Author's response to reviews

Title: Electroacupuncture decreased cognitive impairment and promotes neurogenesis in the APP/PS1 transgenic mice

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Author's response to reviews: see over
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Re: MS: 2228748569968404 - Electroacupuncture decreased cognitive impairment and promotes neurogenesis in the APP/PS1 transgenic mice

Dear Dr. Stephanie Tjen-A-Looi,

Thank you for your letter and for the reviewers’ comments concerning our manuscript entitled “Electroacupuncture decreased cognitive impairment and promotes neurogenesis in the APP/PS1 transgenic mice”. Those comments are valuable and very helpful to improve our paper and to prepare this revised submission. We have carefully considered each of the comments from two reviewers, and made changes to the manuscript accordingly in RED. The main corrections in the paper and the responds to the reviewer’s comments are as flowing:

Responds to the reviewers’ comments:

Reviewer 1

This study evaluated effects of electroacupuncture (EA) on decreased cognitive impairment and neurogenesis in the APP/PS1 transgenic mice. The results from this study suggest that EA may be used to manage Alzheimer’s disease (AD). There are several concerns with this manuscript.

1. The rationale for using Baihui acupoint in this study was not clearly justified. What was the specificity of this acupoint for the present study? How was the control for EA conducted?

Response: We thank the reviewer for the helpful question. Based on meridian theory, an acupoint is relatively specific to certain function or certain organs, and different effects occur when different acupoints are stimulated. The selection of the Baihui acupoint was based on the previous published paper of ours. Generally speaking, the acupoint was chosen based on the effectiveness which we have been proved in our previous published paper [1-4]. The control group of our current paper is defined as mice without acupuncture. We have added more related information about the EA treatment and the control group in the paper on Page 12.

2. This study examined if EA improved cognitive function and the pathological features of AD in amyloid precursor protein (APP)/presenilin 1 (PS1) in APP/PS1 double transgenic mice. However, discussion about why EA had those actions was lacking. In addition, similar changes were found in wild-type mice after EA treatment compared to controls. What was significance of these findings?

Response: Thanks for your valuable comments. The why EA improved cognitive function and the pathological features of AD in APP/PS1 double transgenic mice was added on Page 10 as suggested. In briefly, the current study we focused on the effectiveness for EA on AD. Aβ is not only one biomarker for diagnosing AD, it is also one important target for AD therapy. Thus we believed that an effective method might lower the deposit of Aβ. In the current paper we did not focus on explaining how EA reduced the Aβ level, but we assumed that it might be related to the effect of reducing the production.

We did find the similar changes in wild-type mice in the expression of Aβ and BDNF. We assumed that it might suggest that the effect of EA on Aβ and BDNF could be related to a common signaling pathway but not a specific pathway in AD. It may bring some potential evidence for searching the underlying mechanism for EA treatment. Moreover, the control group was age-matched with the APP mice. Our result might suggest that electroacupuncture could induce the protective effect for the central nervous system of aging and Alzheimer’s disease. Nevertheless,
we added some information on Page 10.

3. The positive labeling shown in the figures should be indicated with symbols. Figure 3 did not support the statement of co-localization of neurons with Aβ1-42. There was a similar concern with Figure 5, Panel B.

**Response:** Sorry for our flaws. For Figure 3 and 5, the co-localization of neurons with Aβ 1-42 or BDNF might not be as clear as the expectation. Thus we have made some symbols of arrows for point out the positive result. We apologize for the negligence.

4. In Figure 1, how could Panel D showing single image indicate statistically significant difference? This statement was addressed in the figure legend and text.

**Response:** We thank the reviewer for pointing out our flaws. For Figure 1, the aim of Panel D was to show that the modeling of AD was successful built. Although we did repeated the immunofluorescence staining from both groups, we did not quantify and analysis the result statistically. Thus we have changed the expression of the figure legends and text accordingly on Page 5 and 23.

5. Figure 2, Panel A appeared to be confusing. In this regard, MWM (Morris water maze test?) needs to be defined. When was BrdU administered at the first time? How many days for an entire EA treatment? 20 or 28 days? and among others.

**Response:** We thank the reviewer for the reasonable suggestion. For Figure 2, the definition of MWM (Morris water maze test) was remarked. The time point for BrdU administration and the duration of EA treatment were defined in a clearer way in the paper on Page 12 and 16. We apologize for the uncleanness of our previous version of the manuscript.

6. In Figure 4, Panel C, the bands demonstrating Aβ 1-42 were questionable when “Con+EA” was compared to “Con”.

**Response:** Sorry for our negligence. For Figure 4, we picked a band for representing the expression of Aβ 1-42. As for the analysis, the statistic result was from 3 different groups. The current
presented western blot might not be perfectly matched the result. However, the figure that we offered in the current version was till now the best quality image. We have no animal tissues left for repeat the experiment and if we do repeat, it would take a lot more time. Thus, we hope that currently the figure would meet the expectation.

7. In Figure 5, the description of “BDNF was attenuated” in the legend did not match the data shown in the figure Panel C.

Response: Sorry for our flaws. In figure 5, we have changed the expression of “BDNF was attenuated” into “BDNF was upregulated” in the legends part on Page 24 considering our result. Thank you again for noticing and we are very sorry for our negligence.

Reviewer 2

In this article, authors demonstrated that EA stimulation in Baihui (GV 20) acupoint ameliorated learning and memory deficits and reduced A #42 deposit in APP/PS1 mice. Their hypothesis is the promotion of neurogenesis and the up-regulation of BDNF expression in the hippocampus and cortex of the APP/PS1 double transgenic mice.

1. It would be helpful if authors could offer the flow chart to clarify the experimental protocol.

Response: Thanks for the helpful suggestion. We have one flow chart of the experimental protocol in figure 2 but we might not be expressing ourselves as clear as we needed to. Thus we have adjusted the chart in the revised manuscript and added more information in the Method section. Hopefully it would meet your expectations.

2. Besides, it would be helpful if authors could mark how many mice they have used, not only in the figure legends, but also in the graphs.

Response: Considering the Reviewer’s suggestion, we double checked the number that we have offered in the figure legends and made up the left ones. However, as we reviewed some of the published paper and we could not figure out a good way for representing the mice number in the graphs. Therefore, we hope currently that the number we offered in the legends could meet the reviewer’s expectation and if not, we hope that the reviewer could give us some clue about how to
add them. We thank the reviewer for the helpful suggestion and we apologize for our current limitations.

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. We appreciate for Reviewers’ warm work earnestly, and hope that the correction will meet with approval. Once again, thank you very much for your comments and suggestions.

Sincerely yours,

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