Peer Review File

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Reviewer A

This study investigated if a specially designed GDNF administration technique would promote parasympathetic regeneration and a recovery from ED in an animal model of bilateral cavernous nerve injury (BCNI). The authors concluded that local continuous GDNF delivery technique promotes parasympathetic nerve fiber regeneration, autonomic balance rehabilitation and sexual function recovery in a rat model BCNI induced erectile dysfunction. However, there are still some major problems which need to be addressed.

[Abstract]
Comment 1: There is no description about the aim of this study in the Background section.
Reply 1: Thank you for your comment. We have added a description of aim in the background section.
Changes in the text: Page 3, line 7

Comment 2: The description of methodology should be improved in the Methods section.
Reply 2: Thank you for your comment. We have added more details of experimental protocols in the methods section.
Changes in the text: Page 3, line 10

Comment 3: Also, the description of the results should be improved in the Results section.
Reply 3: We re-write the results section. Thank you.
Changes in the text: Page 3, line 20

[Text]
Comment 4: The introduction section of this manuscript needs to be clearer and more focused. In particular, the study background described in the Introduction section is not enough to draw the aims of the study. Thus, the introduction section of this manuscript needs to be improved.
Reply 4: Thanks for your suggestion. We have updated the Background section to make

it clearer and more focused especially for the aim of this study.
Changes in the text: Page 5, line 3

Comment 5: A major concern about this manuscript is that rats used for the studies were only 56 days old at the start of the experiments and only 84 days old at the end. This is not adult (P110-120). The experiment was performed during their puberty in the rat when developmental pathways are ramped up to finish tissue morphogenesis. The results may not reflect findings in an adult animal.
Reply 5: This is a very good question. In this project, we focused on the impairment and recovery of sexual function. Most rats become sexually mature at age 6 weeks (P45-48), so we believe that 8 week (P56) old rats are proper for research in sexual function. However, sexual maturity itself does not mark the beginning of adulthood which means more mature behavior and more advanced body weight. It represents the beginning of adolescence to a greater extent. We totally agree that the results in this project may not reflect findings in adult animals, but we believe they reflect findings in sexually mature animals. We have added this information in the methods section and discussion section.

Reference about animal sexual maturity:
The Laboratory Rat: Relating Its Age With Human's. Sengupta P. Int J Prev Med. 2013 Jun;4(6):624-30. PMID: 23930179
References about experiments using 6 to 8 weeks old rats in the field of urology or andrology:
The influence of smoking exposure and cessation on penile hemodynamics and corporal tissue in a rat model. Lin JH, Ho DR, Shi CS, Chen CS, Li JM, Huang YC. Transl Androl Urol. 2020 Apr;9(2):637-645. doi: 10.21037/tau.2019.12.45. PMID: 32420170
Possible therapeutic effects of vindoline on testicular and epididymal function in diabetes-induced oxidative stress male Wistar rats. Oguntibeju OO, Aboua Y, Kachepe P. Heliyon. 2020 Apr 28;6(4):e03817. doi: 10.1016/j.heliyon.2020.e03817. eCollection 2020 Apr. PMID: 32373734
Changes in the text: Page 8, line 6; Page 20, line 18

Comment 7: Also, the Discussion section needs to be further improved and summarized, focusing on the major findings of the study.
Reply 7: Thanks for your suggestion. We have updated the discussion section to focus on the major findings of the study.
Changes in the text: Page 20

Reviewer B

Overall this is a nice study looking at the impact of GDNF treatment in a model of nerve injury-induced ED. Authors demonstrated a dose dependent increase in erectile function in BCNI rats following 4 week infusion of GDNF.

Minor comments:

Comment 1: Please indicate in the methods section how blood pressure was measured. Additionally, please include the MAP tracing in each representative ICP tracing.

Reply 8: Thanks for your comment, we have added a description of how blood pressure was measured in this project. Representative MAP tracing was added in s-Fig 1

Changes in the text: Page 11, Line 10; s-Fig 1

Comment 2: Please indicate in the figure captions how many animals were used in each experiment.

Reply 9: Thank you for your comment, we have added the information of animal numbers in the figure legends

Changes in the text: Page 30

Comment 3: Previous studies have shown that BCNI decreases GDNF gene expression in the MPG. I would consider including it in your discussion doi: 10.1002/jnr.23553

Reply 10: Thank you for your suggestion. We have added this reference

Changes in the text: Page 22, Line 19

Reviewer C

The manuscript in title of Local continuous GDNF release using osmotic pump promotes parasympathetic nerve rehabilitation in an animal model of cavernous nerve injury induced erectile dysfunction aims to explore a new method to deriver GDNF for regeneration of nerve fibers especially for parasympathetic nerves. This is a nice study and the manuscript is well prepared. However, there are several issues should be fixed before it could be accepted for publication.

Comment 1: The abstract should be reorganized. More information should be presented in the abstract such as experimental design and grouping information.

Reply 11: Thank you for your suggestion. We have updated the Abstract section to include more information.

Changes in the text: Page 3

Comment 2: The authors used 8-week old male rats in this experiment. However, those animals were not adult rats. They are not good models for the erectile function study.

Please provide reason why the 8-week male rats were used in this experiment.

Reply 12: This is a very good question. In this project, we focused on the impairment and recovery of sexual function. Most rats become sexually mature at age 6 weeks (P45-48), so we believe that 8 week (P56) old rats are proper for research in sexual function. However, sexual maturity itself does not mark the beginning of adulthood which means more mature behavior and more advanced body weight. It represents the beginning of adolescence to a greater extent. We totally agree that the results in this project may not reflect findings in adult animals, but we believe they reflect findings in sexually mature animals. We have added this information in the methods section and discussion section.

Reference about animal sexual maturity:

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Changes in the text: Page 8, line 6; Page 20, line 18

Comment 3: There are many typos in the main text, such as “vesicular acetylcholine transporter (VaCHT)” it should be “vesicular acetylcholine transporter (VAChT), please revised them.

Reply 13: Thank you for your comment. We checked all the typos and misspellings through the text. We have updated the manuscript and figures throughout.

Changes in the text: Through the text

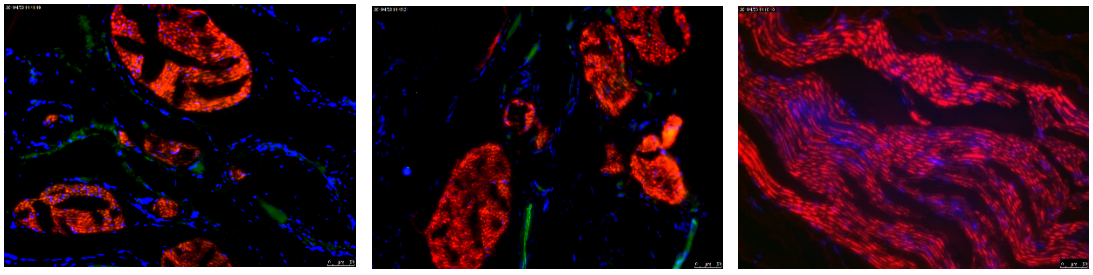
Comment 4: In the Figures 1&2&5, the image resolution is pretty low, please provide images at higher resolution> 300dpi.

Reply 14: Thank you for your comment. The Figures provided for the editorial system is in higher resolution. The system built a PDF for reviewers with limited file size. We will make sure the figures for final publish are in higher resolution. Thank you again.

Changes in the text: All the figures

Comment 15: Please provide better image for Fig 6A.

Reply 15: Thank you for your suggestion. Fig 6A are the representative images of cross section of dorsal penile nerve in higher magnification fields. The nature of nerve tissue makes it fragile in the tissue processing. We have reviewed the remaining pictures from this project, but they all looked similar. The pictures of other nerve tissues in our other projects also have similar issues. Please find the pictures attached. We are really sorry for this technique issues and we will definitely improve our tissue processing procedures in our future projects. As for Fig 6A in this study, we have added some white lines to outline the nerves' transection to make this figure more readable. Thank you and we are sorry for this inconvenient caused by us for your reviewing process.



Changes in the text: Fig 6A