

## Challenges of Using Modified Multiple Platforms to Induce Sleep Deprivation in Experimental Studies

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Since we use the modified multiple platforms (MMP) technique for induction of sleep deprivation in our experimental studies (1, 2), there are two important points in this technique; (1) which sleep stage [rapid eye movement (REM) or non-REM (NREM)] has been deprived using this technique? Although it is stated that this technique eliminates only REM stage, it should be noted that it can significantly reduce NREM sleep (3-5); (2) the second point is about the effect of stress in MMP technique on study results. It seems that this technique is accompanied by significant stress (6). Probably, induction of forced sleep deprivation is an unpleasant process for animals, and causes stress and anxiety that affect the results of study. Furthermore, there are a number of psychological environmental stressors which can be effective factors influencing the results and intensify the challenges mentioned earlier. So, the observed results using this technique may not be solely due to REM sleep deprivation alone.

In this regard, it seems that deletion of environmental stressors can reduce the effect of inappropriate interfering factors on the results. In our studies, we try to reduce stress and anxiety by eliminating environmental stressors as follows. To perform this technique, we use a water tank containing eight platforms with a diameter of 6.5 cm that allows the animal to move freely on all platforms, and

therefore the immobilization stress is controlled. To eliminate the isolation stress and social instability, three animals are placed together inside the tank at the same time, all of which are already kept in a common cage. Before the experiments, the animals are placed inside a water tank (control tank) in which there are four platforms with a diameter of 14 cm that allows animals to sleep. In this way, the stress related to facing the new environment is also controlled. To prevent cold stress (because it is possible for animals to fall into the water during induction of sleep deprivation), the ambient temperature is completely controlled, and the upper part of the platforms is covered with a thermal insulation coating to prevent the transfer of water-induced cold around the platforms.

Finally, it is also suggested that it is better to modify such techniques with alternatives such as using rewards instead of forced sleep deprivation.

### Conflict of Interests

Author has no conflict of interests.

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