

## Answering to the reviewers

### SPECIFIC COMMENTS TO AUTHORS

How to harvest sufficient NSCs is a basic requirement for the study and clinical application of NSCs. This study describes a simplified and efficient method for neonatal rat brain-derived NTC culture and identification comprehensively considering the influencing factors including timing of passaging, passage number, passaging approaches and methods for cell identification. It is essential for research in the field of neural stem cells, as it will provide important insights for understanding and treating brain disorders. Efficient way to harvest sufficient neural stem cells will facilitate the use of NSCs in clinical scenarios. Thank you for a useful and important synopsis of this important topic. I have no specific comments to the authors.

**Answer:** Thank you for your comments.

### SPECIFIC COMMENTS TO AUTHORS

The manuscript is well researched and well written, and may developed a simplified and efficient method for neonatal rat brain-derived neural stem cell culture and identification. Overall, this study was well conducted with good methodology and intelligible English. 1. Title: Proper and cover all the core result from the study. 2. Abstract: Address all the important component from the study. All 'NTCs' should be 'NSCs'. 3. Key words: could cover this study. 4. Introduction: Describe the overall basic knowledge for this study. Moreover, the aim of the study is clear. 5. Method: Primary culture, passage, cryopreservation, resuscitation and identification of NCSs are described in great detail. 6. Results: The results are very good, the figures are very representative, so that we can see clearly at a glance. 7. Discussion: The manuscript clearly interprets the finding adequately and appropriately. In addition, the manuscript highlights the key points clearly. The previous significant paper involved were included in the discussion, I suggest to add the significance of the study, and what further research is required. 8. References: The manuscript reviewed previous related literature.

I recommend that the manuscript can be published after a minor editing. Sincerely

**Answer:** Thank you for your comments. The manuscript has been revised according to your comments.

1. All 'NTCs' have been changed to 'NSCs'.
2. The significance of the study has been added.
3. The references list has been edited and updated.
4. The manuscript was proofed and edited carefully again.