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Title

Comparison of the effects of two therapeutic strategies based on olfactory ensheathing cells transplantation and repetitive magnetic stimulation after spinal cord injury in female mice

Author names

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JOURNAL OF Neuroscience Research

Editor:
Editor Comments to the Author:

Response: First of all, we would like to thank the editors for his comments and questions.

Thank you for appropriately addressing the editors and reviewers comments, however, your manuscript still needs some minor changes.

Please change the graph type in figure 2, JNR does not support the use of bar graphs, also see the comments from the Statistics Editor below.

Please add a comment on the limitation of using only female animals.

Response: We did some changes related to reviewers and editors comments such as:
1. Figures appear now with SD instead of SEM.
2. Dot plots are used in all the Figures without bar.
3. Discussion section has been rewritten to fit with Editor's comments, in particular we have added as a limitation the fact that only female animals have been used in our study.

However, I have some concerns related to the comments of the reviewer 2 and those of the Statistics Editor.

The reviewer 2 is without any doubt a great expert of the olfactory ensheathing cells' field. We know also that many labs published and still publish very good articles related to these cells and their use after peripheral nerve injury or spinal cord injury (https://onlinelibrary.wiley.com/doi/epdf/10.1002/jnr.24817), however to our point of view our paper is not a review of the literature regarding OECs properties. So, without showing any disrespect, we made the choice to not discuss all the points addressed by the Reviewer 2. Indeed, the main purpose of our study was to compare OECs transplantation and RMS treatment individually and the combination of these two therapies. Our study did not explore the precise mechanisms which can explain tissue repair and functional recovery observed within the studied groups. That is why the Discussion part of our manuscript tries to discuss the main results we obtained without talking about all the potential properties of OECs or RMS therapies.

JOURNAL OF Neuroscience Research

We have taken into account the points highlighted by **the Statistics Editor**, and we mainly do agree with him. We changed our figures, they now appears with dot plots (without bar) and with SD instead of SEM.

However, we would to say to the Statistics Editor that our groups of animals are independent with no relationship between members in each group or between groups.

The statistics Editor explains that we should use parametric tests “unless you are convinced that the assumptions for anova are not met (like the assumption of normality”). This is exactly why we made the choice to use non-parametric tests. In fact, before to perform statistical analyses we did Shapiro tests for assessing data distribution. These tests reveal that for Figure 6 our data are not normally distributed. For Figures 4 and 5 our populations are normally distributed according to Shapiro tests, however to our point of view our populations (n=5) are too small to perform parametric tests. Even so, we performed Anova Tests and we compare the statistical results with those obtained with non-parametric tests and it appears that the significant differences between groups were the same for Figures 4 and 5.

These points have been added into the revised version of our manuscript.

JOURNAL OF Neuroscience Research

Statistics Editor: McArthur, David - Comments to the Author:

Response: First of all, we would like to thank the Editor for his comments and questions.

The Kruskal-Wallis test is not a test of means as stated but a test of medians. Note that the test has the underlying assumption that observations should be independent with no relationship between members in each group or between groups. Additionally, all groups should have the same shape distributions. The necessary independence does not appear to be present in your analyses. It would appear that your intent is to test all possible pairwise contrasts in each of the plots in the final three figures; neither the Kruskal-Wallis nor the Mann Whitney are appropriate in that context. (The Mann-Whitney test is not mentioned in the section titled Statistical analysis, nor is the statistical software (and version) noted).

Supplemental Table 1 is not the location in which to place the results of your statistical analyses. This journal prefers they be presented in the Results narrative or Figure captions, and not merely as asterisk equivalents for p-value ranges. Note that you can declare that "all tests except as noted were computed with the XXX test" to greatly simplify the listing of results.

The first column of that table points to "figure and section" but the figure numbers are not correct, and the use of "section" does not match the narrative.

Altogether, in the final three figures it seems that you are attempting to analyze a one-factor design solely with post-hoc comparisons. Kindly start with a simple anova followed by appropriate post-hoc testing (Tukey's method is frequently recommended here). This will more appropriately account for the multiplicity of comparisons -- which if uncorrected can provide erroneous conclusions. Unless you are convinced that the assumptions for anova are not met (like the assumption of normality), nonparametric tests are usually less preferable due to generally being less powerful than corresponding parametric tests.

The use of SEM is not provided with a rationale. SD's are preferred. SEMS's calculated on separate data distributions that all have identical means and identical variances will be the same only if the number of cases in all groups is also the same. Thus SEMs cannot be interpreted as a stable reflection of variability for a given set of data distributions, and the visualizations using SEMs are thus also not stable. It is for these reasons that 21st century statistical thinking has pushed for SDs.

This journal does not accept "dynamite plots" (also called "plunger plots", "toiletbrush plots", or "bogbrush plots") as they are often severe distortions of the actual data distributions. You already use dotplots in most instances; the bars add nothing of intellectual value.

Response: We have taken into account the points highlighted by **the Statistics Editor**, and we mainly do agree with him. We changed our figures, they now appears with dot plots (without bar) and with SD instead of SEM.

However, we would to say to the Statistics Editor that our groups of animals are independent with no relationship between members in each group or between groups.

The statistics Editor explains that we should use parametric tests "unless you are convinced that the assumptions for anova are not met (like the assumption of normality"). This is exactly why we made the choice to use non-parametric tests. In fact, before to perform statistical analyses we did Shapiro tests for assessing data distribution. These tests reveal that for Figure 6 our data are not normally distributed. For Figures 4 and 5 our populations are normally distributed according to Shapiro tests, however to our point of view our populations (n=5) are too small to perform parametric tests. Even so, we performed Anova Tests and we compare the statistical results with those obtained with non-parametric tests and it appears that the significant differences between groups were the same for Figures 4 and 5.

These points have been added into the revised version of our manuscript.

How did Figure 2 end up with N=4 when that number is used nowhere else in this study?

Response: The results of the Figure 2 are related to OECs characterization by flow cytometry (materials and methods page 9).
These experiments have been performed using OECs cultures (n=4 cultures obtained from n=8 WT mice).

JOURNAL OF Neuroscience Research

Reviewer 1: Comments to the Author

Response: First of all, we would like to thank the editor for his comments and questions.

The authors have adequately addressed the review queries. However, the manuscript needs proofreading by someone fluent in English as there are some grammatical and syntax errors throughout the manuscript.

Response: The manuscript has been checked for spelling and grammar.

JOURNAL OF Neuroscience Research

Reviewer: 2 Comments to the Author

Response: First of all, we would like to thank the editor for his comments and questions.

The authors have significantly improved the manuscript by entering much of the information required by the reviewer. The research detailed in the manuscript is original and relevant as a new repair therapy for injured spinal cord, at least at the pre-clinical level. The authors have discussed the results in greater depth. Despite this, the authors still do not discuss in greater depth why the transplanted cells do not survive in the injured parenchyma, as indicated in the first version of the manuscript. This point is relevant, and the authors discuss it very superficially, focusing on only three recent articles. Authors should discuss their results with both articles in their favor and articles against. There is experimental evidence showing that the olfactory ensheathing glia cells have the ability to migrate through the medullary parenchyma as well as to divide in the injured spinal cord parenchyma. In this part of the discussion, the authors should include more information, and not just indicate that their results are similar to what these other three scientific articles indicate. A more exhaustive review of cell survival times should be performed by other authors than if they demonstrate such survival in the injured parenchyma.

On the other hand, the authors indicate "To date, the precise mechanisms by which OECs play their key role during neurogeneration is still poorly described. In fact, OECs transplantation reduces axonal dieback and inflammation at the lesion site (Khankan et al., 2016; Zhang et al., 2021). Moreover, it has been also described that OECs transplantation modulates chondroitin sulfate proteoglycan (CSPG) expression in injured spinal cord (Wang et al., 2021). Several studies have demonstrated as well that OECs secrete different neurotrophic factors (Lipson et al., 2003; Blumenthal et al., 2013; Gu et al.,2017).". The information provided by the authors is true, but they should provide more information. From 2000 to 2015, many scientific articles were published on the characteristics of olfactory ensheathing glia cells in cell cultures, studies that the authors do not cite in their discussion. Likewise, they do not speak of the role of the olfactory ensheathing glia cells in the angiogenesis, factor that can also contribute to neuroregeneration. There are other articles that show that the olfactory ensheathing glia cells promote the infiltration of Schwann cells from the injured spinal cord, and that both glial elements can generate “tunnels” through which axons can regenerate. They also do not speak of the phagocytic capacity of the olfactory ensheathing glia cells to eliminate cellular debris, especially myelin, a fact that can contribute to axonal regeneration, since myelin inhibitory molecules (e.g. NOGO) are a limiting factor of axonal growth in the lesioned spinal cord. In summary, the authors have not conducted a comprehensive review of the topic. In the final version of the manuscript, they must include all this information.

Finally, the authors should indicate whether the treatment proposed in the manuscript may have a clinical translation, since transplants of olfactory ensheathing glia cells are already being applied in humans, and magnetic stimulation of the central nervous system is also applied in humans.

Response: The Discussion part has been rewritten.