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 manuscript entitled " A Sleep Disturbance Method Using Novel Objects in the Home Cage to Minimise Stress " by Egebjerg et al. reported a novel sleep disturbance method with less stress in the home cage. This work provides an exciting topic in the sleep research field and would help establish a golden standard for analyzing sleep disturbance or deprivation effects.

The experiments are well-conceived, and the interpretations are largely sound. However, the reviewer believes the manuscript could be improved to enhance the work's impact. As this is a methodology paper, the Materials and Methods section should be described more precisely. As the other reviewers suggested, the terminology or definition should be precise.

1. In the conceptual aspect, for instance, the authors described a kind of spontaneous sleep deprivation by novel objects. By contrast, the previous studies mainly used forced sleep deprivation protocols by waking up mice during the inactive phase. Those are different paradigms.

In Figure 1, for another example, were the objects tested independently or simultaneously? The reviewer speculated it was probably the latter case. If so, the expression "a set of 13 objects" would be precise.

2. In Figure 2, where is an explanation of "Pair housed"? There is a description that "All mice were group housed (2-5 mice per cage)" in the Materials and Methods section. Does "Pair housed" mean "group housed"? Or was a target mouse housed with another single mouse?

3. At what time was a set of 13 objects introduced in the EEG cage on the first experimental day?

4. In the Discussion section, the authors mentioned, "Over the 24 hours, the mice were also sleep deprived on the first day of SD, but on the seventh day, the mice recovered their lost sleep at other time points, suggesting habituation to the setup." However, the time course shown in Figure 2J seems to show enhanced "daytime sleepiness" between ZT18 and ZT23 (The dark period is the "day" for mice). In addition to ZT2-6, EEG for each quarter (ZT6-12, ZT12-18, and ZT18-24) should be analyzed.

The reviewer would like to enhance the current work's value, and a more careful and precise description throughout the whole manuscript would improve and enhance its impact.