Author’s response to reviews

Title: Repeated electroacupuncture treatment attenuated hyperalgesia through suppression of spinal glial activation in chronic neuropathic pain rats

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Author’s response to reviews:

Re: Manuscript reference number:BCAM-D-17-01063

Dear Dr. Hee Young Kim,

We have submitted the revised version of our manuscript entitled “Repeated electroacupuncture treatment attenuates hyperalgesia through suppression of spinal glial activation in chronic neuropathic pain rats” by Junying Wang, Yonghui Gao, Lina Qiao, Jianliang Zhang, Chenglin Duanmu, Yaxia Yan, Shuping Chen, Junling Liu to the editor office for publication in BMC Complementary and Alternative Medicine. Many thanks to you and the reviewers for the insightful comments, which helps us a lot in improving the quality of our manuscript.
The following pages contain our point-by-point responses to each of the comments. Revisions in the text are shown using highlighting. We have also revised Figure 4 and Figure 6, which Reviewer 1 Weirong Fang pointed out.

We hope that our responses are sufficient to render our manuscript suitable for publication in BMC Complementary and Alternative Medicine.

Thank you once again for considering our submission.

Sincerely yours,

Junling Liu

Author’s Response to Reviewers’ Comments

Manuscript title: Repeated electroacupuncture treatment attenuates hyperalgesia through suppression of spinal glial activation in chronic neuropathic pain rats

Weirong Fang (Reviewer 1):

The authors studied the effect of EA in chronic constrictive injury (CCI) rats by left sciatic nerve ligation. The results indicated that repeated EA treatment significantly relieved CCI-induced mechanical and thermal allodynia on bilateral sides, and downregulated astrocyte and microglia activation. Overall it is an interesting paper, while need further revision.

Main comments:

1. Figure 2C: How to explain there are different tendency of Iba-1 in ipsilateral and contralateral sides?

Response: Thanks for the reviewer’s good question.
A similar tendency of increased Iba-1-positive cells on the ipsilateral and contralateral sides of the spinal cord was found on day 6 and 18 after CCI relevant to the sham group in Figure 2C, suggesting a substance foundation of existence of mirror-image pain in neuropathic pain rats. This phenomenon was also found in other studies as Reference 24, 25, etc. of the present manuscript, but the reasons remain unclear up to now, possibly due to CCI-induced central sensitization, descending regulation of higher central nervous system, …. Nevertheless, the increased number of Iba-1-positive cells on the ipsilateral side was far more remarkable than that on the contralateral side. The sciatic nerve afferents directly brought the nociceptive information to the ipsilateral spinal cord dorsal horn after CCI surgery. Then the ipsilateral microglial cells were activated and increased in the spinal cord dorsal horns. However, due to the mirror-image pain, a delayed activation of microglia occurred in the contralateral side. We had added this interpretation to the Discussion (see Discussion section line 23, page15).

2. Figure 4B: Sham (18), the "D" is missing.

Re: Thanks for the reviewer’s correction, we added the missed “D” in the sham (18D) of Figure 4B.

3. Figure 6B: The signature of Y-axis is missing. Besides, it is better to provide the time course of GFAP expression in CCI group.

Re: The missed signature of Y-axis was added in Figure 6 of the revised version, and the time course of GFAP expression in the CCI group was also added in the Methods section, Western blotting analysis, line 29, page8, (red words) and labeled in the revised Figure 6.

4. English writing need further proved and edit.
Re: Thanks for the reviewer’s criticism, we corrected many wrong words and sentences in the revised manuscript (labeled in red).

5. EA first inhibit microglia activation, and then inhibit astrocyte activation. While there is no difference between 6D and 18D in Iba-1 or GFAP activation. How to explain the switch of EA effect and mechanism?

Re: Similar to the thermal anti-nociceptive effect, EA treatment for twice mainly inhibited spinal microglial activation first, then the astrocytic activity after 2 weeks’ intervention. This finding is probably due to that the microglial activation in the spinal dorsal horns contributes to the initiation of hyperalgesia/ alodynia, while the astrocytic activation contributes to the development and maintaince of chronic pain, which is supported by some researches. However, the underlying mechanism remains unclear up to now and needs to be studied in the future. We added this interpretation to the Discussion.( Discussion section, line36,page16.)

Hsiu-Mei Chiang (Reviewer 2):

The present study aimed to study different roles of astrocytes and microglia of spinal cord in chronic neuropathic pain and electroacupuncture analgesia in chronic constrictive injury (CCI) rats. CCI induced a significant decrease of mechanical and thermal pain thresholds and increase of the number of Iba-1 labeled microglia and fluorescence intensity of glial fibrillary acidic protein (GFAP)-labeled astrocyte. Repeated EA treatment significantly relieved CCI-induced mechanical and thermal allodynia. Microglial activation was suppressed by 2 days' electroacupuncture treatment whereas astrocytes activation suppressed by 1 week's electroacupuncture treatment.

1. The point or location applied electroacupuncture is important. However, authors do not describe how they applied the electroacupuncture and it may cause different results in this study.
Re: Many thanks to the second reviewer’s criticism. We added the locations of the acupoints in the methods of the revised version. (see methods section, electroacupuncture treatment, line18, page 7)

2. How is the quantitation of immunofluorescence labeling done? How many samples get from one spinal cord section? How many spinal cord sections were measured for each animal?

Re: The calculation process of numbers of Iba-1 positive cells and the mean intensity of GFAP immunofluorescence was described in the Methods section of our manuscript, immunofluorescence multiple labeling, line 18, page 8. We selected three sections from each animal spinal cord tissue and five regions in each tissue section were measured for calculating the mean values.

3. The activity of the nerve may record during electroacupuncture to explain the effect and mechanism of electroacupuncture on CCI.

Re: Thanks for the reviewer’s good suggestion. In fact, we have made neuronal firing rates recording in CCI rats in another study (unpublished). We also recorded the electrical activity of neurons during electroacupuncture (EA) in another paper for explaining the effect of EA. See the paper “Electroacupuncture reduces the effects of acute noxious stimulation on the electrical activity of pain-related neurons in the hippocampus of control and neuropathic pain rats. “(PMID:27833763). For this study, we chiefly investigate activities of glial cells mediating the accumulative effect of repeated EA in the chronic neuropathic pain rats.

4. The No. of approved document of animals study must show in the text.
Re: Thanks the reviewer’s opinion, we added the No. of approved document of animals study in the Methods section, Animal, line 20, page 5.

5. There are many grammar and typing errors. Please revise the manuscript carefully.

Re: Many thanks to the reviewer’s criticism. We tried our best to correct many wrong grammar and wrong typing words in our revised manuscript.