Author’s response to reviews

Title: Acupuncture Attenuates Cognitive Deficits and Increases Total Neuron Number in Hippocampal CA1 Area of Vascular Dementia Rats

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Author’s response to reviews: see over
Response to Editor

Dear editor,

Thank you for your time and efforts. We are grateful for your and the reviewers’ comments and feedback on our manuscript entitled “Acupuncture Increases the Total Neuron Number in the Hippocampal CA1 Area of Rats with Multi-infarction Dementia” (MS: 1116072020150091) by Fang Li, Chao-Qun Yan, Li-Ting Lin, Hui Li, Xiang-Hong Zeng, Yi Liu, Si-Qi Du, Wen Zhu and Cun-Zhi Liu. According to the comments, we have finished revisions and polished the manuscript with a professional editing service – Editage. The main amendments have been highlighted in red in the revised manuscript. For details of the revisions please see the Response to Reviews attached.

Thank you again for your cooperation.

Sincerely yours,

Cun-Zhi Liu

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Response to Reviewers

Reviewer #1:

1) Physiologic saline in page 5, line 2 should be normal saline.

Response: We have revised “physiologic saline” to “normal saline” in page 5, line 3.

2) Text in the figure is not readable. In figure 2A, the above charts of synaptic bouton is not the same size as 4* in the neuron and astrocyte. And also, the author should explain clearly which area is enlarged in the above charts shown in the below.

Response: Thank you for your constructive suggestions. We mistakenly wrote the size of synaptic bouton as 4*. We have corrected the size of synaptic bouton to 5* (scale bar =500 µm) in figure legends in page 18, lines 25-28 of manuscript and independent figure 3 file.

3) In figure 2B, the reviewer can’t understand why n=4, since in each group there are 10-11 rats.

Response: When we performed the unbiased cell estimation, we respectively conducted staining for Nissl, glial fibrillary acidic protein (GFAP), or synaptophysin (SYN). Because of involving a variety of staining methods, we made the allocation of rats. According to a systematic random sampling procedure, the sections of immunohistochemistry staining can be conducted using two or more staining methods. However, Nissl staining is a different histochemistry staining method, and we can’t select the same 4 rats to conduct three staining methods. Therefore, we selected 4 rats to conduct Nissl staining, and another 4 rats to conduct GFAP and SYN staining in each group.
Reviewer #2:
The conclusion is too applausive to believe. The title of this paper is not fit for the content. Also some spelling mistakes exist. More evidences are needed to provide. Please think over and revise carefully.

Response: Thank you for your constructive suggestions. We have revised this title to “Acupuncture Attenuates Cognitive Deficits and Increases Total Neuron Number in Hippocampal CA1 Area of Vascular Dementia Rats”. We hope that the revised title will fit for the content. According to editorial comments, we have polished the manuscript with a professional editing service – Editage, and carefully revised the whole manuscript to avoid grammar or spelling errors. In addition, we have supplemented some additional revisions to provide more sufficient evidences in this manuscript. About the conclusion, we have rewritten it to avoid a definitive or bombastic statement. We hope the revised manuscript will provide a scientific and objective evaluation for acupuncture.
Reviewer #3:

1) The authors should identify the increased neuronal type (e.g. pyramidal cells or interneurons) in the hippocampal CA1 area by acupuncture treatment.

Response: In present study, we adopt the unbiased stereology method to quantificationally measure the number of neurons. But this method is not able to identify the increased neuronal type. In the future study, we will continue to study the neuronal type which may relate to cognitive deficits. And thank you for your constructive suggestions.

2) Acupuncture increases total neuron number in the hippocampal CA1 area, however, it is due to a reduction of cell death or an increase in cell proliferation?

The authors should provide direct evidence to distinguish them.

Response: Apoptosis (programmed cell death) is thought to be one of the contributors to cerebral ischemia induced neuronal loss (PMID: 12657369, PMID: 17762207). Our previous study (PMID: 18938189) showed that acupuncture decreased the number of apoptotic cells and expression of the proapoptotic Bax gene; on the contrary, it increased expression of the antiapoptotic gene Bcl-2. So we think that the increased total neuron number in hippocampal CA1 area by acupuncture might relate to a reduction of cell death. We will conduct further verification whether or not the increase in cell proliferation is related to the increase of total neuron number in hippocampal CA1 area for acupuncture in the future study.

3) Long-term potentiation (LTP) is a molecular mechanism underlying the learning and memory. Whether or not the hippocampal LTP is also involved in acupuncture-induced improvement of cognitive deficits in MID rats should be examined.

Response: We really approve this opinion of “Long-term potentiation (LTP) is a molecular mechanism underlying the learning and memory.” Our previous study (PMID: 25481359) have shown that acupuncture reversed cerebral multi-infarction induced impairment of LTP. In this text we have briefly mentioned long-term potentiation (LTP) and long-term depression (LTD) in page 11, lines 4-8.

4) Why the authors select a single acupoint of zusanli (ST36) for acupuncture? What
is the control for acupoint? What are the effects of other single acupoint or multiple acupoints for acupuncture?

Response: In “Discussion section”, we explained the selection of acupoint in page 10, lines 5-9. Based on our clinical trials and experiences, we choose the representative acupoint ST36 to explore the biological mechanisms of acupuncture for improving cognitive function in MID rats. In our earlier studies (PMID: 16181648, PMID: 16236447), multiple acupoints for acupuncture were performed, and we have also indicated that acupuncture improved cognitive impairment, but the mechanism of multiple acupoints is complicated, which may involve in different manipulation of acupoints, different effects of single acupoint, and the synergistic effect of acupoints. Considering the complexity of acupuncture research, we specifically select the representative single acupoint-ST36 for acupuncture. For the control acupoints, we select the non-acupoints beyond meridians at the hypochondrium (10 mm cranial to the iliac crest) bilaterally, which are far away from the verum acupoints. These control acupoints could not produce the interaction with verum acupoints, therefore the selected control acupoints are reasonable.

5) There are a lot of errors for the spelling and phrase in the manuscript, a professional language editing should be done carefully.

Response: According to your suggestions, we have polished the manuscript with a professional editing service – Editage, and carefully revised the whole manuscript to avoid grammar or spelling errors.