Review of: "A Sleep Disturbance Method Using Novel Objects in the Home Cage to Minimise Stress"

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An interesting paper that provides a more welfare-friendly way of determining the impact of sleep loss on mouse stress responses and the results that such mice, as subjects in an experimental set-up, would generate. The main rationale behind this paper is one of enhancing positive welfare in experimental/observational studies, and this is commendable. The paper has a clear 3Rs philosophy behind it, specifically the refinement of how research pertaining to the biological and physiological effects of sleep loss is conducted.

There needs to be more clarity between the use of the terms sleep deprivation and sleep disruption or sleep disturbance. Sometimes these are used interchangeably, but it is clear from the author's aims that they wish to focus on sleep disturbance. I think clarifying this would help the methods and results be easier to follow.

The methods could be improved to make them repeatable and replicable. I have struggled to work out how behavioural data were collected, and this needs to be better defined (and more obviously set out) in the text. Figures 3 and 4 contain the word “bout,” so I am assuming that bouts of activity and inactivity were recorded somehow. I am assuming this was from the video monitoring of the mice? I would like this part of the data collection procedure to be better described.

It would be helpful to know why eight individual mice were decided upon for the sample population. It would be helpful to have more information on Figure 1. After reading several times, it is clear that this figure links to the selection of enrichment items, but at first reading, it appears as if a result is presented in the methods.

Some sections of the method could be better explained to show how the mice were prepared for the experiment. For example, the “behavioural assessment” performed after each mouse had undergone surgery and the habituation period - how was habituation defined and measured?

I don't understand the description of some of the enrichment items. For example, what is a tulle? What is a falcon with beads? Were all of these types of enrichment already in common use for this species? Please provide justification for the items that you provided.

I do not understand this sentence: “8 female adolescent mice (SD start at p36-p42).” What does this mean? It needs clarification. Were all mice adolescent when they entered the experiment? If so, this needs to be explained in section 2.1. It is important that anyone attempting to repeat this study does so with the same type of animal at the same life stage.

The statistical analysis section is a little weak and would benefit from a better description. When you say “graph plotting,”

you mean descriptive analyses. There is scant information on the mixed effects analysis - what is this? A repeated measures ANOVA? If so, what was the random factor? You are repeating observations on the same mice, so are the mice the random factor? What is an ordinary one-way ANOVA, and why was it chosen for cort. concentration analyses? Were data tested for their distribution and equal variance before tests were applied? I would like to see this section written out more clearly and with a better explanation of what testing was chosen and why. Perhaps writing out the data analysis section by aim or prediction would help? “To test the effect of X predictors on Y outcome variable, a Z test was used…”

"Significant ANOVAs are reported with p-values, and p-values < 0.05 were considered statistically significant and noted as *p<0.05, **p < 0.01, ***p < 0.001 on the figures." is a clunky sentence, and I recommend rewriting it for clarity. Why are you deciding to look at each of these levels of significance if you've decided that <0.05 is your alpha level?

You state that an ANOVA was used for the cortisol analysis, but I do not see the results from this testing in the corresponding section of the results. Even if this was non-significant and you ran the test, you should report the outcome. If you did not run the test, then do not include it in the stats section of the methods.

The discussion and conclusion are generally well written. It would be easier to judge the validity of the points made in the discussion if the methods were more clearly explained, and thus the results could be critiqued with more certainty as to what was done. However, I commend the researchers for their attempts at improving the lives of laboratory rodents and feel that this is useful and impactful work. I feel that the execution of the paper could be improved to encourage the reader to fully digest its main points and ensure clarity in what was done and why.

The research is well referenced, for the most part. Some areas with multiple citations would benefit from further evaluation or discussion as to why all of these citations are required to support a specific point.

A few basic errors were noted in the paper that could be corrected to improve the quality of writing. For example, remember that data are plural (so “these data…” not “the data”) and avoid starting a sentence with a numeric. ”Twenty-one…" rather than “21…”