OPEN PEER REVIEW REPORT 1

Name of journal: Neural Regeneration Research
Manuscript NO: NRR-D-18-00869
Title: Pre-treatment with GLYX-13 Attenuates Long-term Isoflurane Exposure Induced Cognitive Impairment in Mice
Reviewer's Name: Elizabeth D Kirby
Reviewer's country: USA
Date sent for review: 2018-12-14
Date reviewed: 2018-12-26

COMMENTS TO AUTHORS

The article is largely acceptable except for how the statistics were done. They need to use two-way ANOVAs with posthoc tests to compare groups. They can use the LSD test, but it should follow a two-way ANOVA, not a one-way ANOVA.

In this study, the authors present the results of two studies investigating how pre-treatment with the NMDAR agonist GLYX-13 alters isoflurane-induced cognitive impairment and decrease in hippocampal NR2B-CamKII-CREB signaling activity. The authors present data from two experiments. In the first experiment, they administer GLYX-13 systemically before prolonged (6h) isoflurane exposure and show that 1 and 3 days after isoflurane, cognitive performance in two tasks is impaired by isoflurane but that that impairment is reduced by pre-exposure to GLYX-13. Similarly, they show that mRNA expression and levels of phosphorylated NR2B, CamKII and CREB are reduced by isoflurane 1 and 3 days after exposure and that GLYX-13 pre-exposure prevents those reductions. In the second experiment, the authors expose all mice to isoflurane anesthesia but some mice are also pre-exposed to a CamKII inhibitor, KN93. They find that while GLYX-13 still improves cognitive performance and increases total mRNA and phosphorylated NR2B, CamKII and CREB protein, pre-treatment with KN93 prevents these effects of GLYX-13, suggesting that activation of CamKII is essential for these improvements caused by GLYX-13. Overall, the studies are well-constructed, the data are well-presented and the findings provide a useful advance in understanding how a systemic compound can help combat anesthesia-induced cognitive deficits. There are some issues of data analysis and some further discussion that need to need to be addressed, as detailed below:

1. Instead of one-way ANOVAs, the data reported throughout the manuscript require two way ANOVAs. In the first experiment, it should be a two way ANOVA with the two factors being isoflurane exposure and GLYX-13 exposure. In the second, it should be a two way ANOVA with the two factors being GLYX-13 exposure and KN93 exposure. The p-values for the interaction and main effects should be reported. These two-way ANOVAs should then be followed by an appropriate post-hoc test (Tukey's, Dunnetts to control group, etc).
2. How long was the isoflurane anesthesia used for ICV injections? The mice in the KN93 study all had two rounds of isoflurane exposure, one for ICV injection of KN93/vehicle and one for the "prolonged" anesthesia manipulation. It would be helpful for interpretation of the findings of this experiment to know how long to first exposure to anesthesia was in comparison to the "prolonged" one.
3. The phospho data presented is normalized to b-actin rather than total protein level. This does not invalidate the data but it does mean interpretation should be done carefully. The increase in phospho-protein level shown here could be driven entirely by an increase in total protein amount or could be the result of changes in phosphorylation rates. This distinction should be discussed in the discussion. Alternatively, the authors could re-run the samples for total protein levels of NR2B, CamKII and CREB to determine if the relative amount of phospho-protein over total protein is

increased or if just total protein (phospho and non-phsopho) is increased.
4. While the CFC task is generally accepted as a hippocampus-dependent task, the NOR task is more controversial. Some studies provide evidence that NOR is hippocampus-independent while others provide evidence for the opposite. The manuscript would benefit from brief discussion of this uncertainty and what it might suggest for these findings.
5. The authors state that GLYX-13 is shown to promote improved cognition in previous work yet they see no effect of GLYX-13 alone on behavior. Some discussion of why the authors found no improvement in cognitive performance after GLYX-13 alone would be helpful.
6. In the discussion, the authors discuss the interaction of NR2B and CamKII. They appear to suggest that these proteins somehow interact directly, in addition to CamKII reacting to the Ca2+ influx controlled by NR2B. This point is a little unclear, though, and could use some further description.
7. When Anes mice are undergoing 6h of 1.5% isoflurane exposure, what are the other mice doing? Remaining in their home cage? Some details on this in the methods would help.