Author’s response to reviews

Title: The interplay of BDNF-TrkB with NMDA receptor in the process of propofol-induced cognition dysfunction in Hippocampus of neonatal rats

Authors:
Fang Wang (grace_wff@hotmail.com)
Junfei Zhou (chow_jf@21cn.com)
Jun Zhang (uuion08@126.com)
Jianfeng Li (jianfengli_sa@sohu.com)
Li Ma (lilima167@sohu.com)
Tieli Dong (mrdongtl@sohu.com)
Zhigang Zhuang (zhuangzg055@sohu.com)

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Responses to reviewers’ comments and the list of changes in text

Dear Editor, We appreciate your response for our manuscript which entitled "The interplay of BDNF-TrkB with NMDA receptor in the process of propofol-induced cognition dysfunction in Hippocampus of neonatal rats" so quickly. On the other hand, we also thank all the reviewers and you very much for the constructive advice and hard work.According to the comments from the reviewers, we do the following explanations and revisions. We wonder if the following replies could meet your requirements. Please feel free to contact with us if there is still something you want us to do. We look forward to hearing from you. Thank you! Best regards! Sincerely yours, Tieli Dong

Response to Reviewer 1: Thanks for your comments on our paper. We have revised our paper according to your comments:1. There are no statements about how the animals did during the anesthesia. What was the mortality rate among the animals treated with propofol, what was the specific propofol dosage given, how did the animals do under anesthesia, for example, what was the respiratory rate, were they monitored for oxygen saturation, were they given supplemental oxygen, did they have their heart rate checked throughout the anesthesia? These are all very important questions, as there may be concerns that the results are secondary to effects of hypoxia, hypercarbia, hypotension, bradycardia, and/or other physiological disturbances during the experiments. We would not be able to understand this, unless there was some additional data reported about the anesthesia model used. If this data is not available for the currently reported animals, we would need to see data from additional experiments with animals undergoing anesthesia using propofol.

Response: How the animals did during the anesthesia was according to the propofol administration as described in a previous study[1]. For the anesthesia procedure, rats were placed in a thermostat incubator...
(28°C) after loss of righting reflex. To avoid the rebreathing of CO2, the inspired CO2 concentration was continuously monitored and maintained a continuous flow supplemental oxygen (2 L / min) and respiratory frequency and skin color were observed until they could perform the righting reflex. The mortality rate was nearly 30%. Rats that failed to restart breathe were excluded from further study.

2. This study is looking at the effects of propofol in the developing brain. The mechanism of anesthesia neurotoxicity in the developing brain is unique to the time period of neuronal development and synaptogenesis. The authors, however, link these findings to post-op cognitive dysfunction (POCD). POCD may be related; however, that is a very different disease process compared to that of anesthesia induced neurotoxicity in the developing brain. If the animal model was that of an older rat with multiple co-morbidities undergoing anesthesia exposures, then this connection would be ok. The model used, however, is that of neonatal animals that are still going through the neurodevelopment process. It is not appropriate, therefore, to correlate the findings in the neonatal brain with those of POCD. From the introduction, it seems that the authors are making a connection that the effects of anesthesia in the developing brain will be the same as that in the older brain that leads to POCD, and this needs to be adjusted. Response: It is estimated that more than 6 million children receive anesthesia every year. In modern anesthesiology, general anesthesia is the most common practice for surgery or relief from procedural pain, particularly in children. Previous studies suggested that anesthetic exposure can lead to neurotoxicity in the developing brain. Similarly, children exposed to anesthetics during early life have been reported to exhibit a higher incidence of learning deficits at adolescence. These results prompt concerns regarding the possible detrimental effects of commonly used anesthetics in the pediatric population. The effects of general anesthetics on the developmental brain have drawn much attention from all sectors of society. Propofol is a commonly used intravenous anesthetic, which is widely used in pediatric anesthesia because of its rapid onset, short acting time, quick and complete recovery. However, a large number of cell experiments and animal studies have found that propofol can lead to cerebral neuronal apoptosis during development, and/or cause developmental abnormalities such as long-term learning and memory in rats. In addition, there are clinical reports of propofol-induced neurological dysfunction and behavioral abnormalities in children. Therefore, actively looking for safe and effective measures to prevent propofol-induced developmental brain damage has become a problem to be solved. According to the reviewer’s thoughtful comment, we have revised the Background section to more consistent with our research for better illustrating and understanding the objectives of our research.

3. The six groups receiving propofol (P, PN, PD, PND, PNK, and PMD groups) were listed but differences between these groups were not defined in the methods section. Response: Thank you for your valuable advice. We have defined these groups clearly in the methods section.

Response to Reviewer 2: Thanks for your comments on our paper. We have revised our paper according to your comments:

1. The nomenclature used throughout the manuscript (e.g., PD, PND, PNK) is confusing and difficult to follow. The authors should relabel the figures and also use simpler nomenclature when referring to the different conditions in the results section of the manuscript. It is difficult for the reader to remember each of the acronyms throughout the manuscript. Response: We greatly appreciate for your suggestion and we think the nomenclature used in the manuscript is reasonable and simple. To better understand and remember these groups, we have provided a clear description of grouping in the Method section.

2. In Figure 2C, the p-TrkB blot shows only 7 samples rather than 8 that are included for all other blots. Additionally, in some cases the blots for the phosphoproteins and total proteins (e.g., p-ERK1 and ERK1 in Figure 2F) appear to be derived from different blots. Please include the data from the same blot for the