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

Juan M. Busso[1]

1 Universidad Nacional de Córdoba, Argentina

Potential competing interests: No potential competing interests to declare.

Qeios ID: PC09D3

The study shows an interesting approach, but with an atypical structure. The authors sometimes showed results on M&M and then repeated these results in the homonymous section. There is no single structure for presenting research, but I strongly suggest using the classic one or improving the presentation. Furthermore, it is not possible to easily follow the use of the experimental units, the application of the treatments, and the number of samples obtained for the analysis of the results. All of this affected my interpretation of the text; that's why I have revised the manuscript up to M&M. I hope you can resolve these major concerns, and then I can discuss the rest if the journal invites me for further reviewing.

I understand that the authors used the rodent model to study a topic/problem about sleeping rhythms in humans. This approach is valid methodologically, but its specificity cannot be ignored in the title and introduction of the abstract. Please, authors should be more specific. I mean, add "mice" (as well as "female," considering that I read later that the authors only used female mice). Please keep in mind that males and females may respond differently to stressful situations such as awake stressors; I would expect this considering a lot of previous reports about sex differences in stress physiology.

In the "new method section," information is missing to interpret the results. For example, when feces were collected to measure fecal glucocorticoid metabolites.

"Comparison with existing methods," this section appears to be the discussion. It is not appropriate to compare present results with other methods if comparisons were not made in the present study. This requires clarification.

Introduction: well done, great!

M&M:

Animals Section. The age of the experimental units is not indicated, and there is no information between the day of purchase of them and the day of application of the protocol. This information is needed.

Selection of objects section. When was the treatment (object exposition) applied by the authors? Light or dark phase? Besides, since the authors tried to study sleeping rhythms, did they check the normal rhythm prior to exposing mice to different treatments?

On the sampling rule, the authors apparently applied a focal sampling method to record interaction with objects. Did the

authors observe all 8 female groups as a focal unit or all individuals? Analyzing the figure, where I did not find error bars associated with each column, I wonder how many measurements (from the experimental unit) are depicted. Another important issue, sleeping behavior must be defined; I later saw a definition, but a behavior could not be defined with a synonym. For example, I understand that sleep is a synonym for resting. Body position and possible actions should be described to allow this behavioral state to be identified. Can authors explain in detail the period of application of the 21 objects? Were 21 objects applied in one night?

I don't understand figure 1c. It seems that the authors applied a different protocol (4hs), and this is not previously explained in M&M. Please explain it.

At this point, I wonder why the authors showed results for the first specific objective in the M&M section. Then, I saw that these results are repeated in the specific section. This needs to be changed, perhaps taking Fig. 1 out from M&M and pasting it in the corresponding section.

Protocol design section. Which female mice were exposed to this protocol? There is no information in this paragraph. If it was applied to the 8 females previously studied, the application does not correspond to the novel object test. I read that “most objects were introduced to different cages,” that would mean several experimental units. Nevertheless, a more detailed description is needed. Again, data is missing on when the rodents were exposed to the objects.

Section 2.4. Looking at figure 2a, I cannot know how many feces per individual were collected. Were the feces identified? What was the collection time? These aspects may be confounding factors for fecal immunoreactive corticosterone measurement. The circadian rhythm is evident even in this type of measurement, since these rodents defecate often and corticosterone is excreted in a pulsatile way. Consider that rodents like mice defecate several times over the day, so it is essential to discriminate fecal samples over the light-dark cycle.

Section 2.5. How much did the authors really weigh of the fecal sample? Was it 1 g per experimental unit/day? It is clear that they used 3 mL/g, but mice excreted pellets, so it is necessary to know precisely the amount or the average. Again, here, it is not clear how many samples were run or how many individuals were studied. Please, may you add this data?