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# A study of rapid eye movement sleep deprivation methods in animals

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## Abstract
Rapid Eye Movement (REM) sleep deprivation methods are extremely important in understanding the importance, mechanisms and effects of REM sleep on animals. A good sleep deprivation technique selectively abolishes the desired stage of sleep without a significant effect on the other stages, does not stress the experimental animals, is humane, limits the contact between the experimenter and experimental animals and allows sleep deprivation for the desired length of time without stressing the experimenter. Many different methods have been developed to study this phase of sleep, each with its own advantages and limitations. Of these, the disk over water method and the flower pot method (and its variants) are the most popular methods for sleep deprivation studies in adult animals. Laboratory shaker method has been developed for sleep deprivation in neonatal animals.

**Keywords:** REM sleep, paradoxical sleep, sleep deprivation method

## Introduction
Most of us think of sleep as a period of rest and recovery from everyday stresses. However, research has shown that sleep is not just a period of decreased brain activity. Sleep has a dynamic phase during which many vital processes take place. Sleep is characterized by two very different states that alternate with one another cyclically, namely, REM Sleep (REMS) and non REM sleep (NREMS) (Dement and Kleitman, 1957) [1]. NREMS is characterized by behavioural and physiological quiescence (Jouvet, 1967) [2]. During NREMS body temperature, heart rate, respiratory rate, and metabolic rate decrease as compared with the waking state (Buchsbaum *et al*., 1989) [3]. There is also an accompanying decrease in resting muscle tone and spontaneous motor activity (Gardner and Grossman, 1967) [2]. The electroencephalogram (EEG) of NREMS shows high voltage, low frequency synchronized activity (Jouvet, 1967) [2]. Hence, NREMS is also called synchronized sleep.
On the other hand, REMS is a seemingly paradoxical state in which behavioural and physiological activities increase in some parts of the body and decrease in others. In contrast to EEG of NREMS, during REMS the EEG shows low voltage, high frequency activity similar to that which occurs during wakefulness (Jouvet, 1967) [2]. Body temperature, metabolic rate, blood flow and CNS neuronal firing show an increase (Tononi and Cirelli, 2006) [5]. Breathing movements become irregular, shallow and rapid. The rectus muscles of the eyes exhibit increased resting tone and phasic bursts of rapid eye movements occur (Jouvet, 1967) [2].
In contrast, during REMS many peripheral structures show decreased activity. REMS is accompanied by atonia, accomplished through the inhibition of motor neurons. At the onset of REM sleep, motor neurons throughout the body undergo hyperpolarization i.e. their already-negative membrane potential decreases by another 2–10 millivolts. This raises the threshold which a stimulus must overcome to excite them. Therefore, during REMS, though skeletal muscles exhibit twitches in conjunction with the phasic events, they are paralyzed most of the time, and spinal reflexes are abolished (Lai and Siegel, 1999) [6]. The activities of peripheral thermoregulatory mechanisms, such as shivering, panting and sweating are largely suspended during REM sleep (Parmeggiani, 1980) [7]. Due to its characteristic physiological parameters REMS is also termed paradoxical sleep or desynchronized sleep.

## REMS Deprivation
To investigate the functions of REMS a positive or negative approach may be employed. A positive approach aims at generating a 'REM sleep like state' by electrical or chemical stimulation and observing the physiological and behavioural changes that accompany it.

Studies on REMS that employ surgical and biochemical techniques are invasive and introduce too many confounding variables that may yield erroneous results (Vogel, 1975; Gulyani *et al*., 2000) [8, 9].

A negative approach that employs sleep deprivation protocols helps to assess the impact of inadequate sleep on health and behaviour. These deprivation studies are aimed at understanding the functional role of sleep per se or specific phases of sleep. In nature everyone experiences sleep deprivation to a lesser or greater extent. Therefore, these experimental deprivation studies are as natural as possible in their approach (Gulyani *et al*., 2000) [9]. This review deals with the various REMS deprivation methods employed and the advantages and disadvantages of each.

1. **Gentle Handling Technique**: The first study on REMS deprivation was carried out by Dement (1960) [10] on human volunteers and involved arousal of the volunteers each time they progressed into REMS, monitored by electrophysiological parameters. In case of experimental animals, the animals are aroused by external stimulation at the onset of each REM period. The control animals, on the other hand, are woken up an equivalent number of times but from NREMS. This technique has been used for rats, mice, rabbits, cats etc. Animals are aroused by acoustic signals, e.g. tapping the cage or tactile signals e.g. gentle prodding with a soft brush or hand.

Though this technique is the least difficult to control in terms of confounding variables, it is not used extensively. First, it requires constant monitoring of electrophysiological recording. Secondly, in animals the number of arousals increases dramatically within two to three days, making the entire exercise practically impossible (Vogel, 1975) [8]. Beyond 24 hours, the intensity and frequency of arousals increase significantly and the experiment becomes highly labour intensive.

The major confounding variable in this study is stress. Rodents have an innate defensive nature due to which mild stroking or cage shaking are perceived as stressors (Longordo *et al*., 2011) [11]. Animals that undergo repeated handling show changes in their locomotor activity and have higher levels of circulating corticosterone as compared to undisturbed control animals [Longordo *et al*., 2011, Meerlo *et al*., 2008] [11]. Kopp *et al*., (2006) compared the effect of two forms of sleep deprivation in mice i.e. mild stimulation by tapping to direct handling at 30 min intervals. They found that corticosterone levels in the mild stimulation group were comparable to those of control animals, but were three times higher in directly handled animals.

Therefore, it is difficult to decide whether activation of stress systems during sleep deprivation is a result of sleep loss per se or is a consequence of the stressful nature of the sleep deprivation technique used in the study (Meerlo *et al*., 2008) [12].

2. **Flowerpot Method/Platform Technique**: One of the most frequently used methods; this was developed by Jouvet in 1964 [14] for cats. Cohen and Dement (1965) [15] adapted this technique to rats. This method involves placing the animals on a small platform, say an inverted flowerpot, surrounded by water in a bucket. In theory, the small size of the platform permits wakefulness and NREMS but not REMS because the muscle atonia that accompanies REMS causes the animal to fall in water and be woken up whenever a REM period is initiated. Ideally, the control animals are placed in similar conditions but on larger platforms that allow the animal to curl up and permit all stages of the sleep wake cycle. Other controls used are caged animals, where the size of the cage is similar to the small platform, restricting the movement of the animal but permitting REM sleep.

This simple technique offers many advantages over the gentle handling method. No implantation of electrodes or polygraphic recording is needed nor does the experimenter have to monitor the EEG of the animal throughout the day. Also, stress caused due to handling of animals is avoided. Moreover, several animals may be REMS deprived simultaneously for several days [Vogel, 1975; Landis, 2004] [8, 12]. Studies show that the degree of REMS deprivation in these animals varies considerably probably as a result of differences in size of the animals with respect to the small platform size [Mendelson *et al*., 1974; Hicks *et al*., 1977] [17, 18]. The control animals, placed on larger platforms are also REMS deprived as much as experimental animals [Stern, 1970] [19] or at least to a certain degree (Machado *et al*., 2004) [20]. During the first 24 hours both experimental and control animals experience sleep deprivation. As the duration of the experiment progresses rats on small platforms are significantly more REMS deprived than their control counterparts (Vogel, 1975; Landis, 1996] [8, 21]. In addition to REMS deprivation, the flowerpot method results in NREMS deprivation also (Mendelson *et al*., 1974; Grahnstedt and Ursin 1985; Kovalzon and Tsibulsky 1984) [17, 22, 23].

A major objection to this technique is that the animal is subjected to many adverse factors such as stress, dampness, confinement, social isolation etc. which could induce many of the effects observed after REMSD (Machado *et al*., 2004) [20]. Large platform controls are useful to rule out the effect of humid conditions. To do away with the adverse water exposure, a few studies employ coldwater stress controls. The control animals are immersed in water twice daily for 1 hour sessions (Mendelson *et al*., 1974; Hartmann and Stern, 1972) [17, 24]. However, as the duration of the experiment increases the animals learns to wake up at the onset of REM and rarely fall into the water (Landis, 2004) [16]. By the fourth day the animal learns to stretch and fall asleep on the small platform. Polygraphic recordings at this stage reveal brief REM episodes (Landis, 2004) [16].

Animals on both large and small platforms showed similar weight loss and symptoms of stress (Vogel, 1975) [8]. The stress induced can be assessed by a decrease in weight of experimental animals by 5- 10%, although food and water are available *ad libitum.* Also, the animals show adrenal hypertrophy and thymus atrophy.

After the period of sleep deprivation, when rats are returned to their cages, they do not immediately fall asleep. Instead the animals show a period of heightened activity, irritability, hypersexuality, aggressiveness etc. which lasts for almost half an hour (Gessa *et al*., 1995; Van Hulzen *et al*., 1981] [26, 27]. This is probably a result of the stress load on the animal caused by many adverse factors including REMS deprivation (Revel *et al*., 2009) [28].

3. **Multiple Platform Method and Modified Multiple Platform Method:** A major drawback of the classical single platform method is immobilization of the animals. Immobilization is known to induce stress in animals and

elevate their circulating corticosterone levels (Dominique *et al*., 1998; Pavlides *et al*., 1993) [29, 30]. To attenuate the immobilizing effect of single platform method Van Hulzon and Coenen (1981) [38] developed the multiple platform method. In this method a large tank with several small platforms is used. This allows the rat to ambulate, thus, preventing confinement on a small platform and the stress resulting from it. Nunes and Tufik (1994) [31] suggested the use of multiple animals on multiple platforms within a large tank to prevent both social isolation and confinement. In this technique multiple animals were sleep deprived on multiple platforms within the same tank. However, animals from different social groups can find it hard to acclimatize within the same tank. Hence, Sucheki and Tufik (2000) [32] proposed the modified multiple platform method (MMPM) which uses rats from a socially stable group in one tank, avoiding stress due to unfamiliarity. Following sleep deprivation, socially stable groups have lower circulating corticosterone levels *viz* a socially unstable groups. The control group is also a socially stable group of animals from one cage, placed in a large tank with environmental conditions but on larger platforms that permit the animal to curl up and theoretically experience all stages of sleep wake cycle. Thus, the MMPM method helps to control two major stressors, namely, immobility and social isolation (Suchecki and Tufik, 2000) [32].

Studies have shown that control animals placed on larger platforms are not an ideal control for REMS deprivation studies (Suchecki *et al*., 1998) [34]. Instead of using larger platforms Suchecki and Tufik (2000) [32] suggest the use of stainless steel grids on the tank floor. The animals may lie down on the grid and sleep without falling into water though their tails may dip into the water. Machado (2004) [20] compared sleep deprivation by both MMPM and classical single platform method. While REM sleep was abolished in both groups over a 96 hour period, there was a concomitant reduction in NREMS too. Also, the control animals, both multiple large platforms and grid, experienced REM deprivation.

4. **Rotation Disk Method/Disk over Water Method:** This method makes use of a horizontal disk placed in a shallow pool of water (Rechtschaffen *et al*., 1983; Rechtschaffen and Bergmann, 1995) [35-36]. Both the experimental animal and the control animal (yoked control) are placed on a divided horizontal disk suspended in a pool of water. The brain activity of the experimental animal is recorded. Whenever the animal begins to fall asleep or a targeted stage of sleep is initiated, the disk begins to rotate automatically within 5 seconds, at a speed of 3.33 revolutions/ minute. The animal has to move in a direction opposite to the movement of the disk or it would be carried into water. As the control animal is housed on the same disk it receives the same mild stimulation as the experimental animal. In this set up, the experimental animal tends to lose sleep per se or a particular stage of sleep. The control animal, on the other hand, can sleep *ad lib* whenever the experimental animal is awake or the disk is still. Thus, control animals experience physiological effects in the same direction as the experimental animals but the intensity of the effects is much less.

Thus, both experimental and yoked control animals are subjected to the same level of activity but the controls are able to rest more than their counterparts (Guzmán-Marín *et al*., 2003) [37]. The rotating disk method and other methods of forced locomotion such as the pendulum method or treadmill method induce a new variable i.e. physical activity, leading to fatigue and confounding stress (Guzmán-Marín *et al*., 2003) [37].

5. **Pendulum or Swing Technique** Van Hulzen and Coenen (1980) [38] developed a new technique for REMS deprivation. They used a swing with room for three rats in separate cages. The cages are moved backwards and forwards like a pendulum. On either side, at the extreme, (42-degree angle to the horizontal plane) the animal is forced to move downwards to the other side of the cage due to postural imbalance. A moving pendulum which does not cause postural imbalance serves as the control. The speed of the pendulum can be varied to increase arousal. During the deprivation period, lasting 72 hours, the group recorded minimal REMS (0-2%) and moderate amount of NREMS (19-30%). During the recovery period a significant increase in REMS was observed.

This technique avoids exposure of the animals to water and a humid environment (Landis, 2004) [16]. No significant difference in amount of REMS deprivation by this method to REMS deprivation by classical single platform and multiple platform methods has been observed (Van Luijtelaar and Coenen, 1986) [39].

6. **Treadmill Arousal technique**: Treadmills, due to their constant movement, can also be used for REMS deprivation. Ferguson and Dement (1967) [40] used a combination of treadmill and arousal technique. In this technique, for 16-22 hours, the experimental animal is placed on a treadmill moving slowly (2m/ min for cats) with a water filled container at the end. When the treadmill belt reaches the other end the animals have to wake up to avoid falling into water. For the remaining 2-8 hours the animals are placed in cages, monitored electro physiologically and gently aroused at the onset of REM sleep. The control animals are placed on the treadmill for the same number of hours as experimental animals and, therefore, experience similar physical activity and stress. When not on treadmill, the control animals are aroused the same number of times as the experimental animals, but from slow wave sleep (Vogel, 1975; Morgane *et al*., 2013) [8].

With time animals learn to run to the other side of the treadmill and sleep (15-20 seconds) till it reaches the other end. Studies suggest that it is possible for animals to have almost 40% of the time spent on treadmill in non-REM sleep (Ferguson and Dement 1967) [40]. The control animals can catch up on REM sleep during the 8 hour non treadmill period.

Guzmán-Marín *et al*., (2007) [37] used a variation of the treadmill method. The experimental rat is placed in a cage such that the vinyl belt of the treadmill forms the floor of the cage. The animal is monitored electro physiologically. At the onset of each REM episode the treadmill is automatically activated (speed 10 cm/second) for 5 seconds, forcing the animal to walk to avoid being carried into the wall of the

chamber. A yoked control rat is placed in the same treadmill and experiences simultaneous movement regardless of its stage of the sleep-wake cycle.

This method does not require constant monitoring by the observer, thereby, making longer REMS deprivation studies easier. Moreover, it is possible to vary the speed of the treadmill to suit the experimental design. However, treadmills cause not just REMS but also total sleep deprivation. They can be best used to study the interplay of substantial sleep deprivation and/ with REMS deprivation (Vogel, 1975) [8].

7. **Head Lifting Method:** Datta *et al*., (2004) [43] introduced the head lift method for REMS deprivation. As in the gentle arousal method, the polygraphic recording of the experimental animal is monitored. The observer, sitting in another room, presses a spring based mechanical lever at the onset of each REMS episode. A system of pulleys and light weight flexible wire is so designed that the downward pressure on the lever causes the rat's head to lift up gently by upto two inches. The observer can thus, terminate a REMS episode within 3-5 seconds of its initiation. Moreover, the experimental animal does not come into direct contact with the observer and extraneous stress is avoided (Colavito *et al*., 2013) [44]. In the 6 hour deprivation done during this study, REMS was reduced by 90- 95%, where as there was no significant reduction in slow wave sleep. Studies of longer duration REMS deprivation, say 48 hours, by this method have, however, not been conducted.

8. **Laboratory Shaker Method for Neonates:** REMS in neonates is many times more than that in adults, suggesting that REMS has developmental functions (Roffwarg *et al*., 1966; Marks *et al*., 1995) [45, 46]. Mirmiran *et al*., (1981) [47] studied the effects of pharmacological REMSD of neonates but the use of drugs for REMS deprivation brings several confounding variables into the picture (Feng *et al*., 2000) [48]. Designing an instrumental REMS deprivation study for neonate rodents is especially challenging because of their small size. Feng *et al*., (2000) [48] introduced the following method for neonatal rats. A small plexiglass chamber is fixed onto the horizontal surface of a general laboratory test tube rack shaker. A vertical wall divides the chamber into two parts- one for housing the experimental neonate and the other for the yoked control neonate. The rats are monitored polygraphically. At the onset of each REM episode in the experimental animal a computer turns on the shaker for five seconds (average speed 250 oscillations/minute) terminating the REMS episode by producing a transition to either wake or slow wave sleep.

In this method the experimental animals are aroused only at REM sleep onsets and are selectively deprived of REM sleep. On the other hand, yoked control rats may be in any stage of the sleep wake cycle when they are aroused. An additional advantage is that it is possible to manually control the shaker's speed. Interestingly, increasing the shaker's oscillation speed increases the amount of REMS deprivation and results in a smaller loss of total sleep than at slower shaking speeds. Other methods of REMS deprivation used for adult rats e.g. Pendulum method, have not been successful with neonates because they resulted in increased total sleep deprivation, confounding the effects of REMS deprivation with that of total sleep deprivation (Feng *et al*., 2000) [48]. The method may be suitably used for large, continuous REMS deprivation of neonatal rats for several days. Whether the shaker technique can be suitably employed for adult rats needs to be evaluated.

9. **Electrical Stimulation:** Kovalzon and Tsibulski (1984) [23] used electrical stimulation of midbrain reticular formation at the onset of each REMS episode for arousal. The arousal stimulus 0.1msec, 100Hz, amplitude 2-12 V, duration 0.5-2 sec was activated when the EEG showed cortical desynchronization, a hippocampal theta rhythm and declining EMG. The intensity of the stimulus was increased if the animals were not aroused from sleep. The animals typically obtained 3-8 sec of REMS prior to arousal.

**Discussion**

Why are sleep deprivation studies so relevant? Owing to changes in lifestyle, a large part of the human population suffers from inadequate sleep, fragmented sleep or state specific disorders. It is, therefore, absolutely essential to assess the impact of total and state specific sleep deprivation on human physiology and behaviour. Secondly, although almost 60 years have passed since its discovery, we are still intrigued by the occurrence and role of REM sleep. To understand the function of REM sleep and why it persists in nature, researchers rely mainly on deprivation studies in animals.

Deprivation studies can be broadly divided into two categories (Landis, 2004) [16]. The first category makes use of arousal at the onset of REM sleep episode e.g. platform method or gentle handling etc. These are confounded by various stressors such as dampness, water exposure, stress due to handling etc. The second category involves forced locomotion techniques e.g. treadmill, rotation disk, pendulum method etc. These methods are confounded by variables such as stress due to physical activity. Sleep deprivation is definitely stressful but the symptoms observed due to sleep deprivation are specifically due to the stress of being awake or due to the process of deprivation is not known (Meerlo, 2002) [51]. The effect of confounding variables can be greatly attenuated by employing yoked controls (Rechtschaffen and Bergmann, 1995) [36]. All sleep deprivation studies aim at eliminating these confounding variables such that the observed outcome can be attributed strictly to sleep deprivation. This may be unrealistic, considering the inevitable interaction between sleep loss and stress response activation (Toth, 1995) [50].

**Table 1:** Salient features of different REM sleep deprivation methods

| Method | Features |
|---|---|
| Gentle Handling Technique (Dement, 1960) [10] | 1. Easy to control,<br>2. Suitable for REMS deprivation of human volunteers. |
| Flowerpot Method /Platform Technique (Jouvet *et al*., 1964; Cohen and Dement, 1965) [14, 15] | 1. Electrodes or polygraphic recording not needed.<br>2. Stress due to handling is avoided. |

| | |
|---|---|
| | 3. Suitable for long term studies. |
| Multiple Platform Method (Van Hulzen and Coenen, 1981) [27] | 1. Prevents stress due to social isolation and immobility.<br>2. Suitable for long term studies. |
| Modified Multiple Platform Method (Schecki and Tufik, 2000) [32] | Avoids stress due to<br>1. Social isolation and<br>2. Unfamiliarity. |
| Rotating Disk Method/ Disk over Water Method (Rechtschaffen *et al*., 1983; Rechtschaffen, and Bergmann, 1995) [35-36] | 1. Experimental and control animals experience the same level of activity, water immersion and stress. |
| Pendulum or Swing method (Van Hulzen and Coenen, 1986) | Avoids stress due to<br>1. Exposure of animals to water and<br>2. Humid environment. |
| Treadmill Arousal Technique (Ferguson and Dement, 1967) [40] | 1. Does not require constant monitoring by the observer.<br>2. Suitable for longer REMS deprivation studies.<br>3. Speed of the treadmill can be varied to suit the experimental design |
| Head Lifting Method (Datta *et al*., 2004) [43] | 1. No direct contact between the experimental animal does not and observer.<br>2. Extraneous stress is avoided |
| Laboratory Shaker Method for Neonates (Feng *et al*., 2004) [48] | 1. Selective REMS deprivation<br>2. Speed of the shaker can be controlled to achieve desired deprivation. |
| Electrical Stimulation (Kovalzon and Tsibulsky, 1984) [23] | 1. No contact between experimenter and animal. |

No investigations in animals have been able to achieve total REMS deprivation (Landis, 2004) [16]. As the duration of deprivation increases, progressively more frequent small periods of micro sleep, lasting as little as a few seconds, may be observed probably due to the accumulation of sleep debt (Durmer and Dinges 2005; Tononi and Cirelli, 2006; Ocampo-Garcés *et al*., 2006) [52, 53, 54, 5]. Also, REMS deprivation is always accompanied by some loss of NREMS, though the extent of NREMS deprivation varies across studies (Mendelson *et al*., 1974; Grahnstedt and Ursin 1985; Kushida *et al*., 1989; Landis *et al*., 1992) [17, 22, 56]. Even though the experiment is carefully planned no sleep deprivation can ever be absolute. Most methods of REMSD are not specific and lack adequate controls (Landis, 2004) [16]. Thus, threats to validity and generalization of the study are always present. Moreover, REM sleep deprivation studies may have other side effects like blood effects on brain function (Gomez-Gonzalez *et al*., 2013) [57], cause oxidative stress (Villafuerte *et al*., 2015) [58] and impair muscle regeneration (Mônico-Neto *et al*., 2017) [33] in animal models.

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