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*Short Communication*

# A Protocol for Conventional Sleep Deprivation Methods in Rats

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## Abstract

Several experimental methods have been developed to restrict the sleep in laboratory animals. Among them, the flowerpot and Modified Multiple Platform (Modified-MP) techniques are widely used largely due to their simplicity and low cost. However, better application of these techniques may need some technical points to be considered. Given this, the present guide besides focusing on the critical points of these techniques gives a brief instruction for setting up and executing them.

***Keywords:*** *Partial Sleep deprivation, Flower pot technic, Modified multiple platform technic, Rat*



## Introduction

Modern life, its emergent technologies and industrialisation have changed the human society and his lifestyle. As an important consequence, modern life has worsened sleep quality and shortened its duration which ensue many medical problems [1-4]. Generally, normal daily patterns of many behaviours and physiological systems are regulated by sleep-wakefulness cycle, that any interruption in this cycle acts as a physiological stressor and metabolic challenge [5]. It impairs energy homoeostasis [6] as well as immune and hormonal functions. It also exacerbates generation of free radicals and inflammatory markers. In this regard, it can be considered as a potential risk for obesity, diabetes, cardiovascular and inflammatory diseases [6].

Apart from these problems, mental abilities are also largely affected by Sleep Deprivation (SD), where it contributes to psychiatric and neurologic complications such as declined alertness, vigilance and reaction time, low concentration, mood disorders and impaired motor abilities and anxiety [3, 7-10]. Reports show that adverse effects of inadequate sleep disturb well-being, productivity and safety that ultimately results in healthcare and economic burdens such as injury and death from the vehicle and workplace accidents [10,11]. Given the critical aspect of SD and its biologic effects, achieving valid models for resembling this state in animals is of a great importance. Till present, several techniques have been developed to model SD in animals. In all these methods, SD is induced by imposing external stimulation on animals. Notably, the majority of SD technics have been adopted from the Flower Pot (FP) technique which has been described originally and for the first time by Jouvet et al. (1964) on the cat [12]. The basis for this method is habituation of animal to the situation, in which an animal being placed on an inverted FP is surrounded by water. This condition decreases the duration of sleep, without altering animal ability to obtain slow wave sleep. In this manner, animal gradually loses muscle tone and falls in the water until being awake [13-15]. Application of platform-based techniques has some advantages over the other methods. Briefly, platform techniques do not require any complex instruments and besides restriction of sleep are able to induce sleep fragmentation which happens as one of the sleep disorders symptoms [16]. So this paper aims to provide a guideline to better explain the techniques that with low cost and high translational applicability recapitulate SD in rats.

## Flower Pot Technique

The conventional FP technique is widely used for selective Rapid Eye Movement (REM) sleep restriction in rodents [15,17]. This technique has been developed to selectivly restrict REM sleep. The following steps are used to induce SD in rat using FP method:

1. Transferring the rats to the SD induction unit for habituation to a new environment, 2 days before initiating the protocol.

2. Monitoring the animals' locomotor activity by placing them in the open field arena: This step is done to ensure locomotor condition normality. The open field can have different shapes, sizes and colours. In the present protocol, we used the homemade PVC 70×70×35 open field device. Animals that show abnormal locomotor activity were excluded from the SD induction (Figure 1).
3. Setting up the flower pot method: Putting a round platform and or inverted pot (10 cm in diameter) in the middle of a round water tank which is filled with 22°C water up to 1 cm of platform upper surface [15]. The wall of the tank should be tall enough to prevent animals jumping and escaping (Figure 2).
4. Placing animals in the pot according to the SD schedule: Subjecting animals to the SD procedure may be associated with anxiety, fear, and excitement. In order to minimize and/or eliminate any possible non-specific effects in SD studies, a separate group of animals was served as SD control group. For this reason, animals were placed on a larger diameter platform (21.5 cm), which simulated the SD set up realistic condition and allowed the rats to sleep without falling into the water [15,18].

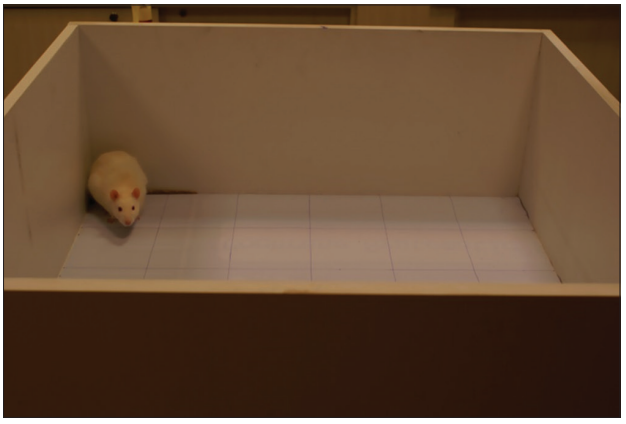

**Figure 1.** Open field test is used to ensure and monitor rats' locomotor activity, before animals introduction to the SD protocol.

**Figure 2.** FP setup, includes a platform at the centre of cylindrical water tank filled with water at the level of 1 cm beneath the platform top surface.

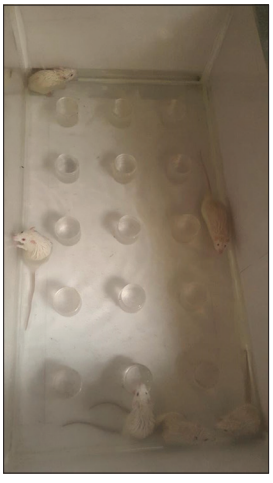

**Figure 3.** Modified-MP tank with a group of rats, water tank contains 15 narrow cylindrical platforms and it is filled with water at the level of 1 cm below the platform upper surface.

## Modified Multiple Platform Technique

Employment of the FP technique restricts animal locomotor activity. Therefore, technical and structural improvements are required to remove this intervening variable. For this, Multiple Platform (MP) technic was first introduced in 1980 [19]. In the primary version of MP technic, only one animal was introduced to the MP water tank environment, therefore elevation in the stress markers was still happening [16]. Given this, a modified version of MP technic (Modified-MP) was approved by introducing a group of animals in the MP water tank to eliminate both isolation and restraint factors [20]. Recently, Modified-MP technique is widely used to induce REM sleep in rats, largely due to its low cost and simplicity.

To restrict sleep in Modified-MP, after transferring the rats and assessment of their locomotor activity, following steps should be considered:

1. Setting up the Modified MP water tank: The box (127.0 cm long ×44.0 cm wide×45.0 cm high) which is used to set up the MP water tank can be made of the tilled water tank and/or Plexiglas material. Affix 15 cylindrical 6 cm high platforms (6.5 cm in diameter, each is horizontally and vertically spaced 13 and 10 cm apart, respectively), to the bottom of the tank [21] (Figure 3).
2. Filling the tank with water up to 1 cm beneath the upper surface of Plexiglas platforms. Notably, bringing animals from different cages to the tank and mixing them inside the MP device can make an unstable group and induce social instability which result in the elevation of stress-related factors. To avoid this unpleasant outcome, place number of animals (4-6 rats) from one cage on the platforms [22].
3. Locating a grid wire on top of the tank for placing chow pellets and water bottle. Notably, animal's introduction to a novel environment and to SD protocol as well as wetness and muscle tiredness could be associated with stressful experience in rats [16,22]; therefore a group of animals can be employed as a control group (or grid control) for the water tank environment [16]. Placing stainless steel wire-mesh grids (126.0 cm long × 43.0 cm wide × 0.9 cm high) over the platforms makes it possible for the animals rest at the tank and freely move to its all areas, without falling into water [22].

## Conclusion

Modified-MP technique saves the manipulation time and manpower, because it contains a group of animals that can be introduced into the modified-MP water tanks at the same time. Also, when a number of animals are introduced to the water tank, it decreases the isolation effects on the animals [23]. Besides, MP does not restrict animal movement throughout the study [22,23]. Generally, animals' exposure to the restricted space that limits their locomotor activities [24] and isolation, can elevate their plasma corticosterone levels, indicating their behavioral and biochemical vulnerability to both immobilization and isolation effects [25,26]. Modified-MP technique can obviate some of the aforementioned behavioral abnormalities [23]. However, apart from the beneficial effects of social interaction that is experienced in Modified-MP, this communication that forces exhausted animals to be awake during the SD induction period, triggers stress-related factors [23]. Furthermore, the outcomes of the SD-related studies are influenced by two crucial parameters including duration of SD [27,28] and induction of sleep restriction in different times of day (during light and or dark cycles) [29]. Also, the recovery period after induction of SD is important. Indeed, SD alters some behavioral and molecular parameters and evidence shows that these changes are affected by recovery time events [30]. Given the advantages and disadvantages of these techniques, it is necessary to improve their methodology and also design more novel and reliable instruments.

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