Animal Models of Sleep Disorder

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1 Introduction

Sleep is a complicated neurological disorder on the conduct of the animals characterized by using altered consciousness with diminished sensory response. The primary feature of sleep is to provide rest and restore the body's energy levels. The duration and pattern of sleep varies considerably among individuals. Age has an important effect on quantity and depth of sleep, and it is recognized as an architecture cyclic process. The sleep cycle characterized by different phases flowed by awake, dozing, unequivocal sleep, deep sleep transitions, cerebral sleep, paradoxical sleep. The changes in normal sleep behavior affected by environmental or psychological parameters are leading causes of sleep disorder. According to American Academy of Sleep Medicine (2014), sleep disorders (Somnipathy) are classified into insomnia, dyssomnias, parasomnias, circadian rhythm sleep disorders involving the timing of sleep, and other disorders including ones caused by medical or psychological conditions and sleeping sickness. Sleep issues especially arise due to alterations in the quality, quantity, and pattern of sleep (Cappuccio et al. 2010).

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Sleep disorders covers a huge spectrum of diseases such as inability to sleep at the desired time, excessive daytime sleepiness, abnormal movements or behavior during sleep. Sleep disorders also include sleep apnea (stops in breathing during sleep), narcolepsy and hypersomnia (excessive sleepiness at in appropriate times), cataplexy (sudden and transient loss of muscle tone while awake), sleeping sickness (disruption of sleep cycle due to infection), sleepwalking, night terrors, and bed wetting.

The pharmacological and therapeutic treatment of sleeping disorders includes barbiturates (phenobarbital, methohexitone), benzodiazepines (diazepam, lorazepam), and newer benzodiazepines (zolpidem, zopiclone). Sleep is a complicated physiological technique; this is stimulated via many factors, such as the problems associated with specific personal situations, and due to insufficient sleep. However, public and individual efforts to limit or manage sleep loss may be offset through the personal or societal desires by means of enhancing our daily recurring works or by way of adapting appropriate behavior to enhance the satisfactory of existence.

To understand this devastating disorder and to develop active therapeutic treatment, there is need to comprehend all animal models which give a wonderful step forward pathway to reveal new strategies for the diagnosis of sleep disorder. In this chapter, we reveal various animal models of narcolepsy like sleep deprivation induced changes in REM and NREM cycles. The different models helped us to reveal the pathophysiology of narcolepsy along with development of more therapeutic treatment.

2 Animal Models of Sleep Disorder

2.1 Sleep Deprivation Induced by the Modified Multiple Platform Technique

Principle

This model is based on principle that when the animals are placed on different platforms, they show variation in sleep cycles (Fig. 1). In this, small platform is used surrounded by water on which animals are placed. The animals enter the paradoxical phase of sleep, lose the postural balance partially or fully slip from the platform into the water and awaken. Now in this, large platform is also used to remove the social isolation dependency of sleep.

Procedure

- Male Wistar rats (250–280 g) are housed under a 12-h light–dark cycle. Room temperature is set at 20 °F, and animals should have free access to water and food at all times.
- The anesthesia is given with ketamine–diazepam, and two ipsilateral stainless steel screws for electroencephalogram (EEG) monitoring are implanted by

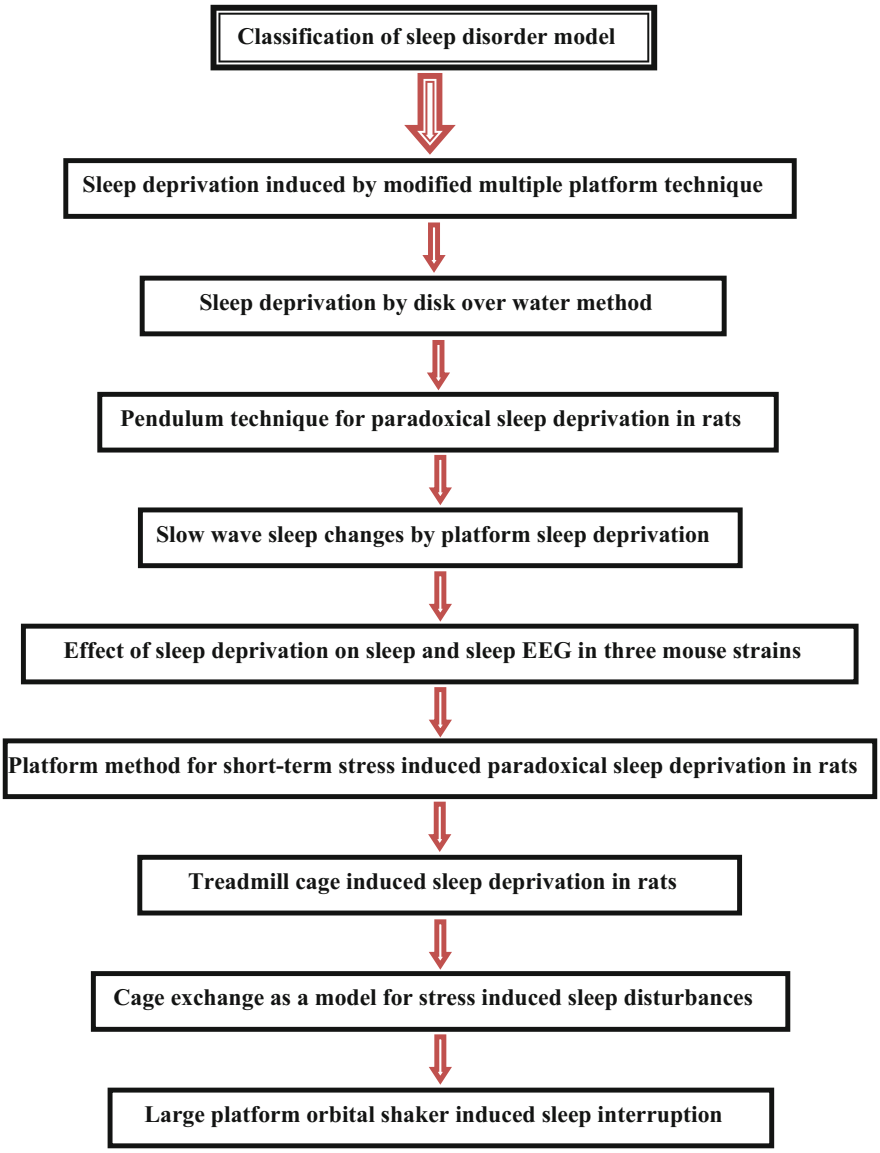

Fig. 1 Classification of animal models of sleep disorders

overlaying the right lateral fronto-parietal area and the left medial parietal cortex.

- Place additional one pair of nickel–chromium fine-wire electrodes in the dorsal neck muscle for electromyogram (EMG) recording. The electrodes are fixed

firmly to the animal cranium with acrylic dental cement. After surgery, penicillin and diclofenac are administered and allow for 15 days for recovery.
- After 15 days, baselines are recorded for 3 days in a group cage (n = 5) for the animal subjected to modified multiple platform (MMP) or in individual home cages for single platform.
- To evaluate the baseline recording, animals are adapted to the sleep deprivation procedure for 30 min on 3 successive days. So, after obtaining the sleep parameters, convert these into percentage of total recording time (usually 23 h) for each day.

Experiment A: *Modified multiple platform (MMP) method*

- In MMP method, rats are tested in socially stable groups of five in separate water tanks (123 × 44 × 44 cm) containing either 18 round platforms of 6.5 cm diameter (small platforms) or 18 platform with 14-cm diameter (large platforms).
- Large platform groups are often used as a control in sleep deprivation experiments. The additional control group is placed in a third tank on a stainless steel wire mesh (2.3-mm openings), which helps in allowing rats to lie down without touching the water.
- This group is referred to as the grid control group. All the tanks are now filled with water up to a level 1 cm below the surface of the platforms or the grid. During experimentation, continuous electrophysiological monitoring is performed in one rat in each group of five.
- Now after 96 h of sleep deprivation, rats are returned to the home cage, where they are continued under recording for 4 additional days. Ten deprivation runs cycles are conducted, with different animals, to achieve a final N of 10 recorded animals per group.

Experiment B: *Single platform method*

- In the single platform procedure, animals are placed on a single small (6.5 cm diameter) platform or large (14 cm diameter) platform in an enclosed tank (23 × 23 × 35 cm).
- As similar to the group procedure, the platform was surrounded or placed above 1 cm to the level of water. Rats are again returned to the home cage after 96 h of sleep deprivation cycle, where they are continued under recording for 4 additional days.
- Twelve animals are tested in each group.

Advantages

- The model reflects the complete sleep cycle occur in the human beings or changes in sleep cycles in pathological conditions.
- This method is useful to identify the effect of social isolation on sleep paradigm.

- The model is also a useful tool for the evaluation of various sedative drugs.

Disadvantages

- Due to the fear of water present under the platform, animal may loss slow wave sleep (SWS) cycles.
- The procedure is lengthy and also needs expert person to carry it smoothly.

2.2 Sleep Deprivation by Disk-Over-Water Method

Principle

The method exhibits the physiological modifications inside the diverse effects of sleep loss by the usage of a gentle bodily stimulation.

Procedure

- The animals of 230–250 g are selected for experimentation and are divided into two groups that are sleeping deprived (SD) rats and a control rats.
- The animals are simultaneously housed each on one side of a divided 46-cm horizontal disk suspended over a shallow tray of 2–3 cm deep water.
- The recordings are monitored continuously by EEG, EMG, and theta (θ) activity to detect sleeps states. The disk is mechanically circled at the low velocity of 3.33 rpm, while the SD rat starts to sleep or enters a 'forbidden' stage. This causes awakening of the rat and forcing it to walk opposite to disk rotation to avoid being carried into the water.
- Similarly, the control rat receives the same mild physical stimulation because they are also placed on the same disk. However, the sleep or targeted sleep stages are severely reduced in the SD rats, than control rats.
- The control rats show significant sleep reduction in comparison with the SD rats because they show physiological effects in the same direction as those of experimental rats, but to a much smaller in degree.

Advantages

- It is a simple method and also uses the physical method to measure the variations in the sleep cycles.
- This model provides easy method to investigate the effect of stress over the sleep.

Disadvantages

- The sleep-deprived rats during the experimentation show reliable syndrome like body temperature changes, weight loss, and increased metabolic rate. So, these consequences can cause alteration in normal sleep paradigm.

- It is difficult to identify the animal behavior whether it is due to loss of sleep or due to change in circadian rhythm.

2.3 Pendulum Technique for Paradoxical Sleep Deprivation in Rats

Principle

The technique is based on the sound evaluation in which animals are placed on the cage which moves in forward and backward directions like pendulum. At extreme motion of cage, the animals show postural imbalance. So this causes awakeness in animals, and they starts to move in opposite direction to the movement of cage.

Procedure

- The rats with body weight 220–250 g are selected are deprived for a night and familiarized with recording conditions prior to experimentation.
- The animals are placed in apparatus consisted of a swing with room for three rats in individual cage (30 × 25 × 35) supported by frame. They are put into recording cages connected to adaptation leads nearly for one week.
- The deprivation treatments are initiated after being positioned in a desk-bound pendulum and connected to the recording leads for one to two days. Animals are divided into two groups (n = 6) and are submitted to a deprivation period of 72 h.
- Give the deprivation into two phases, that is, for one group the deprivation is started at the onset of the light phase (09:30 h) and for the second group at the onset of the dark phase (21:30 h) of the illumination cycle.
- This arrangement helps us to determining the recovery of sleep after deprivation at the beginning of the light period (Group 1) or at the beginning of the dark period (Group 2). The speed of the pendulum is adjusted very carefully to yield sufficient paradoxical sleep (PS) deprivation in the animals.
- Now, set the time of gap between two in such a way that each group takes 12-h period according to the following schedule: 45, 35, 25, 20, 17, and 15 s. Following completion of both deprivation conditions, two baseline conditions (n − 6) are conducted.
- Repeat the same for the next groups in cages when the recovery sleep was monitored in the first and second groups, respectively. Animals are weighed immediately before the deprivation period and after termination of the recovery period.

Advantages

- It is a safe method because the stimulus for the sleep deprivation does not contain nonelectric type, that is, simple cage hanged like pendulum.

- The cages for the animals are covered from all side to avoid the external disturbances.
- This method is helpful to detect the effect of light and dark cycles on the animal sleep activities.

Disadvantages

- There are maximum chances of error because animals while experimentation are judged from adjacent room and movement of animals may be affected by size of cages.

2.4 Slow Wave Sleep Changes by Platform Sleep Deprivation

Principle

The rats are sleep deprived by using different size platforms. The REM and NREM sleep paradigms are recorded in two light cycles of duration 48 h. In both platforms, first light period (0–10 h sleep deprivation) measures REM sleep and second light period (22–34 h sleep deprivation) measures NREM sleep.

Procedure

- Place the rats on inverted flowerpots surrounded by water at room temperature. The flowerpot platform is placed above 2 cm to level of water.
- All the animals are randomly assigned into two groups that is one group having (n = 7) is placed on pots with diameter 15.7 cm. Second group containing (n = 8) is placed on pot with 5.1 cm diameter. The sizes are chosen to maximize the difference between the groups. Food and water is provided continuously through disposers hanging.
- For the first 30–45 min on the platforms, rats are very active and slowly they quieted down. The response is recorded continuously during the platform sleep deprivation. Now, the baseline recordings are made on day 15 following implantation, for 10 h starting at 09.00 h.
- Again the rats are returned to their home cages after first recording until 09.00 h the next morning when they are placed on the platforms for sleep deprivation. After 48 h of platform sleep deprivation with recording, the animals are moved from the platforms to the sleep recording bins. The recovery sleep time is then recorded for 10 h from 09.00 h. The animals did not go to sleep immediately, but spent a considerable part of the first hour grooming.
- The EEG fronto-frontal and fronto-parietal and neck are recorded on paper with speed of 10 mm/s.

Advantages

- The method is a more preferable because it compares the REM and NREM sleep cycles with deep slow wave sleep (SWS) by using platform of different sizes (Alkadhi et al. 2013).
- The changes in slow wave sleep cycles depict those appeared in human beings.
- The apparatus used for measuring sleep deprivation is like animal home cage which does not disturb normal physiology of animals.

Disadvantages

- Error is more during experimentation due to external factors like noise and other environmental changes.
- The protocol is long-lasting because competition of one cycle takes 48 h.

2.5 Effects of Sleep Deprivation on Sleep and Sleep EEG in Three Mouse Strains

Principle

The transgenic animals are used to explore the contribution of genetic makeup to maintaining the sleep cycles (Deboer et al. 2003). The determination of the sleep is done by using animals of different strains to know the underlying genetic cause of variation in slow wave sleep, REM, and NREM cycles.

Procedure

- Adult male mice of three different bred strains are used, 129rOla (Ola; n = 9), 129rSvJ (SvJ; n = 6), and C57BLr6J (C57; n = 11). The animals are maintained in a 12-h light–12-h dark cycle individually, kept in Macrolon cages (36 × 20 × 35 cm), and placed in sound attenuated chambers. Food and water is provided according to need, and animals are adapted for a minimum of 3 weeks to these conditions.
- Now surgically EEG and EMG electrodes are implanted by giving barbital anesthesia with Nembutal sodium (80 mg/kg i.p., volume approximately 0.5 ml). Two gold-plated miniature crews (Ø0.9 mm) served as epidural EEG electrodes. They are placed over the right occipital cortex 2–3 mm lateral to the midline, 2 mm posterior to bregma and the cerebellum at midline, 1 mm posterior to lambda and soldered to a plug with stainless steel wires.
- The EMG was recorded with two gold wires (Ø0.2 mm) inserted into then neck muscle and soldered to the plug, which is anchored to the skull with dental cement. The animals for 3 weeks are allowed for recovery. Now, start the baseline recording at light for 18 h. At least 10 days later, the mice are subjected to 4-h sleep deprivation (SD) and recorded for remain 24 h. The SD is carried

out at light onset by introducing objects (like nesting material) into the cage and later by tapping on the cages. The tapping produces drowsiness, or the EEG exhibits slow waves.
- Again animals are placed into new cages without any disturbance during deprivation. The EEG and EMG signals are amplified by electrodes which help in differentiating the SWS, REM, and NREM movements in different strains.

Advantages

- A transgenic and knockout mouse is an important model to investigate the contribution of genes to behavior.
- It is also helpful in investigating sleep changes at genetic levels.

Disadvantages

- It is very costly method and also needs expert persons for placing electrode surgically into the animals' brain to record EEG and EMG.
- It is not easy to apply the data obtained from rodent species to human's primates.
- Transgenic animals have low survival rate.

2.6 Platform Method for Short-Term Stress-Induced Paradoxical Sleep Deprivation (PSD) in Rats

Principle

The water tank is used to induce stress stimuli to the animals. The animals when loss the consciousness, they suddenly fall into the water and become awakened (Fig. 2).

Procedure

- First rats are placed in a tiled water tank (143 × 41 × 30 cm) for 24 or 96 h. The tank contained fourteen platforms (6.5 cm in diameter) and lies above 1 cm to the water surface, this allowing the rats to move around by leaping from one platform to another.
- At the onset of each paradoxical sleep (PS) episode, the animal experiences a loss of muscle tonus and falls into water, which produces consciousness. So for the evaluation of total suppression of PS over 24 h (PSD-24 h) or 96 h (PSD-96 h) intervals, we uses the multiple platform procedure, which is well-documented to be effective in producing a total suppression of PS. Hence, it is appropriate to consider that these animals are being PS-deprived rather than being completely deprived of sleep.

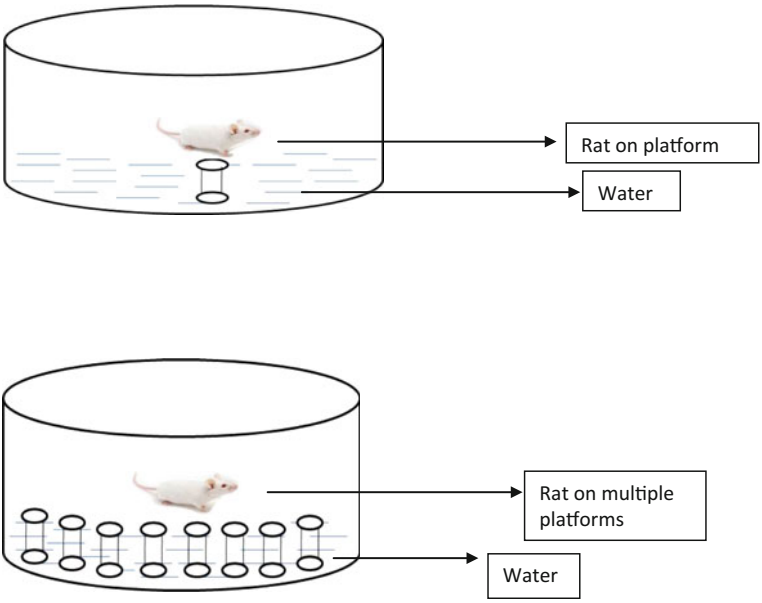

Fig. 2 Platform method for short-term stress-induced paradoxical sleep deprivation in rats

- Maintain the cage control group in the same room likely to the experimental rats for the duration of the study and showed normal sleep patterns, including PS, slow wave sleep (SWS) and wake.
- Again maintain the animal in the experimental rooms at a controlled temperature and light–dark cycle throughout the study along with availability of food and water and chow pellets located on top of the tank. The water in the tank is changed daily throughout the PSD period.

Advantages

- The number of platform used for experimentation is more, so the chances of error are less.
- The source used for stressful stimuli does not produce any harm to animals.
- It is economical, effective, and noninvasive technique to measure neurophysiology of the animals.

Disadvantages

- The aggressive behavior of animals may alter normal physiological responses.
- It does not show specificity in the results because the results vary depending on the size of platform.

2.7 Treadmill Cage Induced Sleep Deprivation in Rats

Principle

Sleep deprivation is defined as lack of restorative sleep over a cumulative period so as to cause physical or psychiatric problem in the animals induced by the physical stimuli like treadmill moving at variable rate with time which affects routine performances of tasks. This stressful movement disturbs the normal sleep regulatory systems and produce awakening by the cortical activation in the brain.

Procedure

- Firstly place the rats in a treadmill cage 50.8 cm (l) × 16.51 cm (w) × 30.48 cm (h).
- Now, switch on the apparatus during which the horizontal belt at floor automatically starts to move slowly at a rate of 0.02 m/s.
- To induce sleep fragmentation firstly, the treadmill ran at slow speed for 30 s. The total sleep deprivation is induced when the treadmill run for 4 s followed by no treadmill movement for 12 s, and these schedules ran continuously for 24 h.
- For habituations of rats in treadmill movement, the treadmills were turned on (5 min on followed by 5 min off) for one hour on each of the 2 days prior to the experiment.

Advantages

- This model is economical and noninvasively effective;
- No evidence for animal suffering from any harmful condition;
- Minimal handling is required while operation.

Disadvantages

- Treadmill procedure is very lengthy because it takes 4 week for the training session (Herman et al. 2010).
- Noise and stressful conditions may produce error in the results.

2.8 Cage Exchange as a Model for Stress-Induced Sleep Disturbances

Principle

The wakefulness in animal is promoted and maintained by multiple arousal systems (de Lecea 2012; Lee 2012). The stressful event during cage exchange paradigm produce continual wakefulness (sleep deprivation), with a pattern of neuronal hyperactivity is prominent causative factor for disturbance in sleep cycle. Specific psychological stressor or extra cellular stimuli increased immediate early

genes (IEGs), i.e., c-Fos which synthesized Fos (neuronal activation) of the medial-parvicellular para-ventricular hypothalamic nucleus (mpPVH).

Procedure

Surgery (implants and lesions)

- All the animals are anesthetized and applied minor cut to explore the cranium.
- The connective tissue is cleaned and four burr holes is drilled with implanting 4 screw electrodes (two on each side) and two EMG electrodes also placed into the nuchal muscles.
- Connect all electrodes to a pedestal socket, which is fixed to the skull with dental cement. Finally, the wound is sutured and applied antibiotic ointment, and the animal is removed from the stereotaxic frame and allowed to recover from anesthesia. After 2 weeks of recovery, rats are connected to the recording apparatus during 3 d for habituation.
- Now, the baseline EEG/EMG recorded for 48 h, rats are placed into a dirty cage previously occupied by another male rat for 1 week (cage exchange). Rats were left undisturbed in the dirty cage until they were killed.
- Control rats are placed in a clean cage at the same time to synchronize the ultradian cycles of both groups.
- To examine the brain circuitry involved in stress-induced acute insomnia, the rats killed at 3:30 P.M., 90 min after the onset of this sleep-disturbed period (5.5 h after cage exchange). This time is selected because animals show sleep fragmentation and decreased sleep beginning 4 h after cage exchange, and 90 min is the optimal time to detect Fos expression associated with a specific stimulus.

Advantages

- It is a noninvasive technique used to measure narcolepsy.
- Sleep changes can easily be identified from EEG wave pattern.

Disadvantages

- Appropriate facilities and trained personnel are required;
- High mortality rate and time-consuming process;

2.9 Large Platform Orbital Shaker Induced Sleep Interruption

Principle

Stress is responsible for the number of biochemical changes at peripheral and central region of brain and also causes permanent neurological and psychological

dysfunction (Moghaddam 1993). Serotonin (5-HT) is a true neuromodulator of sleep because the inhibition of 5-HT synthesis with p-chlorophenylalanine (PCPA) could inhibit tryptophan hydroxylase and impair 5-HT biosynthesis and induced a severe insomnia/sleep deprivation (Jouvet 1999).

Procedure

Surgery (implants and lesions)

- All the animals are anesthetized and applied minor cut to explore the cranium.
- The connective tissue is cleaned and four burr holes is drilled with implanting 4 screw electrodes (two on each side) and two EMG electrodes also placed into the nuchal muscles.
- All electrodes are connected to a pedestal socket, which is fixed to the skull with dental cement. Finally, the wound is sutured, antibiotic ointment is applied, and the animal is removed from the stereotaxic frame and allowed to recover from anesthesia.
- Again after 2 weeks of recovery, rats are connected to the recording apparatus during 3 d for habituation.
- Now, the recording cages are placed on the 27 × 32 cm platform of an analog orbital shaker (VWR Model OS-500; VWRL abshop, Batavia, IL) and affixed the platform, to prevent double-sided mounting tape (3 M, St. Paul, MN). Two cages could be monitored simultaneously.
- The on-off repetitive cycling of the shaker is maintained at 100 rpm and is controlled by a timer on a 120 s cycle (30 s on, 90 s off). A metal cage card holder is also suspended from the top of the cage because this creates an additional audible stimulus when the holder knocked against the side of the cage, once per cycle, during shaking.

Advantages

- It is more effective because sleep deprivation in the rodents is caused by large area platform with additional rotationary movement.

Disadvantages

- Appropriate facilities and trained personnel are required.
- It is a lengthy procedure because animals are used for experimentation after 2 weeks of surgery.

Ethical Statement

All institutional guidelines, national guidelines, state and local laws and regulations with professional standards for the care and use of laboratory animals should be followed. Studies involving animals must state that the institutional animal ethical committee has approved the protocol. For authors using experimental animals, a

statement should be made that the animals' care is in accordance with institutional guidelines and animals used have been treated humanely and with regard to the alleviation of suffering. Researchers should treat animals as sentient and must consider their proper care and use and the avoidance or minimization of discomfort, distress, or pain as imperatives. Animal experiments should be designed only after due consideration of animal health. It should be ensured that all researchers who are using animals have received instruction in research methods and in the care, maintenance, and handling of the species being used. All the surgical procedures should be performed under appropriate anesthesia and follow only those procedures which avoid infection and minimize pain during and after surgery.

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