Reviewer's report

Title: Increased expressions and levels of tachykinin both ipsilaterally and contralaterally in response to unilateral rabbit muscle overuse leading to muscle and nerve affection and inflammation

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Reviewer: Mary F Barbe

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Title:
Increased expressions and levels of tachykinin both ipsilaterally and contralaterally in response to unilateral rabbit muscle overuse leading to muscle and nerve affection and inflammation

Authors: Sture K Forsgren, Per S Stål, Yafeng Song and Jiguo Yu

Reviewed by Mary Barbe

1. Is the question posed by the authors well defined?
Yes. The authors are exploring tachykinin expression bilaterally after a unilateral inducted exercise- (using a rig) and electrically-stimulated myositis. This is an important and current topic in several fields currently (muscle, tendon and bone biology, for example). I have great interest in this question as well, but have not been able to explore this question in my model (since mine is a bilateral use model). Their model is ideally suited to answer this question. Other researchers have postulated that these bilateral changes exist (several of whom are cited in the manuscript), although the final findings of some of these studies were limited by the model used, or by their methodology or its execution. This study systematically studied tachykinin expression in not only nerve cells in the muscles, but also in blood vessels and infiltrating immune cells. Nice.

2. Are the methods appropriate and well described?
Yes, overall, although see minor revisions below.

3. Are the data sound?
Yes. Appropriate controls were used for the immunohistochemistry to show specificity of the antibodies. Two types of antibodies were used to detect tachykinin, and the results of each described and discussed. This was helpful to me as a researcher. Appropriate negative control was used in the in situ hybridization. The quantification of tachykinin in the muscles using EIA and by intensity scoring, and vessel density counts gave strong support to their conclusions. Statistics are appropriate. Please see major essential revisions below, #a.
4. Does the manuscript adhere to the relevant standards for reporting and data deposition? Yes.

5. Are the discussion and conclusions well balanced and adequately supported by the data?
   Yes, except, as pointing out regarding T cells. Please see major essential revisions below, #a.

6. Are limitations of the work clearly stated?
   Yes, although the low number of animals in weeks 1 and 3 need to be highlighted again in the discussion. This is especially true with regard to the ipsilateral versus contralateral examinations (n=2 or n=1 for week 1, for example). This is a weakness for these time points that needs to be repeated. They should then highlight that the number of animal in the 6 week time point was sufficient.

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
   Yes.

8. Do the title and abstract accurately convey what has been found?
   Yes.

9. Is the writing acceptable? Yes, although is it quite long. The English is generally grammatically correct, but could be shortened and compressed from textbook English grammar to condensed science English grammar. That said, this is not a real concern – I was just thinking that a more compressed version might encourage more readers to complete their read of the manuscript. A few typos were noted. Changes are indicated in minor essential revisions:

   Minor essential revisions:
   Methods:
   a. This section could be shorted in several sites by condensing the English.
   b. The number of animals per group needs to be stated more clearly in the expt design section.
   c. What beta tubulin and S100 beta stain needs to be added to page 10. This is described later in the results, but a general reader needs it here too.
   d. Page 5, 2nd paragraph: Please change “patterns and levels were notified” to “patterns and levels were noticed.”
   e. Page 8, first line: add a word so that it reads: “One day after the last bout of exercise…”
   f. Page 8, concerning the cyrosectioning, change to read: “…muscles were directly mounted and frozen as described above,…”
   g. The greatest compressions could take place in the methods.
   h. The discussion could stay as it is.
   i. There are a few typos that need to be corrected.
j. Figure legends 10 and 11 need to have their respective subparts merged into a
one legend per figure. It is a bit confusing to have them separated.
k. Page 30. Please define c.f.
l. Page 34. Last paragraph. I.e. used incorrectly. The first sentence should be
rewritten to read: “A drawback with the setup of the study was the fact that other
muscles in the contralateral side... examined so that changes in the general
circulation could be considered.”

Major essential revisions:
a. Results: One site needs fixing – Page 21 it states that T cells did not express
tachykinin. The figure legend (Fig 10) suggests otherwise. The discussion
suggests otherwise. Please clarify these findings in each section of the
manuscript.
b. Results – Correlations. I got lost in the last paragraph. Please clarify what was
being compared to what in this last paragraph of correlations. The preceeding
paragraph examining bilateral changes and if they correlated was quite clear.
c. Discussion: The low number of animals in weeks 1 and 3 need to be
highlighted again in the discussion. This is especially true with regard to the
ipsilateral versus contralateral examinations (n=2 or n=1 for week 1, for
example). This is a weakness for these time points that needs to be repeated.
They should then highlight that the number of animal in the 6 week time point
was sufficient.

10. Level of interest:
Their systemic and carefully executed approach showing bilateral upregulation of
tachykinins in nerve, blood vessels and immune cells, despite a clearly unilateral
loading/stimulation, will be of interest to muscle, tendon and bone biologists. The
increase in circulating immune cells may even broaden the interest further.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being
published

**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.