

### **Kertas Asli/Original Articles**

## **Sleep Deprivation Models in Rodents (Model Kekurangan Tidur dalam Roden)**

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#### ABSTRACT

*Sleep deprivation has been identified as a risk factor for various diseases. The number of patients suffering from sleep deprivation is increasing daily. Therefore, the risk to develop various diseases, including cardiovascular disease is increasing. However, there is a limitation to elucidate the pathophysiological changes following sleep deprivation in humans. Thus, the need arises for sleep deprivation models using animals, which will serve the purpose of understanding the disease in a better way. Several techniques have been developed to model sleep deprivation in animals, including inverted flowerpot and multiple platforms techniques. Genetic and environmental factors, costs, infrastructure and animal life spans are some of the factors that need to be considered when selecting a particular model. Furthermore, when studying sleep deprivation, tissue samples, such as peripheral blood, brain samples and aorta are used to elucidate the underlying mechanisms of a particular disease. Currently, more than ninety percent of all laboratory animal experiments are performed in rats and mice. This review article focuses on models of sleep deprivation in Rodents, which are generally used in research laboratories. The article also tries to evaluate the advantages and disadvantages of each technique discussed, guides the sleep deprivation model and helps researchers to decide on a specific model for their purpose.*

*Keywords: Sleep deprivation; animal models; inverted flowerpot technique; multiple platforms*

#### ABSTRAK

*Kekurangan tidur telah dikenal pasti sebagai faktor risiko untuk pelbagai penyakit. Jumlah pesakit yang menghadapi masalah kurang tidur meningkat setiap hari, seterusnya risiko mendapat pelbagai penyakit termasuk penyakit kardiovaskular semakin meningkat. Walaubagaimanapun, terdapat limitasi untuk menjelaskan perubahan patofisiologi akibat kekurangan tidur pada manusia. Oleh itu, timbul keperluan terhadap model kekurangan tidur menggunakan haiwan yang boleh digunakan bagi tujuan untuk memahami sesuatu penyakit dengan cara yang lebih baik. Pelbagai teknik telah dibangunkan untuk mencipta model kekurangan tidur dalam haiwan, termasuk teknik pasu terbalik dan teknik menggunakan platform yang banyak. Faktor genetik dan persekitaran, kos, infrastruktur and jangka hayat haiwan adalah antara faktor yang perlu diambil kira apabila memilih sesuatu model. Di samping itu, kajian kekurangan tidur memerlukan sampel tisu seperti darah periferi, sampel otak dan aorta untuk menjelaskan mekanisme sesuatu penyakit. Pada masa kini, lebih daripada sembilan puluh peratus kajian makmal yang melibatkan haiwan telah dijalankan pada tikus dan mencit. Artikel ulasan ini adalah berfokuskan pada Roden, iaitu yang kebanyakannya digunakan dalam makmal penyelidikan. Artikel ini juga cuba menilai kelebihan dan kekurangan setiap teknik yang dibincangkan, juga memberi panduan terhadap model kekurangan tidur dan membantu para penyelidik dalam memilih model tertentu untuk tujuan masing-masing.*

*Kata kunci: Kekurangan tidur; model haiwan; teknik pasu terbalik; platform yang banyak*

## INTRODUCTION

Sleep is crucial for the maintenance of health, emotional and physiological well-being as well as for motor skills and neurocognitive performance (Jiang et al. 2017; During & Kawai 2017). Sleep is a complex physiological process that is influenced by many internal and environmental factors (Toth & Bhargava 2013). Sleep is divided into two broad categories: rapid eye movement (REM) sleep and non-REM (NREM) sleep (Patel et al. 2020), which are physiologically and behaviorally distinct from one another. The NREM phase is also called dreamless sleep where the breathing is slow and the heart rate and blood pressure are normal (Delimayanti et al. 2020). The NREM phase will eventually deepen and lead to REM sleep. REM sleep is also known as “paradoxical sleep”, which is a behavioral state, a brain state and a dream state (Blumberg et al. 2020). It is characterized by a variety of components: muscle paralysis, rapid eye movements, activated cerebral cortex, rapid breathing as well as increased heart rate and blood pressure to near-waking levels.

Sleep deprivation is defined as abnormal sleep that can be described in measures of deficient sleep quantity, structure and/or sleep quality (Krause et al. 2017). Sleep deprivation has become a global public health issue that is associated with a range of negative health and social outcomes (Chattu et al. 2019). Therefore, the study on sleep deprivation has attracted the interest of many researchers for many years. Nurses and physicians are among the health professionals who are vulnerable to sleep deprivation due to fluctuating work schedules (Eanes 2015). Despite the growing number of studies that have emerged, the precise underlying mechanisms for sleep deprivation related diseases remain unknown. Animals and humans experiencing sleep deprivation may exhibit detrimental physiological responses. Long-term comorbidities arising from sleep deprivation include heart disease, stroke and high blood pressure (Javaheri et al. 2018), which have significant morbidity and mortality. Animal-based research plays a critical role in sleep medicine research, particularly in the study of the physiology of sleep and for the delineation of the underlying mechanism of sleep disorders (Toth & Bhargava 2013). Therefore, it is necessary to develop a competent model that can provide a convincing platform to develop the pathophysiology of the disease. There are some limitations of using humans in research, particularly in testing certain compounds or drugs.

It is interesting to note that the first study of sleep deprivation published in 1894 was conducted in puppies (Chen & Kushida 2005). Subsequently, human subjects and various animal models were used. The largest number of animal models used to date is Rodents, including rats

and mice (Shen et al. 2017). Rodents have been used to study all aspects of sleep, such as neuroanatomy, neurophysiology, neurochemistry and gene expression (Hendricks 2005). In addition to Rodents, other species used to study sleep deprivation include monkeys, rabbits, cats and dogs (Hendricks 2005). Many researchers have used animal models in sleep deprivation study to elucidate the pathophysiology of diseases or conditions, including endothelial dysfunction (Jiang et al. 2017; Nawi et al. 2020a; Nawi et al. 2020b), memory impairment (Wiesner et al. 2015), behavioral changes (Hanlon et al. 2015), pain (Siran et al. 2014) and lipid peroxidation (Thamaraiselvi et al. 2012; Nawi et al. 2020a). Animal models, therefore, play a pivotal role as a tool in sleep deprivation research. The samples obtained from animal models include blood and descending thoracic aorta (Nawi et al. 2020a), femoral artery (Nawi et al. 2020b), hippocampus (Alzoubi et al. 2012; Abd Rashid et al. 2017; Raven et al. 2019), brain stem (Ramanathan et al. 2002) and hypothalamus (D'Almeida et al. 2000).

Animal models provide a crucial approach for evaluating molecular and cellular pathways in a controlled environment (Wang et al. 2017). Although animal models have been used to delineate the precise mechanism of pathophysiological changes in sleep deprivation, many factors need to be considered before starting the experiment. This review tries to evaluate the advantages and disadvantages of some animal models, in particular Rodents, in sleep deprivation studies, which will help guide researchers to decide on a specific model for their purposes.

## SLEEP DEPRIVATION MODELS IN RODENTS

Animal-based research has been developed to address the deleterious effects of sleep deprivation, including small and large animal models in sleep deprivation study. Genetic and environmental factors, costs, infrastructure and the requirement for specialized personnel should be taken into account when selecting a specific model (Zaragoza et al. 2011). Animal models are established based on three basic constructs: face validity (a phenotype similar to humans who have the illness), construct validity (processes resulting in human pathology are recapitulated to the model) and predictive validity (sensitivity to pharmacological and non-pharmacological interventions that are effective for the disease or condition in humans) (Nestler & Hyman 2010). Various models have been used in the study of sleep deprivation in Rodents and each model has its advantages and disadvantages, as shown in Table 1. Most sleep deprivation models were based on the

TABLE 1. Sleep deprivation models

Sleep deprivation model	Animal species	Sleep-state deprivation	Advantages	Disadvantages	Authors
Classic single platform	Rats and mice	<ul style="list-style-type: none"> <li>Paradoxical sleep/ REM sleep deprivation</li> </ul>	<ul style="list-style-type: none"> <li>Abolishes REM sleep</li> <li>Simple, easy and cost-effective</li> <li>Continuous monitoring is not required</li> <li>Surgical intervention is not required</li> </ul>	<p>Stress may be developed due to social isolation, immobilization, soaking, and cooling</p>	<p>D'Almeida et al. 2000; Koban et al. 2008; Singh &amp; Kumar, 2008; Thamaraiselvi et al. 2012; Mehta et al. 2018; Pandey &amp; Kar 2018</p>
Double platforms	Rats	<ul style="list-style-type: none"> <li>Paradoxical sleep/ REM sleep deprivation</li> </ul>	<ul style="list-style-type: none"> <li>Abolishes REM sleep</li> <li>Simple, easy and cost-effective</li> <li>Animals can mobilize between the two platforms</li> <li>Continuous monitoring is not required</li> <li>Surgical intervention is not required</li> </ul>	<p>Stress may be developed due to social isolation, soaking and cooling</p>	<p>Siran et al. 2014; Abd Rashid et al. 2017; Tengku Adnan et al. 2017; Nawi et al. 2020a; Nawi et al. 2020b</p>
Multiple small platforms	Rats and mice	<ul style="list-style-type: none"> <li>Paradoxical sleep/ REM sleep deprivation</li> </ul>	<ul style="list-style-type: none"> <li>Abolishes REM sleep</li> <li>Eliminates immobilization and social isolation</li> <li>Save time and manpower</li> <li>Continuous monitoring is not required</li> <li>Surgical intervention is not required</li> <li>Many animals can be deprived of simultaneously</li> </ul>	<p>Stress may be developed due to soaking, cooling and social conflict</p>	<p>Hanlon et al. 2005; Silva et al. 2007; Patti et al. 2010; Suer et al. 2011; Alzoubi et al. 2012; Alzoubi et al. 2013; Zhang et al. 2013; Hirotsu et al. 2013; Lungato et al. 2013; Lima et al. 2014; Mahmoudi et al. 2017; Noorafshan et al. 2017; Ma et al. 2019</p>
Disk-over-water	Rats	<ul style="list-style-type: none"> <li>Paradoxical sleep/ REM sleep deprivation</li> <li>Total sleep deprivation</li> </ul>	<ul style="list-style-type: none"> <li>The researcher can manipulate the sleep phase to be deprived</li> <li>Eliminates immobilization</li> <li>More accurate as the phases of sleep are determined by EEG</li> </ul>	<ul style="list-style-type: none"> <li>Stress may be developed due to social isolation</li> <li>Invasive due to electrodes insertion</li> <li>High cost as it requires a sophisticated computer-based controller and EEG machine</li> </ul>	<p>Ramanathan et al. 2002; Gopalakrishnan et al. 2004; Everson et al. 2005; Chang et al. 2006; Jiang et al. 2017</p>

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Electrical stimulation	Rats	<ul style="list-style-type: none"> <li>Paradoxical sleep/ REM sleep deprivation</li> <li>Total sleep deprivation</li> </ul>	<ul style="list-style-type: none"> <li>The researcher can manipulate the sleep phase to be deprived</li> <li>More accurate as the phases of sleep are determined by EEG</li> </ul>	<ul style="list-style-type: none"> <li>The duration and intensity of the stimuli need to be adjusted regularly</li> <li>Invasive due to electrodes insertion</li> <li>Mechanical damage to neurons and fibers if continuous stimulation is applied</li> </ul>	Singh & Mallick 1996; Kovalzon & Tsubulsky 1984; Cirelli & Tononi, 2005
Pendulum/ swing technique	Rats	<ul style="list-style-type: none"> <li>Paradoxical sleep/ REM sleep deprivation</li> <li>NREM sleep deprivation</li> </ul>	<ul style="list-style-type: none"> <li>Eliminates a wet and humid environment</li> <li>No surgical intervention is required</li> </ul>	Inadequate feeding due to constant swinging motion	Vogel 1975; Van Hulzen & Coenen 1980; Coenen & Van Luijckelaar 1985
Cold ambient environment	Rats	Paradoxical sleep/ REM sleep deprivation	Abolishes REM sleep	Invasive due to electrodes and thermistor insertion	Rousseu et al., 1984; Amici et al. 2008
Gentle handling	Rats and mice	<ul style="list-style-type: none"> <li>Total sleep deprivation</li> <li>Short sleep deprivation</li> </ul>	<ul style="list-style-type: none"> <li>Less stressful environment</li> </ul>	<ul style="list-style-type: none"> <li>Stress may be developed due to social isolation and immobilization</li> <li>Needs trained experimenter</li> <li>Animals may adapt to the novel stimuli</li> <li>Continuous monitoring is required</li> <li>Involves surgical intervention</li> </ul>	Gopalakrishnan et al. 2004; Colavito et al. 2013; Hirotsu et al. 2013; Melgarejo-Gutierrez et al. 2013; Kalinchuk et al. 2010; Patti et al. 2010; Raven et al. 2019

Abbreviations: REM, rapid eye movement; NREM, non-REM; EEG, electroencephalogram

flowerpot technique that has been described for the first time by Jouvet et al. in 1964 on cats (Mahmoudi et al. 2017).

### CLASSIC SINGLE PLATFORM

Classic single platform or inverted flowerpot is known as one of the most popular and effective methods for selective REM sleep deprivation in rats (Lungato et al. 2013; Mehta et al. 2018). This model is also known as the water tank, platform, or pedestal technique (Gulyani et al. 2000). In this model, a rat is placed in a tank, chamber, or container containing a small platform, often an inverted flowerpot, approximately 4.5-10 cm in diameter (Villafuerte et al. 2015). The wall of the tank should be high enough to prevent the animals from jumping and escaping (Mahmoudi et al. 2017). After the period of adaptation in a dry tank, the water is added and the height of the platform is 1 cm above a pool of water as shown in Figure 1a.

The basic concept of this model is that during REM sleep, animals lose their muscle tone and are unable to maintain their extended, relaxed body posture on the small platform, causing the rat to fall from the platform into the water, hence, waking up instantly (Pandey & Kar 2018). As the period of deprivation is prolonged, the rat may fall into the water more frequently (Cirelli & Tononi 2005). At any time, only one rat is placed in the tank and maintained on the platform, which was small enough with reference to the rat's body size. In all sleep deprivation models, animals were given access to food and water *ad libitum*. This model allows animals to obtain NREM while REM sleep is restricted (Tufik et al. 2009).

Furthermore, this model is simple and cost-effective (Mahmoudi et al. 2017). This model was used to study behavior (Hanlon et al. 2005), lipid peroxidation (Thamaraiselvi et al. 2012) and oxidative stress in the hypothalamus (D'Almeida et al. 2000). However, isolation, immobilization, soaking and cooling may induce stress to

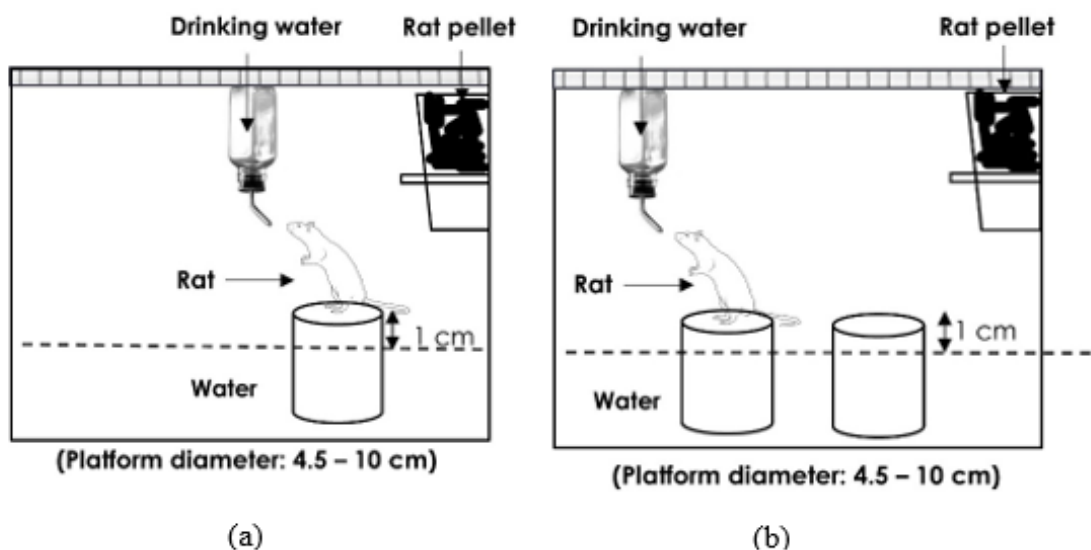


FIGURE 1. Schematic diagram of sleep deprivation models, (a) Classic single platform (b) Double platforms. Water is added and the height of the platform is 1 cm above a pool of water

the rat when using this method (Kovalzon & Tsibulsky 1984; Villafuerte et al. 2015).

### DOUBLE PLATFORMS

This model has been modified from the classic single platform. This model has two platforms instead of a single platform, as shown in Figure 1b. A wire mesh was used to cover the tank for ventilation and to prevent the rats from jumping and escaping from the tank (Nawi et al. 2020a).

This model was used to study the effects of REM sleep deprivation on pain-related genes (Siran et al. 2014), endothelium (Tengku Adnan et al. 2017; Nawi et al., 2020a; Nawi et al., 2020b), memory (Ab Rashid et al. 2017), oxidative stress in the plasma (Tengku Adnan et al. 2017) and oxidative stress in aortic tissues (Nawi et al. 2020a). The methods used during the intervention are similar to the classic single platform with a similar basic concept. The classic single platform restricts animal locomotor activity (Mahmoudi et al. 2017), thus, this model has the advantage of the rat being able to mobilize from one platform to another.

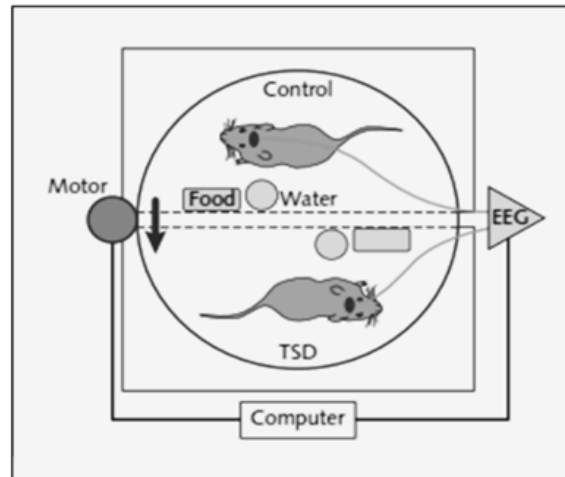


FIGURE 2. Diagram of disk-over-water model. An experimental rat and a control rat are placed on either side. A continuous electroencephalogram (EEG) recording is linked to a computer programmed to trigger rotation of the disks depending on the type of desired sleep deprivation. When the experimental rat starts to enter sleep deprivation, the disk is automatically rotated at a low speed that awakens the rat (Taheri, 2007)

#### MULTIPLE SMALL PLATFORMS

In contrast to single and double platform methods, this model uses a single tank or chamber that has multiple small platforms with the size of 3-5 cm each and a space between the platforms of 7 cm (Alzoubi et al. 2012; Lima et al. 2014). Multiple rats are placed in the tank, for example, 5 rats with 7 platforms or 10 rats with 18 platforms filled with water at the level of 1 cm below the platform's surface (Cirelli & Tononi 2005). Mahmoudi et al. (2017) have fixed 15 narrow cylindrical platforms (6.5 cm in diameter each) at the bottom of the tank. The tank is filled with water up to 1 cm beneath the upper surface of platforms and 4 rats were added to the tank. The basic concept of this method is similar to a single and double platform model where the loss of muscle tone during REM sleep results in rats touching the water and waking up.

This model was used to study the effects of REM sleep deprivation on oxidative stress in the frontal cortex (Hirotzu et al. 2013), the hippocampus (Alzoubi et al. 2013) and the whole brain (Suer et al. 2011). It has also been used to study the effects of REM sleep deprivation on oxidative stress in the hippocampus and prefrontal cortex in mice (Silva et al. 2007; Lima et al. 2014). This model prevents immobility due to many platforms and also prevents social isolation, as more rats can be placed at one time (Landis 2005; Villafuerte et al. 2015). This model also saves the manipulation time and manpower, as many rats can be evaluated at the same time (Mahmoudi et al. 2017). However, it has been reported that this technique has shown an increased social conflict between rats (Suchecki et al. 1998) and therefore, the number of rats to be placed in the tank is approximately 4 to 6 (Mahmoudi et al. 2017).

#### DISK-OVER-WATER

In this model, two rectangular clear plastic chambers are placed side by side. A disk of 40 cm in diameter is built in the middle of the lower quarter of the chambers, which serves as the rat carrier platform, as shown in Figure 2. The experimental rat and the control rat are placed on either side of the disk, suspended over a shallow tray of 2-3 cm deep water with separate food and water containers. A surgical procedure was performed on both rats to implant the electrodes through the skull before the intervention. During the intervention, there is a continuous electroencephalogram (EEG) recording, which is linked to a computer programmed to trigger disk rotation, depending on the type of deprivation desired, REM sleep deprivation, or total sleep deprivation. When the experimental rat starts to sleep or enters REM sleep, the disk is automatically rotated at a low speed at 3.33 revolutions per minute (rpm), which awakens the rat and forces it to walk to the opposite direction of the disk rotation to avoid falling into the water (Taheri 2007). The yoked control rat receives the same physical stimulation as the experimental rat because it is on the same disk. The control animal can sleep whenever the experimental animal is awake as the disk does not rotate (Colavito et al. 2013).

This model was used to study the effects of REM sleep deprivation on endothelium (Jiang et al. 2017) and whether total sleep deprivation causes oxidative stress in the hippocampus, cortex, cerebellum, hypothalamus and brain (Ramanathan et al. 2002). The advantage of this method is the ability for the researcher to manipulate the desired sleep phase to be evaluated and immobilization is eliminated. Isolation stress may, however, appear in this

method (Villafuerte et al. 2015).

#### ELECTRICAL STIMULATION

Brain electrical stimulation methods are usually used for larger animals, such as cats and dogs (Cirelli & Tononi 2005). Animals are monitored by EEG and electromyography (EMG) in this model. EMG is used to record the electrical activity produced by the skeletal muscles. Direct brain stimulation is introduced when the rat shows the features of REM sleep (Landis 2005).

Electrical stimulation was also performed by direct electrical stimulation of the midbrain reticular formation (MRF) during the onset of REM sleep deprivation, which was determined polygraphically (Kovalzon & Tsibulsky 1984). However, the duration and intensity of the stimuli applied need to be adjusted regularly, almost every hour if the animals failed to arouse. This method is invasive where the insertion of the electrode during implantation can cause mechanical damage to the neurons and fibers (Singh & Mallick 1996).

#### PENDULUM OR SWING TECHNIQUE

The pendulum technique was designed to avoid the stress experienced by the platform technique (Van Hulzen & Coenen 1980). This model involved a slowly rotating apparatus like a pendulum, consisting of three separate compartments that resembled a swing (Landis 2005). The apparatus continuously oscillates back and forth, which causes the rat to wake up. The posture imbalance caused by the swing caused the animal to remain awake to maintain balance (Mehta et al. 2018). The speed of oscillations can be adjusted to increase the number of awakenings to prevent REM sleep from occurring. The animals experienced minimal REM sleep (0–2 percent) and lost significant NREM sleep (Gulyani et al. 2000). The floor of the cage was covered with a rough surface to prevent the rats from sliding while the top was covered with a wire mesh to prevent escape (Cirelli & Tononi 2005).

This model was used to determine the effects of paradoxical sleep deprivation on depression (Vogel 1975), intracranial self-stimulation (Van Luijtelaar et al. 1982) and stress (Coenen & Van Luijtelaar 1985). Short wave sleep (Stage 3 of non-REM sleep) also decreased over 3 days of deprivation (Van Hulzen & Coenen 1980). The disadvantage of this method is that the animals may have inadequate feeding due to the constant swinging motion of the pendulum.

#### COLD AMBIENT ENVIRONMENT

Cold is more disruptive to sleep than heat (Haskell et al. 1981). This model uses cold temperatures between 10°C and -10°C for 1-2 days to deprive the REM sleep phase in rats (Amici et al. 2008). Two stainless steel electrodes for EEG recording and a thermistor to measure hypothalamic temperature were implanted epidurally. REM sleep deprivation is almost complete at -10°C compared with 0°C. Exposure to sub-thermoneutral ambient temperatures has been shown to reduce sleep in mice (Rousseau et al. 1984). REM sleep can be selectively deprived using extreme ambient temperatures as the physiological thermoregulatory defences and acclimatization processes are disturbed (Landis 2005). Nevertheless, the mechanism involved in thermoregulatory loss during REM sleep has remained unknown. The major brain region; the preoptic area/anterior hypothalamus (POAH), plays an important role in the regulation of sleep due to heat loss (Van Someren 2000).

#### GENTLE HANDLING

This is the most common method of assessing the effects of total sleep deprivation, which is based on the direct interaction with the experimenter, who actively keeps the animal awake (Colavito et al. 2013; Raven et al. 2019). To use this technique, electrodes need to be surgically implanted to record the electrophysiological signals to objectively define and identify the sleep and waking states (Mehta et al. 2018). Multiple techniques may be applied to keep the rats awake, including gently touching their tails or whiskers, introducing objects in chambers, shaking their cages and brushing their fur (Villafuerte et al. 2015; Raven et al. 2019). The ultimate aim of this technique is to diminish the simulated effects of stress and force the locomotor activity intruded by other methods.

This model was used to determine oxidative stress in the frontal cortex (Hirotsu et al. 2013), hippocampus (Melgarejo-Gutierrez et al. 2013) and the cerebral cortex (Gopalakrishnan et al. 2004). Raven et al. (2019) have shown that five hours of sleep deprivation reduces spine density in the dentate gyrus with a prominent effect on the branched spines. The stressful environment is low in this method but the isolation and immobilization stress to the rats may develop (Villafuerte et al. 2015). This method requires the constant physical presence of dedicated and fully trained experimenters that the animals must be familiar with before the experiments (Colavito et al. 2013). However, when the animals have adapted to the novel stimuli, the duration of sleep loss will be significantly limited (Li et al. 2009).

## DISCUSSION AND CONCLUSION

Everyone is aware of the harmful effects of sleep deprivation. Research in sleep deprivation has received considerable attention from several researchers worldwide. Creating valid models resembling this state in animals is of great importance. Taken together with those models, in this review, we addressed the evidence that researchers are highly dependent on the animal models to avoid obstacles in human studies and to obtain samples. The application of platform-based techniques has some advantages over other methods, as no complex instruments are required, as well as being simple, readily available and low-cost. Among the platform methods, the multiple small platforms save time and manpower, decrease social isolation and prevent immobilization.

The use of animal models has increased our knowledge and improved the diagnosis and treatment of certain diseases. Although each model has its advantages and disadvantages, it can be used to understand the pathophysiological changes following sleep deprivation.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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