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

Dietmar Zechner[1]

1 Universitätsmedizin Rostock, Rostock, Germany

Potential competing interests: No potential competing interests to declare.

The manuscript entitled "A Sleep Disturbance Method Using Novel Objects in the Home Cage to Minimise Stress" describes a novel subchronic sleep disturbance method using daily four-hour exposures to novel objects. Based on no significant changes when analyzing fecal corticosterone metabolites, the authors conclude that their method does not cause substantial stress to mice. Although I consider this an interesting topic, I would like to see the following points addressed:

Major points:

Material and methods section:

1. The Material and Method section contains partially insufficient information regarding material and methods, but at the same time describes some results of experiments and contains data figures. It would be better to clearly separate these two issues (Material and Methods versus Results).
2. I do not understand the message of Fig 1C. Are the mice independent of which objects they received falling asleep later during the inactive phase? Or were the objects offered to the mice more boring 6 hours after the start of the inactive phase? Where is the information on how this experiment was conducted (were the objects given to the 8 mice in the same sequence or not? and what was the sequence?). Or is this already an experiment that was done according to supplementary table 1 and supplementary table 2? Then it might make it easier for the reader if you just include this supplementary information in figure 1 or together with figure 1C as a separate figure.
3. Please inform the reader of the minimum detection limit and/or the limit of detection of the assay used for determining the FCM concentration and show the linear range of the assay in the form of a standard curve. Without this information, it is impossible to judge how well this assay works.

Results section:

1. Fig. 5: Please also provide data that prove that this assay is sensitive enough to measure increased FCM concentration when comparing any stressful situation to a not stressful situation (e.g., before and after surgery). Without such information, it is in my opinion impossible to conclude that this sleep disturbance method minimizes stress, because the same results could be observed when the mice experience a lot of stress but the FCM assay is very insensitive to measure it.
2. To address the same fundamental limitation of this study, it would have been great to measure distress by any other

methods in addition to FCM concentration. Are there at least body weight data available that could support the conclusion that the mice did not experience too much distress? Please provide the body weight data or other data indicating stress on these mice during your experiment.

Discussion:

1. I think it is incorrect to conclude that this method is not a method for sleep deprivation beyond day 1. Or did you do any EEG recordings on day 2, day 3, day 4, day 5, or day 6 of sleep deprivation? If you did not do any measurements on those days, you cannot conclude anything about those days. Please adjust the discussion section.

Minor points:

Material and methods section:

1. Please use the correct nomenclature without contradictions to define the mouse strain and reduce redundancies in the text (that female mice were used is mentioned 2x in the material and methods section - in a contradictory manner: C57bl/6JTac female mice (purchased at Taconic) were used in these experiments versus eight female C57BL/6Rj were single housed?).
2. Evtl. please improve your English, e.g.: The administration of the objects was done with minimum disruption of the cage? Do you mean with minimal disruption for the mice?
3. Material and Method section: please provide information on what objects you exactly bought at which company, so that other scientists have a better chance to reproduce your data.
4. Please provide information on how the selection of objects for experiments (data in Fig 1A and Fig 1B) was done. When (starting 2 hours after the begin of dawn? Starting at dawn?) and in which order were the objects added to the cage, and on how many mice was this done?
5. Please clarify in each figure legend: On how many mice are these data based? In Fig. 1 A, also only 1 mouse, or is this the average of 8 mice?
6. I assume the word “Zeitgeber” has to be removed from the x-axis in Fig. 1C; please also define the units of each axis of each graph (the unit for the x-axis in Fig. 1C is in hours?).
7. Material and Method section 2.3: Please provide the information on which objects were offered on which day at which time point to the mice and if/how this differed between the 8 animals or 4 cages (because mice were pair-housed according to Fig 2A?).
8. It might be helpful to give an experimental overview in a figure that includes all main elements of the entire experimental setup: when were the animals exposed to the objects, when was the EEG/EMG surgery done, when were the fecal samples taken, when were the EEG recorded, and when was the body weight taken (you mentioned this in the material and methods part).
9. Fig 2B: what does ZT2-6 mean? Please define all abbreviations in a figure always in the relevant figure legends or alternatively mark what ZT2-6 would be in Fig2H, 2I, and 2J. What is the shaded green rectangle on the left of Fig 1I and Fig 1J (was this the attempt of indicating ZT2-6?)?

10. Figure legend to Fig. 2B-G: Are the data really the data on all days or just on the indicated days?
11. Was meloxicam and not, as claimed in the manuscript, mexilocam used?
12. Please correct the English in incorrect sentences such as “One mouse where euthanized between the first day and the seventh of SD due to a loose head mount, reading to an n of 7 for the last recording,” and check the entire text.