

# Variation in Behavior and Corticosterone Levels Following the Moving Bar method of Sleep Fragmentation vs. the Inverted Flowerpot Method of Sleep Deprivation

Michael B. Roberts, Yonglin Gao, Rif S. El-Mallakh

Department of Psychiatry and Behavioral Sciences, University of Louisville School of Medicine, Louisville, Kentucky



## Introduction

In a previous study, sleep fragmentation using the moving bar method was used as a stressor for mice and corticosterone levels following sleep fragmentation were unexpectedly below baseline. Following this, comparisons of corticosterone and behavior between this method and the inverted flower pot method were performed to check for differences in effect on behavior and physiology resulting from either the pattern of sleep loss or common test conditions.

## Methods

Black Swiss mice were stressed with one of two treatments: the inverted flowerpot method of sleep deprivation or the moving bar method of sleep fragmentation. The mice used for behavior data were tested in an activity monitor before and after the treatment to provide a baseline and experimental result. Mice used for corticosterone levels had serum collected at the end of the treatment, with baselines taken from mice that were not exposed to either method. Corticosterone was measured with a corticosterone immunoassay (DetectX from Arbor Assays, Ann Arbor, Michigan). The moving bar method was performed using a sleep deprivation test chamber (Lafayette Instrument Company, Lafayette, IN).

Both treatments were used in 1 day and 3 day variations. The inverted flowerpot method used 1 mouse/cage with unlimited water and 30 minute feedings 3 times/day. For the moving bar method, 3-5 mice were used/cage with unlimited food and water available.

## Disclosure Statement

The authors do not have any conflicts of interest in connection with this study.

## Results

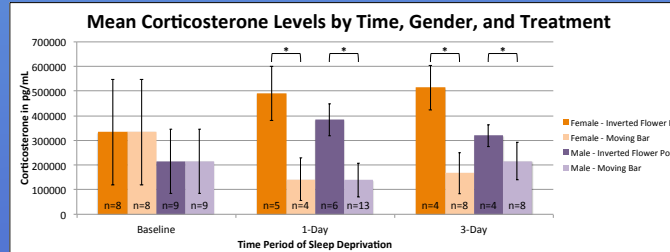


Fig. 1 Serum corticosterone levels at baseline and after either sleep deprivation or fragmentation. \* $p < 0.05$ ; analyses were performed between treatments in same gender/time groups with a 2-tailed T-test.

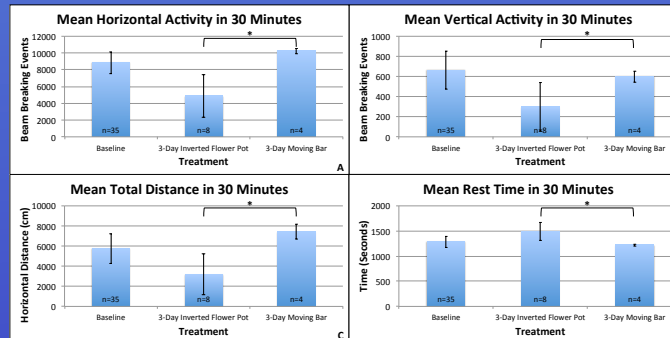


Fig. 2 Behavior measurements at baseline and after 3 days of either sleep deprivation or fragmentation. Center time (not shown here) was not significant between treatments. \* $p < 0.05$ ; analyses were performed between treatments at the 3 day time period with a 2-tailed T-test. Males and females were grouped together to produce the samples.

## Conclusions

- The inverted flower pot method raises corticosterone levels and lowers activity measures, whereas the moving bar method lowers corticosterone levels and raises or does not effect activity measures.
- These data clearly indicate that the treatments as applied have different effects on physiology and behavior.

## Discussion

Previous studies have found differences between sleep fragmentation and deprivation, but the tendency in both is still for corticosterone levels to increase or, in a few studies, stay the same. These results indicate that the difference found here is not likely to be caused by differences in the animal model used (Black Swiss mice) or a unique feature of the laboratory environment. The opposite operator for corticosterone and behavior measures for the different treatments may most likely then be attributed to either intrinsic properties in the pattern of sleep available and/or to the confounding variables of limited food and group size in this social animal model. While this study cannot separate these two variables, either would be significant. If the effect is mediated by sleep as intended then the moving bar method may present a distinct physiological effect in stress hormones that may be relevant medically (the device was designed to mimic sleep apnea without oxygen deprivation). If the effect is mediated by test conditions, then the moving bar method may be a way to limit confounding factors in some sleep studies by providing mice with an environment that is more similar to standard lab rearing cages.

## Acknowledgements

Special thanks to the KBRIN program under grant # 5P20GM103436-13