Author's response to reviews

Title: Electro-acupuncture decreases 5-HT, CGRP and increases NPY in the brain-gut axis in two rat models of Diarrhea-predominant irritable bowel syndrome (D-IBS)

Authors:

Jianhua Sun (81485166@qq.com)
Xiaoliang Wu (wxl20021116@aliyun.com)
Yunfang Meng (34651929@qq.com)
Jie Cheng (384820666@qq.com)
Houxu Ning (ninghouxu@sina.com)
Yongjun Peng (zylgmr@163.com)
Lixia Pei (11801758@qq.com)
Wei Zhang (582595828@qq.com)

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Author's response to reviews: see over
Dear professor,

On behalf of my co-authors, we thank you very much for giving us an opportunity to revise our manuscript, and appreciate reviewers very much for their positive and constructive comments and suggestions on our manuscript entitled “Electro-acupuncture decreases 5-HT, CGRP and increases NPY in the brain-gut axis in two rat models of Diarrhea-predominant irritable bowel syndrome (D-IBS)”.

We have revised the manuscript which marked in red in the paper, according to previous comments and suggestions of reviewers, and responded, point by point to, the comments as listed below.

We would like to re-submit this revised manuscript to BMC Complementary and Alternative Medicine, and hope it is acceptable for publication in the journal. Looking forward to hearing from you soon.

With kindest regards,

Correspondence should be addressed to Xiaoliang Wu, wxl20021116@aliyun.com, Chinese Medicine Hospital of Jiangsu Province, 155 Hanzhong Road, Nanjing 210029, Jiangsu Province, P.R.China.

Response to reviewer 1:

1. **Response to comment**: The INTRODUCTION is too short, failing to provide sufficient information concerning benefits of acupuncture on IBS, role of abovementioned neurotransmitters in IBS process particularly in the mediation of acupuncture induced therapeutic efficacy.

   **Response**: We are ashamed for our unsatisfactory writing level and thank you very much for your cautious correction. After receiving your email, we spent a lot of time making some small changes marked in red in the paper to improve the INTRODUCTION and writing quality.

2. **Response to comment**: The authors gave a detailed description of the METHODS, though inappropriate in sequence. Notably, only ELISA was invoked to assess the changes of the neurotransmitters in this study. Apparently, additional means like immunohistochemical and or Western blotting are required, as it usually goes, to support the reliability of the data.

   **Response**: In fact, we have made the test on immunohistochemistry, EMG measurement, PCR assay and confocal Cajal cells, etc., but for the words limited, part of the data have been submitted, and the main purpose of this paper is intended to explore the role of three neurotransmitters in the electric acupuncture regulating in IBS brain-gut axis through data analysis. We found that 5-HT related between EA and the brain-gut axis most closely, and the importance of 5-HT in IBS has also been recognized internationally at present, analysis of the test data also provided evidence for our ongoing research about acupuncture regulating 5-HT receptor gene knockout IBS rat model, we hope to have the opportunity to further share relevant results.
3. **Response to comment**: It is of great significance to link the bioactive molecules with behaviors. In the RESULTS, the authors presented by and large a comprehensive profile of the multiple variables and their potential correlations. Due to the lack of bar figures, however, it is not readily for the readers to get to the point.

**Response**: Considering the limitation of the article page, we didn't put the graph in the paper of the data part, but with the form to provide the real data. Thank you very much for your advice, we replace the data table with digital bar graphs in this paper to better illustrate enrollment the data results.

4. **Response to comment**: The DISCUSSION is not deep-going. It is generally held that the vagus nerve takes critical role in the brain-gut axis. What are the impacts of 5-HT, CGRP and NPY on vagal activity and its neurotransmitter (Ach)? Why the lumbar intumescentia (L4-6) was targeted in this work? It should be noted that these spinal segments have no much relationship with the distal colon, either sympathetically or vagally or parasympathetically. In addition, the limitations of the study were not fully stated.

**Response**: The results in this study show that 5-HT, CGRP and NPY contents of in the IBS model rats distal colon, hypothalamus and Spinal Cord have the abnormal change all, and in three different tissues have the same trend which means the EA can achieve a therapeutic effect on IBS by adjusting brain-gut axis. In this study design, lower thoracic intumescentia associated colon observation may be better, but at least the results of this paper confirmed the distal lower limb acupoints exist the overall regulation of intestinal by electrical stimulation.

5. **Response to comment**: Quality of written English: Needs some language corrections before being published.

**Language**

*Overall, the manuscript would need some editing.*

Line 77: Part of the rat subjects received…

Line 83-84: NanJing JiSheng Medical Treatment Science and Technology…

Line 101: Rats were sacrificed on the second after…

Line 110: Numerical variables are expressed as means±SD, and used analysis of variance…

Line 122: In the head column of table 1, “times” ;

Line 141-142: Both the CAS and ISC conditions can decreased CRD…

Line 160: …content of spinal root…

The title of table 2: …between arious factors…

Line 176: In combined with correlation matrix…

**Response**: We are ashamed for our unsatisfactory English and thank you very much for your cautious correction. The above mentioned language corrections have also been edited. We made some small changes marked in red in the paper to improve the English expression.
Response to reviewer 2:

1. **Response to comment:** 1) The use of pellet output / fluid volume by AWR may not be an accurate method to rate the CRD due to the fact that pellet may be watery and AWR is highly dependent of the observers. Electromyogram (EMG) signals are more objective measurements. EMG from the oblique muscles should be used for the correlation with the expression of 5-HT, CGRP, NPY content in these experiments. 3)How many observers do you have for the AWR?

   **Response:** Because of our initial experiments plan limited, in this test section, two professional training observers were to record and collect data, and by calculating the average of the two to reduce the error, and we have in the current experiment using Electromyogram (EMG) signals recognized authority at present. Of course, the methods in the process of the experiment are also susceptible to the interference of other factors, which influence the result data.

2. **Response to comment:** For logistic regression, although the fluid volume required to reach an AWR value is nominal data. However, AWR is an ordinal rating by different observers. It may be inappropriate to correlate the relationship of fluid volume through logistic regression. Each categories should be replaced by Spearman's correlation by non-parametric analyses.

   **Response:** In fact we have not found significant correlation by spearman correlation analysis? in this data analysis. Spearman correlation analysis are non-related parameters, because of the reduced conditions of application, the scope has expanded, and it cannot mean the actual value of the raw data, by calculating the correlation coefficient using only the size of the order (with the rank) of the original data. We observed AWR data by the box diagram, making a box for each class diagram hierarchically arranged from low to high, the average number of each class or median connection. Considering this line trend and each level of AWR data analysis meets the condition of bivariate normal distribution and combination with fitting linear model, we carried on the linear regression analysis to all the data.

3. **Response to comment:**: Minor Essential Revision:1) typo mistake on Table 2 "X # CGRP in spinal cord"; 2) Table 1 is unable to show all the p-Values from the Pdf File.

   **Response:** We are ashamed for our unsatisfactory English and thank you very much for your cautious correction. The above mentioned language corrections have also been edited. We made some small changes marked in red in the paper, and we replace the data table 1 with digital bar graphs in this paper to better illustrate enrollment the data results.

**Discretionary Revision**

1) Did you actually rate the state of the stool? A rating should be used to record the state of stool as well? Water content of feces expelled by the rats may be measured for a better correlation to the IBS-D symptoms.

   **Response:** Thank you for this good advice, and we will pay more attention to the subjective index in experiment to analyze from the aspects of rating.