JOURNAL OF Neuroscience Research

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

Guérout, Nicolas; Delarue, Quentin; Robac, Amandine; Massardier, Romane; Marie, Jean-Paul

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Revision Received: 8 March 2021
Accepted: 12 March 2021

Editor 1: Bradley Kerr
Editor 2: Cristina Ghiani
Reviewer 1: James St John
Reviewer 2: Enrique Verdu Navarro

1st Editorial Decision

Decision letter

Dear Dr Guérout:

Thank you for submitting your manuscript to the Journal of Neuroscience Research. We've now received the reviewer feedback and have appended those reviews below. As you will see, the reviewers find the question addressed to be of potential interest. Yet, they do not find the manuscript suitable for publication in its current form.

If you feel that you can adequately address the concerns of the reviewers, you may revise and resubmit your paper within 90 days. It will require further review. Please explain in your cover letter how you have changed the present version and submit a point by point response to the editors' and reviewers' comments. If you require longer than 90 days to make the revisions, please contact Dr Cristina Ghiani (cghiani@mednet.ucla.edu). To submit your revised manuscript: Log in by clicking on the link below https://wiley.atyponrex.com/submissionBoard/1/c5c54ebd-76a1-43b1-910e-3ca71d56d12d/current

(If the above link space is blank, it is because you submitted your original manuscript through our old submission site. Therefore, to return your revision, please go to our new submission site here (submission.wiley.com/jnr) and submit your revision as a new manuscript; answer yes to the question "Are you returning a revision for a manuscript originally submitted to our former submission site (ScholarOne Manuscripts)? If you indicate yes, please enter your original manuscript's Manuscript ID number in the space below" and including your original submission's Manuscript ID number (jnr-2020-Dec-9350) where indicated. This will help us to link your revision to your original submission.)

Thank you again for your submission to the Journal of Neuroscience Research; we look forward to reading your revised manuscript.

Best Wishes,

Dr Bradley Kerr
Associate Editor, Journal of Neuroscience Research

Dr Cristina Ghiani
Editor-in-Chief, Journal of Neuroscience Research

JOURNAL OF Neuroscience Research

Editor Comments to the Author:

Did the authors only use females in this study? The sex of the animals is not clearly defined in the title or abstract, the authors mentioned 'females mice' once at the beginning of the methods.
SEX DIFFERENCES
The National Institutes of Health now mandates the inclusion of sex as a biological variable. To conform with this mandate, the Journal of Neuroscience Research has established new policy (please see our editorial: http://onlinelibrary.wiley.com/doi/10.1002/jnr.23979/full) requiring all authors to ensure proper consideration of sex as a biological variable. Please ensure that: 1) Any paper utilizing subjects of one sex state the sex of the sample in the title and abstract; 2) The number of samples/subjects of each sex used in the research must be clearly stated in the methods section; 3) The inability for any reason to study sex differences where they may exist should be discussed as a study limitation. We are also encouraging authors to report exploratory analyses of potential sex differences in studies not explicitly designed to address them

There are no sham animals in this work, please explain/address this point properly.

Please properly described in the methods and confirm that the cells were transplanted during the same surgery to perform SCI, but how does this translate to a possible use for therapy?

When did the stimulation begun after SCI? the authors mentioned that they were applied for 14days, but it is unclear when the stimulations were started, and were the 14 days consecutive?

Language:
The verb tense should be kept consistent.
'mice were analgesia' should probably be 'mice were anesthetise'?
"bOECs transplanted was taken from LUX+/- mice" would read better as "transplanted OECs were purified from LUX+/- mice."

SIGNIFICANCE STATEMENT
Authors must submit, in the main text of the document, a 100-word-maximum statement about the significance of their research paper written at a level that is understandable to the general public and to scientists outside their field of specialty. This statement will be distinct in purpose from the abstract, with the primary goal of broadly explaining the relevance and importance of this work and how this work contributes to different diseases.

RESOURCE IDENTIFICATION INITIATIVE (Abbreviated Text)
Please incorporate Research Resource Identifiers (RRIDs) in your citation of all resources used in your manuscript (antibodies, software tools, databases, model organisms) where applicable in the text, exactly as you would a regular citation or Genbank Accession number. Please also be sure these RRIDs are included in your keywords list in addition to the required keywords. For any antibodies, we ask that you also include the RRIDs in your antibody table, in addition to citing them in the text. An example of how to list RRIDs in your antibody table can found in the example antibody table attached and for more information about how to obtain and cite RRIDs within your text, please visit the "Resource Identification Initiative" section of our author guidelines: http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1097-4547/homepage/ForAuthors.html

Furthermore, in accord with JNR requirements, you will have to provide additional information on each of the antibodies that you use. This must include the source (not just the manufacturer and catalog or lot number, but the species it was raised in, and the EXACT structure of the immunizing antigen - including the amino acid sequence for peptide antigens); characterization (e.g., what does it recognize on immunoblots); and appropriate controls (i.e. the effect of blocking peptides for immunohistotochemical localization).

GRAPHICAL ABSTRACT
Please upload a graphical abstract, which we are asking of all authors submitting original research articles. This is intended to provide readers with a visual representation of the conclusions and an additional way to access the contents and appreciate the main message of the work. What we require is a .tif image file and a .doc text file containing an abbreviated abstract. For the image, labels, although useful, must be kept to a minimum and the image should be 400 x 300, 300 x 400, or 400 x 400 pixels square and at a resolution of 72 dpi. This can be one of the figures from your article, or something slightly different, as long as it

represents your study. Instructions for this can be found in our author guidelines online at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1097-4547/homepage/ForAuthors.html

Reviewer: 1

Comments to the Author
This manuscript reports the effects of two potential treatments for spinal cord injury in a mouse model. The two treatments, (i) magnetic stimulation and (ii) transplantation of olfactory ensheathing cells, have each separately been previously demonstrated to improve recovery after spinal cord injury. In this study, they test whether the combination of the two treatments further improves outcomes. The findings demonstrate that while each treatment improves outcomes, the combined treatment does not provide additional benefits. The authors propose that each treatment targets different stages of recovery.

Overall, the manuscript is well written and the results are convincing, and the manuscript provides an important contribution to the field which will help others to design new therapeutic approaches.

Suggestions for improvement:

Methods. The details of the injection of the cells are perhaps incorrect. They state that the bOECs were injected at "1 mm depth, 1.5 mm from the midline, 5 mm rostrally and caudally from the lesion site". A female C57BL6 mice typically has a cord depth at T10 of 0.8-1 mm and a cord width of 1.2 mm. So an injection 1 mm deep would put it at the very base of the cord if not outside the cord. 1.5 mm from the midline – does this mean a unilateral injection from the midline and if so which side? And 1.5 mm lateral would certainly put it outside the cord. Perhaps the authors have used the injection locations and dimensions from a rat study by mistake? Can this description be clarified.

Results: Figs 3-4. It would be useful to have some high magnification images of the cells of interest as these will enable readers to understand the cell morphology. The morphology of the cells can provide insights into how they are reacting to the environment and treatment. Including these along with an interpretation of the outcomes would improve the impact of the manuscript.

The manuscript needs to be edited and proofread for English language expression as there are some minor errors throughout the manuscript.

Reviewer: 2

Comments to the Author
Manuscript # jnr-2020-Dec-9350

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

Comments of the reviewer

In the aim to improve the manuscript, several changes should be included in the final version of the manuscript.

INTRODUCTION
1. The authors indicate "To date no cure can be offered to injured patients". This statement is not completely true, since various repair strategies have been tried in humans, including spinal cord repair with nerve grafts and ensheathing glial cell transplantation (Cell Transplant. 2014;23(12):1631-55.) or the application of stimulation neuroprosthesis (Science. 2012 Jun 1;336(6085):1182-5; Nature. 2018 Nov;563(7729):65-71.), with very promising results in patients in whom these strategies have been tried. Authors should correct this phrase, contributing these strategies in the final version of the manuscript.
2. The authors indicate "Spinal cord damages induce a cascade of secondary events which include inflammation, cell death or release of inhibitory factors leading to scar formation (Grégoire et al., 2015; Stenudd et al., 2015)". The authors should describe in greater detail the cellular and molecular phenomena involved in the secondary injury, especially those that will cause the separation of the glial scar, since one of the objectives of the study is the modulation of this glial scar. Please include this information in the final version of the manuscript.

3. The authors indicate "This scar which takes place after SCI, initially described as a glial scar, is composed of several cellular types which play a dual inhibitory/permissive role (Sabelström et al., 2013; Anderson et al., 2016). The authors should describe in greater detail the cellular and molecular components of the glial scar, indicating in what phase of the spinal cord injury this glial scar begins to form, and what are the factors that induce its generation, as well as the meaning glial scar function. Please include this information in the final version of the manuscript.
4. The authors indicate "To do so, different therapies have been developed and tested in rodents and also in Human (Ahuja et al., 2020)". The authors must provide more detailed information on the therapies studied in animal and human models that comply with the two approaches described in the previous paragraph. This information is relevant and should be included in the final version of the manuscript.
5. The authors indicate "As we described above, mainly two populations of cells constitute the spinal scar; astrocytes and fibroblasts (Göritz et al., 2011; Dias et al., 2018)". Are they (astrocytes, fibroblast) the only cells present in the glial scar? Where do the fibroblasts that are part of the glial scar come from? What role do these fibroblasts play in the glial scar? In which species are fibroblasts and astrocytes described as the main cells of glial scar? Traumatic spinal cord injury in rats and mice has different consequences regarding glial scar formation. What is the main difference between these two animal models regarding glial scar formation? In humans, is a glial scar similar to rats or mice also generated? Authors should provide answers to these key questions, and should add this information in the final version of the manuscript.
6. The authors indicate "It has been also reported that modulation of these cellular populations in particular, by reducing the fibroblastic component of the scar can induce axonal regrowth and enhance functional recovery (Dias et al., 2018). Please describe in detail what strategy these authors of the bibliographic citation have used to modulate the fibroblasts of the glial scar, and what are the main results described in this scientific article. This information is relevant and should be included in the final version of the manuscript. Is the only strategy to modulate the glial scar? If there are more strategies, the authors should indicate them in this introductory section.
7. In the last paragraph of the introduction, the authors should include more detailed information on the mechanisms by which the applied therapy reduces fibrosis and demyelination, and increases cell survival. This information is relevant, and should be included in the final version of the manuscript.
8. What is the novelty of the work presented? There is already previous evidence that the combination of cell transplantation and magnetic stimulation after spinal cord injury may be a promising therapy to repair the spinal cord (e.g. Med Sci Monit. 2020 Aug 20;26:e924445). This information is relevant, and should be included in the final version of the manuscript.

MATERIALS AND METHODS
9. Why do the authors use LUX mice? What advantages and disadvantages do these animals have compared to other strains of mice? Are the strains used in the present study the most commonly used as an animal model of spinal cord injury? Is there prior evidence that these animal strains present histopathological signs similar to those observed in humans after spinal cord injury? Please clarify all these points and include all this relevant information in the final version of the manuscript.
10. The authors indicate that the cells are resuspended in DF10S. What previous evidence do the authors have that serum injected with cells does not induce inflammation and / or exacerbation of inflammation after spinal cord injury? Please clarify this point and include this relevant information in the final version of the manuscript.
11. How many histological images of each animal and for each marker have been taken and analyzed? Include this information in the final version of the manuscript.
12. The authors indicate that a minimum of 3 sagittal sections of the spinal cord have been analyzed, but they should also indicate the maximum number of sagittal sections analyzed per animal.
13. The authors indicate that each experimental group consists of 10 animals. However, histological studies have been carried out with 5 animals per experimental group. Why was the histological study carried out with half of the animals in each experimental group? What has been the criterion used to select the animals of each experimental group that have been analyzed histologically?
14. In the methodology section, it is not clear at what point in the experimental follow-up the histological studies are carried out, as well as the functional study of locomotion. Was the locomotion study carried out during the 14 days of magnetic stimulation? It has been done at the end of it? How many functional assessments were carried out in the study? Also indicate the post-injury day on which the histological study was performed. All this information is relevant and should be included in the final version of the manuscript.
15. In the methodology section, it is not clear if the statistical analysis of the results has been carried out blindly, that is, if the researcher who has analyzed the data is different from the researcher who has operated on the animals and performed the transplants and magnetic stimulation. This point is relevant and this information should be included in the final version of the manuscript.

RESULTS

16. The authors indicate that primary olfactory bulb cultures contain approximately 65% fibroblasts and 35% cells of the ensheathing glia of the olfactory bulb. This information is very interesting, but how do the authors know that they have really injected only cells of the ensheathing glia? Is the flow cytometry technique sufficient to guarantee that 100% of the transplanted cells are OECs? In addition, in primary cultures they indicate that there are two subtypes of OECs, which of them have been injected into the animals? Please, clarify these points and include this information in the final version of the manuscript.

17. The authors indicate "Before to compare the effects of the two therapies and there combined effects on functional recoveries and tissue repair, we analyzed the impact of rTSMS treatment on the survival of transplanted bOECs. We performed bOECs primary culture with cells obtained from LUX+/-mice. Then, using bioluminescence imaging system we followed the transplanted cells overtime. We transplanted the same number of cells (100.103 cells) in twenty mice divided in two groups of ten and in one of it, rTSMS treatment has been applied during 14 consecutive days (Fig. 2A, B). By quantification of emitted photons, we measured in each group the number of remaining cells at 9 days, 14 days and 16 days after SCI (Fig. 2C-E)". Everything that has been described in this paragraph, constitutes a new study carried out with animals other than the groups described in the methodology? In case of an affirmative answer, include all this information in the methodology section, and describe in more detail how this study of the survival of transplanted cells was carried out. What is the criteria for selecting days 9, 14 and 16 post SCI? Please, clarify these points and include this information in the final version of the manuscript.

DISCUSSION

18. The authors indicate "Indeed, immunohistological results indicate that only rTSMS treatment modifies the spinal scar by decreasing fibrosis and demyelination and increasing gliosis, whereas bOECs transplantation did not have a strong effect on these parameters (Figs. 3 and 4)". There are previous experimental evidences suggesting that the transplantation of OECs reduces glial scarring and reduces gliosis after spinal cord injury (e.g. J Comp Neurol. 2004 May 17;473(1):1-15; Neurobiol Dis. 2006 Dec;24(3):443-54; Neurobiol Dis. 2006 Jan;21(1):57-68; Glia. 2007 Feb;55(3):303-11; J Neurosci. 2016 Jun 8;36(23):6269-86). Why the results of the present study are inconsistent with other previous studies showing that the transplantation of OECs has effects on astrogliosis and glial scarring? Authors should make a more in-depth discussion on this point, and include it in the final version of the manuscript.

19. By what mechanisms does rTSMS treatment modify glial scar, astrogliosis and microgliosis? The authors must explain in depth the mechanisms involved in this therapy on the histological and functional changes observed. All this information is very relevant and should be discussed in depth by the authors, and should be included in the final version of the manuscript.

20. The authors indicate "in particular bOECs transplantation enhances functional recovery (Li et al., 1997; Watzlawick et al., 2016)". Authors should specify whether these citing authors also show functional recovery of locomotion based on the same technique used by the authors in the present study.

21. Why have the authors used this method of assessing the recovery of locomotion and not the most widespread method of locomotion in open field and BBB scale? It is essential that the authors discuss this point in depth, and that this information be included in the final version of the manuscript.

22. The authors indicate "In fact, OECs transplantation reduces axonal dieback and inflammation at the lesion site, however as we described here they do not modulate strongly the glial or the fibrotic scar (Khankan et al., 2016)". This sentence is not true, the authors have not carried out an exhaustive review of the previous bibliography, where they will be able to find both in vivo and in vitro studies that contradict what they indicate in the previous sentence. Please modify the previous sentence, including more bibliographic evidence and discussing the results with all these new previous scientific evidences. Include this information in the final version of the manuscript.

23. The authors indicate "our bioluminescence results are in agreement with previously reported studies describing that OECs survival is poor in lesioned spinal cord microenvironment (Khankan et al., 2016; Watzlawick et al., 2016; Delarue et al., 2020). This phrase is very relevant, but it is very poorly discussed by the authors, who must give more detailed and precise information on the survival of the OECs in the injured parenchyma of the spinal cord. These results must be discussed in more detail and depth, and in a more critical way. Please include all this information in the final version of the manuscript.

24. The authors indicate "We can hypothesize that rTSMS, via reduction of apoptosis, exerts its main effects shortly after the beginning of the treatment whereas, at the opposite bOECs transplantation through neurotrophic factors secretion induces its main properties at later time point". On what scientific evidence are the authors based to write the previous sentence? Please include this scientific evidence and better discuss this sentence based on this evidence. Include all this information in the final version of the manuscript.

25. In general, the discussion is very poor, the authors do not discuss the results in depth, and they do not justify their results with previous evidence that may be contrary to what they have found in these experiments. However, some of the results of this manuscript are very relevant, but they are very poorly

discussed and justified. The authors should make a significant effort to better discuss these relevant results. The authors also do not highlight which results are relevant with respect to previous similar studies.

Authors' Response

Editor:

Editor Comments to the Author:

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

Did the authors only use females in this study? The sex of the animals is not clearly defined in the
title or abstract, the authors mentioned 'females mice' once at the beginning of the methods.

SEX DIFFERENCES

The National Institutes of Health now mandates the inclusion of sex as a biological variable. To conform with this mandate, the Journal of Neuroscience Research has established new policy (please see our editorial: http://onlinelibrary.wiley.com/doi/10.1002/jnr.23979/full) requiring all authors to ensure proper consideration of sex as a biological variable. Please ensure that: 1) Any paper utilizing subjects of one sex state the sex of the sample in the title and abstract; 2) The number of samples/subjects of each sex used in the research must be clearly stated in the methods
section; 3) The inability for any reason to study sex differences where they may exist should be discussed as a study limitation. We are also encouraging authors to report exploratory analyses of
potential sex differences in studies not explicitly designed to address them.

Response: We have added this information into the manuscript (title, abstract and materials and methods).

There are no sham animals in this work, please explain/address this point properly.

Response: We have discussed this point in the Discussion part.

Please properly described in the methods and confirm that the cells were transplanted during the same surgery to perform SCI, but how does this translate to a possible use for therapy?

Response: This part of the methods has been clarified and is now discussed in the Discussion part.

When did the stimulation begun after SCI? the authors mentioned that they were applied for 14days, but it is unclear when the stimulations were started, and were the 14 days consecutive?

Response: This part of the methods has been clarified.

Language:

The verb tense should be kept consistent. 'mice were analgesia' should probably be 'mice were anesthetized'? "bOECs transplanted was taken from LUX+/- mice" would read better as "transplanted OECs were purified from LUX+/- mice."

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

SIGNIFICANCE STATEMENT

Authors must submit, in the main text of the document, a 100-word-maximum statement about the
significance of their research paper written at a level that is understandable to the general public and to scientists outside their field of specialty. This statement will be distinct in purpose from the
abstract, with the primary goal of broadly explaining the relevance and importance of this work and how this work contributes to different diseases.

Response: Significance statement has been added to the revised version of the manuscript

RESOURCE IDENTIFICATION INITIATIVE (Abbreviated Text)

Please incorporate Research Resource Identifiers (RRIDs) in your citation of all resources used in
your manuscript (antibodies, software tools, databases, model organisms) where applicable in the text, exactly as you would a regular citation or Genbank Accession number. Please also be sure these RRIDs are included in your keywords list in addition to the required keywords. For any antibodies, we ask that you also include the RRIDs in your antibody table, in addition to citing them in the text. An example of how to list RRIDs in your antibody table can found in the example
antibody table attached and for more information about how to obtain and cite RRIDs within your
text, please visit the "Resource Identification Initiative" section of our author guidelines:
http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1097-4547/homepage/ForAuthors.html

Furthermore, in accord with JNR requirements, you will have to provide additional information on each of the antibodies that you use. This must include the source (not just the manufacturer and
catalog or lot number, but the species it was raised in, and the EXACT structure of the immunizing
antigen - including the amino acid sequence for peptide antigens); characterization (e.g., what does
it recognize on immunoblots); and appropriate controls (i.e. the effect of blocking peptides for immunohistotochemical localization).

Response: RRIDs have been added to the revised version of the manuscript, however we did not find the amino acid sequences for the different antibodies.

GRAPHICAL ABSTRACT

Please upload a graphical abstract, which we are asking of all authors submitting original research
articles. This is intended to provide readers with a visual representation of the conclusions and an additional way to access the contents and appreciate the main message of the work. What we require is a .tif image file and a .doc text file containing an abbreviated abstract. For the image, labels, although useful, must be kept to a minimum and the image should be 400 x 300, 300 x 400,
or 400 x 400 pixels square and at a resolution of 72 dpi. This can be one of the figures from your article, or something slightly different, as long as it represents your study. Instructions for this can
be found in our author guidelines online at
http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1097-4547/homepage/ForAuthors.html

Response: Graphical abstract has been added to the revised version of the manuscript

Reviewer 1: Comments to the Author

This manuscript reports the effects of two potential treatments for spinal cord injury in a mouse model. The two treatments, (i) magnetic stimulation and (ii) transplantation of olfactory ensheathing cells, have each separately been previously demonstrated to improve recovery after spinal cord injury. In this study, they test whether the combination of the two treatments further improves outcomes. The findings demonstrate that while each treatment improves outcomes, the

combined treatment does not provide additional benefits. The authors propose that each treatment
targets different stages of recovery.
Overall, the manuscript is well written and the results are convincing, and the manuscript provides
an important contribution to the field which will help others to design new therapeutic approaches.
Response: First of all, we would like to thank the reviewer for his comments and questions.
Suggestions for improvement:
Methods. The details of the injection of the cells are perhaps incorrect. They state that the bOECs were injected at "1 mm depth, 1.5 mm from the midline, 5 mm rostrally and caudally from the lesion site". A female C57BL6 mice typically has a cord depth at T10 of 0.8-1 mm and a cord width of 1.2 mm. So an injection 1 mm deep would put it at the very base of the cord if not outside
the cord. 1.5 mm from the midline – does this mean a unilateral injection from the midline and if so which side? And 1.5 mm lateral would certainly put it outside the cord. Perhaps the authors have used the injection locations and dimensions from a rat study by mistake? Can this description
be clarified.
Response: We agree with the Reviewer. It was a mistake, this description comes from a study performed in rats.
Injections were delivered at 0.6 mm depth, 0.4 mm from the midline at the left, 5 mm rostrally and
caudally from the lesion site.
Results: Figs 3-4. It would be useful to have some high magnification images of the cells of interest
as these will enable readers to understand the cell morphology. The morphology of the cells can provide insights into how they are reacting to the environment and treatment. Including these along
with an interpretation of the outcomes would improve the impact of the manuscript.
Response: We agree with the Reviewer. Figures 3 and 4 have been remade and are now Figures 4 and 5 and higher magnifications images have been added to these Figures.
The manuscript needs to be edited and proofread for English language expression as there are some
minor errors throughout the manuscript.
Response: The manuscript has been checked for spelling and grammar.
Reviewer: 2
Comments to the Author
Manuscript # jnr-2020-Dec-9350
Comparison of the effects of two therapeutic strategies based on olfactory ensheathing cells transplantation and repetitive magnetic stimulation after spinal cord injury
Comments of the reviewer
In the aim to improve the manuscript, several changes should be included in the final version of the manuscript.
Response: First of all, we would like to thank the reviewer for his comments and questions.
INTRODUCTION

Response: We agree with the Reviewer.
The Introduction part has been completely rewritten to fit with Reviewer's comments.
The question 5 is also discussed with more details in the Discussion part.

1. The authors indicate "To date no cure can be offered to injured patients". This statement is not completely true, since various repair strategies have been tried in humans, including spinal cord repair with nerve grafts and ensheathing glial cell transplantation (Cell Transplant. 2014;23(12):1631-55.) or the application of stimulation neuroprosthesis (Science. 2012 Jun 1;336(6085):1182-5; Nature. 2018 Nov;563(7729):65-71.), with very promising results in patients
in whom these strategies have been tried. Authors should correct this phrase, contributing these strategies in the final version of the manuscript.

2. The authors indicate "Spinal cord damages induce a cascade of secondary events which include
inflammation, cell death or release of inhibitory factors leading to scar formation (Grégoire et al., 2015; Stenudd et al., 2015)". The authors should describe in greater detail the cellular and molecular phenomena involved in the secondary injury, especially those that will cause the separation of the glial scar, since one of the objectives of the study is the modulation of this glial scar. Please include this information in the final version of the manuscript.

3. The authors indicate "This scar which takes place after SCI, initially described as a glial scar, is
composed of several cellular types which play a dual inhibitory/permissive role (Sabelström et al.,
2013; Anderson et al., 2016). The authors should describe in greater detail the cellular and molecular components of the glial scar, indicating in what phase of the spinal cord injury this glial
scar begins to form, and what are the factors that induce its generation, as well as the meaning glial
scar function. Please include this information in the final version of the manuscript.

4. The authors indicate "To do so, different therapies have been developed and tested in rodents and also in Human (Ahuja et al., 2020)". The authors must provide more detailed information on the therapies studied in animal and human models that comply with the two approaches described
in the previous paragraph. This information is relevant and should be included in the final version
of the manuscript.

5. The authors indicate "As we described above, mainly two populations of cells constitute the spinal scar; astrocytes and fibroblasts (Göritz et al., 2011; Dias et al., 2018)". Are they (astrocytes,
fibroblast) the only cells present in the glial scar? Where do the fibroblasts that are part of the glial
scar come from? What role do these fibroblasts play in the glial scar? In which species are fibroblasts and astrocytes described as the main cells of glial scar? Traumatic spinal cord injury in
rats and mice has different consequences regarding glial scar formation. What is the main difference between these two animal models regarding glial scar formation? In humans, is a glial

scar similar to rats or mice also generated? Authors should provide answers to these key questions,
and should add this information in the final version of the manuscript.
6. The authors indicate “It has been also reported that modulation of these cellular populations in particular, by reducing the fibroblastic component of the scar can induce axonal regrowth and enhance functional recovery (Dias et al., 2018). Please describe in detail what strategy these authors of the bibliographic citation have used to modulate the fibroblasts of the glial scar, and what are the main results described in this scientific article. This information is relevant and should
be included in the final version of the manuscript. Is the only strategy to modulate the glial scar? If there are more strategies, the authors should indicate them in this introductory section.
7. In the last paragraph of the introduction, the authors should include more detailed information on the mechanisms by which the applied therapy reduces fibrosis and demyelination, and increases
cell survival. This information is relevant, and should be included in the final version of the manuscript.
8. What is the novelty of the work presented? There is already previous evidence that the combination of cell transplantation and magnetic stimulation after spinal cord injury may be a promising therapy to repair the spinal cord (e.g. Med Sci Monit. 2020 Aug 20;26:e924445). This information is relevant, and should be included in the final version of the manuscript.
MATERIALS AND METHODS
9. Why do the authors use LUX mice? What advantages and disadvantages do these animals have
compared to other strains of mice? Are the strains used in the present study the most commonly used as an animal model of spinal cord injury? Is there prior evidence that these animal strains present histopathological signs similar to those observed in humans after spinal cord injury? Please
clarify all these points and include all this relevant information in the final version of the manuscript.
Response: We are sorry, it was not clear enough in our manuscript.
The LUX mice have been initially obtained from Jackson Laboratory. Then, we crossed this mouse
line with C57BL/6 mice 3 times consecutively to be sure to avoid any problem related to the strains.
We discussed this specific point into the Discussion part.
10. The authors indicate that the cells are resuspended in DF10S. What previous evidence do the authors have that serum injected with cells does not induce inflammation and / or exacerbation of inflammation after spinal cord injury? Please clarify this point and include this relevant information in the final version of the manuscript.
Response: FBS used is heat inactivated (30min at 56°C) to inactivate complement proteins which
could increase inflammation. Moreover the final FBS volume injected per mouse is 0.4 µl.
We have previously injected DF10S alone in injured and uninjured mice and compared these groups to control SCI and control uninjured mice respectively. These data are under review but are not published yet. Based on these results we presume that FBS does not induce inflammation.
11. How many histological images of each animal and for each marker have been taken and

analyzed? Include this information in the final version of the manuscript.

Response: For immunochemistry analyses, one image per animal for each marker has been taken and analyzed which represents five images by group for each marker.

12. The authors indicate that a minimum of 3 sagittal sections of the spinal cord have been analyzed, but they should also indicate the maximum number of sagittal sections analyzed per animal.

Response: We are sorry, it was not clear enough in our manuscript.

Three to five sections per animals were observed in order to determine the epicenter of the lesion.

Then, an image of the epicenter was taken and analyzed.

13. The authors indicate that each experimental group consists of 10 animals. However, histological studies have been carried out with 5 animals per experimental group. Why was the histological study carried out with half of the animals in each experimental group? What has been
the criterion used to select the animals of each experimental group that have been analyzed histologically?

Response: All mice were euthanized and fixed 60 days after spinal cord injury. For behavioral analyzes, our statistical analyses are based on previous studies performed in our laboratory showing that 10 animals per group are needed for these experiments. In the same way in our previous studies, 5 animals per group were needed to obtain significant differences between groups. That is why, in the present study we analyzed 5 mice per group (randomly chosen among the 10), and keep the other samples frozen in order to be able to conduct additional experiments if
needed.

14. In the methodology section, it is not clear at what point in the experimental follow-up the histological studies are carried out, as well as the functional study of locomotion. Was the locomotion study carried out during the 14 days of magnetic stimulation? It has been done at the end of it? How many functional assessments were carried out in the study? Also indicate the postinjury
day on which the histological study was performed. All this information is relevant and should be included in the final version of the manuscript.

Response: We agree with the Reviewer.

An overview of the paradigms used in this study is now presented as Figure 1.

All the time points have been also added into their respective parts of the Materials and Methods.

15. In the methodology section, it is not clear if the statistical analysis of the results has been carried out blindly, that is, if the researcher who has analyzed the data is different from the researcher who has operated on the animals and performed the transplants and magnetic stimulation. This point is relevant and this information should be included in the final version of the manuscript.

Response: Statistical analyses have been carried out blindly. All the experiments were carried out
and distributed between 3 different researchers. SCI, OB cultures and transplantations have been performed by one researcher (QD) who performed also histological experiments and statistical analysis, whereas magnetic stimulation, bioluminescence and locotronic experiments have been performed independently by two other researchers (AR and RM).

RESULTS

16. The authors indicate that primary olfactory bulb cultures contain approximately 65% fibroblasts and 35% cells of the ensheathing glia of the olfactory bulb. This information is very interesting, but how do the authors know that they have really injected only cells of the ensheathing
glia? Is the flow cytometry technique sufficient to guarantee that 100% of the transplanted cells are OECs? In addition, in primary cultures they indicate that there are two subtypes of OECs, which of them have been injected into the animals? Please, clarify these points and include this information in the final version of the manuscript.

Response: We are sorry, it was not clear enough in our manuscript.

In our study we have injected primary olfactory bulbs cultures and not purified OECs, we discuss that specific point into the Discussion part. It means that we have injected a mixture of OECs containing the two p75 populations and fibroblasts.

We have changed "bOECs" and replace it with "primary bOECs" throughout the manuscript.

17. The authors indicate "Before to compare the effects of the two therapies and there combined effects on functional recoveries and tissue repair, we analyzed the impact of rTSMS treatment on the survival of transplanted bOECs. We performed bOECs primary culture with cells obtained from LUX+/-mice. Then, using bioluminescence imaging system we followed the transplanted cells overtime. We transplanted the same number of cells (100.10^3 cells) in twenty mice divided in two groups of ten and in one of it, rTSMS treatment has been applied during 14 consecutive days (Fig. 2A, B). By quantification of emitted photons, we measured in each group the number of remaining cells at 9 days, 14 days and 16 days after SCI (Fig. 2C-E)". Everything that has been
described in this paragraph, constitutes a new study carried out with animals other than the groups
described in the methodology? In case of an affirmative answer, include all this information in the
methodology section, and describe in more detail how this study of the survival of transplanted cells was carried out. What is the criteria for selecting days 9, 14 and 16 post SCI? Please, clarify these points and include this information in the final version of the manuscript.

Response: We are sorry, it was not clear enough in our manuscript.

An overview of the paradigms used in this study is now presented as Figure 1.

Our time line is based on the analysis of the literature and our own studies. Indeed, Khankan et al.,
2016, have described that bOECs survived 2 weeks after transplantation into lesioned spinal cords.

In our lab we have also demonstrated that primary bOECs survived up to 2 weeks after SCI and transplantation (Delarue et al, 2020).

This specific point is discussed into the Discussion part.

DISCUSSION

Response: We agree with the Reviewer.

The Discussion part has been completely rewritten to fit with Reviewer's comments.

18. The authors indicate "Indeed, immunohistological results indicate that only rTSMS treatment modifies the spinal scar by decreasing fibrosis and demyelination and increasing gliosis, whereas bOECs transplantation did not have a strong effect on these parameters (Figs. 3 and 4)". There are

previous experimental evidences suggesting that the transplantation of OECs reduces glial scarring
and reduces gliosis after spinal cord injury (e.g. J Comp Neurol. 2004 May 17;473(1):1-15; Neurobiol Dis. 2006 Dec;24(3):443-54; Neurobiol Dis. 2006 Jan;21(1):57-68; Glia. 2007 Feb;55(3):303-11; J Neurosci. 2016 Jun 8;36(23):6269-86). Why the results of the present study are inconsistent with other previous studies showing that the transplantation of OECs has effects on astrogliosis and glial scarring? Authors should make a more in-depth discussion on this point, and include it in the final version of the manuscript.

Response: We agree with the Reviewer, gliosis has been replaced by glial scar and we discuss about the difference between "glial component of the scar" and "gliosis and hypertrophic astrocytes".

19. By what mechanisms does rTSMS treatment modify glial scar, astrogliosis and microgliosis? The authors must explain in depth the mechanisms involved in this therapy on the histological and
functional changes observed. All this information is very relevant and should be discussed in depth
by the authors, and should be included in the final version of the manuscript.

20. The authors indicate "in particular bOECs transplantation enhances functional recovery (Li et al., 1997; Watzlawick et al., 2016)". Authors should specify whether these citing authors also show
functional recovery of locomotion based on the same technique used by the authors in the present
study.

21. Why have the authors used this method of assessing the recovery of locomotion and not the most widespread method of locomotion in open field and BBB scale? It is essential that the authors
discuss this point in depth, and that this information be included in the final version of the manuscript.

22. The authors indicate "In fact, OECs transplantation reduces axonal dieback and inflammation at the lesion site, however as we described here they do not modulate strongly the glial or the fibrotic scar (Khankan et al., 2016)". This sentence is not true, the authors have not carried out an
exhaustive review of the previous bibliography, where they will be able to find both in vivo and in vitro studies that contradict what they indicate in the previous sentence. Please modify the previous sentence, including more bibliographic evidence and discussing the results with all these
new previous scientific evidences. Include this information in the final version of the manuscript.

23. The authors indicate "our bioluminescence results are in agreement with previously reported studies describing that OECs survival is poor in lesioned spinal cord microenvironment (Khankan et al., 2016; Watzlawick et al., 2016; Delarue et al., 2020). This phrase is very relevant, but it is very poorly discussed by the authors, who must give more detailed and precise information on the survival of the OECs in the injured parenchyma of the spinal cord. These results must be discussed in more detail and depth, and in a more critical way. Please include all this information in the final version of the manuscript.

24. The authors indicate "We can hypothesize that rTSMS, via reduction of apoptosis, exerts its main effects shortly after the beginning of the treatment whereas, at the opposite bOECs

transplantation through neurotrophic factors secretion induces its main properties at later time point". On what scientific evidence are the authors based to write the previous sentence? Please include this scientific evidence and better discuss this sentence based on this evidence. Include all
this information in the final version of the manuscript.
25. In general, the discussion is very poor, the authors do not discuss the results in depth, and they
do not justify their results with previous evidence that may be contrary to what they have found in
these experiments. However, some of the results of this manuscript are very relevant, but they are
very poorly discussed and justified. The authors should make a significant effort to better discuss these relevant results. The authors also do not highlight which results are relevant with respect to previous similar studies.

2nd Editorial Decision

Decision Letter
Dear Dr Guérout:

Thank you for resubmitting your manuscript to the Journal of Neuroscience Research. We've now received the reviewer feedback and have appended those reviews below. I'm glad to say that the reviewers are overall very enthusiastic and supportive of the study. They did raise some concerns and made some suggestions for clarification, but I expect that these points should be relatively straightforward to address. If there are any questions or points that are problematic, please feel free to contact me. I am glad to discuss. .

We ask that you return your manuscript within 30 days. Please explain in your cover letter how you have changed the present version and submit a point-by-point response to the editors' and reviewers' comments. If you require longer than 30 days to make the revisions, please contact Dr Cristina Ghiani (cghiani@mednet.ucla.edu). To submit your revised manuscript: Log in by clicking on the link below https://wiley.atyponrex.com/submissionBoard/1/1ce08316-0529-439d-8780-cf5f05383a55/current

(If the above link space is blank, it is because you submitted your original manuscript through our old submission site. Therefore, to return your revision, please go to our new submission site here (submission.wiley.com/jnr) and submit your revision as a new manuscript; answer yes to the question "Are you returning a revision for a manuscript originally submitted to our former submission site (ScholarOne Manuscripts)? If you indicate yes, please enter your original manuscript's Manuscript ID number in the space below" and including your original submission's Manuscript ID number (jnr-2020-Dec-9350.R1) where indicated. This will help us to link your revision to your original submission.)

The journal has adopted the "Expects Data" data sharing policy, which states that all original articles and reviews must include a Data Availability Statement (DAS). Please see https://authorservices.wiley.com/author-resources/Journal-Authors/open-access/data-sharing-citation/data-sharing-policy.html#standardtemplates for examples of an appropriate DAS. Please include the DAS in the manuscript as well.

Thank you again for your submission to the Journal of Neuroscience Research; we look forward to reading your revised manuscript.

Best Wishes,

Dr Bradley Kerr
Associate Editor, Journal of Neuroscience Research

Dr Cristina Ghiani
Editor-in-Chief, Journal of Neuroscience Research

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

JOURNAL OF Neuroscience Research

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.

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

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

Reviewer: 1

Comments to the Author
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.

Reviewer: 2

Comments to the Author
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.

Authors' Response

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.

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.

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 posthoc
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).
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.
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.

3rd Editorial Decision

Decision Letter

Dear Dr Guérout:

Thank you for submitting your manuscript "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" by Delarue , Quentin ; Robac , Amandine ; Massardier, Romane ; Marie, Jean-Paul; Guérout, Nicolas.

You will be pleased to know that your manuscript has been accepted for publication. Thank you for submitting this excellent work to our journal.

In the coming weeks, the Production Department will contact you regarding a copyright transfer agreement and they will then send an electronic proof file of your article to you for your review and approval.

JOURNAL OF Neuroscience Research

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Congratulations on your results, and thank you for choosing the Journal of Neuroscience Research for publishing your work. I hope you will consider us for the publication of your future manuscripts.

Sincerely,

Dr Bradley Kerr
Associate Editor, Journal of Neuroscience Research

Dr Cristina Ghiani
Editor-in-Chief, Journal of Neuroscience Research

Authors' Response

4th editorial decision

Decision Letter

Author response