REVIEW ARTICLE

Rapid Eye Movement Sleep and Significance of its Deprivation Studies - A Review

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Rapid eye movement (REM) sleep is a unique phenomenon within sleep-wakefulness cycle. It is associated with increased activity in certain group of neurons and decreased activity in certain other group of neurons and dreaming. It is likely to have evolved about 140 million years ago. Although mention of this stage can be traced back to as early as 11 century BC in the Hindu Vedic literature, the Upanishads, it has been defined in its present form in the mid-twentieth century. So far, neurobiology of its genesis, physiology and functional significance are not known satisfactorily and mostly remains hypothetical. Nevertheless, more and more studies have increasingly convinced us to accept that it is an important physiological phenomenon which cannot be ignored as a vestigial phenomenon. Although there are articles where different aspects of REM sleep have been dealt with, a review where the knowledge gathered by REM sleep deprivation studies to understand its significance is lacking. There is a need for such a review because a major portion of the knowledge about various aspects of REM sleep, specially its functional significance, has been acquired mostly from the REM sleep deprivation studies. Hence, in this review the knowledge gathered by REM sleep deprivation studies have been colated along with their importance so that it may be useful and referred to for information as well as while designing future studies. (Sleep and Hypnosis 2000;2:49-68)

Key words: deprivation, flower pot, REM sleep

INTRODUCTION

Sleep-wakefulness, a behavioral phenomenon, is a modified form of basic rest-activity cycle occurring in the lower species. Sleep is a reversible behavioral state of disengagement from, and unresponsiveness to, the surrounding environment. The threshold for most of the responses, including arousal, is higher during sleep. Since ancient times sleep was considered to be a passive phenomenon i.e. after a period of awakefulness one falls asleep because one cannot keep oneself awake. It was also viewed as a monolithic and homogenous state, however, further studies have established that sleep is an active and a non-monolithic phenomenon (1). The precise role of sleep and its mechanism of action are yet to be fully understood. Nevertheless, its importance in maintaning normal physiological processes is beyond doubt. It is true in principle that for a good sleep, both quality as well as quantity must be present to lead a normal life. However, requirement of a minimum and maximum amount of sleep necessary to maintain a healthy normal life is yet to be conclusively proved.

Since, sleep is a behavioral phenomenon, detail understanding on this subject could not be extended till an objective and unbiased physiological criteria exclusively identifying the state could be identified. The objective criteria were also necessary to understand the depth and variations in sleep.These were only possible once the state specific electrophysiological parameters could be identified, defined and recorded. Subsequently, its unbiased classification and quantification could be done with the help of the following electrophysiological parameters viz. electrical activity of the brain recorded from the scalp, the electroencephalogram (EEG); eye movements as reflected in the electroocculogram (EOG); muscle tone as reflec-

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ted in the antigravity muscles (usually recorded from the neck muscles) the electromyogram (EMG). In a broader sense none of the above mentioned characteristic electrophysiological signals are present or absent either during sleep or wakefulness in an all-or-none manner. Here, we are not including rapid eye movement (REM) sleep stage during which, as we shall see later, some of the signals (other than those mentioned above) are present apparently exclusively. The relative frequency and voltage of the electrophysiological signals mentioned above alter during different behavioral states including sleep and wakefulness. Although the changes in frequency and voltage of the signals exist across species, the absolute values may differ among species. Based on the electrophysiological signals, the sleep and wakefulness states could be objectively classified and therefore, could also be quantified for evaluation. However, a closer look through the electrophysiological parameters subdivided both the sleep and the wakefulness states further. In addition, another state viz. REM sleep was identified, which is unique in the sense that some of the signals viz. lateral geniculo-ponto-occipital waves (LGN/PGO) and muscle tone are exclusively present and absent, respectively, in this state compared to other states. Thus, based on the electrophysiological parameters the sleep-wakefulness states have been classified into wakefulness, non-REM sleep and REM sleep and the former two being further subdivided into two stages each as mentioned below.

I) Active wakefulness (AW) - This stage corresponds to attentive and/or psychomotor active waking state and is characterized by the presence of low voltage (20-50 V) and high frequency (30-50 Hz) desynchronized waves in the EEG (2,3). The EOG shows frequent and irregular pattern of eye movements, whereas EMG shows high muscle activity.

II) Quiet wakefulness (QW) - Quiet wakefulness is representative of non-attentive waking or a stage of non-motivated motor activities. This stage is characterized by the presence of desynchronized EEG along with occassional (<20% of the recording time) spindles in the EEG. The EOG shows fewer or no eye movement, whereas the EMG shows reduced muscle tone than AW.

III) Slow wave sleep (SWS) - This first stage of sleep is characterized by the presence of low frequency (6-18 Hz) synchronized waves in the EEG with a progressive increase in amplitude (50-300 V) for 25-50% of the recorded time. Muscle tone is further decreased as compared to that of quiet awake period and there is fewer or no sign of eye movement in the EOG.

IV) Deep sleep (DS) - In this stage the EEG is synchronized which is characterized by the presence of low frequency (6-18 Hz) and high amplitude (50-300 V) waves in the EEG for most of the recording period or for at least more than 50% of the recorded time. There is near absence of eye movement and the muscle tone is very low.

V) Rapid eye movement (REM) sleep - During REM sleep some of the electrophysiological signs of the animal/subject resemble that of the awakefulness state although the subject remains in deep sleep state to the extent that the threshold for arousal is maximum during this state. The EEG is apparently desynchronized and eye movements are present (both signs of awakefulness) although the EMG shows atonia in the antigravity muscles. Added to that, other marked electro-physiological signs associated with this phase are presence of characteristic field potentials in the pons and lateral geniculate nucleus, the LGN/PGO waves and theta rhythm recorded from the hippocampus. Thus, based on these characterestic features it is a paradoxical state within sleep. Hence, in addition to the term REM sleep, this stage is also known as "paradoxical sleep" or "desynchronized sleep" or "active sleep" or "dream sleep" since dreams are associated with this stage of sleep. Although formally it has been identified and defined by the classical electrophysiological signs and symptoms in the mid-twentieth century (4-6), knowledge about this state can be traced back in the ancient Hindu Vedic literature, the Upanishad (7). In addition to the signs and symptoms mentioned above other characteristic features of REM sleep are occassional myoclonic twitches (most apparent in facial and distal limb musculature), decreased sympathetic tone, pronounced cardiovascular and respiratory fluctuations (8,9), changes in brain metabolism (10,11) increase in brain blood flow (12-16), rise in arterial pCO2 (17), increase in brain oxygen consumption (18) and brain temperature (19,20). Some of these events are tonic, while the others are phasic in nature.

REM SLEEP ACROSS SPECIES

A large amount of REM sleep is found in foetus and newborn of most mammals. The newborn humans spend nearly half of sleep time in REM sleep (21). The REM sleep at this stage lacks characteristic cortical EEG desynchronization and muscle atonia but is identified by the presence of muscle twitches and eye movements. Nevertheless, there are differences between mammals depending on how well developed they are at birth. Mammals born precocious (antelope, goats, cattle, etc) i.e. with their brain and physical abilities in

fairly advanced state, have relatively lesser REM sleep at birth (22). Contrast is with kitten and rat pup, born blind and helpless, where REM sleep makes up nearly 100% of sleep at birth, but falls quite rapidly to 30-40% within a month. Normal newborns (animals or humans) spend one third of their day time sleep and one half of their night time sleep in REM sleep. This high ratio diminishes progressively with maturation. By virtue of this pattern REM sleep can be considered as ontogenetically primitive sleep. However, this may not be uniformly true because the birds which are born immature have less REM sleep while the echidna, which is immature at birth, does not exhibit all the signs of REM sleep. It has been suggested that REM sleep and non-REM sleep might have evolved as a differentiation of a primitive state (23). Although REM sleep appears to be present in all marsupials and terrestrial placental mammals, it is believed to be absent in echidna, a monotreme (egg laying mammal). Thus, REM sleep appears to have evolved some 140 million years ago as a common ancestor of marsupial and placental mammals (24,25). However, recently it has been shown that REM sleep is present in platypus, although all the signs may not be expressed as those in other higher species (26). This suggests that REM evolved much earlier. The REM sleep, a cyclic phenomenon, is a function of sleep cycle length, increases with size of the brain and is negatively correlated with the basal metabolic rate. The average length of sleep cycle in humans, starting from non-REM sleep is approximately 90 min. The length of non-REM sleep is longer during the initial sleep stage which reduces with progress of sleep. The duration of REM sleep is inversely related to that of the non-REM sleep. A 45-85 min of non-REM sleep (progressing from longer to shorter duration) is interrupted by 5-65 min duration of REM sleep throughout the night in humans.

REM SLEEP ACROSS AGE

Although the REM sleep is identified by a number of signs, they do not develop simultaneously during the developmental course of the brain. By three months postterm age all parameters of REM sleep appear selectively in REM sleep (27). As NREM sleep and wakefulness emerge with maturation, REM sleep time is reduced. The human neonate sleeps for 16 to 17 hours per day, after which sleep gradually decreases with age. At 8 months of age only 33% of total sleep time (13-14 hours) is spent in REM sleep. The value declines to 20% to 25% in the adult and to less than 15% in late adulthood. It occupies 90% in thi kittens of the time during first 5 days of life and even more in the infant rat (28).

NEED FOR STUDY OF REM SLEEP

It has been mentioned above that REM sleep is an instinct phenomenon present across age and species. There is a compulsion within the physiological system to recover the lost REM sleep and if adequate recovery is not allowed, death may follow after prolonged deprivation. It is also reported that REM sleep is affected in several diseased conditions. These indicate that nature regards REM sleep absolutely necessary to maintain normal physiology and life processes. Nevertheless, even after about half a century of its discovery it continues to be a challenge to the sleep-wakefulness researchers. Evidence so far suggests that although the precise mechanism and significance of REM sleep are not known, it is one of the very important physiological processes (and not a vestigeal phenomenon) and hence cannot be ignored. Unless its basic mechanisms of generation and functions are known, causes and results of its disturbance would not be known. Several types of studies including stimulation, lesion, chemical injection as well as deprivation have been conducted to investigate and understand its mechanism of generation, functions and mechanism of action. However, unlike most others, the REM sleep deprivation method is non-invasive, involves the least, if at all, external treatment and also simulates near natural situation in the sense that often one experiences certain amount of REM sleep loss during one's normal day to day life.

FOCUS OF THIS REVIEW

Several reviews, articles and monographs are available where neurophysiology of REM sleep and its regulation by different brain structures (29-33), functions of sleep including REM sleep (22), molecular biology of circadian clock and sleep (34), involvement of neurotransmitters and neurochemicals in relation to REM sleep (35-42), REM sleep deprivation methods (43-44) and other related knowledge (45-49) have been dealt with. Earlier studies have established that REM sleep is generated within the brain stem and particularly in the pons. An interaction between the norepinephrinergic and cholinergic systems in the generation and modulation of this state has been proposed. However, recent studies also suggest that GABA is likely to play an important role, in addition to those classical neurotransmitters, in the genesis of REM sleep (50-53). At the cellular level it has been shown that there are specific groups of neurons in the brain stem which are active during REM sleep, the REM-ON cells (54,55), while another group of neurons which are inactive/silent during REM sleep, the REM-OFF cells (56,57), and their interaction is important for REM

sleep regulation (58). Nevertheless, our overall knowledge and understanding about REM sleep generation and function continue to be far from satisfactory. So far most of the data about its physiologial significance and functions have been gathered primarily by investigating the effects of REM sleep deprivation on different physiological parameters viz. gross and cellular behavior, brain biochemistry and so on. In fact, the deprivation method has been extensively and maximally used for the purpose. As mentioned above although several reviews are available, there has not been a serious attempt to highlight the importance and information gathered by the deprivation studies which has contributed probably maximally to understand the overall knowledge about REM sleep. Therefore, the primary focus of this review is to colate the knowledge gathered by REM sleep deprivation studies, specially in relation to functions and changes in the brain, with an aim to appreciate the significance of deprivation studies in understanding various aspects of REM sleep. The importance and relevance of such a review is increasingly felt to realise the role played by the deprivation studies in understanding REM sleep and to eliminate the lacunae as well as limitations of earlier studies while further designing future experiments to reveal the significance of REM sleep. However, before going into specific studies the methodologies involved for REM sleep deprivation along with their advantages and disadvantages will be discussed for a better and meaningful understanding.

METHODS USED TO STUDY REM SLEEP

Ever since the discovery of REM sleep attempts have been made to investigate its physiological significance, mechanism of generation and maintenance by using transection, stimulation (electrical and chemical), lesion and deprivation techniques/studies. Each of the methods has its own merits and demerits. The transection, lesion and stimulation approaches helped in elucidating the gross brain structures responsible for REM sleep. However, in such studies although the role of the brain area under study in either the tonic or the phasic events could be investigated; the effects of recovery could not be studied; the direct and/or the indirect effects, if any, could not be dissociated; and the associated effects could not be prevented. The deprivation experiments, a powerful strategy to study its functions (59), on the other hand, takes care of most of those disadvantages to a reasonable extent and probably therefore, has contributed significantly in elucidating and understanding the functional significance of REM sleep.

Since REM sleep episodes are short lasting and their effects are likely to be cummulative, it is important to amplify the effects on the parameters under study. Accordingly, two approaches viz. either by increasing or by decreasing the amount of REM sleep, positive and negative effects, respectively, if we may so term them, may be used. In the first one, REM sleep can be induced and during this condition its functions can be studied. However, it would suffer from several limitations. As it is known that different components (signs) of REM sleep are generated by different brain areas or groups of neurons, it is very difficult to stimulate (chemically or electrically) simultaneously and precisely all those areas to generate all the signs expressed during spontaneous REM sleep. Only the REM sleep like state with one or more of its major components (signs or symptoms) can be made to express for varying duration. It is also true that normally REM sleep follows deep sleep. However, during induced REM sleep although signs of REM sleep may be expressed, the timing and sequence of its occurrence (in relation to sleep-wakefulness paradigm) is lacking. It is probably difficult to term that as a naturally occuring REM sleep phenomenon and hence it has often been termed as "REM sleep like state". Moreover, chemicals have to be injected either systemically or locally into the brain. On induction of REM sleep like state by a chemical it is almost impossible to know whether the observed effects were due to REM sleep or an effect of the chemical used per se and the drawback of local injections is that it is an invasive technique. Similarly, there are limitations with the electrical stimulation studies also. First, it is generally an invasive technique. The insertion of the electrode during implantation is likely to cause a mechanical damage to the neurons and fibres resulting in secondary effect; second, it is difficult to know the strength of the stimulating current which may be considered normal/natural; third, the extent of stimulating current cannot be localized precisely; fourth, accommodation of the stimulation cannot be taken care of, if at all; and fifth, there are not many reports of induction and reduction of REM sleep by electrical stimulation of the brain (60,61). Added to these it may be argued that in nature, the duration and frequency of any physiological phenomenon has evolved to an optimal level after a great deal of trial (and error) which would be ideal and best suited for that particular system. An increase in the phenomenon, REM sleep in this case, may not necessarily precipitate the disturbance to an extent similar to that of withdrawing the phenomenon. This is because the intensity of the increase in the parameter under study may not be significant beyond the optimum level which already exists. Hence, experimental study by increasing the REM sleep may not be a very effective approach.

On the other hand, the deprivation or the so called negative approach has the advantage of overcoming the draw backs of the so called positive approach by increase in REM sleep (mentioned above) and help understanding the functions of REM sleep. The major advantages of this method of study are that it is a non-invasive technique; the parameters under study, if related to REM sleep, are likely to be amplified proportional to the period of deprivation (which quantitatively being a small change could have been missed otherwise in normal situation); and most important is that REM sleep loss (to different degree) is experienced by almost everybody and thus, probably can be considered a near natural phenomenon in terms of applicability of the knowledge for the ultimate goal, human welfare. However, this method also has its own limitations. For instance, it is difficult to quantify the amount of stress (if at all), the small loss of slow wave sleep during REM sleep deprivation and to determine the optimum period of REM sleep deprivation required to obtain the significant effects. Nevertheless, these limitations are overcome to a great extent by the use of appropriate and effective control (yoke) experiments. The effect of possible stress factor is most often the major criticism for the REM sleep deprivation studies and one wonders if induction of REM sleep could be an effective alternative to it. A counter argument to that can be that since in the nature any phenomenon including its duration has evolved to benefit as per the requirement of the living being/organism, increasing the duration of that phenomenon by external means, REM sleep for instance in this case, may also be stressful. The quality and the intensity of stress in cases of deprivation and induction of REM sleep may, however, differ and may be argued. Nevertheless, since we have not come across any study where effects have been studied with increase in REM sleep, and since this review concentrates on REM sleep deprivation studies, the latter will be discussed here. However, since various techniques have been used for REM sleep deprivation, their merits and limitations will be discussed first for convenience and ease of understanding.

METHODS USED FOR REM SLEEP DEPRIVATION

I) Hand arousal technique:

Dement (62) first applied the hand arousal technique in humans for REM sleep deprivation. Electrophysiological parameters signifying sleep-wakefulness were constantly monitored from human subjects and as soon as rapid eye movements as well as an "activated" EEG pattern appeared (characteristics of REM sleep), the subjects were awakened. This technique was subsequently applied on animals also. Experimental subjects/animals were aroused by external stimulation at the onset of each REM sleep episode. Immediately after awakening the experimental subjects were allowed to resume sleep. Nonspecific concomitants of this technique were reasonably controlled by "yoked" control animals which were aroused concurrently with the experimental animals. The limitations and disadvantages of this technique are, first, it needed constant monitoring and second, that although initially this technique could effectively prevent the animals from entering into or continue REM sleep, the frequency of awakening (vis-a-vis going into REM sleep by the animals) increases rapidly with increase in length of deprivation making it extremely difficult to continue deprivation for more than a few hours.

II) Flower-pot method:

A popular and rather effective technique for long term REM sleep deprivation is the Flower-pot technique. This method is also known as water tank, platform, or pedestal technique. It was first used in cats (63) and has since been extensively used on cats, rats and mice for REM sleep deprivation (43,44). In this method animals are maintained on small raised platforms, typically inverted flower pots, surrounded by water. On this artificial island the animals can sit, crouch and move around freely. However, the animals cannot have enough room for complete relaxation. As soon as the animals enter into REM sleep, due to atonia in the postural muscles, they cannot stay on the small platform and fall into the surrounding water. Consequent to coming in physical contact with the surrounding water the animals wake up. Thus, although the animals can enjoy sleep, occurrence of REM sleep is prevented. In this method the animals quickly learn to wake up at the onset of REM sleep and hence do not frequently fall into the water. Thus, in this method the animals can have non-REM sleep but not the REM sleep. This technique is very effective, inexpensive, procedurally simple and at the same time allows a large number of animals to be deprived of REM sleep simultaneously.

A large platform enabling the animals to curl up and have both slow wave sleep as well as REM sleep without falling into the water is most effectively used as control. To rule out the effects of restricting the movement of the animals on small platforms, movement restriction controls are carried out. In this the animals are maintained in a smaller cage, ideally of similar size to that of the small platform. In this control animals can have both the slow wave sleep and REM sleep. Another control which has frequently been used to

eliminate the effect of increased muscular activity on the small platforms is the swimming control. However, it is difficult to equate the increased muscle activity on the small platforms and that of by swimming. This platform technique is most widely used for REM sleep deprivation studies. Mendelson (64) recorded the EEG for 96 hrs from four groups of rats a) normal or baseline, b) on small platform (diameter 6.5 cm), c) on large platform (diameter 12.5 cm), and d) swimming (1hr). He reported that rats on small platform had 57% as much REM sleep as baseline during the first 24hrs, without any change in non-REM sleep, while rats on large platform, on the first day had 55% REM sleep (compared to baseline). The REM sleep increased to baseline value by the fourth day in the large platform control animals. On the fourth day the rats on the small platform continued to have significantly reduced REM sleep. The swimming control rats had no reduction in REM sleep or non-REM sleep at any stage. It was concluded that it is the ratio of platform size to the body weight of animal which decides the amount of reduction in REM sleep (65).

III) Treadmill arousal technique:

In this method the animals are maintained on a treadmill where due to continuous movement of the latter the rats cannot sleep. In this method theoretically the animals should not get any form of sleep (i.e. total sleep deprivation) since they are always in motion. However, in practice after a short while the animals learn to run in the opposite direction to that of the movement of the treadmill and have a nap till it reached the other end of the treadmill. This time the animals must wake up to avoid falling. The time of sleeping depends on the speed of treadmill. It has been found that the animals could have almost 40% of the time spent on treadmill in non-REM sleep. Hence, a combination of treadmill and hand arousal was used (66) for REM sleep deprivation. The animals were kept for 16-22 hrs per day on the treadmill. The remaining 2-8 hrs were spent in cages from which polygraphic recordings were possible. Thus, in treadmill-arousal studies, the experimental and control animals differ in REM sleep, but as this technique deprives the animal of a large amount of total sleep, this technique has not frequently been used for REM sleep deprivation studies.

IV) Pendulum technique:

To avoid the restriction and other stress experienced by the platform technique, the pendulum technique was designed (67). In this method the animals were kept in their home cages (thus they were not transferred to a new environment as in water tank procedure). This arrangement along with the cages were made to swing like a pendulum. At the extremes of motion due to postural imbalance the animals are forced to walk downward to the other end of cages. A minimal amount of REM sleep (0-2%) and a moderate amount of non-REM sleep (19-30%) were detected by the authors during 72 hrs deprivation by using this technique.

V) Multiple platform technique:

Subsequently, the classical platform technique was modified with multiple platforms (68) in order to reduce immobilization stress in the platform technique and to avoid problem of inadequate feeding in pendulum technique due to constant swinging motion. In this, seven small (6.5 cm) platforms were placed (separated from each other) in one water tank, permitting movement of the animals from one platform to another thus reducing forced immobility. The effects on sleep in this condition were identical to that of the classical platform technique, since only one platform can be used for sleeping purpose. In the multiple platform method, one platform was made a little bigger to serve as control so that the animals could have REM sleep as well.

VI) Rotating Disc method:

More recently Bergmann et al. (69) designed a computer aided elaborate procedure for REM sleep deprivation, the rotating disc technique. In this technique a pair of clear, smooth plastic cages house the experimental rat and its yoked control (in different cages). A smooth plastic disc, with its center in an alley between the cages, protrudes under each cage to provide a partial floor. Beneath each side of the disc and beyond it upto the walls of each cage is a tray of water. Both the rats are connected to polygraph for continuous recording of EEG and other electrophysiological signals signifying sleep-wakefulness. This polygraph is linked to a computer programmed to trigger rotation of the disks at randomly chosen direction at a rate of 3.33 rev/min whenever an experimental animal entered into REM sleep or non-REM sleep, depending on the type of deprivation is desired, and the animal wakes up. The rotating disk awakens the rat which walks opposite to disc rotation to avoid being carried into the water and is deprived of the desired sleep.

COMPARISON OF DIFFERENT METHODS FOR REM SLEEP DEPRIVATION

In all the above mentioned methods for REM sleep deprivation, the challenge is to achieve a total REM

sleep deprivation without affecting other forms of sleep and to eliminate the effects due to non-specific factors. The hand arousal technique suffers from a major draw back that after sometime the frequency of awakening increases to such a level that it becomes almost impossible to continue deprivation in the animal/subject. Due to frequent awakening the slow wave sleep is also affected significantly. Besides, in this technique the experimenter also undergos forced sleep deprivation. Ofcourse, it may be reduced by shift arrangements among the experimenters and consequently demands more number of personnel to undertake such an experiment. Thus, this technique is not the best choice for longer duration of deprivation studies. The treadmill method can hardly be used for selective REM sleep deprivation. In the pendulum technique although the REM sleep was deprived maximally, presence of only a small amount of non-REM sleep (19-30%) is not desirable. The rotating disc technique is a recent and rather selective method for REM sleep deprivation but needs an elaborate instrumentation and arrangement which may not be easily accessible always. A major draw back of this method is total dependency on the instruments to decide about REM sleep and accordingly to awaken the animal. Special care and precaution must be taken to eliminate the artifacts interfering with the analysis for awakening the animal and also to avoid the loss of recording due to unplugging by the animals (which may happen frequently). Besides, it is unlikely to run multiple animals and more than one control simultaneously. In addition, since the animal head is plugged for electrophysiological recording, it is difficult to take out the brain quickly and with equal ease as when the head is not plugged primarily for brain neurochemistry studies. Thus, although this method may not be as good for classical method of studies where brain needs to be taken out quickly, is reasonabl good for other chronic studies where blood, CSF, etc are needed for study. Another significant limitation of this method is that the yoke control animals are also awakened for equal number of times when the experimental animals are awakened from REM sleep. Thus, yoke control animals are also deprived of REM sleep or atleast the sleep is fragmented in them.

The platform method, on the other hand, has its own advantages which takes care of the draw backs present in other methods mentioned above viz. i) this technique induces almost total REM sleep deprivation without significantly affecting other sleep states; ii) it does not require elaborate and sophisticated instrumentation; iii) suitable controls/studies can be designed to rule out the non specific effects; iv) many experimental and control experiments can be run simultaneously; v) electrophysiological analysis suggested that this method is best among all other available methods (70,71). Nevertheless, this method is not so effective for REM sleep deprivation for 24 hr or less deprivation since it is difficult to design appropiate control because 24 hr large platform control animals are also deprived of REM sleep to an extent comparable to that of the experimental animals on the small platform. However, beyond 24hr, it has been shown that rats, mice and cats could be very effectively deprived of REM sleep, without significant loss of non-REM sleep, by this method (72).

Because of its advantages, this flower pot technique has been the preferred method and has most widely been used for REM sleep deprivation studies across the globe. For instance, this method has been used to study the effects of REM sleep deprivation on brain and neuronal excitability, on behavioral changes, on memory processing, on biochemical changes viz. alterations in neurotransmitter levels, receptor binding, brain biochemistry and so on. Now, we will discuss the significant knowledge gathered so far by the REM sleep deprivation studies with a view to appreciate their significance in understanding REM sleep in its overall perspective.

REM SLEEP DEPRIVATION AND BEHAVIORAL CHANGES

Dement performed the first REM sleep deprivation experiment on humans (73). Classical electrophysiological parameters of sleep-wakefulness were monitored in humans and as soon as the subjects displayed "activated EEG" patterns and rapid eye movements, they were awakened manually. It was observed that the frequency of occurrence of REM sleep was directly proportional to the length of deprivation. In addition, during the recovery period, subjects spent a larger percentage of their sleeping time in REM sleep compared to baseline. Thus, this was probably the first experimental evidence to show that there is a need for compensating the lost REM sleep vis-a-vis the significance of REM sleep. This increased REM sleep after the deprivation has been termed as "rebound effect". Dement and Fisher (74) deprived 21 subjects of REM sleep for 2-7 nights and all showed signs and symptoms of anxiety, irritability, inability to concentrate. An increase in appetite and irritability after 3-4 days of REM sleep deprivation in different subjects has been reported (75). They also found that all the subjects showed signs of confusion and withdrawal.

The REM sleep deprivation has been shown to alter behavioral effects of some drugs. Heise and Boff (76) studied the effects of REM sleep deprivation on rate of shock avoidance in control and in rats treated with D-amphetamine. It has been reported that amp-

hetamine (which releases brain catecholamines) significantly enhanced the rate of avoidance in control rats but not in REM sleep deprived rats, thus, concluding that catecholamine systems are impaired by loss of REM sleep. In another experiment both control and REM sleep deprived rats were given passive avoidance training. After training retention test was given in which REM sleep deprived rats showed poorer retention than normal rats. But when these rats were injected with either catecholamine potentiating drugs i.e. imipramine (blocks the neuronal reuptake of monoamines) or pargyline (blocks the degradation of these amines by MAO), these drugs significantly enhanced retention performance in REM sleep deprived rats without any change on normal rats. It has been concluded from these experiments that pharmacological agents which elevate catecholamine activity can reduce impairment in passive avoidance retention which occur following REM sleep deprivation without improving retention in control subjects (77). Since the catecholamine system is affected, it is reasonable that cardio-pulmonary systems also would be affected by REM sleep deprivation (78-80) though the cause and effect relationship is yet to be confirmed.

The REM sleep deprivation affects several instinctive and motivational behaviors including fighting (81) and nesting (82). These are likely to be natural protective behavioral phenomena. Sleep deprivation (including REM sleep deprivation) has been found to impair the control of postural balance (83). The REM sleep deprivation has been shown to facilitate self stimulation (84) and an increase in locomotor as well as exploratory activity. It results in hypersexuality and modulates the effects of testosterone on male sexual behavior in rats (85,86). In female rats, REM sleep deprivation alters drug induced behaviors like increase in apomorphine induced stereotypy and decrease in pilocarpine induced hyperthermia (87). Recently it has been shown that REM sleep deprivation impairs ACTH induced yawning behavior (88) and 24 hr recovery was enough to display number of yawns similar to those in control animals.

The REM sleep deprivation has been shown to improve endogenous depression (89). In rats, REM sleep deprivation has been shown to have the same effect as antidepressant drugs. REM sleep deprivation renders serotonergic dorsal raphe neurons less sensitive to the inhibitory potency of serotonin reuptake blockers. Hence REM sleep deprivation might alleviate depression through neurophysiological mechanisms similar to antidepressants (90). All these studies showed that there are alterations in instinct as well as acquired behaviors after REM sleep deprivation signifying the importance of the latter in maintaining normal physiology and behavior.

The above mentioned studies suggested that i) REM sleep is an important physiological phenomenon; ii) it is important for normal life processes; iii) within limit the body has a mechanism to compensate for the lost REM sleep; iv) its deprivation may cause disturbance in the physiological processes leading to disorder/disease and as a corollary it suggests that some of the diseases may be ameliorated or worsened by an alteration in REM sleep. These findings led to further exploring the underlying biochemical, cellular and molecular mechanisms of REM sleep deprivation induced changes.

REM SLEEP DEPRIVATION AND BRAIN EXCITABILITY

In one of the earlier studies when a cat was deprived of REM sleep for more than 30 days, its initial periods of REM sleep were dramatically enhanced by overt manifestations. Against the background of muscle atonia there were episodes of violent facial and limb twitches interspersed with convulsive movements of the entire body. The latter was so intense that the animal appeared to be in a state of seizures. During the recovery period there was rebound or compensation for lost sleep that was roughly proportional to the length of deprivation and the intensity of phasic motor activity subsided (91). In another study accelerated auditory recovery in cats deprived of REM sleep for 5 or more days has been reported (92). In that study, cortical responses evoked by paired acoustic clicks separated by 25 msec were recorded during wakefulness. The ratio between amplitude of potentials, evoked by the pair of clicks, was greater in the non-deprived than in the deprived rats. These changes were reversed when the animals were allowed to make up for the lost sleep i.e. after recovery. In other study the threshold for electro-convulsive shock was found to drop significantly after REM sleep deprivation (73,93). These findings along with the earlier mentioned behavioral changes studies led us to hypothesise that REM sleep deprivation is likely to result in a generalized increase in neural excitability (94,95).

The loss of REM sleep for as short as 12 hr and as long as 140 hr enhanced responsiveness of the brain as measured by a decrease in threshold to seizures (96). In a related study the effect of progressive deprivation on neural responsiveness of different areas of the brain using evoked potential as an indicator of neural excitability was studied (97). It was reported by those authors that entorhinal potentials gradually increased in amplitude from day 1 to day 7 of deprivation. In contrast, the cats showed a decrease in excitability of primary sensory afferent pathways. Thus, it was concluded that REM sleep deprivation might not

lead to a generalized increase in responsiveness but restricted to paleocortical excitability and an increase in inhibition responsible for sensory filtering. In other words, REM sleep deprivation is thought to sharpen the focus on external sensory stimuli by hind brain inhibition of internally generated signals. It may probably be said that function of REM sleep may be to maintain neuronal excitability status. Total sleep loss has been found to induce an increase in susceptibility to generalized kindled and penicillin seizures during all waking and sleep states but did not alter the temporal pattern of seizure susceptibility (98). Both, before and after sleep loss, kindled cats showed maximum seizure susceptibility, indexed by lowest seizure thresholds, during slow wave sleep and transition from slow to REM sleep.

Although there are several studies showing gross behavioral changes after REM sleep deprivation, fewer studies have been conducted to show its (REM sleep deprivation) effect at the cellular/neuronal level and their mechanism(s) of action. Alterations in the tone of muscles (the excitable tissue other than neuron) have also been reported (99). Similarly, REM sleep deprivation is also expected to alter individual neuronal excitability since their firing rates altered with changes in normal sleep-wakefulness cycle. In an attempt to find an answer to this question, effect of REM sleep deprivation on single neuronal responsiveness were studied in freely moving behaving cats. It was found that the deprivation resulted in a decrease and an increase in the waking discharge rates of pontine 'REM-OFF' and 'REM-ON' neurons, respectively (100). It also reduced the auditory evoked inhibition of unit discharge in dorsolateral pontine region (101). The neuronal firing rates however, returned to the baseline after recovery from REM sleep deprivation.

Thus, all these studies suggest that REM sleep deprivation exerts a generalized effect on the brain. It influences individual neuronal as well as brain excitability and their responsiveness is proportional to the period of deprivation. Since the deprivation induced effects were reversed after recovery, it will not be wrong to assume that at least one of the functions of REM sleep could be to maintain the neuronal excitability status (94,95). Ideally, transmembrane potential study in free moving animals before, during and after REM sleep deprivation needs to be conducted to provide a direct evidence as a proof of changes in absolute level of neuronal excitability. Such study could not be conducted yet, primarily due to technical difficulty. Nevertheless, in the absence of intracellular study, the effect of REM sleep deprivation on some of the factors known to maintain neuronal transmembrane potential viz. ATPases, calcium levels, etc. were investigated and have been discussed later in this review in the biochemical study section.

REM SLEEP DEPRIVATION AND MEMORY

Since the classical demonstration by Jenkins and Dallenbach (102) that sleep facilitates memory, many studies were done to know the role of sleep in this process. More specifically it has been suggested that REM sleep promotes memory consolidation. Dement (91) concluded from his studies that REM sleep deprivation affects performance of a learning task in cats. In other studies it was observed that mice trained for passive avoidance before the deprivation exhibited impairment in long term memory trace though the short term memory and acquisition of new memory were not affected (103,104). Similar observation was reported in the rats (105,106). There has been a variety of studies that investigated the effects of REM sleep deprivation on memory acquisition. Several studies (107) demonstrated that REM sleep deprivation impairs the acquisition of active avoidance conditioning; while others reported that the deprivation affects memory consolidation (108). Fishbein and Gutwein (104) hypothesized on the basis of REM sleep deprivation experiments that REM sleep is important for memory in two ways: first, REM sleep phase provides conditions which facilitates the conversion of labile short term memory trace, requiring extensive adaptational changes into a stable long term memory. Second, following initial fixation, it serves to promote stable memory into long term storage. In the above mentioned studies animals were deprived of REM sleep for a longer duration. In a controlled study it was found that 3hr REM sleep deprivation was ineffective although long term deprivation slowed down the stabilization of memory (109). In a series of recent studies, it was found that REM sleep deprivation after training in the Morris water maze decreased spatial memory retention (110,111). The same investigators (112) concluded that brief REM sleep deprivation impairs reference but not working memory in radial arm maze task. It was also shown that the effect of REM sleep deprivation on spatial learning is not prevented by dietary valine which does not effect serotonin metabolism though it reduces brain tryptophan transport (113).

There are many reports which showed an increase in REM sleep after learning task (104,114,115). A recent report indicated that learning in humans at the level of primary visual cortex was disrupted by REM sleep deprivation (116). Hars et al. (117) in their study supported the role of REM sleep in information processing during post learning phase. It was observed that in rats trained for active avoidance task, a conditioned stimulus given as reminder during REM sleep, greatly improved the retention performance than the non-reminded ones. The reminder was inef-

fective when given in wakefulness and it had negative effects when given during other stages of sleep.

Hippocampal theta rhythm, which occurs throughout REM sleep in all marsupial and terrestrial placental species, plays an important role in hippocampal processing of memory. Givens and Olton (118) reported that elimination of theta rhythm in the rat, via lesioning or infusion of cholinergic antagonists into medial septum, produced spatial memory deficits. Based on these findings it has been hypothesized that "A necessary aspect of mammalian memory processing is the integration of individual experience into a strategy for future behavior. In lower mammals, species specific behaviors most important for the survival of each species are accompanied by theta rhythm. In these behaviors theta rhythm acts to parcel incoming information and allows its storage of memory in hippocampus. Further experience gained during these species specific waking behaviors is reassessed and integrated into an animal's behavioral strategy during REM sleep" (25). Support of this aspect of the hypothesis is provided by single unit studies (119) in which CA1 neurons in hippocampus, that have previously encoded spatial information during the waking state, have been shown to fire preferentially during REM sleep. These studies emphasize on the role of REM sleep in memory retention and consolidation. Studies on the effect of REM sleep deprivation on memory, bring out the importance of REM sleep on memory processing and consolidation.

REM SLEEP DEPRIVATION AND BIOCHEMICAL CHANGES

The need for REM sleep and its rebound increase after deprivation have been expressed in almost all marsupial and placental mammals. Both the slow wave sleep and the REM sleep can be altered by drugs known to affect one or many aspects of metabolism. Since neurons function by releasing neurotransmitters and biochemical processes play an important role in normal physiological functions, to understand the neurochemical basis of REM sleep and also to gather knowledge of consequences of its deprivation, biochemical investigations were carried out.

I) Studies on brain:

After 10 days of REM sleep deprivation in rats the blood and brain potassium concentrations decreased (120). Brain glycogen (total and free) content decreased in different regions of the brain after 72 hr of REM sleep deprivation (121). To understand the probable pathways of altered glucose metabolism in rat brain during REM sleep and its deprivation, the effect of the latter was studied on activities of hexokinase and glucose-6-phosphatase (122). An increase in activity of hexokinase, a rate limiting enzyme in glycolytic pathway, and a decrease in were observed glucose-6-phosphatase activity in all the brain regions, after 4 days of REM sleep deprivation. From this study it was concluded that the deprivation induced increase in hexokinase activity is likely to enhance glucose metabolism and thereby it is likely to increase the energy production. An increased energy consumption after REM sleep deprivation may be supported by other studies (47). Generation of sleep including REM sleep has been correlated with brain energy production and temperature (124,125). Effects of REM sleep deprivation on cerebral amino acids have been studied and it was found that almost all the amino acids in the brain showed an increase after REM sleep deprivation (126-128). The most significant changes occurred in serine, glycine, alanine, leucine and phenylalanine. An increase in uptake of 3-H labelled amino acids in in vitro brain stem slice preparation in rats deprived of REM sleep has been reported though no change in protein synthesis could be detected (129). An increase in the brain catabolism was reflected by alteration in brain ammonia (130) and nitrogen (131) metabolism.

The REM sleep deprivation resulted in an increase in uptake or accumulation of exogenous norepinephrine (NE) in the diencephalon and the telencephalon regions of rats but produced no significant change in endogenous NE (132). These authors also reported an increase in synthesis and utilization of exogenous NE during rebound of REM sleep after deprivation in brain stem, mesencephalon, diencephalon and telencephalon regions. Monoamine concentrations have been reported to change after REM sleep deprivation have been reported (133). However, in other studies, after the deprivation no change in turnover of exogenous (134) or endogenous NE in different brain regions (135) could be detected. In one study, NE concentration was seen to increase after REM sleep deprivation as compared to yoked control rats (136) and returned to near baseline values after recovery from REM sleep deprivation (137). Tyrosine hydroxylase is a rate limiting enzyme in the pathway of NE biosynthesis. Its activity increased after 96 hr of deprivation in lower brain stem and cerebral cortex, but no change was seen in upper brain stem (138). An increase in tyrosine hydroxylase gene expression in locus coeruleus (139) and a decrease in NE concentration in neocortex, hippocampus and posterior hypothalamus (140) in REM sleep deprived rats have been reported. Although the effect of REM sleep deprivation on the level of neurotransmitter and its synthetic enzymes were studied, the information regarding the effects on the degrading enzymes were lacking without which the knowledge

remained incomplete. Therefore, to bring out a clearer picture about the effects of REM sleep deprivation on aminergic mechanism we (141) reported that the deprivation decreased the activity of rat brain Monoamine oxidase (MAO), specifically MAO-A, an enzyme primarily responsible for breakdown of NE. Thus, these studies supported that REM sleep deprivation is likely to alter NE level although may be indirectly. Changes in these enzymes in the brain stem supported the role of brain stem in REM sleep regulation (142).

An inhibitory role of NE in REM sleep has been supported by a recent report that mild stimulation of the locus coeruleus induced an effect similar to that of REM sleep deprivation (60). Also we reported that NE is likely to play an important role in REM sleep deprivation induced increase in Na-K ATPase activity (143). A significant decrease in cortical §-adrenergic receptor binding after 72 hr REM sleep deprivation has been reported (144). Troncone et al. (145) used NE stimulated cyclic AMP accumulation as an index of NE receptor status. They found a dramatic increase in cAMP accumulation in slices from rat cortex after 96 hr of REM sleep deprivation. Nevertheless, no change in noradrenergic binding densities and affinities could be detected in rat brain after total sleep and selective REM sleep deprivations (146).

Since both the aminergic and the cholinergic mechanisms are important for REM sleep, studies have been conducted to understand the effects of its deprivation on acetylcholine (ACh) and its degrading enzyme, acetylcholinesterase (AChE). The REM sleep deprivation has been found to decrease the level of ACh in the rat telencephalon though the levels of NE and serotonin (5HT) remained unaltered (147). In an attempt to understand the mechanism, we observed that there is an increase in AChE activity in the cerebrum after 8 days and in brain stem after 4 days of REM sleep deprivation (148). In an extension of this study it was observed that even short term (24 to 48 hr) deprivation increased AChE activity in the medulla of rats (149). Since AChE exists in two forms, membrane bound and cytosolic, and the former is primarily responsible for ACh breakdown in the brain, in order to understand the specificity of cholinergic mechanism the effect of REM sleep deprivation was studied on different molecular forms of AChE. The study revealed that there was an increase in the activity of membrane bound form of AChE in the pons (150). Thus, the results of deprivation studies on the AChE activity supported earlier finding of changes in ACh activity after REM sleep deprivation. Nevertheless, these studies now raise the question whether the increase in the level of AChE could be the cause of the decrease in ACh or vice versa or the deprivation may affect both the changes independently. In another study the effects of chronic sleep deprivation on cholinergic receptors in rat brain were studied but no significant difference in the nicotinic receptor binding was found in any of the brain regions even after 10 days of total or REM sleep deprivation; significant differences, however, in muscarinic receptor binding sites were seen only in the septal area after REM sleep deprivation (151). Downregulation of M2-type muscarinic receptors in rat brain was demonstrated by autoradiography after REM sleep deprivation (152).

To understand the correlation, if any, between cerebral serotonin and REM sleep the effect of 96 hrs deprivation of the latter on the synthesis, utilization and accumulation of serotonin in rat brain were studied (153,154). It was observed that intracisternal administration of tryptophan resulted in a marked increase in formation of [3H] serotonin in REM sleep deprived than in control rats. No effect was seen when [3H] 5-hydroxytryptophan was used instead of [3H] tryptophan, both in vivo and in vitro experiments. There was also a significant increase in accumulation of [3H] tryptophan in the deprived rat brain tissues. Thus, it was suggested that increased [3H] serotonin synthesis in the deprived animals seemed to result from both an increased transport and an increased rate of conversion of [3H] tryptophan to [3H] 5-hydroxytryptophan. They also concluded that increased turnover of serotonin in the deprived animals could be related to impossibility of triggering REM sleep. Serotonin receptor activation in rats deprived of REM sleep has been shown (155). Serotonergic responses (serotonin syndrome and head shakes) after administration of precursors and agonists of serotonin in control and REM sleep deprived rats were studied. The latter showed a larger incidence of the serotonin syndrome and greater number of head shakes in comparison to control animals, when challenged with serotonin precursors. The same group studied the central cholinergic responses after REM sleep deprivation in rats (156). It was seen that cataleptic behavior induced by cholinomimetic drugs pilocarpine, oxotremorine and eserine was not modified by previous REM sleep deprivation. But the intensity of oxotremorine and eserine induced tremors and nicotine induced convulsions were potentiated by REM sleep deprivation. Recently, changes in monamines and their metabolic concentrations in REM sleep deprived rat forebrain nuclei were studied (157). After 96hr REM sleep deprivation concentration of serotonin and its metabolite HIAA were reduced in the frontal and parietal cortices while a significant increase was observed in the concentrations of dopamine metabolites in the striatum. The REM sleep deprivation is reported to induce changes in responses to dopaminergic drugs

(158) and also alter dopamine metabolism as well as receptor sensitivity in the rat brain (159). It also upregulates (increased Vmax) adenosine A1 receptors (65). This upregulation in the adenosine receptor may be supported by the finding that there is a decrease (160) in the activity of 5'nucleotidase, an enzyme known to produce adenosine from AMP at the synaptic cleft. Although dopamine may not have any direct effect on REM sleep, its deprivation is reported to reduce the sensitivity of dopamine receptors also (161). REM sleep deprivation has been seen to increase dopaminergic receptor D2 binding but not D1 receptor binding in the rat brain (162).

Therefore, these studies confirm that at least adrenergic and cholinergic mechanisms are involved in REM sleep regulation while other neurotransmitters may not have a direct role in the regulation of REM sleep although they may play a modulatory and/or permissive role.

II) Studies on other organs:

Although in this review the primary emphasis is on the changes in the brain after REM sleep deprivation, a brief mention will be made here to show that the deprivation also affects other organs as well showing its generalised effects. Kushida and coworkers (163) in their exhaustive studies reported the effects of REM sleep deprivation in rats and summarized their results as follows: 1) all the rats showed progressively debilitated, scrawny appearance with brownish, dishevelled fur; 2) severe ulcerative and hyperkeratotic skin lesions localized to tails and plantar surfaces of paws and the tails; 3) increased food intake which registered an 100% above baseline values; 4) weight loss where group means differed from baseline by 21%; 5) an increase in energy expenditure; 6) a decrease in body temperature beginning near the middle of survival period; 7) an increase in plasma NE levels; 8) a decrease in plasma thyroxine (T4) and an increase in the ratio of plasma triiodothyronine (T3) to T4; and finally 9) death after prolonged deprivation (rats died after 16-54 days of REM sleep deprivation). Accelerated protein catabolism was indicated by an increased level of plasma urea nitrogen. During the last four days of deprivation, the rats showed reduced urine pH, suggestive of metabolic acidosis and a large amount of blood indicating kidney and urinary tract damage. Although the physiological mechanism of the above mentioned changes are not known, the results of those studies indicate that REM sleep is essential for normal physiological processes and its prolonged deprivation may eventually be fatal. Nevertheless, studies so far have also shown that if REM sleep deprivation is not continued for prolonged period, part of the altered functions may return to normal level after recovery from moderate period of REM sleep deprivation.

The REM sleep deprivation is likely to have a synergistic effect due to simultaneous changes in other factors which may affect the system. Thus, it is reasonable to understand that malfunctioning in REM sleep may lead to a disease and vice versa. Although it is known that REM sleep is affected in different diseased conditions and vice versa, definite correlation as well as cause and effect relationship between them are not known in majority of the instances. It is tempting to know more about the relationship between the diseases and their relation with REM sleep. However, this aspect is being kept out of this review for two reasons, one, several articles are available in the literature covering this topic and two, considering its importance and the depth of knowledge, justice will not be done if detailed discussions are not presented. Nevertheless, it is worth mentioning that REM sleep is affected in different psychiatric disorders (164) and at the same time at least in case of depression some relief has been reported after REM sleep deprivation treatment (165). Therefore, it may be suggested that experimental model may be designed properly to investigate the relationship between REM sleep and different diseases. Thus, REM sleep deprivation studies may play a significant role in advancing the knowledge and bridge the existing gap at present. However, it is important to note that the period of REM sleep deprivation which may be considered to be within biological limit and non-harmful is not known yet.

FUNCTIONAL SIGNIFICANCE OF REM SLEEP

Studies remain incomplete till they are correlated with functional significance. Hence, the functions of REM sleep, as proposed by several workers based on REM sleep deprivation studies will be reviewed here in brief. The REM sleep, which is a part of sleep-wakefulness, is a basic and an instinct behavior. Nevertheless, even after about half a century of its discovery the knowledge regarding its functional significance is generally hypothetical in nature. Three approaches have primarily been used to understand its functional significance viz. phylogenetic approach or study of evolution, developmental or ontogenic approach and the deprivation studies. Results obtained from all these methods of study provided significant information which need to be colated and must be understood in its totality to get a complete picture. The first method depends primarily on historical observations, literature and their interpretation while the latter two provide scope for experimentation. Among the latter two approaches the former is relatively more time consu-

ming and one must be extremely cautious to rule out the effects of non-specific factors. The last method (i.e. the deprivation method), on the other hand, gives enough scope for experimentation and means to control the variations as well as the effects due to non-specific factors.

One hypothesis is that during non-REM sleep since the state of vigilance (cortical tonus) is reduced, which could be potentially dangerous for the sleeper, the REM sleep excites the cerebrum to restore the tonus (166,167). Snyder (168) extended it further and hypothesized that REM sleep also takes the organism to have a glimpse of the external world for a quicker response against any danger. It has been observed that the REM sleep is more in the young than in the adults. This led Roffwarg and Dement (21) to propose the 'Ontogenic hypothesis'. These authors suggested that REM sleep is probably required for proper development and maturation of the nervous system. Once the nervous system is matured in the adults, the REM sleep is reduced. The brain maturation function of REM sleep has been proposed earlier and reinforced recently (169-171). Brain maturation is proportional to that of synthesis and turn over of proteins. It has been hypothesised that REM sleep probably maintains the functioning of the aminergic system and protein synthesis in the central nervous system (77). A possible role of REM sleep in maintaining neuronal excitability has also been proposed (94). The role of REM sleep in motivation (123), in preventing sudden infant death (172), in life sustaining (173) and in maintenance of body temperature, specially the brain temperature (174) has been proposed. Although EEG and body temperature changes are seen simultaneously, they have been proposed to be independent processes (175,176). However, simultaneous changes in EEG (desynchronization), along with an alteration in body temperature, is likely to maintain the latter within physiological limit (177,178). The role of sleep in body temperature regulation and vice versa has also been proposed (124). Sleep-Wakefulness and body temperature are modulated by various neurotransmitters including adrenergic (179,180) and cholinergic (181). It has been reported that adrenergic neurotransmitter may affect sleep and body temperature by acting on different subtypes of receptors (182). An interaction between adrenergic and cholinergic systems for the regulation of sleep and body temperature has also been shown (183,184). Thus, it is likely that alterations in either the neurotransmitters or their receptors or the function may affect physiological functions including sleep and REM sleep. The importance of REM sleep in maintaining body temperature can be ascertained from the fact that REM sleep deprivation alters thermoregulation during sleep (185). The role of REM sleep in learning and memory has been discussed above. Crick and Mitchison (186) hypothesised that 'reverse learning' or 'unlearning' takes place during REM sleep when the unwanted and useless information acquired during awakefulness are removed. This may be compared to that of deleting unwanted information, which might have been stored temporarily or which is no more necessary, from a computer diskette to make memory space available for newer information to be saved.

Although the physiological significance of REM sleep is known to a limited extent (mentioned above), very little is known about the cellular mechanism/s of those changes which need to be investigated. Nevertheless, studies in that direction have started using modern technology and there are isolated studies where the effects of REM sleep and total sleep deprivation on cellular changes have been reported. Regulation of adrenergic receptor sensitivity during REM sleep has been proposed (187). An increase in the level of tyrosine hydroxylase and norepinephrine transporter mRNA was also seen (188). Total and REM sleep deprivation are reported to induce c-Fos and IEG expressions in selected regions of the brain (189-194). After REM sleep deprivation mRNA coding for GHRH decreased, while that of somatostatin increased (195). Expression of some molecules like neurogranin and dendrin were also seen to alter after sleep (including REM sleep) deprivation (196,197). Similarly, galanin gene expression also increased (198) after REM sleep deprivation. REM sleep deprivation has also been seen to increase the density of VIP receptors in several regions in the brain stem and forebrain (199). Some investigators have seen the effect of REM sleep deprivation on the size of lateral geniculate nuclei cells after monocular deprivation (200,202). Although the molecular basis of these changes are not known, alterations in membrane fluidity (203) and synaptosomal calcium levels (204) as reported by us may help in explaining the phenomena. The REM sleep deprivation was reported to alter brain (73) and single neuronal responsiveness (100,101). Based on this we proposed that one of the basic functions of REM sleep is to maintain neuronal excitability (94,95). In a series of relatively elaborate studies, we have investigated the possible cellular and molecular mechanism of action of increase in neuronal excitability after REM sleep deprivation. It was shown that following REM sleep deprivation, there was a generalized increase in the Na-K-ATPase activity, an enzyme which is known to maintain neuronal excitability (205). The increase was observed throughout the brain, the pontomedullary area being first to be affected (206). REM sleep deprivation had also been shown to increase chlo-

ride sensitive Mg-ATPase activity although the chloride insensitive Mg-ATPase activity did not change (207).

Further, our studies showed that the increase in Na-K-ATPase activity was mediated by NE acting through -adrenoceptors (143). At the molecular level, our recent data suggests that the action of NE acting on 1A adrenoceptors releases membrane bound Ca^{++}(208,209), which possibly acts on calcium dependent calmodulin leading to dephosphorylation of Na-K-ATPase and an increase in its activity (210). It has also been shown that REM sleep deprivation is likely to alter ATPase synthesis as well as conformation because REM sleep deprivation has been shown to induce an uncompetitive stimulation of Na-K-ATP ase activity in the rat brain (211).

Thus, these intracellular changes after REM sleep deprivation studies are fragmented and needs to be followed with the use of modern technology. Besides, since there is a lack of proper model to fit all these findings and hypotheses, it is the need of the hour to identify the lacunae and accordingly design the expeirments precisely to fit in reasonable model. While doing so the REM sleep deprivation studies, in addition to other studies, should undoubtedly play an important role in advancing the knowledge. Future studies are likely to concentrale on the molecular mechanism of changes due to REM sleep deprivation.

CONCLUSION

In conclusion, it may be said that REM sleep is an essential component of life process which reversibly affects primarily the brain functions. The deprivation studies have contributed significantly in understanding the functions of REM sleep and has also provided supporting evidence towards understanding its mechanism of generation and maintenance. The platform method of REM sleep deprivation is the method of choice used by a majority of investigators for exploring and understanding the physiological significance of REM sleep because of its ease of use and minimum limitations. Of course, suitable controls which can be conducted with relative ease, must be carried out to rule out the effects due to non-specific factors. So far mostly the effects of REM sleep deprivation on gross behavior have been studied. Although isolated studies have been conducted to show the effects of REM sleep deprivation on cellular responses, a major deficiency is that there has been a lack of systematic study, in most of the instances, to investigate the cellular mechanism and changes which could be possible causes for a gross physiological changes due to REM sleep deprivation or vice versa. The possible role and mechanism of action of REM sleep at the cellular level and their correlation with gross physiology are yet to be investigated systematically. Finally, the knowledge gathered so far needs to be fitted in properly designed models for effective understanding and use by the mankind.

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