Author's response to reviews

Title: Activation of Amygdala Opioid Receptors by Electroacupuncture of Feng-Chi (GB20) Acupoints Exacerbates Focal Epilepsy

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Author's response to reviews: see over
We thank the referees for finding our work of interest and for agreeing with us that this work is a valuable contribution to the field. The referees spent considerable effort in the review of this manuscript, and we thank referees for their comments and suggestions. We have incorporated many of suggestions into this revised version of the manuscript. Specific changes are delineated in the text of manuscript by red font and highlighted in yellow, as per instructions from the editor. We provide a detailed list of our responses here:

Reviewer #1:

Minor Essential Revisions
The rat pilocarpine epilepsy model is usually induced through systemic administration of pilocarpine, but, in this article, it is done through the administration of pilocarpine into the left CeA. How about the reliability of this kind of rat epilepsy model? Is it related to the kind of the model that the treatment is invalid? Or is it related to the stimulus parameter such as the stimulus time and frequency? I suggest that the author should explain these problems carefully.

Response: We have validated the reliability of focal epilepsy induced by administration of pilocarpine into the left CeA by multi-electrode EEG recording in this study. The predominant epileptiform EEGs were recorded from the left parietal electrode and some epileptic activities were also acquired from the left occipital electrode when administration of pilocarpine (1 mg) into the CeA in rats of group 1, whereas no epileptic activity was recorded from the rest of four electrodes (Figure 2). These statements were found on page 11, lines 6-9. The reason for us focusing on the effect of EA on suppressing focal epilepsy is that we would like to preliminarily determine the EA effect on epilepsy with subtle epileptiform activities (when compared with generalized epilepsy), since there is no scientific evidence to prove the epileptic suppression by EA of Feng-Chi acupoints. If EA of Feng-Chi acupoints exhibits suppression in focal epilepsy, then the effect of EA on generalized epilepsy would be evaluated. Unfortunately, EA of Feng-Chi acupoints exhibits no epileptic suppression in focal epilepsy. We added the detail to explain why we targeted on focal epilepsy in the discussion section on page 14, lines 1-12. “The goal of this study is to elucidate the effect of EA stimulation of Feng-Chi acupoints on epileptic suppression. Epilepsy can be divided into focal and generalized epilepsy according to classification proposed by International League Against Epilepsy (ILAE). Focal epilepsy is usually subtle and the epileptiform activity starts in one area of brain and may spread to other brain regions. In contrast, generalized epilepsy, which is more severe than focal
epilepsy, is result of abnormal brain activity in both hemispheres. In this study, we would like to first determine whether EA of Feng-Chi acupoints suppresses focal epilepsy. Systemic administration of pilocarpine in rats leads to a pattern of generalized seizure and status epilepticus [25]. However, the reliability of focal epilepsy induced by administration of pilocarpine into CeA has been confirmed in this study (Figure 2). We found that epileptiform EEGs were primarily recorded from the left parietal electrode near left CeA, but were not acquired from electrodes implanted on the right hemisphere, when EEG signals were acquired by multiple electrodes on both hemispheres.”

Discretionary Revisions

1. The authors suggest that high-frequency (100 Hz) EA stimulation of bilateral Feng-Chi acupoints has no effect to protect against pilocarpine-induced focal epilepsy. However, the acupoints of Feng-Chi had been indicated the effect of epileptic suppression documented in the Lingshu Jing. The authors should explain the reason in detail.

Response: We have added the statements “These observations, since they subvert the functions of Feng-Chi acupoints documented in the Lingshu Jing, surprise us. The possible reasons of contradiction between our findings and the documentation in Lingshu Jing are as follows. First, with or without delivering electrical currents into Feng-Chi acupoints is a fact. The effect of epileptic suppression documented in Lingshu Jing is manipulated by dry needling, whereas the exacerbation of epilepsy we observed in this study was the results after EA with delivering currents into acupoints. Second, different stimulation frequencies may differ the outcomes. It is worthy to investigate the effect of different EA stimulation frequencies, especially for the lower frequency (e.g., 10 Hz), on the epileptic activity.” on page 15, last line and on page 16, lines 1-8 to explain in detail.

2. In my opinion it will be better to illustrate the Experimental protocol of all the 9 groups with a table, so it can be understood easily.

Response: We added Figure 1 to depict the schematic representation of the experimental protocol.

Quality of written English: Needs some language corrections before being published

Response: We revised the language errors throughout the text.
Reviewer #2:

The manuscript is well written and easy to follow in spite of some awkward sentences and terms every now and then—I’ve marked some of these and they should be fixed before publication. The experimental work is very good and the authors managed to communicate very well their results.

**Response:** We corrected those sentences and terms according to the reviewer’s suggestion and revised the language errors throughout the text.

We believe these answers could respond to most of the referees’ suggestions and the changes in text would make this manuscript suitable for published in *BMC Complementary & Alternative Medicine*.

Best regards,

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