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:

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Dear Dr. Hee Young Kim,

We have submitted a clean 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 for the comments which helps us a lot in improving the quality of our manuscript.

We could not open the address of English language editing service you recommended, so we used the free assistance from your English language tutorial. The following pages contain our point-by-point modification for the typing and grammatical errors.

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.

In the abstract

1) Line 3, page 2, “… its effect in downregulating glial cell activation of the lumbar spinal cord, ……”

Revised: “glia cell activation”.

2) Page 2 line 6 “…the roles of microgliacytes and astrocytes of spinal cord in the process of EA analgesia remains unknown.”

Revised: the letter ‘s’ of the word ‘remains’ should be removed.

3) Page 2 line 25 “The increased of number of microgliacytes was markedly suppressed by 2 days’ EA”

Revised: the first ”of” in “The increased of number of microgliacytes…” should be removed.

4) Page 2, line 27, “The expression of GFAP were down-regulated…”

Revised: “The copula “were” should be “was”.

In the “Background”

5) Page 3, line 12 “However, in recent years, it was found that glial activation as hypertrophy of astrocytes and ameboid shaping of microgliacytes ….. nerve injury or inflammation, tumor development, and many other neurological disruptions also contributes to … [1, 2].”

Revised: “However, in recent years, it was found that glial cell activation shown by hypertrophy of astrocytes and ameboid shaping of microgliacytes ….. nerve injury, inflammation, tumor development, etc. also contributes to … [1, 2].”

6) Page 3, line 19, “... synapses and an aberrant…”
Revised: “...their synapses and processes and an aberrant”

7) Page 3, line 16, “...interleukin-1 beta (IL-1β), interleukin-6 (IL-6), etc. which are also important in mediating pain [5,6].”

Revised: “... interleukin-1 beta (IL-1β), interleukin-6 (IL-6), etc. in the dorsal horns (DHs) of the spinal cord and brain, and thereby initiating the pain process [5,6].

8) Page 4, line 3, “...underlying mechanism requires further investigation.”

Revised: “... the underlying mechanisms are far more unclear”.

9) Page 4, line 3 “Previous studies showed that the analgesic effect of EA is mediated by descending pain inhibitory systems,...”

Revised: “Previous studies showed that the analgesic effect of EA is mediated by the descending pain inhibitory pathways ...”.

10) Page 4, line 6 “It was reported that..... partly mediated by inhibition of inflammator and … [13].”

Revised: “It has been revealed that ...... partly mediated by inhibition of inflammation and … [13].”

11) Page 4, line 11 “...produced a stronger anti-allodynia induced by tetanic stimulation of sciatic nerve than propentofylline or EA alone”

Revised: “... produced a stronger anti-allodynia relevant to propentofylline or EA alone”.

12) Page 4, line 22 “...in the spinal cord among which IL-1β, NGF and BDNF were involved in spinal cord ...... [17, 18]

Revised: “...in the spinal cord, some of which have been demonstrated to be involved in spinal cord ...... [17, 18].”

13) Page 4, line 25, “...sciatic nerve have not been known.”

Revised: “... sciatic nerve have not been determined.”
In the “Methods”

14) Page 5, line 17, “The section 1 was...”

Revised: “The 1st section was...”

15) Page 5, line 19, “... divided into 3 groups: sham CCI control (n=12), CCI model (n=12), and EA (n=12).”

Revised: “...divided into 3 groups: sham CCI control, CCI model and EA (n=12 in each).”

16) Page 5, line 19, “The section 2...”

Revised: “The 2nd section...”.

17) Page 5, line 24, “The 3rd section containing 30 rats were randomized into 5 groups”

Revised: “The 3rd section designed to observe the effect of EA on the expression of glial fibrillary acidic protein (GFAP) of the lumbar spinal cord. Thirty rats were randomized into 5 groups”.

18) Page 6, line 12, “The paw withdraw latencies...”

Revised: “The paw withdrawal latencies...”

19) Page 6, line 19, “Mechanical PWLs (response time, s; and force threshold, g.) of each rat’s plantar surface of hind paws were measured by using a DPA...”

Revised: “Mechanical PWLs (response time, s; and force threshold, g.) of the plantar surface of hind-paws were measured by using the DPA...”

20) Page 6, line 25, “...enclosure compartment on a glass pane of the aesthesiometer for 20 minutes

Revised: “...plastic enclosure compartment on the polymethyl methacrylate pane of the plantar tester for 20 minutes,...”
21) Page 6, line 25, “...the hind paw and the PWLs of the bilateral footplates were recorded, which was repeated 3 times, with an interval of about 5-10 mins between every two detections.”
Revised: “...hind-paw to detect the PWLs. The detection for each paw was repeated 3 times, with an interval of about 5 mins between every two tests.”

22) Page 7, line 20, “... then 4°C 4% paraformaldehyde solution in 0.1M phosphate buffer (PB, pH7.4).”
Revised: “ then 4% paraformaldehyde solution in 0.1M phosphate buffer (PB, pH7.4, 4°C).”

23) Page 7, line 23, “The lumbar spinal cord was sectioned...”
Revised: “ The spinal cord tissue was sectioned...”

24) Page 7, line 23, “...immunofluorescence labeling”.
Revised: “...immunofluorescence staining”.

In the “Results”

25) Page 7, reciprocal line 11 to the bottom: “The mechanical and thermal PWLs were approximate before surgery ...”.
Revised: “The mechanical and thermal PWLs were comparable before surgery...”

26) Page 10, reciprocal line 10 to the bottom: “…contralateral DHs were still markedly increased in the CCI group compared to that of the normal (P<0.01) and the sham groups (P<0.05, Fig 2 C).”
Revised: “…contralateral DHs were considerably increased in the CCI group compared with that of the normal and sham control groups (P<0.01, P<0.05, respectively)”.

27) Page 10, reciprocal line 3 to the bottom: “…suggesting no signs of activation, but ...”
Revised: “…suggesting no activation, but...”.

28) We removed the sentence of “... suggesting that the inhibition of spinal astrocytic activation plays a more important role in EA analgesia following CCI.” in line 15, Page 11.
29) Page 11, reciprocal line 4 to the bottom: “EA treatment down-regulated CCI induced GFAP over-expression”. Revised: “EA treatment down-regulated CCI induced over-expression of GFAP”.

30) Page 12, line 1, “…CCI+EA2W groups compared with that of…” Revised: “…CCI+EA2W groups relevant to that of that of…”

In the “Discussion”

31) We deleted the words “levels of” in page 12, line 14.

32) Page 12, line 17, “…spinal cord dorsal horns” Revised: “…spinal cord DHs”

33) Page 12, line 28, “…being different from the thermal hyperalgesia” Revised: “…and is different from the thermal hyperalgesia”

34) Page 13, line 7, “…pain (both time and force responses)” Revised: “…pain (shown by both time and force responses)”

35) Page 13, line 21-22 “The activated microglia and astrocytes synthetize and release some mediators including proinflammatory cytokines,…” Revised: “The activated microgliacytes and astrocytes synthetize and release some mediators as proinflammatory cytokines,…”

36) Page 13, line 26-29, “…microglial cells (increase in number, and ameboid shape with hypertrophic and hyperplastic bodies and fewer processes) and astrocytes (increase in immunofluorescence intensity of GFAP and hypertrophic body with thicker processes) was found…”
Revised: “...microglial cells (shown by increase in number and appearance of ameboid shape with hypertrophic and hyperplastic bodies and fewer processes) and astrocytes (marked by increase in immunofluorescence intensity of GFAP and occurrence of hypertrophic body with thicker processes) was found...”

37) Page 14, line 2 “The sciatic afferent nerve directly brought the nociceptive information to the ipsilateral spinal cord DHs after CCI surgery. Then the ipsilateral microglial cells were activated and increased in the spinal cord DHs. However, due to the mirror-image pain, a delayed activation of microglia occurred on the contralateral side.

Re: “On day 6 and 18 after surgery, along with the decrease of PTs, the number of microglacytes and the fluorescence intensity of GFAP were apparently increased in DHs on the contralateral side despite of being markedly lower than on the ipsilateral side.

Theoretically, the sciatic primary sensory afferent nerve fibers may take priority to carry the nociceptive information of the focus to the spinal cord DHs on the ipsilateral side and then to the higher nerve center after CCI surgery, inducing pain and protective motor reflex. It is reasonable that the neurons, microgliacytes and astrocytes were activated first in the ipsilateral DHs, followed by mirror-image pain-induced activation of glia cells in the contralateral DHs and ventral horns.”

38) Page 14, line 19, “...and regulated the time of induction...”

Revised: “...activation to mediate an induction...”

39) Page 14, line 23, “Previous study...”

Revised: “A previous study..”

40) Page 14, line 25, “... repeated EA as a longer intervention measure may be useful for”

Revised: “... repeated EA may be useful for...”

41) Page 14, line 26, “...it was demonstrated that the effects...”

Revised: “...it was found that the effects...”
42) Page 15, line 3, “...treatment in CCI rats.”
Revised: “...in CCI rats in the present study.”
43) Page 15, line 8, “...neuropathic pain, while the inhibition...”
Revised: “...neuropathic pain and the inhibition...”
44) Page 15, line 9, “...phase of neuropathic pain, which are similar...”
Revised: “...phase, which is similar...”
45) Page 15, line 10, “...astrocytic activation may be responsible ...”
Revised: “...astrocytic activation is responsible ...”
46) Page 15, line 13, “...longer, in parallel with a decreased nociceptive threshold”
Revised: “...longer, and was in parallel with the hypersensitivity.”
47) Page 15, line 17, “Astrocytic activation...”
Revised: “Astrocytic inactivation...”
48) We added “... which is basically identical to Mi’s and colleagues’ results that the analgesic effect of EA was closely related to the upregulated expression of spinal neurotrophin-3 protein and gene via inhibition of spinal GFAP (astrocytic marker) in inflammatory pain rats [47]” in the page 15, line 19 behind “the present study”.
49) Page 15, line 21, “Repeate EA for over 1 week suppressed the activation of astrocytes, but 2 days’ EA could not, which is in parallel with changes of mechanical pain”
Revised: “Moreover, repeated EA for over 1 week (not 2 days) suppressed the activation of astrocytes, which is in parallel with an increase of mechanical...”
50) We deleted the paragraph “How the glial cells play its role in the process of EA analgesia. Our previous study showed that cytokines and neurotrophic factors in the spinal cord may be closely associated with astrocytes in the spinal cord in the process of EA-induced relief of chronic pain [6]. Spinal NT-3 was involved in the analgesic effect of EA via inhibition of
expression of spinal GFAP (an astrocytic marker), OX-42 (a microglial marker) as well as proinflammatory cytokine IL-1β, IL-6 and TNF-α [47]. EA also mediated its analgesic effect by attenuating interferon-γ (IFN-γ) release and subsequent generation of P2X4 receptor in spinal cord microgliacytes [48].” in page 15.

51) Page 15, line 26, “In the present study, EA treatment for twice or 2 weeks had no effect on the resting astrocytes or microglia on day”

Revised: “In the present study, EA treatment for twice or 2 weeks in sham CCI rats had no apparent effect on the activities of astrocytes and microgliacytes of DHs of the lumbar spinal cord on...”

52) We removed the paragraph “… indicating that the analgesic effect of EA is definite and repeated EA intervention has few side effects. Intrathecal or intraperitoneal injection of glia inhibitor propentofylline relieved tetanic stimulation of sciatic nerve induced mechanical hyperalgesia and inhibited tetanic stimulation of the sciatic nerve (TSS)-induced activation of microglia and astrocytes. EA combined with low dosage of propentofylline produced more potent anti-allodynia than propentofylline or EA alone [15]. For its advantage, EA treatment combined with low dose of medicine may be a prospective clinical therapy to forestall the side effects of long-term pharmaceuticals for chronic pain.”

Revised: “Therefore, for chronic conditions, a longer period of EA intervention is necessary and has fewer side effects.” in page 16, line 1.

53) Page 15, line 26, “…treatment for relieving chronic…”

Re: “… EA treatment procedure for relieving...”.

54) The last reference 48 was deleted.

55) The word “microgliacyte” was instead of “microglia” in the manuscript.