Author's response to reviews

Title: Effect of Apoptosis in Neural Stem Cells Treated with Sevoflurane

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Author's response to reviews:

Reviewer(s)' Comments to Author:
The authors thank the reviewer's for sparing their valuable in reviewing our manuscript.

Answers to Reviewer Pisano Pascal:

1. The Background/Introduction section does not show enough the main interest of this study. The first paragraph of the Discussion section "Sevoflurane anesthesia in infant rats can result in long-term cognitive impairment, possibly by inhibiting neurogenesis [13]. The hippocampus is critical for memory consolidation and is one of only two mammalian brain regions where neural stem cells (NSCs) are renewed continuously throughout life. Thereby, further studies with sevoflurane exposure of NSCs are necessary to confirm whether sevoflurane can influence hyperplasia and apoptosis of neural cells" better shows the aim of the paper and it seems more appropriate, in the reviewer view, to place this at the end of the Introduction. Therefor the first sentence of the discussion should be revised.

Response: We agreed with the reviewer's view, and we have changed this section on line 50-55, page 3. We have revised the first sentence of the discussion on line 201-207, page 10.

2. Material and methods: please justify the concentration of sevoflurane used (1MAC) which is higher than this reported by Pellegrini et al. (Paediatr Anaesth. 2014 Jul;24(7):749-59 (0,5 MAC) in neonate rats.


3. Discussion, line 189 : please cite the work of Pellegrini et al. (Paediatr Anaesth. 2014 Jul;24(7):749-59) who showed sevoflurane-induced brain
apoptosis in brain of neonate rats.
Response: We have cited the article for [24] on line 221, page 11.

4. In figure legends please type GABAA receptor with A subscripted.
Response: We were sorry to make a mistake, and have corrected the errors on line 410-414, page 17.

Answers to Reviewer Monika Berns:

1. NSC culture
There should be a paragraph for the cell isolation from the removed brains. Did they take the whole brains or only specific regions as the hippocampus? Or is the medium they used a selection medium for specific cells?
Response: We have added a paragraph for the cell isolation from the removed brains on line 93-96, page 5. We cultured the NSCs which were derived from the hippocampus. The NSCs we cultured were used a selection medium which mixed with DMEM/F12, 1:1, 20 ng/ml bFGF, and 20 ng/ml EGF.

2. There should be a paragraph concerning the method used for sevoflurane exposure. Did they use a billups chamber?
Response: We have added a paragraph concerning the method used for sevoflurane exposure on line 101-115, page 5.

3. The method for the administration of bicuculline is completely unclear. What was the concentration used?
Response: The concentration of bicuculline we used was 10uM.

4. CCK-8 Assay and LDH Assay
What is the CCK-8 assay measuring exactly? Even if an assay is used the reader would like to know briefly how it works? LDH release is measured in the supernatant. Make this clear.
Response: We used the CCK-8 assay to measure the viability of NSCs. We have introduced the CCK-8 assay and LDH release how they work on line 118-124, page 6.

5. Results
Page 8 “Expression of GAGAA receptor with the treatments of sevoflurane” – What does that mean? Were the cells for this experiment exposed to sevoflurane for 2 days?
Response: We are sorry to make a mistake, and we have changed the mistake on line 186-192, page 9. What we wanted to express was the expression of GABAA receptor in Group S and Group S+B. The NSCs were not exposed to sevoflurane for 2 days, but for 0 h, 3 h, 6 h, 12 h.

6. Discussion
In general there are unclear statements and conclusions. The assumed
conclusion of hyperplasia is poorly explained by the results and/or not discussed. So the title of the paper might be wrong.
Response#We have changed the title in the revised manuscript, we deleted the part of hyperplasia.

7. The authors conclude that the proliferation of NSCs exposed to sevoflurane decreased, but there is no experiment in their work that examines that. All experiments deal with cell death and apoptosis. Where the cells from the hippocampus?
Response#Yes, we derived the NSCs from the hippocampus, and we have changed this part on the proliferation of NSCs.

8. Many grammatical errors or incomprehensible sentences.
Page 9 line 175: Behide “the study” is missing “of Statmann et al.”
Page 9 line 182: expose “to” sevoflurane
Page 10 line 198: “exposure” instead of “exposed”, “way” is missing behind time-dependent
Response#We have added “of Sratmann et al” on line 208, page 10.
We have added “to” on line 214, page 10.
We have changed “exposure” to “exposed” on line 230, page 11, and added “way” behind time-dependent.

9. Conclusion
In my opinion the conclusion has to be formulated more cautiously. Bicuculline is fare away from being a medication in clinical use.
Response#We agreed with your view, and have changed the conclusion more cautiously on line 268-270, page 13.

10. Specific comments
Other grammatical examples
Page 3 line 49: “caused” instead of “causing”
Page 3 line 50: “system” is missing after nervous
Page 3 line 62: insert “the” before central
Page 4 line 69: “lead” not “leading”
Response#We have changed “causing” to “caused” on line 49, page 3.
We have added “system” behind “central nervous” on line 50, page 3.
We have added “the” behind “amio acid of” on line 61, page 3.
We have changed “leading” to “lead” on line 68, page 4.

Answers to Reviewer You Shang:
1.To add the control group in this paper would be better. Because the GABAA inhibitor bicuculine was dissolved in DMSO, DMSO may affect apoptosis of cells. To declare whether or not the DMSO cause the change, and then to ensure the
effects of sevoflurane on the neural stem cells.

Response: We agreed with you, and have added the data of the viability and cytotoxicity of NSCs exposed DMSO on Figure 1.

2. The data described Group B in the results of viability and cytotoxicity, but did not be found in Annexin V/PI staining and western blots. Please explain this phenomenon.

Response: We have added the interpretation why we delete the Group B and Group D on line 165-169, page 8, and line 175-179, page 9.

3. Please merge the Fig.3A with Fig.3B. As the the same to merge the Fig.4A with Fig.4B, and Fig.5A with Fig.5B.

Response: We have merged these figures.

4. There are some spelling errors in manuscript. Please check and correct them.

Response: We have changed a lot of errors, and would have the language service to promote our manuscript.

All of the indicated errors and omissions have been corrected in line with the referee’s comments.