

Reply to REV 1

This study investigated the effect of probiotics supplementation on chronic sleep restriction inflammation and oxidative stress in mice. Overall, the work done is exhaustive, however, as explained below, I raised some questions and suggestions that need to be addressed.

Major points

- Why do the authors claim probiotics have preventive effects and exclude therapeutic effects? Yet the treatment was continued during the week of sleep restriction. In order to differentiate these two distinct effects, two groups of mice need to be added to the experiments. The first group should be treated during the two months preceding the CSR and the second group only receive the treatment during the week of CSR. If this is not possible, the authors should revise the use of the term "preventive" throughout the manuscript and highlight this point in the limits and future direction.

Thanks to the reviewer for raising this point. We avoided the use of the term "preventive" throughout the manuscript. In addition, we also added a small paragraph in the discussion highlighting this limitation as follows: *"Since we administered probiotics before and during the CSR, we currently cannot distinguish whether probiotics have preventive or therapeutic effects on CSR-induced inflammation and oxidative stress, and future research with a different experimental design is needed to clarify this ambiguity."*

- Was the CSR procedure applied for 3 hours a day? This needs to be mentioned clearly in the CSR procedure section.

No, the CSR procedure was active for 24h/day for 7 seven days consecutively. Mice were gently touched by the bar twice per minute. By contrast, control mice were exposed to the bar movement for 3 hours/day during the dark period when they were spontaneously awake. This was done to control for the potential stress induced by the bar movement. We have clarified this procedure in the text as follows: *"Mice were sleep restricted for 7 consecutive days. To ensure a modest but persistent sleep restriction, mice were exposed to the rotating bar for 24h/day at a velocity of 2 rpm (one turn each 30s). Control mice were placed in the same sleep deprivation chambers and allowed to sleep undisturbed, except for 3 hours/day (during the dark period, when mice are usually awake) during which the bar rotation was activated to expose the mice of this group to the experience of the bar movement and to the stress associated with it."*

- SLAB-51 administration should be further detailed. What is the concentration of these compounds in the water?

The concentration is 200 billion bacteria/Kg/day. Before starting the experiment, we have estimated the daily water intake and dissolved a proper amount of probiotics into the drinking water to reach the desired concentration. We added this brief explanation in the text.

- The statistical analysis section should be more detailed. For instance, it should be indicated for which analysis the one-way ANOVA and for which analysis the two-way was used. Moreover, why the authors used different post hoc analyses (Bonferroni or Tukey)?

Thanks for this suggestion. We added more specific statistical information, and we also clarified the post-hoc test used. Since there was no statistical reason to use different post-hoc test, we applied Tukey's, and we updated p-values accordingly.

- ANOVA main effect should be added to all figures and table 1.

Added in the text.

Minor points

- Abstract: Line 1: please remove the indentation.

We think that it is generated by the system according to the manuscript formatting.

- Discussion, paragraph 3, Line 8: please explain further the differences in sleep deprivation procedures reported in the literature.

We added a sentence to explain the different sleep deprivation procedures as follows: “*This discrepancy has been ascribed to the different sleep deprivation procedures (e.g., gentle handling vs disk over the water) and the different duration of the sleep deprivation*”