Author's response to reviews

Title: Notch signaling activation is critical to the development of neuropathic pain

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Author's response to reviews:

Dear Tom Rowles, Dae-Hyun Roh and Gabriel Rusanescu:

Thank you very much for giving us a chance to revise our manuscript (MS: 203156558125747). We would like to express our appreciation for the constructive comments provided by the referees. Their suggestions and criticisms are very helpful for the improvement of the manuscript. A substantial revision and additional experiments have been made to the manuscript accordingly. We responded point by point to each referee’s comments as listed below.

Reviewer: Dae-Hyun Roh

1. In this study, the authors showed that single or repetitive administration of DAPT, an inhibitor of notch signaling pathway, prevented or reversed the decrease of mechanical PWT after spared nerve injury. Especially, both early and late administration of DAPT dose-dependently produced the anti-allodynic effect. In addition, single administration of Jagged-1 peptide, a ligand of notch signaling pathway, decreased the mechanical PWT of normal rats. Overall experimental design is reasonable and results showing the powerful effect of DAPT are interesting. However, there are some important concerns that should be addressed for the possible publication in BMC Anesthesiology.

Answer: Thanks very much for this comment.

2. Although the authors titled the “notch signaling activation” and addressed this extensively, there is no data or literature related to this notch signaling activation after SNI in rats. The authors need to present obvious evidences showing whether notch signaling pathway in the lumbar spinal cord was activated after SNI surgery using several experiments such as western blot assay or immunohistochemistry.

Answer: This comment is very constructive. We added an additional experiment according to this comment. We measured the NICD expression, which indicates
the level of notch signaling activity, in the dorsal horn of lumbar spinal cord after SNI operation by western blot assay. We found that notch signaling pathway in the lumbar spinal cord was activated after SNI surgery (Figure 1). Thank you.

3. In figure 1, the injection of DAPT 30 min before (A) or after (B) SNI surgery produced different effect in development of mechanical allodynia at 21 or 28 days after surgery. In addition, the effect of repetitive daily DAPT injection for 3 days (C) was similar to the effect of single injection of DAPT 30 min before SNI (A). The purpose of these experiments or the significance of these findings should be discussed in detail.

Answer: This comment is also very constructive. Revised as suggested. In our preliminary experiments, we did a lot of experimental protocols including DAPT intrathecally (i.t.) administered at different dosages and time points. In this revised manuscript, we only remained the important results. And the significance of these findings was discussed in detail. Thanks again.

4. Address the precise time point when the experiments in Figure 2 and Figure 3B were performed after SNI surgery.

Answer: Thanks for this comment. Revised as suggested.

5. The authors need to show when the decreased PWT after the injection of Jagged-1 peptide was restored.

Answer: Thanks for this comment. Revised as suggested. Additional experiment was observed. Thank you.

6. In abstract, line 20, the word “increased” should be corrected to “decreased”.

Answer: Revised as suggested. Thanks.

Reviewer: Gabriel Rusanescu

1. Considering the role that Notch signaling plays in a variety of cellular functions, the authors investigate the possibility that Notch is also involved in the development of chronic neuropathic pain using a rat model of spared nerve injury (SNI). The authors demonstrate that the inhibition of Notch signaling with DAPT, an inhibitor of gamma-secretase, also inhibits allodynia in SNI rats. Conversely, the activation of Notch pathway in normal rats with a Jag1 peptide that binds and activates Notch receptors is shown to induce allodynia.

Answer: Thanks very much for this comment.

2. DAPT is a non-specific inhibitor of Notch receptors. The Notch family includes 4 receptors with different functions, as indicated by the phenotypes of various Notch transgenic mouse lines. These 4 receptors are expressed unequally in various cell types. Therefore the study does not specify which Notch receptor(s) are involved in allodynia. The authors should discuss this idea in the discussion and in the “Limitations of the study” section.

Answer: This comment is very good. Revised as suggested.

3. Jag1 is also a non-specific ligand of Notch receptors. Moreover, Jag1 may
have different affinities for different Notch receptors, depending on cellular context and post-translational modifications. Also, the extracellular Jag1 peptide will activate Notch signaling indiscriminately in neurons (both inhibitory and excitatory) and glia. This also needs to be discussed and mentioned in the study limitations.

Answer: This comment is also very good. Revised as suggested.

4. Please refer to a recently published study, “Notch3 is necessary for neuronal differentiation and maturation in the adult spinal cord.” J Cell Mol Med. 2014 Aug 28, for a new neurogenesis-based model of chronic pain that should be mentioned among the other models of pain. This may also help in discussing the role of Notch signaling in allodynia and may clarify some of the results.

Answer: Revised as suggested (Ref. 12). Thank you very much.

5. Notch receptors and ligands have variable expression in various cell types. The authors should discuss whether Notch effect on nociception is the result of Notch expression in neurons or in other cell types.

Answer: This comment is constructive. Revised as suggested.

6. In discussing the potential role of Notch as “a new therapeutic target”, one must keep in mind the widespread and varied expression of Notch receptors and ligands and the potential to harm other processes. What will be the effect of a systemic inhibition of Notch signaling on other events where Notch plays a key role in stem cell renewal? For example hippocampus neurogenesis, that plays a key role in memory, will be also inhibited by Notch inhibition. Please include this in discussion.

Answer: This comment is very constructive. Revised as suggested.

Editorial comments:

1. Thank you for including an ethics statement in your manuscript. However, we would ask you to please include the full name of the committee that approve your study in this statement.

Answer: Revised as suggested. Thanks.

2. Please move your Competing Interests and Acknowledgments section from their current location to after your Conclusions section.

Answer: Revised as suggested. Thanks.

3. Please ensure that separate and individual email addresses are uploaded into our submission system for each manuscript author. At present, the email address of Yanyan Sun is clearly derived from the name of author Kieliang Xie. It is highly important that we be able to contact each author separately, if necessary.

Answer: Revised as suggested. Thanks.

Sincerely yours,
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