Author's response to reviews

Title: Acupuncture at homotopic acupoints exerts dual effects on bladder motility in anesthetized rats

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

We are submitting a revision of manuscript (ID: 1230726949149816) entitled “Acupuncture at homotopic acupoints exerts dual effects on bladder motility in anesthetized rats” for your consideration of publication in the BMC Complementary and Alternative Medicine.

In the revision, we revised the manuscript based on the reviewers’ comments. Kristin Schoepfer, a native English speaker who focuses her research on Neuroscience, went through the manuscript and corrected the grammar errors. To express our appreciation for her work, we added her name in “Acknowledgements”.

To express the scientific significance better, in the revision, the title was revised as “Acupuncture at homotopic acupoints exerts dual effects on bladder motility in anesthetized rats”.

Regarding the detailed responses point by point to the reviewers’ comments, please see the Responses to the reviewers attached.

Thank you in advance for your kind consideration. We look forward to hearing from you.

Sincerely,

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Authors’ answer to reviewers’ comments

Reviewer 1

Major Compulsory Revisions

1. Acupuncture stimulation is not in detail. Whether was there an interval time when the next acupoint was acupunctured after the prior acupoint?

Answer: It is good question. Yes, there was an interval between acupoints stimulations. I already added “In the present study, acupuncture was performed at the above acupoints in a random order. After baseline recovered, next acupoint was stimulated.” to “Acupuncture stimulation” in the revised manuscript.

2. In the study 6 points were acupunctured on the same animal, including heterotopic and homotopic points. Whether would these acupoints interfere with each other?

Answer: No interference happened between different acupoints, because the baseline recovered before stimulating next acupoint. Please see “Answer” to “Question 1”.

3. Please explain in detail how you defined the static state.

Answer: Static state and active state are two basal motilities observed by the manometric balloon recording system in the present study and indicate inactive and active state of the bladder. We already defined the static state and active state. Please see the part 1 of results. Generally, in active state: the amplitude of contractive wave is 10-30 mmH₂O, duration 10-30 s, motility index (MI) 573.86 ± 199.45 mmH₂O*s, frequency 10.10 ± 4.27 units/min; in static state: the amplitude of contractive wave was less than 2 mmH₂O, duration less than 10 s, MI 25.30 ± 17.62 mmH₂O*s, frequency 8.50 ± 2.21 units /min.
4. In the references, there was only two references published in nearly five years.

**Answer:** Regarding the mechanisms responsible for the effect of acupuncture on visceral organs, particular in somato-visceral reflex, most studies were conducted in vivo before 2000. In this study, our purpose was to elucidate whether acupuncture could produce dual effects on bladder motility and exhibit site-specific. We thus had to cite the references which had been published earlier.

5. The name of acupuncture points should be written in the right format.

**Answer:** We thank you for your reminder and already changed to the same format “acupoint” which is widely accepted by many investigators and has been used in many published papers (the references are too many to be listed here).

6. The heterotopic and homotopic points should be described clearly in the abstract.

**Answer:** We already added “homotopic acupoints contain afferent innervation in the same segment from which efferent fibers innervate target visceral organs, and heterotopic acupoints utilize different spinal segments to innervate target visceral organs than the segment receiving the afferent signal.” to the abstract.

**Reviewer 2**

1. The authors need to address the issue of inadequate control. They used multiple homotopic acupuncture points and a single, heterotopic, common acupuncture point, BL-23 as control. It does not answer the question whether the dual effects they observed on the homotopic points are acupuncture point specific. In other words, would needle stimulation at any point within the bladder dermatome lead to the same duel effect on bladder pressure. I realize the experiments are finished, but the authors should at least address this possibility and propose follow up studies with better control.
Answer: Thank you for your suggestion. In the current study, we selected heterotopic acupoints like LI11 (Quchi), SP6 (Sanyinjiao) and GB34 (Yanglingquan) in addition to BL23 (Shenshu), and found that MA at LI11, SP6 or GB34 produced similar results as MA at BL23. Because only BL23 is one of the most common heterotopic acupoint among the above acupoints for bladder disorders, we selected it as a control in the manuscript. Now we already added sentence “Similar data were observed in other heterotopic points like LI11 (Quchi), SP6 (Sanyinjiao) and GB34 (Yanglingquan) (data not shown)” to the “Discussion”. In addition, we would like to show the statistic results of MA at LI11, SP6 and GB34 in the Figure R1. This figure shows that MA at BL23, LI11, SP6 and GB34 increased intravesical pressure when in static state while the increased pressures are different.
Figure R1 Effects of MA at BL23, LI11, SP6 and GB34 on intravesical pressure MA at BL23, LI11, SP6 and GB34 increased the intravesical pressure when in the static state. The intravesical pressure after acupuncture was normalized by that before acupuncture which was shown as dashed line and set as 100%.

2. Poor introduction. While the authors did an adequate job on acupuncture and how it may exert dual effects on body function, it did not provide ANY background on bladder function and innervation. I had a hard time fishing out from Methods and Results on what static and active state of bladder refer to. The authors need to present some info on bladder innervation (somatic, visceral, and motor) and how that relate to the acupuncture points used. The authors also need to provide clear, concise definition of bladder motility and HOW it relates to bladder pressure, and the active and static state of the bladder.

**Answer:** We thank you for your suggestion. In the revised manuscript, we rewrote the introduction and added a paragraph to describe bladder innervation. The paragraph is:

Parasympathetic fibers are the primary form of innervation to and from the urinary bladder. Presynaptic fibers arise from neurons in spinal cord segments S2–4 and project via pelvic splanchnic nerves and the inferior hypogastric and vesical plexuses to the bladder. They form synapses with postsynaptic neurons that are found on or near the bladder wall. Parasympathetic fibers provide motor innervation to the detrusor muscle and inhibit the internal urethral sphincter. Sympathetic fibers arise from neurons in spinal cord segments T11–L3, project via lumbar splanchnic nerves, and synapse on hypogastric plexuses [7]. Sympathetic nerves play little role in bladder motor activity, but
they do appear to heavily innervate the neck and trigone of the bladder. Sympathetic stimulation allows for bladder neck closure, which is crucial for bladder filling. Somatic fibers to the external urethral sphincter arise from motor neurons in spinal cord segments S2–4 and project to the bladder via the pudendal nerve [8].

Regarding the relation between bladder innervation and the acupoints in this study, we have a detailed description in the “Discussion”.

Regarding HOW bladder motility relates to bladder pressure, we added several sentences to “Intravesical pressure recording” in Materials and Methods. These sentences are “As previously reported [12], bladder motility was assessed via cystometrogram, which measures pressure within in the bladder while being slowly filled (0.05 ml/min) with warm water using a transurethral catheter.”

3. Unclear explanation of outcome measures (methods): bladder pressure, vs motility vs filling. From what I can see, the authors used bladder pressure as their key outcome measure. However, throughout the manuscript, the term "motility" and "pressure" was used indistinguishably. For example, Line 170 from page 8, "whereas bladder motility was not significantly changed...." Another example is line 217 to 220 on page 10: "stimulating skin could excite the activity for pelvic nerve... thus increase the contraction of the bladder when it is empty." Note the entire paper primarily talked about how at different state (defined by motility and pressure), acupuncture changed the intravesical pressure, not contraction. In short, the authors need to be consistent with their language, currently, it is confusing. I would suggest limit your terms to ONLY bladder (or intravesical) pressure and motility, and eliminate other terms, such as contraction etc.
Also the authors should better define the motility index, with more intuitive details (ie what factors are included in calculating the index, bladder volume? Pressure? Etc)

**Answer:** we thank you for your suggestions. In the revised manuscript, we already use consistent bladder motility which could be assessed through intravesical pressure. In the published papers, intravesical pressure is the most common index to assess bladder motility (here we listed several references). We added several sentences to “**Intravesical pressure recording**” in Materials and Methods to describe the relation between bladder motility and pressure. These sentences are “As previously reported [12], bladder motility was assessed via cystometrogram, which measures pressure within in the bladder while being slowly filled (0.05 ml/min) with warm water using a transurethral catheter.” Here we also listed several published papers in which bladder motility was assessed via intravesical pressure.

References:


4. Discussion needs to be tightened. The authors discussed several interesting concepts in the Discussion, all of which should be tightened and clarified. For example, visceral-somatic convergence was discussed in paragraph #2 then again in #3. The section on the functional neuroanatomy of micturition should be tightened, clarified with a picture, and ideally should appear in the intro, along with a picture of the acupuncture points chose side-by-side with the neuroanatomy of the bladder.

**Answer:** We thank you for your suggestion. We already rewrote the Discussion and made it tightened.

Regarding the diagram with bladder innervation along with acupoints, we tried it but gave it up finally, because the neuroanatomy of bladder in male and female is different, and acupoints in this study locate at abdominal wall and low back, and we at least had
to use two diagrams to show the relation between bladder innervation and acupoints. To make it clearer, we described this relationship in details in the Discussion. Please see the second paragraph.

5. Inaccurate conclusion – see my edits below “Manual acupuncture at homotopic acupoints exerts dual effects on bladder PRESSURE. When the bladder is active, acupuncture at homotopic locations reduces the intravesical pressure; while the same treatment increases bladder pressure when the bladder is in a resting state.”

Basically, the authors confused motility (which they did not present in the result section) with pressure (the outcome measure in all figures and the text) again in the conclusion.

It is also repetitive to define “homotopic;” which was defined earlier in the paper and which most people understands.

**Answer:** We thank you for your reminder. Regarding the relation between bladder motility and pressure, please see Answer 2 and 3. In the revised manuscript, we already used the consistent motility and deleted the repetitive items and corrected the conclusion.

6. Major editing is needed.

The very interesting topic and good science presented in this manuscript is overshadowed by redundant and confusing language. Macroscopically the structure of the intro and discussion are suboptimal and littered with unnecessary repetition. Microscopically, the terms are loose and inconsistent. For example, line 148, page 7, the term “EA” was used and I assume this paper is all about manual and not electrical acupuncture. Another example is the indiscriminantly mixing of “pressure,” “contraction,” and “motility,” as outlined above. Also, I am just not sure if “duel effect” is the best term
to describe the findings in this paper. Perhaps a different term, such as “opposite effect,” would be clearer. Finally, at line 69 of page 4 (second line of Background), I would NOT use “motivating or inducing,” which mean the same. Rather, I think the authors meant “facilitating or inhibiting.” Similar language problems are littered throughout the manuscript.

To address these numerous problems, the authors need to hire a native English speaker to go through the entire manuscript to insure a) its structural soundness b) its language clarity.

In summary: Interesting paper with good science but suboptimal presentation.

Mandatory changes: all of the above Discretionary changes: 1) the authors may consider, after streamlining their discussion, adding the potential clinical application of their findings (e.g. acupuncture can assist with bladder autonomic dysfunction and be potentially useful in neurogenic bladder conditions, cauda equine etc based on results from the current study. 2) I think it would be great to include a picture in the intro highlighting the functional neuroanatomy of the bladder, and alongside, draw the location of the acupoints in the study.

**Answer:** we thank you for your nice comment and suggestions. We have corrected EA to MA and kept bladder motility and pressure, because motility could be assessed through pressure. We also use “facilitating or inhibiting” to replace “motivating or inducing” in the revised manuscript.

Regarding the language, the manuscript was polished by a very professional native English speaker Kristin Schoepfer. We also added her name in the Acknowledgements.
Regarding the potential significance, we already added “The above findings will provide valuable information for treatment of functional bladder disorders.” to the Discussion.

Regarding the diagram, please see Answer 4.