Reviewer's report

Title: Notch signaling inhibition ameliorates mechanical and thermal allodynia in a rat model of neuropathic pain

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Reviewer: Gabriel Rusanescu

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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.

Major Compulsory Revisions

1. 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.

2. 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.

3. 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.

Discretionary revisions:

1. 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.

2. 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.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.