

OPEN PEER REVIEW REPORT 1

Name of journal: Neural Regeneration Research

Manuscript NO: NRR-D-22-00009

Title: Chronic spinal cord compression associated with intervertebral disc degeneration in aging SPARC-null mice

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Reviewer's country: USA

COMMENTS TO AUTHORS

Summary: In this work, the authors studied the effects of an established disc degeneration model on spinal cord compression. They are proposing this as a model for nontraumatic chronic spinal cord compression-related injuries. They evaluate the model for motor and pain behaviours, as well as with extensive histological analyses. I commend the authors on their thoroughness. This work is important because half of all spinal cord injuries are non-traumatic, and the field will benefit from an animal model. I have many suggestions below that will help improve readability, understanding, and replication.

General Comments and Suggestions:

1. In the introduction, it would be helpful and more impactful to include some information about prevalence of CSCC
2. In the results section, please provide score numbers and statistical outcomes in more detail, instead of just saying that SPARC was worse than wildtype and referencing the figures.
3. There is a nice discussion on different compression models, but the manuscript lacks any other discussion
4. There are a lot of results, and within each section there is some interpretation of the results, which is great. However, I would like to see some more elaboration on the results in the discussion section.

Specific Comments and Suggestions:

1. Please reword this: "is a matricellular collagen-binding protein implicated in fibrillar collagen assembly in the ECM of connective tissue" because it is a replication of the text in Riley and Bradshaw, 2020
2. Why was this limited to only male mice? What is the prevalence of CCSC in males vs females?
3. The description for the pain threshold test should indicate that you were looking for the withdrawal threshold
4. What is UGO, Italy? I was unable to locate this source.
5. Tissue preparation: Did you cut along the sagittal plane, or the axial/transverse plane?
6. I don't understand this: "The whole spinal cord of other mice was taken, and wrapped in tinfoil, and stored at -80°C." It's very unclear how the tissue analysis was done and on what and how many mice.
7. Please provide the sources for your H&E dyes and other markers
8. Western blots: is this correct? "anti-tyrosine kinase B (rabbit anti-ASC monoclonal antibody, ab134155, 1:1000)". Shouldn't it be anti-TrkB?
9. Anti-C3 is missing from your Western Blot methods section
10. MRI is not a method of radiographic imaging because it does not use radiation.
11. TdT mediated dUTP Nick End Labeling (Tunel) assay: Please define these acronyms and techniques before using them, especially in a title. Perhaps a different subtitle for this section would be better.
12. What is the BMS sub score? This was not described well in the methods. Please indicate which sub

score(s) you are looking at and why.

13. Is Tukey's post-hoc test the most appropriate choice for these data? If the sample sizes in the pairwise comparisons are not the same, Tukey-Kramer method should be used. Alternatively, Bonferroni method would be better for detecting a true difference between groups.
14. Why was there more compression of lumbar segments compared to other segments?
15. Please make the axis labels and font sizes in the figures consistent
16. Figure 1G-J is barely readable
17. Figure 1G-J - if stance times lower for all limbs, does that even make sense? And swing is either lower or the same?
18. What was the variability between the BMS scores between the 2 evaluators?
19. Results, page 13, lines 46-52: you say that the H&E stain indicates a significant reduction in neurons in the gray matter of the SPARC mice compared to the WT, but what is missing is an actual count of the neurons and a statistical comparison. Without these, I don't think you can make that claim.
20. Figure 3A: it would be helpful if you pointed to several neurons with an arrow in the magnified slices
21. Figures 3B, 5D: the scatter plots are too small
22. Results, page 15, line 12: I like that you give a brief description of what MBP stains for, and it would be helpful to include this type of information for all stains. It would preferably be in the methods section, and perhaps a brief mention of the stain target in the results to avoid any confusion.
23. Results, page 18, line 12: "compression" is spelled incorrectly
24. The scalebars in the immuno figures are unreadable or missing
25. BDNF background details in the results section should be moved to the methods
26. Figure 8: Missing labels A, B, C. In A, the BDNF is decreased in the SPARC-null mice, but is increased in B. Is this correct? The text says that it was decreased.
27. Discussion, page 21, line 17: missing a space between "and" and "significant"
28. Discussion: Please provide references for the other types of compression models. Later you reference studies from the 1950s. Are there more recent studies?
29. Strengths and limitations. This is a bit sparse. Also, the last sentence needs revising.