the definition of asterisks and # should be provided in all figure legends. The title of Figure 3 should convey the main message rather than the test used. The title of the figure has been edited.

Minor points 1. All abbreviations should be defined at first used and should be used in the abbreviated form thereafter (e.g. CAE, ALC). There is no point in abbreviating words that are only used once (e.g. BDNF). This has been changed except in cases when molecules are better known by their abbreviation (e.g. BDNF).

2. CAE induced acute recurrent pancreatitis should be written with a hyphen (CAE-induced...). This has been changed.

4 LANGUAGE QUALITY

Please resolve all language issues in the manuscript based on the peer review report. Please be sure to have a native-English speaker edit the manuscript for grammar, sentence structure, word usage, spelling, capitalization, punctuation, format, and general readability, so that the manuscript's language will meet our direct publishing needs.

5 EDITORIAL OFFICE'S COMMENTS

Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:

(1) *Science editor:*

- (2) 1 Scientific quality: The manuscript describes a basic study of the brain microglial response to recurrent caerulein pancreatitis. The topic is within the scope of the WJG. (1) Classification: Grade B; (2) Summary of the Peer-Review Report: This interesting study set out to investigate the effects of acetyl-L-carnitine on secondary hypersensitivity and anxiety-like behavioral changes in caerulein-induced recurrent acute pancreatitis. However, some questions

raised by the reviewers should be answered; and (3) Format: There are 9 figures. A total of 130 references are cited, including 10 references published in the last 3 years. There is 1 self-citation. 2 Language evaluation: Classification: Grade A. 3

- (3) Academic norms and rules: The authors provided the Institutional Animal Care and Use Committee Approval Form or Document. However, the Biostatistics Review Certificate, and The ARRIVE Guidelines are not qualified. **The authors should provide the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement.** No academic misconduct was found in the CrossCheck detection and Bing search. 4 The Conflict-of-Interest Disclosure Form does not open on the website. We have tried from 3 different computer locations and were unable to get access to the form. We therefore wrote one ourselves based on a published one from your website.

Due to the present pandemic, it is impossible for all authors, presently located in 3 different states separated by over 1000 miles, to sign the copyright license agreement in person. A version with electronic signatures has been attached.

- (4) Supplementary comments: This is an unsolicited manuscript. The study was supported by United States Department of Veterans Affairs and United States National Institute of Health. The topic has not previously been published in the WJG.
- (5) Issues raised: (1) **The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s);** Approval documents have been uploaded.
- (6) **(2)** The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor

The PowerPoint files of all figures have now been attached.

- (7) The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text. An "Article Highlights" section has now been included at the end of the manuscript. The instructions for a Basic Science Manuscript describe that an Abstract but not Research Highlights are required. We now have both.
- (8) 6 Re-Review: Required. 7 Recommendation: Conditional acceptance.

(2) Editorial office director: I have checked the comments written by the science editor.

(3) Company editor-in-chief: I have reviewed the Peer-Review Report, full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Gastroenterology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors.

Round-2

2 Peer-review report

Reviewer #1: The manuscript has been improved, but unfortunately some of my points have not been adequately addressed or were completely neglected.

1. Most importantly, it would be essential to quantify the severity of pancreatitis in the various groups in more detail. Determining the translucence of pancreatic tissue is not enough; other histological and/or laboratory parameters need to be measured to confirm the effect of ALC. Without this, I do not recommend the acceptance of this paper. **The requested histology and its quantification have been added to Figure 3.**
2. The revised title stated in the rebuttal letter does not match that of the auto-edited manuscript file (circuitry vs neuraxis). Also, it is inappropriate to use non-standard abbreviations like ALC in the title. **The title has been edited accordingly.**
3. On page 7, the daily dosing of ALC should be written as 2x100 mg/kg instead of 200 mg/kg. **This has been changed.**
4. On page 22, conclusions, there is still nothing stated on the effect of ALC. **This has been changed.**
5. Even though the manuscript includes many different research areas, I think that the number of references is way too many and could be reduced. **The number of references has been reduced by 20.**
6. n numbers and the definition of asterisks and # have not been provided in all figure legends. **This has been changed.**

Reviewer #2: This interesting study set out to investigate the effects of acetyl-L-carnitine (ALC) on secondary hypersensitivity and anxiety-like behavioral changes in caerulein (CAE)-induced recurrent acute pancreatitis. Morphological changes indicative of microglial activation in pain and anxiety-related brain regions were quantified after 6 weeks of CAE induced recurrent acute pancreatitis. Efficacy of ALC treatment to alleviate these symptoms in the last 3 weeks of CAE pancreatitis was determined. Mice with pancreatitis had significantly reduced mechanical withdrawal thresholds and heat response latencies, indicating ongoing pain. Treatment with ALC attenuated hypersensitivity.

Major points

1. It would be much better to start the results section with characterization of pancreatitis severity in the various groups (Figure 4 is not enough). Please show histological pictures (with quantification). Do you have characteristic features of chronic pancreatitis? Would it be possible to demonstrate the extent of fibrosis as well? **The manuscript describes pancreatitis as it occurs, first the effects on the living animal and then the histopathological findings. Former figure 4, now figure 3, has now been expanded to include histology and its quantification.**
2. The title needs to be more specific and should reflect the main findings of the study. **The title has been changed to "Effect of acetyl-L-carnitine on hypersensitivity in acute recurrent caerulein-induced pancreatitis and microglial activation along the brain's pain circuitry"**
3. The aim in the abstract is too general (pathological changes) and does not refer to the brain. **This has been changed.**
4. The introduction is a bit redundant (e.g. the antioxidant and free radical scavenger activity of ALC is mentioned in two sentences). **This has been changed.**
5. Methods. On page 7 it would be better to indicate that daily doses of 2x100 mg/kg ALC was administered to mice. It is unclear to me why this dose was chosen. On page 9, the method of euthanasia should be stated. **This has been changed.**
6. On page 10, the first part of the results is really a repetition of the methods, it should be deleted. **This has been changed.**
7. I think that it is pointless to provide results to the decimals when the values are in the hundreds or thousands (see main text and suppl. table 1). **This has been changed.**
8. Figure 1 is not essential and could be moved to the supplementary part. **This has been changed. It is now a supplemental figure.**
9. Figs. 5-6 and 7-8 could perhaps be combined in two figures. **Each figure corresponds to a different brain region. It is less confusing and keeps the attention of the reader to keep them separate. Therefore, the figures have not been combined.**
10. On page 19, conclusions, there is nothing stated on the effect of ALC which is odd. **This has been changed.**
11. There are far too many references (more than double) in this paper. **The number of references has been reduced by 20.**

12. In general, the size of figures is too small. In figure 4, the n=3/timepoint could be moved to the legends. **This has been changed. The PowerPoint files have been uploaded separately files.**

13. n numbers and the definition of asterisks and # should be provided in all figure legends. The title of Figure 3 should convey the main message rather than the test used. **This has been changed.**

Minor points

1. All abbreviations should be defined at first used and should be used in the abbreviated form thereafter (e.g. CAE, ALC). There is no point in abbreviating words that are only used once (e.g. BDNF). **This has been changed except in cases when molecules are better known by their abbreviation (e.g. BDNF).**

2. CAE induced acute recurrent pancreatitis should be written with a hyphen (CAE-induced...). **This has been changed.**