

## ANSWERING REVIEWERS



July 25, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 4079-review.doc).

**Title:** A common genetic mechanism underlying susceptibility to posttraumatic stress disorder

**Author:** Zhen He, Li Cui, Bei He, Sherry A. Ferguson and Merle G. Paule

**Name of Journal:** *World Journal of Neurology*

**ESPS Manuscript NO:** 4079

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

*List for revision following the Editorial Format*

- 1) The title of the manuscript has been shortened to 10 words: "A common genetic mechanism underlying susceptibility to posttraumatic stress disorder"
- 2) A running title has been added: "Aberrant neuronal gene expression and PTSD"
- 3) Author contributions have been added
- 4) Corresponding author information has been reformatted
- 5) The abstract has been shortened to less than 200 words
- 6) A core tip has been added
- 7) The font type and size as well as the line spacing have been changed
- 8) References have been formatted, both in the text and in the reference section to comply with the regulations of WJN. For example, the name of the first author has been bolded and the DOI and PMID numbers have been added, where applicable.

2 Revision has been made according to the suggestions of the reviewer

*Responses to reviewer comments and critiques*

Reviewer #1

Authors In the review article "A common genetic mechanisms underlying susceptibility to posttraumatic stress disorder- a potential approach with combination of laser-assisted microdissection and microarray techniques", He Z et al discussed the evidences supporting that susceptibility to PTSD may be determined in part by aberrant microtubule-associated protein tau (MAPT) expression in neurons of critical brain structures. This article is well written, and the cited references are updated, i.e., 19 cited paper (23%) were published in and after year 2010.

The weakness of this article, however, is shown in the following points:

1. The conclusion in lines 17-20, page 2, was not useful to interested readers. The usefulness of laser capture microdissection (LCM) and microarray analysis of gene expression (MAGE) have been proved and in providing specific insights in every discipline of neuroscience. There is no need to add another positive statement for LCM-MAGE. Instead, interested readers of the Journal would like to learn: what new mechanisms have been derived by using this combination.

**Response:** The text has been revised as follows: “To detect the potential molecular mechanisms underlying PTSD episodes, laser-assisted/capture microdissection can be used with microarray analysis as an alternative approach to identify changes in gene expression in excitatory and/or inhibitory neurons in critical brain structures (i.e., hippocampus and amygdala) in response to the onset of PTSD.” (page 2). In addition, the following descriptions have been added on page 11 (highlighted in red): “Practically, a subset of neurons that express the targeted proteins-- such as excitatory glutamatergic neurons with CamKII $\alpha$  as a marker or inhibitory GABAergic neurons with GAD67 as a marker[85]--can be selectively collected using an optimized immunohistochemical labeling technique followed by the LAM/LCM procedures. Presumably, the altered gene expressions in the excitatory and/or inhibitory neurons may indicate the signaling pathways accountable for the vulnerability to and onset of PTSD”. A new reference (#85) has also been added.

2. Similar to the aforementioned comment, Figures of this review article appear superfluous, because they were used only to depict the procedures of LCM and the typical analyses of MAGE, which have long become the standard procedures. I suggest that authors use Figures and add Tables for summarizing key evidences to support their hypothesis.

**Response:** See the above response. In addition, a new figure (Fig. 4 in the revised manuscript, the previous Fig. 4 is now labeled as Fig. 5) with its relevant text has been added to address the amount and quality of LAM-derived cellular RNA before processing via microarray hybridization.

#### Reviewer#2

##### General comments

(1) The importance of the research and the significance of the research contents The research about posttraumatic stress disorder (PTSD) is very important because of the recent increase of PTSD upon natural disaster and modern problems and stress. The attempt to reveal the common genetic mechanism of PTSD is highly significant to resolve the complex situation of PTSD. (2) The novelty and innovation of the research The approach to reveal the gene expression of PTSD is unique and the importance of the research should be emphasized. (3) Presentation and readability of the manuscript Presentation and readability of the manuscript are quite acceptable. (4) Ethics of the research There seems to be no relevant problem in ethics of the research

##### Specific comments

Title: It accurately reflects the major topic and contents of the study.

Abstract: It describes about the aim of the research to reveal gene expression in PTSD and the methods using laser-assisted/capture microdissection with microarray to reveal the mechanism of PTSD are innovative.

The result should be described more in detail to emphasize the importance of the research.

**Response:** A new figure (Fig. 4 in the revised manuscript, the previous Fig. 4 is now labeled as Fig. 5) with its relevant text has been added to address the quality and amount of LAM-derived cellular RNA that is used for the microarray hybridization studies.

Material and methods: Material and methods are needed to explain figures.

**Response:** The present manuscript is targeted for categorization as a WJN “Frontier” article which is intended “to review the most representative achievements and comment on the current research status in important fields, and propose directions for future research”. Adding a “Materials and Methods” section would be beyond this intended effort and thus, we have not added a “Material and Methods” section.

Results: “Future studies” includes the recently revealed data, so the heading of the section might be changed to describe the gene expression in PTSD, or semi-sections may be added.

**Response:** We have not made any changes in response to this comment.

In page 16, line12, "Forty-nine of the 50 1000-neuron RNA samples were amplifiable" is hard to be interpreted. Does this mean that 501000 samples have been examined? It seems like figure 4 plots signal intensity in each gene.

**Response:** The text has been revised to read "Forty-nine out of 50 sets of the 1000-neuron RNA samples were amplifiable ..." for clarification.

Discussion: Discussion section may be added.

**Response:** We have not made any changes in response to this comment.

References: Shin LM et al, 2006 might be cited.

**Response:** This has been done.

Tables and figures: Legends are well described. It should be clarified to which panels the equation indicating the correlation of samples corresponds in figure 4.

**Response:** This has been done.

Material and method section to describe the figures should be added.

**Response:** See response above.

Reviewer #3

This manuscript is acceptable for publication with minor changes as follow:

1. The authors should complete the name of peoples in Acknowledgements. (There is a blank in the last line of the Acknowledgements)

**Response:** "Jyotshnabala Kanungo" has been added to fill the blank.

2. The authors should make the reference style consistency and meet the journal format.

**Response:** Please see "List for revision following the Editorial Format" above. Briefly, the references have been formatted per WJN regulations.

3. There are 2 journals list in Reference no. 14, so the total references should be 84.

**Response:** This has been changed as suggested.

Reviewer #4

He et al: A common genetic mechanism underlying susceptibility to posttraumatic stress disorder--A potential approach with combination of laser-assisted microdissection and microarray techniques This is an article can be clarified either as a review with a much narrowed topic, or an argument for a research proposal (with the first sentence such as "We hypothesize that susceptibility...". If this is a review paper, authors should extensively research literature and summarize the most recent development in the field, thereby giving audiences a brief but updated view of PTSD research.

**Response:** This manuscript includes information from the recent literature: 19 of the cited papers (22% of the total 85 references) were published after January 2011. According to the "Aims and scope"--WJN aims to rapidly report new theories, methods and techniques for.... Our manuscript is in compliance with the "Aims and scope" of WJN because it proposes a new theory along with potential approaches, namely methods to test that theory.

Please also see the "Core tip" section of the manuscript.

On the other hand, if this is a research proposal, "a potential approach with LAM/LCM technique and microarray..." seems not working very well even in author's laboratory as stated in the paper that "In

order to maximize the likelihood of obtaining quality data, the quality of the RNA obtained using the LAM/LCM technique should be carefully examined...". Therefore, a backup approach should be in place.

**Response:** A new figure (Fig. 5) with its relevant text has been added to address the quality and amount of LAM-derived cellular RNA that is obtained before processing via microarray hybridization.

3 References and typesetting were corrected  
(See above section)

Thank you again for publishing our manuscript in the *World Journal of Neurology*.

Sincerely yours,

A handwritten signature in black ink, consisting of a series of loops and a horizontal line extending to the right.

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