Author's response to reviews

Title: Intra-peritoneal Pretreatment with Intrathecal Amitriptyline Potentiates Anti-Hyperalgesic effects of Post-Surgical Systemic Amitriptyline following Spinal Nerve Ligation

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Author's response to reviews: see over
Dear editor:
The authors are glad to have a good response from the reviewer who is an expertise in this field. We appreciate reviewer Sawynok’s kindness to correct our manuscript. Here are point-by-point responses to the comments.

**Responses to the reviewer Sawynok:**

- This study uses “intra-abdominal” to refer to systemic administration of amitriptyline, yet the more common expression for such delivery is “intraperitoneal” administration. Please use this expression.
  
  **Answer:** Appreciate the valuable comment. The word “intraperitoneal” is instead of “intraabdominal” in the manuscript. However, group A still represent the rats of spinal nerve ligation and the intra-peritoneal (intra-Abdominal) amitriptyline administration post-injury shown in the section of material and method.

- At the start of the Introduction, there needs to be a clear distinction made between clinical and preclinical references.
  
  **Answer:** The text of introduction section is revised under the reviewer’s suggestions. Thanks a lot.

  Injured DRG neurons are likely to have a more hyperpolarized threshold for overshooting action potential with the presence of Nav1.3 in place of Nav1.8 after peripheral nerve axotomy. **The hyperpolarized threshold** brings the neurons requiring less depolarization for activation and easy firing under the actions of Nav1.3 and Nav1.7. (Rush AM, et al. J Physiol. 2007 15;579:1-14)

- References. Throughout, there were several references cited that did not seem appropriate for the point being made. Please check the accuracy of all citations.
  
  **Answer:** The references were cited in accordance with the reviewer’s suggestions. Adding references

- p.19, line 16. This should refer to a review of amitriptyline actions. A detailed consideration of this was included in the response to the referees, but it really belongs in the manuscript.
  
  **Answer:** The paragraph has been revised and corrected under reviewer’s suggestion and cited a new reference in line of the point of pre-emptive and post-injury amitriptyline administration are needed to prevent sensory changes and to blunt neuropathic pain.

- Why was 90 ul used for the i.t. injections? This is a large volume compared to the overall CSF volume. Rostral spread of the drug is inevitable with this volume, and this must be considered directly.
  
  **Answer:** Appreciate reviewer’s valuable comment. Intrathecal 90 µl amitriptyline is a large volume as compared to the overall CSF volume in rats of body weight 300-350 g. However, intrathecal 90 µl (7.5mM) amitriptyline has been demonstrated a spinal anesthetic effect with potent blockade of motor and sensory function and full recovery from Chen et al. The authors are not able to confirm whether the injectate spreads rostral to cervical or to brain stem or not. However, rats with spontaneously breathing did not suffer from cardiac arrest or respiratory arrest during surgery in the present study.

- Need to quote the Gebhart and Eisenach 1994 study which showed analgesia by i.t. amitriptyline. 200 ug is a very large dose in comparison with that dose. Why was such a high dose chosen?
  
  **Answer:** We appreciate reviewer Sawynok’s delicately viewing the manuscript. Eisenach and Gebhart demonstrate that intrathecal amitriptyline (60 µg in 3 µl) administration prior to carrageenin intra-plantar injection attenuate carrageenin induced inflammatory pain. Their results have been included in our discussion section.” Intrathecal amitriptyline 60 µg in 3 µl was administered via a catheter showing no immediate sensory or motor functional impairment” in page 23. However, there are some different points between the study of Eisenach and Gebhart and the present study.
The First, amitriptyline was administered intrathecally via a PE-10 catheter in study of Eisenach and Gebhart. In our study, intrathecal amitriptyline was administered with a no. 30 needle to approach subarachnoid space blindly. Therefore, we are not able to identify the true volume into the subarachnoid space though the technique is in accordance with the clinical approach to produce spinal anesthesia. In addition, supra-spinal analgesic effect may occur in the present study; Further research should be undertaken to elucidate whether the spinal or supra-spinal effects of intrathecal amitriptyline.

The second, intrathecal amitriptyline (60 µg in 3 µl) was administered via a catheter to produce analgesic effect without lagging withdrawal latency in the study of Eisenach and Gebhart. In the present study, intrathecal amitriptyline (200 µg in 90 µl) was administered to block sensory and motor function. It indicates that intrathecal amitriptyline with 90 µl (7.5 mM) reveals regional anesthetic effects but not low volume of intrathecal amitriptyline (60 µg in 3 µl).

The third: intrathecal amitriptyline (60 µg in 3 µl), a high concentrations of amitriptyline (68 mM), may hurt spinal neuronal or glial cells easily (debate in the discussion section). However, the concentration of amitriptyline (200 µg in 90 µl) showed no obvious abnormal histo-pathological finding in the present study.

In addition, rats undergoing surgery with intrathecal amitriptyline 200 µg in 90 µl recovered fully and detected no apparent side effects or behavioral abnormalities. There is no rat suffering from cardiac or respiratory arrest during surgery in the present study. We think the intrathecal dose should be safely given; however, further research should be undertaken to elucidate its toxicity in neuron cells.

- Other minor corrections have been done in accordance with review’s suggestions.