AUTHORS' RESPONSE TO REVIEWER'S COMMENTS:

We would like to thank the reviewer for the insightful comments that allowed us to provide further clarification to our study, ultimately improving the clarity of the manuscript. We also used this opportunity to edit the manuscript to improve readibility, as requested. The modified sections of the manuscript are highlighted in yellow. We have also added a flow chart of the study, which now appears as a new Figure 1, and added a section summarizing the results to the Results. Please find below the point-by-point response to your questions:

1. Was there a sample size planned, as there was a significant body of work in this area including the authors group?

We apologize for omitting the details on this facet of the study. We have based our sample size based on our prior results from this model, specifically refs 1, 2 and 62 of this manuscript. We have targeted n=8 survivors in each group to allow for evaluation of neuronal death. This was based on power sample size calculation for a continuous parameter (neuronal count), using two independent sample analysis with alpha=0.05, power = 0.8, to detect 20% reduction in neuronal death.

These additional details were added to the "Statistical Methods" section of the manuscript.

2. From my understanding of the presented details, it appears to me that the study had a simple randomisation sequence. However, the authors present that "block randomisation" was used. If this is the case, please clarify how the blocks were generated.

You are correct that we used block randomization to reduce the possibility of bias and confounding, or unrecognized problems with a certain batch of rats. We have used similar method of randomization before. Rats were block-randomized to blocks of fixed n=4, with two rats in each block assigned to receive minocycline and two rats assigned to receive vehicle treatment. The chosen block size corresponded to the number of rats in each shipping container, to reduce the possibility of bias and confounding.

These comments were added to the "Experimental protocol" section of the manuscript.

3. Randomisation was performed after ROSC. I am not sure of the first paragraph in the results that states that three did not achieve ROSC and three others were excluded for technical reasons. Are these exclusions post randomisation or pre randomisation? A flow chart may help in improving the clarity of the process where the number of rats shown at each step including initial induction of VF, ROSC and inclusion in the study details.

A randomization schedule was created prior to study commencement with balancing for each sequential groups of 4 rats, with two rats in each block assigned to receive minocycline and two rats assigned to receive vehicle treatment, in order to balance the number of ratsallocated for to each condition for each shipping container, thus reducing the possibility of bias and confounding. Rats that either did not achieve ROSC, or died prior to the scheduled time-point of sacrifice were replaced at the end of the study following the same randomization protocol. The ongoing block was finished as originally designed.

These clarifications were added to the "Experimental protocol" section of the manuscript. Per your suggestion, we have also added a flow-chart diagram of the study, which now appears as a new Figure 1.

4. Please also summarise the results in the first paragraph of the results in the main text.

Thank you for the suggestion. We have added a brief summary to the first paragraph of the "Results" section of the manuscript. The section now reads as follows:

"A total of 39 rats was used (Figure 1). Prolonged CA resulted in significant early biochemical derangements heralded by marked metabolic acidosis and significantly increased lactate levels that clearly indicated a severe insult. Post-resuscitation treatment with minocycline did not change survival rate or survival time, neurologic outcome or histological damage at 72 h that included marked neuronal degeneration and microglial activation in multiple selectively vulnerable brain regions."