Review

Role of norepinephrine in the regulation of rapid eye movement sleep

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Sleep and wakefulness are instinctive behaviours that are present across the animal species. Rapid eye movement (REM) sleep is a unique biological phenomenon expressed during sleep. It evolved about 300 million years ago and is noticed in the more evolved animal species. Although it has been objectively identified in its present characteristic form about half a century ago, the mechanics of how REM is generated, and what happens upon its loss are not known. Nevertheless, extensive research has shown that norepinephrine plays a crucial role in its regulation. The present knowledge that has been reviewed in this manuscript suggests that neurons in the brain stem are responsible for controlling this state and presence of excess norepinephrine in the brain does not allow its generation. Furthermore, REM sleep loss increases levels of norepinephrine in the brain that affects several factors including an increase in Na-K ATPase activity. It has been argued that such increased norepinephrine is ultimately responsible for REM sleep deprivation, associated disturbances in at least some of the physiological conditions leading to alteration in behavioural expression and settling into pathological conditions.

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1. Introduction

Sleep and wakefulness are instinctive rhythmic biological phenomena. They are unique physiological behaviours that are characterized by changes, probably most interesting among them being alterations in the level of consciousness. The mechanisms of initiation and maintenance of sleep and wakefulness, as well as the interaction between them are highly complex neurophysiological processes. Although these phenomena may be identified by external behavioural manifestations, the observations often suffer from subjective bias. Also, some of the behavioural expressions may vary with time, circumstances, environmental conditions, differences between species etc. The above referred difficulties have reasonably been overcome by recording and analysing the electrophysiological signals associated with sleep-waking states. The electrophysiological signals are recorded from the skull to reflect the brain electrical activity as the electroencephalogram (EEG); from the muscles to reflect the muscle tone as the electromyogram (EMG) and from the eye muscles to reflect eye movements as the electrooculogram (EOG). Based on the characteristic features of these electrical signals, the state of wakefulness has been divided into two stages viz. quiet wakefulness and active wakefulness while the sleep state has been divided into three stages viz. slow sleep, deep sleep and rapid eye movement (REM) sleep.

2. Rapid eye movement sleep

Aserinsky and Kleitman (1953) first identified REM sleep based on its present electrophysiological characteristics. However, mention of expressions resembling such

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Abbreviations used: EEG, Electroencephalogram; EMG, electromyogram; LC, locus coeruleus; NE, norepinephrine; PGO, pontogeniculooccipital; REM, rapid eye movement.

behavioural phenomenon can be found in ancient literatures viz. the Greek epic, Virgil's *Aeneid* (19 BC), *the Mandukya Upanishads* (11 BC) as well as in the hunters' description of the behaviour of their dogs during rest and sleep inbetween hunting episodes. During REM sleep, some of the electrophysiological signals of the animal/ subject resemble wakefulness, although behaviourally the subject remains in deep sleep. Thus in REM sleep, the EEG shows desynchronization accompanied by eye movements, both signs of wakefulness, as compared to EEG synchronization with no eye movements during deep sleep. Additionally, the EMG shows atonia (loss of muscle tone in antigravity muscles) and there is presence of pontogeniculooccipital (PGO) waves. Therefore, it is a paradoxical state within sleep. This stage is also known as 'paradoxical sleep', or 'desynchronized sleep' or 'active sleep' or even 'dream sleep' since dreams are associated with this stage of sleep. This phenomenon with its characteristic features has attracted the attention of researchers, since its discovery. Nevertheless, even after almost half a century, our lack of knowledge regarding the generation, maintenance and physiological significance of REM sleep continues to be a challenge to sleep researchers.

Normally, REM sleep follows deep sleep. Although it does not follow wakefulness, it may end either in a sleeping or waking state. The quantity of REM sleep may vary in different species with age, as well as with the level of maturity of the brain. It is a rhythmic phenomenon and episodes of REM sleep are repeated several times within a single sleep cycle. In normal cases, the duration of REM sleep per episode may vary from a few seconds to several minutes. In normal human adults, on an average, REM sleep is repeated once every 90 min during sleep; the duration of REM sleep increases as sleep progresses. Adult humans spend about 20% of sleep time in REM sleep.

Patterns of sleep among mammals are diverse. Human non-REM sleep is divided into four stages (stages 1–4), while in cats it has been divided into two categories, slow wave sleep (quiet sleep or slow sleep 1) and deep sleep (slow sleep 2). Many mammals possess only a single quiet sleep stage. Cats, dogs, hedgehogs, foxes and cattle show a unique state of drowsiness with very low response thresholds. Sleep habits have been modified depending on the surroundings and the environment that the species living in the ecosystem have to face. Broadly, it has been found that species that spend a longer time in sleep tend to be smaller in size. Also, another view is that animals that are predators (e.g. cats) are usually good sleepers with REM sleep occupying 15% or more of sleeping time; conversely, species that are subject to predation (e.g. rabbit) are poor sleepers, with reduced REM sleep (Bert *et al* 1977).

REM sleep time is positively correlated with total sleep time. Since non-REM sleep and total sleep time are related to body weight, larger animals spend less time in non-REM sleep and consequently experience less REM sleep. For example the rat, chimpanzee and cow spend about 14, 10 and 4 h in sleep per day that includes 2, 1·5 and 0·76 h in REM sleep (Aserinsky 1999). Also, the duration of REM sleep is related to maturity at birth. Thus, animals that are immature at birth spend longer total duration in REM sleep. It may also be noted that since REM sleep is part of the sleep process, nocturnal animals (e.g. rats) experience it during daytime when they sleep normally. Similarly, the REM sleep is distributed among the sleep periods in the polycyclic species (who sleep several times a day), as well as in monocyclic species (who sleep once a day).

3. Brain areas controlling REM sleep

It has been established that the brain controls the basic sleep-waking phenomena (Jouvet 1972). Attempts have been made to identify areas in the brain responsible for the control of REM sleep (Sakai 1985; Siegel 1983, 1989). Recently, a chronicle of progress and development in research for identification of brain areas regulating REM sleep has been described by Jouvet (1999). In most of those studies the method of elimination has been adopted i.e. specific brain regions have been transected and/or lesioned and the effect on REM sleep has been studied. In other words, the signs and symptoms characterising REM sleep were recorded before and after transection/lesion of specific areas in the brain. Jouvet and his group termed such sleep as "rhombencephalic or paradoxical sleep" and identified areas in the brainstem between pons to lower brainstem as being responsible for such phenomenon (Jouvet and Mounier 1960; Jouvet 1999). In a similar and earlier study of isolated preparation of the brain, the pons continued to generate periodic episodes of rapid eye movements and PGO spikes in a pattern which, in an intact animal, were seen only during REM sleep (Matsuzaki 1969). Subsequently, by transecting the brain stem at different levels, Siegel *et al* (1986) showed that the signs of REM sleep were expressed within the structures with which the pons remained connected. In experiments where the transection was made above the pons i.e. the pons remained connected with the medulla and the spinal cord—most of the REM sleep defining signs were expressed in the structures caudal to the cut. However, when the transection was made caudal to the pons—i.e. the pons remained connected to the mid- and fore-brain—REM sleep signs were expressed in the latter structures. Thus, based on the results obtained from the studies mentioned above, Siegel (1989) in his review commented that "the pons is both necessary and sufficient to generate the basic phenomenon of REM sleep".

Subsequently, several reviews have dealt with the role of peribrachial (Datta 1995) and pedunculopontine (Rye 1997) regions in the pons for the regulation of REM sleep.

4. Localization of the area/region within the pons responsible for REM sleep

It has been reported that lesion of the pontis reticularis oralis permanently eliminated all signs resembling REM sleep (Carli and Zanchetti 1965). Some reports suggested that medial pontine reticular formation (within 2 mm from the midline) was critical for REM sleep (Jones 1979), while other reports (Drucker-Colin and Pedraza 1983; Friedman and Jones 1984; Sastre *et al* 1981) led us to conclude that the lateral portion of the pontine structure was critical. Cytotoxic lesion (using kainic acid) showed that neurons located in the lateral pontine reticular structure were essential for REM sleep (Sastre *et al* 1981). The importance of the pontine structure in REM sleep was further substantiated by the demonstration of presence of REM sleep related neurons, the REM-ON and REM-OFF neurons, in that area. Thus, although it was demonstrated that the pontine area was regulating REM sleep, the precise area and neurotransmitter specific nuclear group within the pons responsible for such regulation had not been identified. This, as put forward by Jouvet (1999), was due to non-availability of current modern techniques in those days. Specific nuclear groups viz. locus coeruleus (LC), etc. or neurotransmitter specific neurons could neither be clearly and specifically identified nor were they described in the then available brain atlas.

5. Locus coeruleus and REM sleep

An important structure in the dorsolateral tegmentum of the pons is LC. It consists of predominantly norepinephrine (NE)-ergic neurons (Foote *et al* 1983; Jones and Moore 1974). The LC was so named by Wenzels in 1811 because of the dark bluish colouration it exhibited in man and primates (Chu and Bloom 1974). This group of neurons is the primary site for providing NE projections to most of the brain structures of the higher nervous system (Foote *et al* 1983; Moore and Bloom 1979). Depending on the size of the cells and their organization, the LC has recently been subdivided into LC-principal, LC*a*, peri-LC*a* and subcoeruleus (Sakai 1980; Sakai *et al* 1981). In addition, this area also receives cholinergic (Aston-Jones *et al* 1986; Jones 1990; Luppi *et al* 1995) as well as GABAergic projections (Ennis and Aston-Jones 1989; Aston-Jones *et al* 1991) from other parts of the brain and it has GABAergic interneurons (Iijima and Ohtomo 1988). LC-neurons receive moderately dense projections from the galanin and GABAergic neurons in the ventrolateral preoptic (VLPO) area (Sherin *et al* 1998; Steininger *et al* 2001). Since the LC is the primary site for providing NE to the brain, researchers interested in studying its role in REM sleep regulation. The role of LC in REM sleep has been studied by lesion (permanent inactivation), reversible inactivation and stimulation of this area. Further, at the cellular level it has supported by the findings that the activity of the neurons in LC altered in relation to REM sleep or its loss.

6. Locus coeruleus lesion studies

There are a number of lesion studies suggesting that the NE containing neurons in LC within the pons is the key element in the organization of both the tonic and the phasic events of REM sleep (Jouvet and Delorme 1965; Jouvet 1972; Roussel *et al* 1967). Destruction of the ventral part of the LC (i.e. LC*a* and peri-LC*a*) was followed by irreversible disappearance of atonia associated with REM sleep (Henley and Morrison 1974; Jouvet and Delorme 1965; Sakai 1980). Sastre *et al* (1979) demonstrated that REM sleep was completely suppressed during two post lesion months after the destruction of both the LC-proper and the LC*a* along with peri-LC*a*. Subsequently, Braun and Pivik (1981), from their lesion studies in rabbits suggested that the LC region was essential for the integrity of sleep and was especially important for the control of motor expressions during sleep. Besides, there were studies where the LC-principal was locally cooled and led to increase in sleep and REM sleep (Cespuglio *et al* 1982). Those authors reported that local cooling of LC*a* and that of peri-LC*a* induced wakefulness as well. Also, an increase in REM sleep was seen after 6-hydroxydopamine lesion of LC (Farber *et al* 1983). These studies suggested an inhibitory role of LC for the generation of REM sleep. Nevertheless, another study where the lesion was restricted to LC-principal did not show a lasting effect (more than 48 h) on slow wave sleep and REM sleep (Jones *et al* 1977). Further, Jones (1979) from her lesion studies suggested that the gigantocellular tegmental neurons and not the NE containing LC-neurons were responsible for REM sleep. In yet another study, it was shown that depletion of NE from the NE-ergic LC by local injection of 6-OH dopamine did not prevent REM sleep (Laguzzi *et al* 1979). Thus, although the studies mentioned above supported the fact that the LC may be involved in REM sleep, the detailed mechanism was not clear. Nevertheless, it was necessary to confirm the role of LC in REM sleep regulation using other methods and also to understand the mechanism of action.

7. Locus coeruleus stimulation studies

In a study by Cespuglio *et al* (1982) the LC was transiently stimulated in two cats and that induced arousal and wakefulness. It is difficult to comment on the stimulation results as the study was limited to two cats and that too in an isolated manner. Besides, although the authors did not mention the strength and frequency of stimulation, it is possible that it might have activated the brain stem reticular formation resulting in wakefulness and EEG desynchronization (Mallick *et al* 1986; Moruzzi 1972). Moreover, the study was not done on free moving animals, hence, it is difficult to comment on the role of LC on sleep-wakefulness-REM sleep which are behavioural phenomena. Nevertheless, our study in chronic preparation of normally behaved free moving rats showed that mild, low frequency but long term bilateral stimulation of the LC reduced REM sleep significantly and there was a rebound increase in REM sleep during the post-stimulation period (Singh and Mallick 1996). The results of that study also showed that the reduction of REM sleep during the stimulation period was due to reduction in the frequency of generation of REM sleep. This suggested that if the LC-neurons could be kept active *vis-a-vis* not allowed to stop firing, REM sleep was not generated. In other words, it also confirmed that if the LC-neurons were active, it prevented REM sleep generation. As a corollary, the results also suggested that the neurons in LC must cease activity for the generation of REM sleep – possibly a withdrawal phenomenon. These lesion and stimulation studies suggested that the NE-ergic neurons in the LC were likely to play a crucial role in REM sleep regulation, however, it needed to be confirmed further by studies at the cellular level. Since the LC-neurons are NE-ergic, it is likely that NE would play a significant role in REM sleep regulation. Hence, before dealing with the LC-neuronal activity, the chemical regulation of REM sleep with emphasis on adrenergic mechanism will be discussed in brief.

8. Effect of norepinephrinergic agonists and antagonists on REM sleep

Several neurotransmitters and small molecular weight peptides as well as neuropeptides may influence sleep-wakefulness including REM sleep (Inoue 1989). However, cholinergic and NE-ergic mechanisms have been studied more extensively. Alpha-1 adrenergic antagonist, prazosin and intraperitoneal (i.p.) injection of NE degrading enzyme increased REM sleep (Depoortere 1985; Gaillard 1985; Hilakivi *et al* 1980; Wauquier *et al* 1985). Alpha-1 adrenoceptors stimulated by methoxamine have been reported to decrease REM sleep in rats (Pellejero *et al* 1984). The acetylcholine and its agonist as well as acetylcholinesterase and its antagonist are reported to affect REM sleep (Amatruda *et al* 1975; Baghdoyan *et al* 1984; Gillin *et al* 1985; Velazquez-Moctezuma *et al* 1991; Wauquier *et al* 1985). It has also been reported that chemical stimulation of the dorsolateral pontine area including the LC by cholinergic agonist increased REM sleep (Amatruda *et al* 1975; Baghdoyan *et al* 1984; Gillin *et al* 1985). This suggested that the cholinergic stimulation must somehow, directly or indirectly, inhibit the LC-neurons for the initiation of REM sleep. It is likely that these two mechanisms work hand in hand and it was proposed that acetylcholine was probably is responsible for triggering REM sleep while NE played a permissive role.

Local injection of clonidine – an $\alpha 2$ agonist – in LC was seen to strongly reduce REM sleep (Tononi *et al* 1991). In another study, it was demonstrated that NE caused dose dependent inhibition of REM sleep when applied unilaterally to the caudal peri-LCα of the mediodorsal pontine tegmentum that contains mainly non-cholinergic and non-noradrenergic neurons. This effect was also mimicked by clonidine and antagonised by selective $\alpha 2$ adrenoceptor antagonists, rauwolscine and RX 821002 (Crochet and Sakai 1999).

9. Locus coeruleus single unit studies

In general, the LC-neurons have a slow and rhythmic firing rate of about 2 Hz (Jacobs 1986), which gradually decreases during slow wave sleep and ceases during REM sleep (Chu and Bloom 1974; Jacobs 1986). Hence, these neurons have been classified as 'REM-OFF' cells. The LC-neurons respond to various environmental conditions as well as external stimuli and also increase activity in anticipation of changes in sleep-wakefulness (Aston-Jones and Bloom 1981; Jacobs 1986). The activities of the NE-ergic neurons in the LC have been correlated with the simultaneous changes in intensity of activation of the sympathetic nervous system of the same animal (Reiner 1986). According to Reiner the activity of the LC-NE neurons increases with an increase in discharge in the sympathetic nervous system. Sympathetic activation is normally accompanied by EEG desynchronization. Thus, studies suggested that the activity of LC-neurons was related to sleep-wakefulness and REM sleep. However, it was also necessary to study the behaviour of individual LC-neurons, especially in relation to REM sleep.

Cessation of activities of LC-neurons during spontaneous REM sleep suggests that there should be reduced release of NE during REM sleep in areas of the brain where the LC-neurons project. The effect of deprivation of REM sleep at the single neuronal level has been studied: in cats, the 'REM-OFF' neurons never cease firing (i.e.

keep on firing continuously) upon REM sleep deprivation (Mallick *et al* 1989). The results showed that normally the NE-ergic neurons in the LC ceased firing during REM sleep and they continued firing during REM sleep deprivation. This observation led us to hypothesize that cessation of LC-neurons was a pre-requisite for the generation of REM sleep. The hypothesis was confirmed by the results of a study where continuous activation of LC-neurons simulated an REM sleep deprivation like condition: that is, there was significant decrease in REM sleep during stimulation, followed by rebound increase in REM sleep during the post-stimulation period (Singh and Mallick 1996). REM sleep deprivation was found to block the evoked inhibitory responses of the dorsolateral pontine neurons induced by external auditory stimulation (Mallick *et al* 1991). Whether the LC-neurons influenced sleep-wakefulness or were influenced by the latter was not clear, as also the cellular mechanism of inhibition of the LC-neurons during REM sleep. It was necessary to investigate those at length in order to understand the detailed mechanism underlying the genesis and maintenance of REM sleep, as well as to know the role of NE in such regulation. At this point it is also important to note that there are neurons ventral to LC that show activity exclusively during REM sleep. Those neurons are presumably cholinergic and have been classified as 'REM-ON' neurons (Hobson *et al* 1974; Sakai 1988; Steriade and McCarley 1990).

10. Interaction between REM-ON and REM-OFF neurons for the regulation of REM sleep

In has been mentioned above that there are two types of REM-sleep related neurons, the cholinergic REM-ON and the NE-ergic REM-OFF types. Activation of the former increased (Thakkar *et al* 1996) while that of the latter (Singh and Mallick 1996) decreased REM sleep. These neurons fire in temporal sequence so that when one of them is active the other ceases firing. This is true during spontaneous REM sleep (Hobson *et al* 1975; Mallick *et al* 1998), REM sleep deprivation (Mallick *et al* 1989) as well as carbachol induced REM sleep generation (Shiromani and McGinty 1986; Thankachan *et al* 1997). Thus, there is a close interaction between these two groups of neurons for the generation of REM sleep. The existence of such an interaction was first proposed by Hobson and his group (1975) and subsequently modified by Sakai (1988). However, those authors did not consider the neurotransmitter type in their models. Hence, it was difficult to explain the mechanism of generation and regulation of REM sleep. Recently Mallick *et al* (2001) have considered the neurotransmitter content in the neurons and have further modified the model that overcomes the drawback of earlier models.

10.1 *Reciprocal interaction model*

The identification and recording of REM-ON and REM-OFF neuronal activities from anatomically distinct areas resulted in a paradigm shift in concept from gross centers to neuronal mechanism of REM sleep regulation. The model proposed by McCarley and Hobson (1975) envisaged a reciprocal interaction between the cholinergic REM-ON and noradrenergic REM-OFF neurons. They proposed that the REM-OFF neurons in the LC were inhibitory to the REM-ON neuronal population, while the REM-ON neurons exerted an excitatory effect on the LC REM-OFF neurons. In this model, it was proposed that the LC-neurons were active throughout, except during REM sleep, and their continuous activation inhibited the activity of cholinergic REM-ON neurons. During REM sleep the LC-neurons cease firing, resulting in withdrawal of the tonic inhibition from the REM-ON neurons. Cessation of noradrenergic neuronal activity allows an increased number of REM-ON neurons to escape from inhibition resulting in generation of REM sleep. The activation of REM-ON neurons exerts an excitatory effect on the LC-neurons resulting in inhibition of REM-ON neurons and termination of REM sleep episodes.

This model, based on the interactions between REM sleep related specific neuronal groups for the generation of REM sleep was significant being the first model. However, it had limitations as it offered no explanation for the initiation and continuation of REM sleep.

10.2 *Mutual inhibitory model*

Sakai (1988) proposed that both the REM-ON and REM-OFF neurons inhibit each other and he thus differed from the model proposed by McCarley and Hobson. This model hypothesized that REM-OFF neurons are active throughout except during REM sleep and keep the REM-ON neurons inhibited (except during REM sleep), disallowing induction of REM sleep. During REM sleep there is a progressive decrease in the firing rate of the NE-ergic neurons and a consequent withdrawal of inhibition from the REM-ON neurons. This disinhibition (or withdrawal of inhibition) initiates firing of the REM-ON neurons, leading to induction of REM sleep. It was also proposed that this increased activity of the REM-ON neurons simultaneously inhibited the REM-OFF neurons.

This model suggested that the REM-ON and the REM-OFF neurons inhibited each other. Based on this model, it may be said that since REM-ON neurons are cholinergic, acetylcholine released from these neurons would inhibit the NE-ergic REM-OFF neurons and NE released from the latter would inhibit the former. The report that the rat LC-neurons were depolarized (excited) and not hyper-

polarized (inhibited) by iontophoretic application of acetylcholine (Egan and North 1986), however, could not support the above view. Therefore, the above two models could not be sustained because they could not explain the mechanism of action. This is primarily because the models did not consider the type of neurotransmitter released and their mechanism of action on the projected neurons. This deficiency was overcome by recent studies by Mallick and group.

10.3 *GABAergic interneuron based model*

The two models mentioned above are important and are significant initial attempts to explain neural control of REM sleep. Although conceptually justified, they are grossly limited because they have not considered the neurotransmitter type of the neurons and their mechanism of action. They cannot explain the initiation and maintenance of REM sleep. Hence, there has been a pressing need to have a fresh look and possibly refine the model of neural mechanism of REM sleep generation.

It was mentioned earlier that the REM-OFF neurons in the LC must stop firing during REM sleep but on the other hand, that the cholinergic REM-ON neurons simultaneously increased firing during REM sleep (Hobson *et al* 1975; Mallick *et al* 1998). Acetylcholine increased around LC during REM sleep (Kodama 1990) and microinjection of acetylcholine agonist in LC increased REM sleep (Baghdoyan *et al* 1984; Quattrochi *et al* 1998; Vanni-Mercier *et al* 1989). However, since acetylcholine did not hyperpolarise the LC-neurons (Egan and North 1986), it was proposed by Mallick and group (Alam *et al* 1993) that although acetylcholine might trigger the action, the actual inhibition of REM-OFF neurons in LC could be induced by an inhibitory neurotransmitter leading to the generation and regulation of REM sleep.

GABA is an inhibitory neurotransmitter. GABAergic interneurons and terminals are present in LC (Iijima and Ohtoma 1988; Jones 1990, 1991; Sherin *et al* 1998; Steininger *et al* 2001) and GABA receptors are also present in LC (Luque *et al* 1994; Olpe *et al* 1988)**.** GABA levels in LC increased during REM sleep (Nitz and Siegel 1997) and REM sleep has reduced when picrotoxin, a GABA-A receptor antagonist, was microinjected into the LC (Kaur *et al* 1997); GABAergic neurons were reported to be active during REM sleep (Maloney *et al* 1999) and noradrenergic neurons in the LC were inhibited by GABA (Gervasoni *et al* 1998). Also, it was reported that cholinergic receptors (Mash and Potter 1986; Baghdoyan *et al* 1994) were present on the neurons in LC, acetylcholinesterase is present in LC (Albanese and Butcher 1980) and acetylcholnesterase activity in the brainstem was increased after REM sleep deprivation (Mallick and Thakkar 1991, 1992; Thakkar and Mallick 1991). Hence, Mallick and co-workers hypothesized that although cholinergic stimulus may trigger the process, GABA possibly inhibited the LC-REM-OFF neurons leading to initiation and generation of REM sleep.

To test the hypothesis, experiments were conducted on normally behaving freely moving chronic preparation of rats. Sleep-wakefulness was recorded before and after microinjection of receptor agonist or antagonist into the LC bilaterally (Mallick *et al* 2001). Cholinergic agonist was microinjected in the presence of GABAergic antagonist, or GABA was injected in the presence of cholinergic antagonist or antagonists of both GABA and acetylcholine. The results of those experiments showed that acetylcholine action in the LC was mediated through GABA for the regulation of REM sleep. It was further shown that the LC may receive GABAergic inputs from prepositus hypoglossi as well (Kaur *et al* 2001; Mallick *et al* 1999b). Based on the results of those studies we present a modified model (figure 1) of neuronal connections and interactions between the cholinergic, noradrenergic and GABAergic neurotransmitters in the LC for the generation and maintenance of REM sleep (Kaur *et al* 2001; Mallick *et al* 2001). Although the model overcomes the deficiencies of the two earlier models, it still cannot explain the mechanism of inhibition of REM-OFF neurons during wakefulness and non-REM sleep. Subsequent studies mentioned below, have helped in explaining the mechanism further.

11. Influence of waking areas on the REM-ON and REM-OFF neurons

It is known that REM sleep normally appears at a certain depth of sleep and it does not follow wakefulness in the normal condition. It was hypothesised that the wake areas of the brain might influence REM sleep related neurons (viz. the REM-ON and REM-OFF neurons) to keep off REM sleep from appearing during wakefulness. Studies were conducted on normally behaved free moving cats (Thankachan *et al* 2001) which it was observed that the brain stem reticular wake inducing area had an excitatory influence on the REM-OFF neurons and inhibitory influence on the REM-ON neurons (figure 2). These results along with other reports mentioned earlier suggest that during wakefulness, the wake-inducing area keeps the REM-OFF neurons active and the REM-ON neurons inhibited. The REM-ON neurons are kept inhibited by the wake area as well as by the REM-OFF neurons. Since the wake active neurons are inhibited during sleep (Moruzzi 1972; Oakson and Steriade 1982; Steriade and McCarley 1990) there is a withdrawal of excitation and inhibition from the REM-OFF and the REM-ON neurons, respec-

tively. It is known that REM sleep appears at a certain depth of sleep, though each sleep episode is not followed by REM sleep. It is also true that a REM sleep episode may terminate into either a sleep or a wake state. At this stage, it is not completely understood if the sleep area(s) has any role to play and if it has any active excitation on the REM-ON neurons. Our preliminary data (unpublished) suggest that at a certain depth of sleep, the sleep area excites the REM-ON neurons for the initiation of REM sleep (Mallick 1999). Thus, based on these as well as the results mentioned above, the working principle of mechanism of generation of REM sleep can be explained as given below.

11.1 *Working principle of the GABA mediated model for REM sleep generation*

During wakefulness the wake center keeps the NE-ergic REM-OFF neurons active and cholinergic REM-ON neurons inhibited. Hence, the NE-ergic LC REM-OFF neurons continues firing and releases of NE by collaterals onto themselves (Aghajanian *et al* 1977) causing the neurons to fire rhythmically. The NE-ergic projections on to the cholinergic REM-ON neurons cause an increase in NE around them. The increased NE *per se* inhibits the cholinergic REM-ON neurons (Greene and Carpenter 1985) or the inhibition could be mediated through GABA (Mallick *et al* 2001). With the onset of sleep, there is an increase in sleep related neuronal activity and a decrease in wake related neuronal activity. It is suggested that some of the sleep related neurons are GABAergic (Ali *et al* 1999; Gallopin *et al* 2000). GABAergic neurons are active during REM sleep (Maloney *et al* 1999) and GABA levels in LC increase during REM sleep (Nitz and Siegel 1997). As a consequence of increased GABA in and around LC the REM-OFF neurons decrease firing (Gervassoni *et al* 1998). This decreased activity of REM-OFF neurons causes withdrawal of inhibition from the cholinergic REM-ON neurons and at least some of them start firing, resulting in pre-REM sleep period. The cholinergic input excites both the NE-ergic and the GABAergic neurons in the LC as well as in the prepositus hypoglossi resulting in an increase in concentration of both NE and GABA in LC. This causes hyperpolarization and cessation of the REM-OFF neurons in LC. This cessation of LC-neuronal activity removes the inhibition by collaterals on itself; the system remains inhibited due to the effects of GABA. This inhibition of most of the REM-OFF neurons, if not all, will withdraw inhibition from most of the REM-ON neurons resulting in continuation of REM sleep duration (episode). The length of the REM sleep episode is deter-

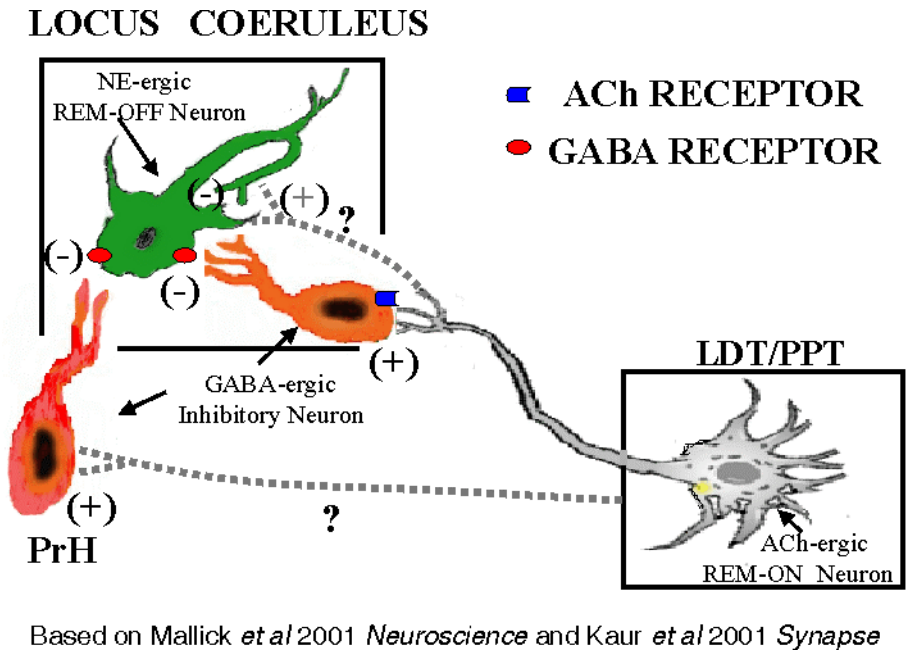

Figure 1. Diagrammatic representation of a model showing likely connections between the cholinergic REM-ON, noradrenergic REM-OFF and GABAergic neurons in the brain stem for generation and maintenance of REM sleep. An anatomically unknown connection has been represented by a broken line (ACh, acetylcholine; GABA, gama-amino butyric acid; LDT/PPT, laterodorsal tegmentum/pedunculopontine tegmentum area; NE, norepinephrine; PrH, prepossitus hypoglossus nucleus; (+), excitation; (-), inhibition).

mined by the period for which sufficient GABA is available in the system around LC (Mallick *et al* 2001). Thus, inhibition of NE-ergic neurons is the key factor for initiation of REM sleep.

This model also helps in explaining muscle atonia during wakefulness as expressed in narcolepsy that has been attributed as a REM sleep related disorder. It has been found that wakefulness inducing area inhibits the REM-ON neurons. Any disturbance in this circuitry (e.g. neural connections or neurotransmitter release or at the receptor level) that results in the REM-ON neurons becoming active during wakefulness, will produce atonia during wakefulness – a symptom of narcolepsy. This is also supported by the implication of REM-ON neurons in REM sleep associated atonia (Sakai 1988). Since narcolepsy is a genetic disorder (Guilleminault 1989), it is possible that the gene responsible for narcolepsy might have a negative role to play in the organization of the neural connections between the wake neurons and REM-ON neurons.

12. REM sleep deprivation studies

It is evident from the above that the NE-ergic REM-OFF neurons must cease firing for the initiation and maintenance of REM sleep. As a corollary, it may be said that continuous activity of those neurons will lead to REM sleep deprivation, and there should be continuous activity of those neurons at that time. These propositions may be supported by the fact that NE-ergic REM-OFF neurons continued firing on REM sleep deprivation (Mallick *et al* 1989) and activation of LC-neurons stimulated a REM sleep deprivation like condition (Singh and Mallick 1996). Hence, it was logical to hypothesize that after REM sleep deprivation, there should be an increase in NE *per se*, or factors that would increase NE in the brain, the increased NE should play a significant role in the physiological manifestations of expressions associated with REM sleep deprivation.

The metabolism of NE remained elevated (Pujol *et al* 1968) and NE turnover increased after REM sleep depri-

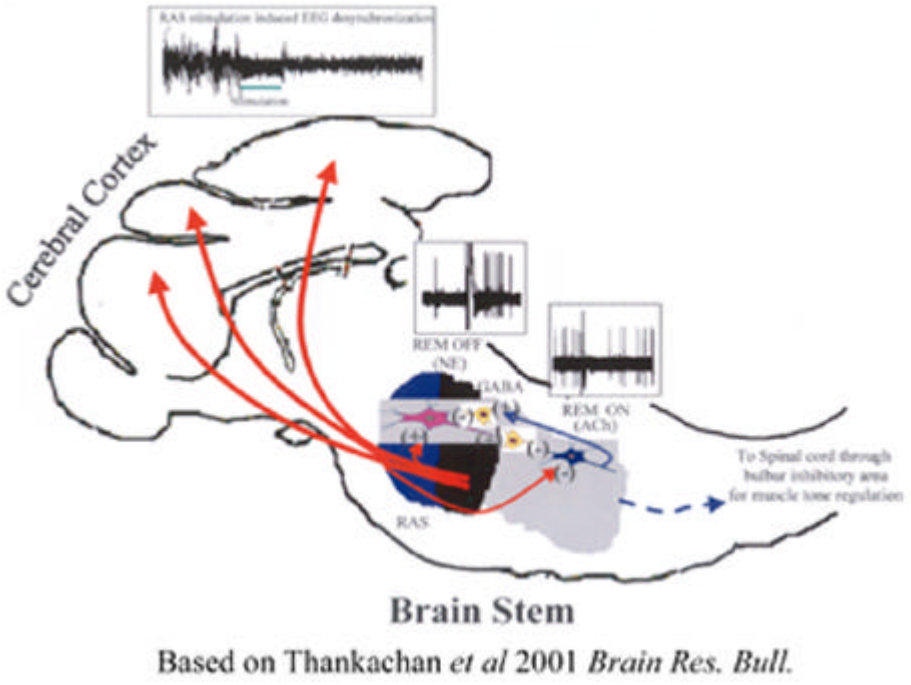

Figure 2. The stimulus bound overlapped responses of a cholinergic REM-ON neuron (blue) and a norepinephrinergic REM-OFF neuron (pink) to stimulation of reticular activating wakefulness inducing area (RAS) stimulation are also shown in insets. The GABAergic neurons are shown in yellow. The upper inset shows that high frequency stimulation of the RAS (green bar) induced desynchronization of the cortical EEG that outlasted the period of stimulation. The REM-ON neurons were inhibited, while the REM-OFF neurons were excited by RAS stimulation. It is unlikely that the same neuron in the RAS would excite REM-OFF neurons, while inhibiting the REM-ON neurons. Although the inter-neural connections and their mechanism of action for such opposite responses need to be studied, it is being suggested that an inhibitory neuron is possibly involved in the inhibition process. The abbreviations are as in figure 1.

vation (Porkka-Heiskanen *et al* 1995). Activity of monoamine oxidase-A (MAO-A), an enzyme responsible for NE degradation, decreased after REM sleep deprivation (Thakkar and Mallick 1993). This suggested that there would be an increase in NE after REM sleep deprivation. Tyrosine hydroxylase (TH) catalyzed reaction is the rate-limiting step in the pathway of NE biosynthesis. There was an increase in TH-activity and its mRNA levels after REM sleep deprivation (Porkka-Heiskanen *et al* 1995; Sinha *et al* 1973). In another study, there was a significant increase in TH-mRNA and NE-transporter mRNA after three and five days of REM sleep deprivation (Basheer *et al* 1998). After increased waking (i.e. reduced sleep, including reduced REM sleep) several transcription factors were expressed at higher levels than that during sleep. This increased expression was found to be regulated by LC, the primary site for providing NE in the brain (Cirelli *et al* 1996). Furthermore, Cirelli (2002) in her recent review, suggested that since increased wakefulness (i.e. reduced sleep including REM sleep) induced an increase in arylsulfotransferase, an enzyme responsible for catabolism of catecholamines, the role of sleep is to interrupt continuous activity of the catecholaminergic system in the brain. These results indirectly suggested that REM sleep deprivation increased NE in the brain.

The above mentioned studies were also supported more directly by a recent report where levels of NE were estimated during REM sleep or its deprivation. The levels of NE increased in different regions of the brain after 72 h of REM sleep deprivation, however there was a slight decrease in NE concentration after 24 h of deprivation (Porkka-Heiskanen *et al* 1995). Also, it was reported recently that NE concentration in the pons and amygdala decreased gradually from spontaneous waking, to slow sleep, to REM sleep stages (Shouse *et al* 2000). An increased concentration of NE in the urine has been correlated with increased sleep onset REM sleep latency and a decrease in REM sleep (Netzer *et al* 2001). After REM sleep deprivation experiments in rats, it was seen that NE level in the blood increased (Bergmann *et al* 1989). Thus, although it was shown that NE decreased during REM sleep and it increased during REM sleep deprivation, whether the increase in NE during REM sleep deprivation was the cause of associated changes in physiological parameters needed to be confirmed.

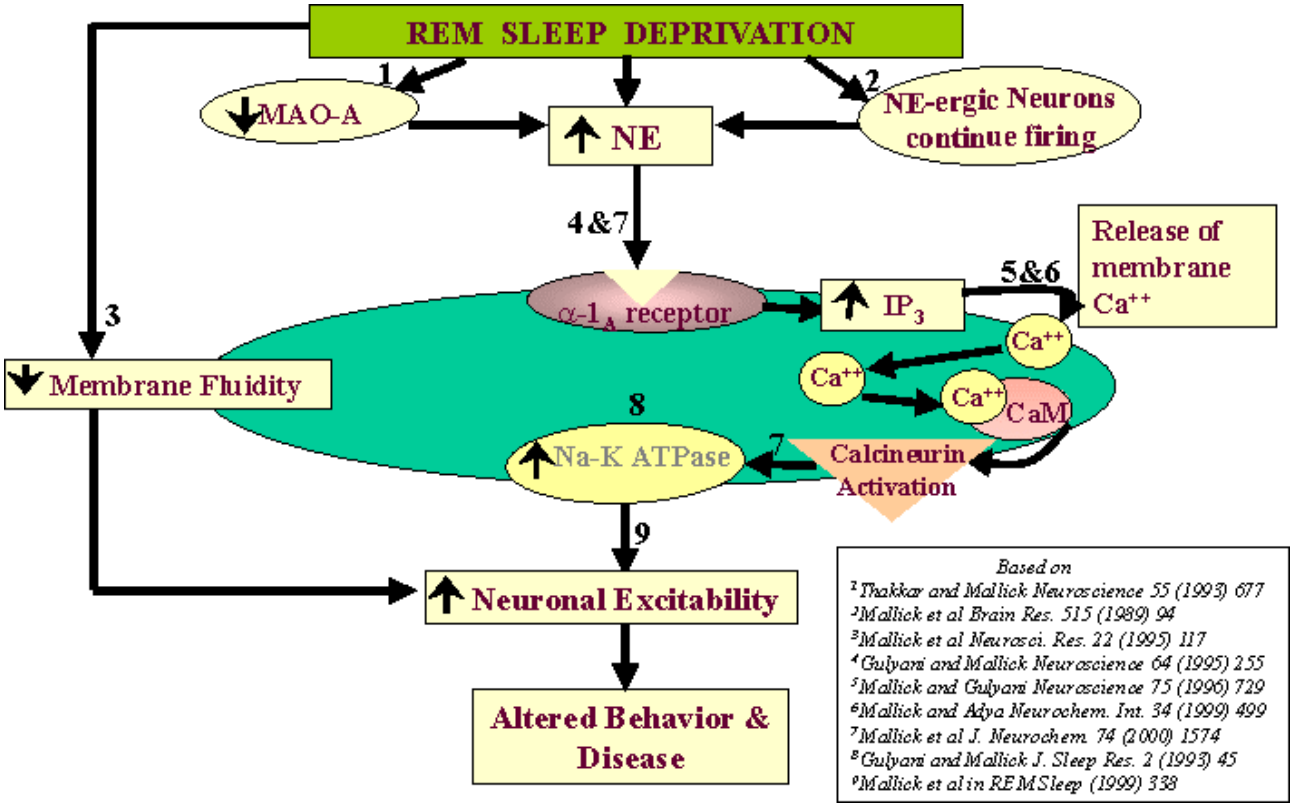

Figure 3. The figure represents the cellular mechanism of REM sleep deprivation induced alterations in neuronal excitability that in turn might lead to behavioural disorders.

13. REM sleep deprivation associated change is mediated by increased NE

REM sleep deprivation is reported to increase aggressiveness, sexuality, excitability, loss in memory consolidation, etc. (Vogel 1975; Gulyani *et al* 2000). It was hypothesised that one of the functions of REM sleep was to maintain the excitability status of the brain and, consequently, it was proposed that deprivation was likely to affect a factor that was crucial to maintain brain and neuronal excitability (Mallick *et al* 1994, 1999a). Since Na-K ATPase is a key enzyme that maintains neuronal excitability it was hypothesised that REM sleep deprivation might affect the enzyme. Experiments with rats showed that REM sleep deprivation was found to increase Na-K ATPase (Gulyani and Mallick 1993) and Mg-ATPase (Mallick and Gulyani 1993) activities in the rat brain. Further, the increase in Na-K ATPase activity was mediated by NE acting through α1-A adrenoceptors (Gulyani and Mallick 1995; Mallick *et al* 2000). It was also observed that REM sleep deprivation reduced calcium concentration in the synaptosome (Mallick and Gulyani 1996) and the deprivation-induced increase in NE released the membrane bound calcium (Mallick and Adya 1999). The calcium then activated calmodulin and increased the Na-K ATPase activity, possibly by dephosphorylation of the enzyme (Mallick *et al* 2000) (figure 3). Notwithstanding, it has further been shown that after REM sleep deprivation the NE-induced increase in Na-K ATPase activity was an uncompetitive stimulation phenomenon (Adya and Mallick 2000). We propose that after REM sleep deprivation, there will be increased synthesis of Na-K-ATPase molecules. Additionally, since REM sleep deprivation was reported to decrease membrane fluidity (Mallick *et al* 1995), it was hypothesised that it might affect neuronal membrane lipid peroxidation. Our preliminary data (unpublished) shows that after REM sleep deprivation, there was a decrease in neuronal membrane lipid peroxidation and this was also mediated by NE acting through α1 adrenoceptor.

14. Conclusion

In conclusion, the results so far suggest that the NE in the brain prevents generation of REM sleep; REM sleep deprivation increases NE in the brain. This increased NE is responsible for REM sleep deprivation associated increase in Na-K-ATPase activity in the brain. This increased Na-K-ATPase activity causes changes in neural and brain excitability leading to alterations in behaviour (figure 3). Nevertheless, although REM sleep deprivation increases NE as well as Na-K ATPase activity and decreases membrane fluidity and lipid peroxidation, the causal relation between these changes are unknown. Further, investigations are in progress to understand this at the cellular and molecular levels.

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