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

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Potential competing interests: No potential competing interests to declare.

The authors developed a sleep disturbance protocol using a set of novel objects to disrupt sleep in mice. The authors applied this protocol for 7 days, 4 hours each day, and the efficiency of the method was verified by EEG/EMG recordings on the baseline day (pre-disturbance), the first day, and the last day of sleep disturbance. Four hours of exposure to the novel objects in the home cage decreased the duration of NREM and REM sleep on the first and the last day. The decrease in NREM sleep time persisted over the 24 hours after the first session of sleep disturbance, but not after the last session.

The manuscript is overall well-written, and the topic is interesting. However, several aspects are in need of careful revision to support the interpretation of results. The major areas of concern are outlined below.

1. Exclusive use of female mice in this study would limit the generalizability and applicability of the findings. Growing evidence suggests sex differences in sleep-related parameters, circadian phenotypes, and susceptibility to sleep loss in rodents. A group of male mice should be included in the study.
2. The authors should clearly state in the manuscript, including the title, that adolescent mice were used to validate the sleep disturbance method. Adolescent rodents exhibit an increase in novelty-seeking behavior compared to adults. Sleep disturbance methods using novel objects in adult rodents might not be as effective as they could be due to the decreased level of novelty-seeking behavior after repeated exposures to the objects.
3. Regarding comparisons of the corticosterone level, the low number of mice (n=4) in the early sleep disturbance condition limits the interpretability of the results. Additionally, the authors stated that all samples were collected at ZT2, which was the starting time point for object exposure in the early and late sleep disturbance conditions. However, samples were collected from home cages in the early condition but from the EEG cages in the late condition. Why are they different? It is still not clear at what time points fecal boli were collected for corticosterone measurements (see Figure 2B). In my view, the corticosterone level should be measured immediately after the sleep disturbance protocol on each day (and collected from the same environmental context) to systematically investigate whether this method induces any changes in the corticosterone level. Furthermore, a direct comparison of fecal corticosterone measurements between this method and the gentle handling method, with a separate group of mice, should be conducted to verify that the present protocol does not cause substantial stress compared to the more commonly used sleep deprivation protocol.

Minor points:

1. The age of the animals should be clearly specified in the methods section.
2. Exact coordinates for EEG recordings should be specified, and the method for sleep-stage classification should be clearly stated.
3. Pg. 12: only the mean REM sleep bouts became longer on the seventh day, but not NREM sleep.

Miscellaneous:

1. Figure 1B legend: should it be n=3?
2. Figure 1C legend: Please clarify “n=1 was tested once on eight mice”; what is n?
3. SD should not be used as an abbreviation for sleep disturbance. SD is more commonly referred to as sleep deprivation.