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 present study developed a novel object based Sleep disturbance model that aims to eliminate the confounding factor induced by stresss of most of the sleep restriction, sleep deprivation rodent models. There are several problems with the study, the major are:

1. The first issue of the manner the authors present the justification for the developmento of their model is that stress is defined as a physiological response to stumuli that alter homeostasis, in humans sleep deprivation, sleep restriction and the sleep alterations like apnea restless leg syndrome etc are considered non-physiological non-homeostatic conditions therefore, are stressful situation that may cause the devolpment of diseases. It is not posible to separate stress from sleep alteration, the authors should review DOI: 10.1007/s11154-022-09755-4, DOI: 10.1016/j.bjorl.2015.09.004, DOI: 10.3109/07420528.2013.795155 as well as references that report elevated plasmatic cortisol data in humans that have been sleep deprived, sleep restricted or with affections that alter the sleep pattern.
2. Another problem is that the authors indistinctly employ the terms sleep disturbance and sleep deprivation without a clear definition. Sleep disturbances can be alterations in the quality and amount of sleep as insomnia, apnea, parasomnias or excessive sleep duration. Sleep derivation is the total elimination of sleep in one or more sleep wake-cycles meanwhile, sleep restriction is the reduction in the amount of sleep (for example, restricting per 4h every 24h for 7 days). The authors should properly define the concepts along the manuscript and refere them to the adequate bibliography.
3. The authors indicate “This method is easy to implement at a low cost, but it is highly dependent on the experimenter, increasing the risk of variability and decreasing reproducibility” without specifying the particular examples in which the results of this protocol have not been reproduced. I find this statement inaccurate since both sleep restriction and sleep deprivation rodent models are highly reproducible sinbce most of them report: decreased memory and learning capacity, increased inflammation, increased macrophage markers in the central nervous system, increased blood brain permeability, increased beta amyloid expression. The main problem of the different protocols are the variations in: restriction/deprivation method (Platform, Novel enriched environments, Rotating drum, Cylindrical apparatus with bar spinning, MMPM), the amount of restriction per sleep wake cycle (8, 18, 12, 24h per day) and the amount of days of the protocol (3 days per week/2 moths-1month, 21 days, 7 days, 6 weeks) https://doi.org/10.1093/sleep/zsab057, https://doi.org/10.1016/j.brainresbull.2006.04.001, https://doi.org/10.1016/j.brainres.2005.10.020, https://doi.org/10.1016/j.bja.2023.04.044, https://doi.org/10.1016/j.physbeh.2020.113128
4. The authors should consider a different approach to justify the development of this protocol that is not a novel method

https://doi.org/10.1093/sleep/zsab057

5. The authors state: “Very small platforms have also been used for total sleep deprivation[5]. A confounding factor with this method is forced immobility” The ref 5 (doi:10.1016/j.nbd.2019.104517) employs the MPMM method that prevents immovility and isolation stress, since the animals have many platforms at least 2 per animal and the protocolo is performed with more than one animalper cage.
6. The methods lack the detail and clarity for the study to be reproducible.
7. Did the animals had water and food during the presentations?
8. Did the object presentations implied the constant removal of the lead to present each object? did the objects were placed all at once? How many animals were employed? it seem that there are several cohorts
9. The authors fail to indicate the time of the day in which lights were on and off and did not mention the exact moment of the LD cycle the animals were sleep disturbed.
10. No indications of the temperature and humidity levels in which the room was mantained.
11. The authors fail to indicate why did they use C57bl/6JTac and C57BL/6Rj mice.
12. The authors fail to provide the brand of the running discs.
13. The authors fail to indicate if the sleep disturbance protocol was performed in an special room or in the normal vivarium.
14. The authors fail to indicate what was the purpose of the paper towels if the objects were not cleaned after each introduction.
15. The authors fail to indicate why does the presentation lasted 4h when the standarization lasted only 30 min? did the animals remain awake and exploring the objects for 4h?
16. This protocol includes a social component which is not considered in the title nor the introduction since the objects hace mice odor.
17. The authors fail to indicate if the EEG animals were somehow differently treated as compared to the other animals in the study, it seems that there are different cohorts in which some animals were used for specific evaluations.
18. Did the bedding material was changed or clean one prior the protocol? How can you enssure that the fecal matter evaluated in the present study did not included the one excreted by the animal before the proptocol?
19. What is the difference between the fecal boli obtained from EEG or home cages?
20. What does before SD, early SD and late SD states for?? in which day did the fecal boli were obtained? in day 1 or 7? every day? how long after the SD begun did the fecal boli were obtained? min? hrs? the indication “All samples were collected at ZT2” is not coherent with the previous three time points.
21. How long does it takes for cortisol/corticosterone to be metabolize and apear in the fecal matter? there are studies that indicate that cort is detected in the fecal matter between 5 and 24 hours after a stressful event https://doi.org/10.7717/peerj.3648.
22. Why does the authors did not obtained blood samples so their data could be compared to the literature presented in the introduction.
23. How can the authors ensure that the lack of changes in cort are not associated with the inespecific detection of the elisa kit, specially for progesterone in females.

24. Did the phase of the estrous cycle was determined to control this aspect?