Author's response to reviews

Title: The Effect of Long vs. Short Duration of Electroacupuncture on Analgesia and Sensory Thresholds: A randomized study for acupuncture analgesic mechanisms

Authors:

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

Thank you for the recent review. We appreciate the reviewers’ constructive input. Based on the reviewers’ comment and recommendation, we have added additional clarification and information to the manuscript. Changes in the manuscript were underlined in bold italic format. Our responses to the reviewers’ comment are discussed as follows:

Reviewer #1: Sabine Sator-Katzenschlager
1) Is there a gender difference in analgesia and cold and warm threshold?

Response: With only 6 male subjects enrolled, this study was not specifically powered to detect gender differences, but as an exploratory analysis, we attempted to identify possible differences between males and females in either a) baseline thresholds and VAS ratings or b) response to stimulation. This analysis revealed no reliable gender differences, and accordingly subjects were pooled for all subsequent statistical analyses.

Reviewer #2: Zhen Zheng

Reviewer’s report:

Title
It is adequate. However the part “a randomized study for acupuncture analgesia mechanisms” can be deleted as the word “randomisation” often indicates more than one group being involved. In addition, this is not exactly a mechanism study. If this was one of the aims, then it has not been well articulated in the manuscript. Minor revision

Response: The title of the manuscript was revised as per reviewer’s recommendation.

Abstract
Again, I recommend deleting "randomized study". Also the conclusion needs to be revised. Please see my comments on the Conclusion.

Response: The abstract was revised as per the reviewer’s recommendation.

Introduction
This section needs major revision. Although the benefit of acupuncture for clinical pain and the use of Ting point stimulation were explained, this section did not give a clear rationale why the duration of EA should be studied and why thermal sensory detection thresholds should be assessed when the main aim was to study the analgesic but anaesthetic effect of EA. Compulsory changes

Response: We have conducted several studies in the past to assess the effect of a short duration of EA in the TMM system on the peripheral sensory thresholds which serve as a way to revealing the potential underlying neuronal mechanisms. This study expanded our ongoing investigation by changing the stimulation duration parameter. By doing so, we were able to assess the corresponding change of peripheral sensory thresholds (thus the underlying neuronal mechanisms) in correlation with the behavioral response. The INTRODUCTION section of the manuscript was revised with additional information to support the rationale to study the effect of acupuncture duration on analgesia and modality specific peripheral sensory thresholds as a means to assess the underlying analgesic mechanisms.

Methods
My main concerns about this section are statistics and the temperature of the testing room. The choice of four-way ANOVAs with repeated measures is correct. However, it is not clear what post-hoc analyses were used.
Response: Information in regards to the post-hoc analyses and testing room temperature was added to the manuscript.

In the Results, the authors did mention that post-hoc ANOVAs were used with Bonferroni corrections. If so, then p value should be divided by the number of comparisons. Please add the description of post-hoc analyses to the Methods.

Response: P-value was held at a constant overall (family wise) error rate of 0.05, and this is now explicitly stated in the results (overall p < 0.05); the reviewer is correct that this necessitates the actual p-value to be divided by the number of comparisons made, but convention in the literature is typically to report the overall error rate that was maintained via the Bonferroni correction, and this is how we now present it. The von Frey data are not fully continuous, but others have published with ANOVA/ t-test analysis of the data. In our study, the effects on von Frey thresholds were essentially undetectable. To maintain consistency in the manuscript, and avoid complex nonparametric modeling that could mimic the 4-factor ANOVAs we performed (and complication of the manuscript by explanation of that modeling), we chose to report the data consistent with all other data in the manuscript. ANOVA and t-test can be fairly robust even when the assumption of continuity is mildly violated as it is by Von Frey threshold calculations.

Finally was the room temperature controlled during the tests? It has a strong impact on the thermal detection and pain thresholds. - Compulsory changes
Response: Information in regards to ambient temperature control was added to the Method section of the manuscript.

My other comments are as follows. 🔄 minor essential revision
◆ Please include the name of the human subject review committee.
◆ Please move the additional information about inclusion and exclusion criteria to this section.

Response: Name of the human subject review committee was added. And additional inclusion and exclusion criteria information was added.

◆ Were subjects screened for a history of chronic pain? Were female subjects tested during pre-menstrual stages? As we know, both chronic pain and menstrual cycles impact on pain thresholds.

Response: All subjects were screened for a history of chronic pain. Female subjects were screened for pregnancy. However, we did not limit the study to any particular menstrual stage given that this is a cross-over study.
◆ Were people with cardiac pacemakers excluded?

Response: Yes. None of subjects participated in the study had a pacemaker.

◆ Include the name of the manufacturer of Von Frey filaments.
◆ Delete repeated description of EA treatment.

Response: The manufacturer information of von Frey Filaments was added to the manuscript and similar description of EA treatment was deleted.

Results
My main comments here are related to the presentation of the data. 

â¢ In the figures, it is important for the readers to understand what the comparisons are. Location? Time? Duration? I suggest you use different symbols representing various types of comparisons.

â¢ Are significant results, indicated in the figures, the outcomes of post-hoc analyses?

â¢ In the PDF version I have, Figure 5 missed a few indicators on the X axis. In addition, what is the comparison indicated by the perpendicular line?

â¢ Only means were presented. Standard Deviations or Standard Error of Means need to be presented in the figures or in tables.

Response: For each dependent measure (Hot Pain VAS, Warm Threshold, Cold Threshold, etc) we have calculated an average standard deviation for each Duration of Stimulation (5, 15, 30 min). The average standard deviation is based on all data points across time (pre-, post-0, post-30, etc) and location (ipsilateral/contralateral, thigh/calf) at a given duration of stimulation. These average standard deviations are an excellent approximation of the actual standard deviation for any given data point on our figures, because the standard deviations for individual data points are all very close to each other. These average deviation values have been inserted into each figure for convenient reference by the reader. We did not wish to add standard errors to each data point because when we did, the figure was rendered virtually unreadable from an endless series of vertical lines representing the error bars, making the data very difficult to appreciate. Because standard deviation is a between subjects measure of variation, and our entirely within-subjects analysis depends more on within-subjects variance across repeated measurements (e.g. location, time pre/post-stimulation) than it does on between subjects variation, we did not wish to obscure the figures with error bars that do not in fact represent the relevant source of error for our statistical analysis methods, and have
instead placed the average deviation values into the figure as an excellent approximation of actual error bar for any given data point. Figures were relabeled and clarified as per reviewer’s recommendation.

*I suggest removing the figure about De Qi.*

Response: The figure of about De Qi was to illustrate the effect of acupuncture analgesia outlasted the sensation of acupuncture and also to showed that the pattern of the De Qi sensation has a gradual decline pattern in light of no change in the stimulation parameter. In order to illustrate this effect, we believe the figure may be necessary.

Why were the data of cold pain threshold not included?

Response: IRB stands for Institutional Review Board which is the same as Human Subject Review Committee. Clarification was made in the manuscript. Due to large variance in the cold pain data, we were not confident that we could adequately draw a conclusion based the cold pain data set. Therefore, since there are quite a few other figures already presented with the manuscript, the authors choose no to show the cold pain figure.

Discussion

This part requires major revision. There is little discussion on the effect of different durations of EA. I. Being the main aim of the study, this finding should be compared with previous studies on this topic. compulsory changes

Response: Similar studies which assess the effect of duration in both human and animal models were included in the discussion.

Furthermore, the finding of increased warm and hot pain thresholds and decreased cold detection and pain thresholds after EA requires further
explanation. The analgesic effect of EA on heat pain is consistent with the findings of other studies. I am, however, surprised to see that EA induced a widespread cold hyperalgesia in this study. This unexpected finding has not been explained. Was this due to the testing time or day, the room temperature or the effect of EA on sympathetic nervous system? - Compulsory changes

Response: Information regarding room ambient temperature control was added to the manuscript in the method section. In the study result section, we reported a main effect of location, time and side. In the cold pain threshold, we reported effects of time and duration based on the ANOVA analysis. However given the large variance of cold pain (c-fiber mediated) threshold among subjects as observed in previous studies as well, we did not feel we can draw a meaningful conclusion from this particular set of data and therefore, no figure was shown. On the hand, since the data from the hot pain and warm thresholds, both of which are c-fiber mediated as well, and consist of much less degree of variance than the cold pain threshold. Therefore, we feel we can confidently make our mechanistic interpretation based on these data sets instead.

The authors intended to explore the mechanisms of the spread of EA analgesia and the different effects on cold and warm detection and pain thresholds. However the proposed mechanisms were not clearly explained. For instance A-delta mediated C-fibre modulation implies that EA stimulation in this study mainly activated A-delta fibers. There is however no evidence to support this hypothesis. It is commonly accepted that the sharp needling sensation upon insertion is likely to be mediated by A-delta fibres. The authors did not provide any data to support the claim that the tingling sensation was also mediated by A-delta fibres. Â Compulsory changes

Response: With the result of the current study, we felt that the initial acupuncture effect was mainly A-delta mediated as reflected by the sharp
The needling sensation of the de qi components. However, the sustainable analgesic effect of EA long after the de-qi sensation has subsided is most likely a centrally mediated mechanism as reflected in change of both A-delta and C-fiber mediated thermal threshold. Additional discussion and information was added to the manuscript to support the assertion that tingling component with the EA de qi sensation is mostly likely mediated by A-delta afferents.

The recommendation of CV 2 is not necessary since the additional effect of this point was not one of the aims of the study. ***minor essential changes***

**Response: We have deleted this section in the manuscript.**

**Conclusion**

Please limit this section to the findings and main aims. Some sentences can be deleted. ***Compulsory changes***

**Response: We have revised the conclusion section accordingly as per the reviewer’s recommendation***

We believed we have made all the necessary change to the manuscript based on the reviewers’ recommendation.

We thank you for time for reviewing this manuscript. If there is anything else that you think I should do to further improve the manuscript, please advise.

Sincerely,

Albert Leung, MD

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