

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 tried to develop a standardized protocol for sleep disturbance in mice. I have the following remarks:

- 1. Why were females used? There is uncertainty with their cycling, as mice cycle every four days. In which phase of the cycle were the females? Have you checked this? Have you verified it using vaginal lavage? Were the females separated from males? Please state all these details.
- 2. Have you used this protocol in males? Why? Please state all these details.
- 3. The title should contain the strain and sex of the animals used.
- 4. The fecal corticosterone is not a good indicator of stress. You can use the ECG record instead to indicate if there is an acute HR increase.

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