

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.

2.1 Animals: Typo: C57bl/6JTac must be C57BL/6JTac.

(purchased at Taconic) is redundant, already visible in the name C57BL/6JTac.

Please stick to the ARRIVE guidelines: Caging system is missing, cage size, food and water, bedding. Please indicate temperature and humidity. The light program (on/off) is important as far as the experiment starts at 9 am (according to Supplementary Table 2).

2.2 Selection of objects: Why do you use mice from a different vendor here (C57BL/6Rj)? The substrain is missing.

Figure 1 A,B: Please explain the indicated values: Sum? Mean (in this case, SD or SE are missing)?

Figure 1C: Please explain "Zeitgeber time."

Figure 2B-J: Values from 8 mice are presented. As mean and SD? Or mean and SE?

Same for Figure 3A-F.

Same for Figure 4A,B,D,E,G,H.

Figure 4C,F,I: There are bars in the columns but no indication if they are SD or SE.

Figure 2H-J: The light/dark phase would be important related to Zeitgeber.

2.5 Fecal corticosterone measurements: How do you know that the fecal pellets you pick out of the cage are freshly excreted? Excretion of stress metabolites is dependent on the time of the day. There are diurnal oscillations in stress hormone metabolite excretion (see Touma et al., 2003, 2004, Palme et al., 2013). You have to collect samples excreted at exactly the same time of the day for comparison.

Fecal stress hormone metabolites are excreted 4 hours after stress (during the active phase/night) or 6-8 hours after stress (during the resting phase/day). If you sample the feces as described, it is no wonder that you did not find any differences (Before SD, fecal boli were sampled from EEG cages just before novel object exposure on the first day. Early SD fecal boli were sampled from home cages when returning mice into EEG cages for the last recording. Late SD fecal boli were sampled from EEG cages after the last recording).

3.3 Validation of stressless protocol: Like mentioned in the first sentence, you measure only fecal corticosterone metabolites (FCM), not corticosterone at all. So please use this wording (FCM instead of corticosterone), also in Figure 5!

According to the introduction, the aim of the study was to create a standardized method using novel objects to induce SD. According to the discussion, the aim was to develop a less stressful SD model. Now, what was the aim? If the latter, it failed because of the wrong sampling method. I would skip this point and concentrate on the aim to develop a standardized, easy, low-cost SD protocol.

Yours sincerely.