Author’s response to reviews

Title: Electroacupuncture at LI11 Promotes Jejunal Motility via the Parasympathetic Pathway

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Author’s response to reviews:

Dear editor:

We appreciate sincerely the comments and suggestions made by you and the reviewers on our manuscript "Electroacupuncture at LI11 Promotes Jejunal Motility via the Parasympathetic Pathway" (BCAM-D-16-01212). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have carefully revised the manuscript accordingly and hope you would find this version acceptable for publication. Point-by-point responses to reviewers’ comments are attached.

Thank you very much for your favorable consideration of this manuscript.
Best regards,
Hu xuanming and Xu bin

To reviewer 1:

Comment 1: The vagal fibers release ACh at the enteric ganglion, which acts on nicotinic receptors of these neurons. These neurons then release ACh to stimulate the muscarinic receptors of the jejunal smooth muscle. It would be better to use nicotinic antagonists to block communication between vagal nerve and enteric neurons.

Response to Comment 1:

According to your suggestion, we supplement the experiment that used rats with gastric vagotomy. After separate and cut off the gastro-vagal nerves, we observed the movement of jejunum with the same method. The result (see Figs. 4 A/B/E) showed that baseline of intestinal pressure lowered and the pressure could not be changed by EA at LI11 with the same intensity and during when gastro-vagal nerves were cut off (Page 6, line 28). We believe this result can further complement the rationality and integrity of our whole study, and make the results more credible.

Comment 2: Why is the percentage change in motility in the beta1/2 knockout more robust in terms of maximal response compared to wild type? The curve is also S shaped.

Response to Comment 2:

We used the gene knockout mice (β1β2/-/- mice, M2M3/-/- mice) to further verify our hypothesis. The result of this section was presented in Figs.6 D/E/F and G by fitting curve. It is believed to be the exciting effect when the change rate of jejunum movement exceeds 105%. The result showed that in WT group, the effect of EA started to appear at intensity of 1mA, reached a plateau region and did not change with the enhancement of EA intensity anymore when the intensity increased to 4 mA. (Page 7, line 13)

In β1β2/-/- mice, the change rate was similar to that of WT mice at intensity of 1mA to 4mA. There was a sudden increase in change rate when EA intensity increased to 4mA. But it also
reached a ‘plateau region’ and did not change with the enhancement of EA intensity anymore when the intensity increased to 6mA. (Page 7, line 19)

In M2M3/- mice, it had a slight change (did not exceed 105%) when EA intensity is at 1mA to 2mA and did not change anymore when intensity is higher than 2mA (Page 7, line 19). According to our result, we believe the effect of stimulation at LI11 is mainly through the parasympathetic-M receptor. The mutual antagonistic effects between sympathetic and parasympathetic nerves may be the reason why the change rate of beta receptor knockout animals caused by acupuncture is stronger than that of WT rats. In this study, we mainly observed the parasympathetic pathway that involved in EA at LI11, but not carried out further research on the sympathetic pathway. We will discuss it in the further research.

Comment 3: Is there any evidence that EA inhibits sympathetic activity?

Response to Comment 3:

In this study, we mainly focused on the parasympathetic pathway that involved in EA at LI11. We can only prove that sympathetic nerve did not take the predominant role when EA at LI11, but no sufficient evidence was found to prove whether stimulating at LI11 could inhibit sympathetic nerve activity.

However, our previous work showed that stimulating at ST37 (shangjuxu) could excite parasympathetic nerve[1] and stimulating at ST25 (Tianshu) could excite sympathetic nerve[2]. All these results could supplement and verify the scientific theory of “homotopic” and “heterotopic” acupoints proposed by Zhu Bin et al. [3-7]

Reference


Comment 4: The text in results and the legend for figure 2 do not match the arrangement of the data in Figure 2 where tracings A and B are from rat and C and D are from mouse.

Response to Comment 4:

We are sorry that we made a mistake in Fig2. The labels on y-axis of mice in Fig 2-5 were wrong, because we directly copy the labels of rats when we made the figures. We have corrected it in the revised manuscript. The picture below is a screenshot during our experiment in WT mice group with EA stimulation at LI11 (2 mA, 1 min).
To reviewer 2:

Comment 1: In order to claim the involvement of parasympathetic nerve pathway as mediator of EA, authors should provide direct experimental data on vagus nerve activity; specifically, the requirement of vagus nerve activity for EA-regulated GI motility can be examined in vagotomized animals.

Response to Comment 1:

We had some similar experiments about the law of effect about EA at acupoints, such as ST37 (Shangjuxu) and ST25 (Tianshu) [1,2]. Refer to the use of agonist and antagonist, we realized that intravenous administration we used makes the drug acting on the whole body, rather than the target nerves that we focused on [3-8]. Therefore, it could not specifically verify the role of target nerves we want to observe. However, the effect of Electroacupuncture on gastrointestinal motility has been fully confirmed.

Fig 3 and Fig 4 showed EA has the same law of effect in different basal states of animals after the application of clenbuterol, propranolol and Ach. We want to verify the law of effect and mechanism of stimulating LI11 from this result. In another word, we believe that EA at LI11 could promote the movement of jejunum through parasympathetic nerve.

According to your suggestion, we supplement the experiment that used rats with gastric vagotomy (Page 6, line 28). After separate and cut off the gastro-vagal nerves, we observed the movement of jejunum with the same method. The result (see Figs. 4 A/B/E) showed that baseline of intestinal pressure lowered and the pressure could not be changed by EA at LI11 with the same intensity and during when gastro-vagal nerves were cut off. We believe this result can further complement the rationality and integrity of our whole study, and make the results more credible.


Comment 2: Another issue is that authors did not provide experimental evidence properly demonstrating the specificity of administered drugs on their target nerves. There is no indication how specifically or nonspecifically iv injection of individual agonists and antagonists affected their target tissue and other tissues. Moreover, a rationale for the use of β1β2-/- mice and M2M3-/- mice needs to be specified clearly along with relevant references.

Response to Comment 2:

In our experiments we used intravenous administration that makes the drug acting on the whole body, rather than the target nerves that we focused on. Therefore, it could not specifically verify the role of target nerves we want to observe. To make up for this deficiency, β1β2-/- mice and M2M3-/- mice were used as supplements. The model we used is very common and mature in our related experiments and its reliability has been demonstrated by other researchers [1]. We added the related references into our revised manuscript. Meanwhile, we supplemented PCR experiment to verify the reliability of gene knockout animals we used and the result was showed in revised manuscript (S Fig. and S Files).

Reference


Comment 3: There are some uncertainties on data presented in the manuscript. First, statistical data comparing pre-EA and dur-EA groups (Fig 2 and 3) are not quite convincing to this reviewer; the difference seems to be quite small although authors claim that differences are significant. Also, a statistical comparison among control, agonist and antagonist groups (One-way ANOVA) should be presented in the figures. Finally, diverse symbols indicating statistical significance in several graphs are very confusing.
Response to Comment 3:

Refer to the data statistics, we verified and analyzed our data again under your advice. We also made some adjustments in result reporting and cartography to make it clearer and easier to understand. We have shown the statistical comparison among control, agonist and antagonist groups used “change rate of jejunal motility” shown in Fig 4E and Fig 5E, because the baseline of each group was different and we mainly focused on the D-value between pre-EA and dur-EA.

Comment 4: There are several parts that need to be corrected.

Response to Comment 4:

We are sorry that we made a mistake in Fig2. The labels on y-axis of mice in Fig 2-5 were wrong, because we directly copy the labels of rats when we made the figures. We have corrected it in the revised manuscript. The picture below is a screenshot during our experiment in WT mice group with EA stimulation at LI11 (2 mA, 1 min).

Besides, we also corrected other mistakes in our manuscript.