Review Article

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Neural mechanism of rapid eye movement sleep generation with reference to REM-OFF neurons in locus coeruleus

Dinesh Pal & Birendra Nath Mallick

School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

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The noradrenergic (NA-ergic) rapid eye movement (REM)-OFF neurons in locus coeruleus (LC) and cholinergic REM-ON neurons in laterodorsal/pedunculopontine tegmentum show a reciprocal firing pattern. The REM-ON neurons fire during REM sleep whereas REM-OFF neurons stop firing during REM sleep. The cessation of firing of REM-OFF neurons is a pre-requisite for the generation of REM sleep and non-cessation of those neurons result in REM sleep loss that is characterized by symptoms like loss of memory retention, irritation, hypersexuality, *etc*. There is an intricate interplay between the REM-OFF and REM-ON neurons for REM sleep regulation. Acetylcholine from REM-ON neurons excites the GABA-ergic interneurons in the LC that in turn inhibit the REM-OFF neurons. The cessation of firing of REM-OFF neurons withdraws the inhibition from the REM-ON neurons, and facilitates the excitation of these neurons resulting in the initiation of REM sleep. GABA modulates the generation of REM sleep in pedunculopontine tegmentum (PPT) by acting pre-synaptically on the NA-ergic terminals that synapse on the REM-ON neurons whereas in LC it modulates the maintenance of REM sleep by acting post-synaptically on REM-OFF neurons. The activity of REM sleep related neurons is modulated by wakefulness (midbrain reticular formation/ascending reticular activating system) and sleep inducing (caudal brainstem/medullary reticular formation) areas. Thus, during wakefulness the wake-active neurons keep on firing that excites the REM-OFF neurons, which in turn keeps the REM-ON neurons inhibited; therefore, during wakefulness REM sleep episodes are not expressed. Additionally, the wakefulness inducing area keeps the REM-ON neurons inhibited. In contrast, the sleep inducing area excites the REM-ON neurons. Thus, the wakefulness inducing area excites and inhibits the REM-OFF and REM-ON neurons, respectively, while the sleep inducing area excites the REM-ON neurons that facilitate the generation of REM sleep.

Key words GABA - locus coeruleus - noradrenaline - pedunculopontine tegmentum - REM sleep

As with most of the great discoveries in experimental science, the discovery of rapid eye movement (REM) sleep was more an act of serendipity than a concerted effort to look for something as complex and astonishing as REM sleep. While working on humans towards the quantification of eye movements as an index of brain activity, Aserinsky and Kleitman[1] stumbled upon a stage within the state of sleep during which the behavioural observation and electro-oculographic recordings showed rapid, jerky and binocularly symmetrical eye movements with low voltage fast waves in the electroencephalogram (EEG). Increased respiration and heart rate were associated with this electrophysiologically identified state. Additionally, the subjects reported vivid dreams,

if awoken during this stage of sleep. Furthermore, in humans this state repeated itself several times within sleep at an approximate interval of 90 min[2]. Subsequently a similar state was identified in cats[3]. Jouvet and Michel[4] in cats and Berger[5] in humans later showed that complete loss of muscle tone in the anti-gravity muscles forms one of the hallmarks of this stage of sleep.

Aserinsky and Kleitman[1] coined the term"REM sleep" because of the presence of rapid eye movements during this unique phase of sleep while Dement[3] used the term "active sleep" to define the same state because the EEG pattern resembled that of an active awake state. Rapid eye movements, low voltage fast waves in the EEG and increased respiration as well as heart rate indicate a behaviourally aroused state, whereas high voltage slow waves in the EEG and significantly reduced muscle tone along with reduced respiration and heart rate are characteristic of deep sleep state. On the basis of co-expression of such behavioural features belonging to mutually exclusive states of consciousness, Jouvet and his colleagues termed this state as "paradoxical sleep"[6]. The REM sleep state is also known as "dream sleep" because 70-80 per cent of awakenings from this state were accompanied by dream recollections. In contrast, the subjects reported either absence of dream or recollection of a fragmentary and thought-like mention, when awakened from non-REM sleep[7]. Although description of a similar behavioural state finds mention in the ancient Greek (*Virgil's Aeneid*, 19 BC) and Hindu Vedic literatures (*Mandukya Upanishad*, 400 BC-200 BC), classification of such a sleep state on the basis of objective electrophysiological criteria was made possible only in the mid-twentieth century. The period following the discovery of REM sleep was marked by an ever-increasing interest in the subject. However, it was in the mid-1960s that a virtual explosion in the field of REM sleep research was observed, which with the aim of unraveling the mechanism of REM sleep generation as well as its function continues with a greater vigor today[8].

REM sleep has been objectively identified and quantified in many species including humans[1,9], cats[3,10], monkeys[11] and rats[12]. REM sleep duration has a positive correlation with the total sleep time (REM sleep + non-REM sleep) and the latter has a negative correlation with body size. Thus, larger animals spend less time in non-REM sleep and consequently experience less REM sleep, *e.g.*, rat, chimpanzee and cow spend about 14, 10 and 4 h respectively in total sleep per day that includes 2.0, 1.5 and 0.76 h of REM sleep, respectively[13]. It has also been found that predators (such as cats) are usually good sleepers as compared to prey species (*e.g.*, rabbit) with REM sleep occupying 15 per cent or more time of the total time spent in sleep[14].

REM sleep: sleeping body-waking mind?

REM sleep is a unique phenomenon marked by indices of highly active brain while the subject/animal experiences probably the deepest phase of rest and sleep. The EEG pattern and the eye movements that occur during REM sleep are apparently similar to that observed during wakefulness. The autonomic tone increases resulting in fast and irregular respiration, increased heart rate and elevated brain temperature. The brain glucose metabolism increases significantly in the thalamus, the limbic system and the pontine reticular formation during REM sleep as compared to quiet wakefulness[15]. The neuroimaging studies have provided vital evidence showing that there is indeed increased activity in specific brain areas during REM sleep as compared to non-REM sleep and functional changes occur in extensive neuronal circuitry across the sleep-wake cycle. It was shown that non-REM sleep is characterized by a widespread decline in the activity in association-cortex of the frontal, parietal and temporal lobes, as well as in the thalamus, dorsal pons, mesencephalon, cerebellum, basal ganglia and basal forebrain[16,17]. A relative decrease in the activity during REM sleep occurs in dorso-lateral prefrontal cortex, parietal cortex, posterior cingulate cortex and precuneus. REM sleep is associated with an increase in the function of the limbic and paralimbic cortex relative to waking. It is also associated with a significant increase in the activity of pontine tegmentum, thalamic nuclei, amygdaloid complexes, hippocampus, anterior cingulate cortex and the temporo-occipital areas of the posterior cortices[16,17].

Is REM sleep of recent phylogenetic origin?

Basic rest-activity cycle (BRAC), which also follows circadian rhythmicity, can be found ubiquitously across the animal species through their life; sleep-wakefulness is a modified form of BRAC. The EEG is characteristically found in species with neocortex or well-developed brain. The EEG being one of the primary criteria to identify REM sleep, severely restricts the number of animal species in which REM sleep can be studied because a fully developed brain is the characteristic of higher order animals in the evolutionary ladder. A comparative study across the animal kingdom

shows that REM sleep is definitely present in mammals[18]. Avians also exhibit a similar state, though the average duration of REM sleep-like episodes (often less than 10 seconds) and the total percentage of time spent in such a state is less as compared to mammalian REM sleep (about 5% of the total sleep time as compared to 15-30 % in mammals)[19]. Further, unlike mammalian REM sleep, there is no rebound increase in the REM sleep-like state in birds following its deprivation suggesting that REM sleep-like state in birds may not be strongly homeostatically regulated[20-22]. Among the mammals, humans along with some other laboratory animals like cats and rats are few of the most studied species. Together these form a miniscule number of species studied from among more than 4000 extant mammalian species. There is no report of the presence of REM sleep or a similar state in fish, amphibians and reptiles though a non-REM sleep-like state has been reported in these[18].

There are three extant groups of mammals, namely, monotremes (echidna, platypus), marsupials (opossum, koala, and kangaroo) and placentals (human, ape, dog, rat, whale). Sleep is a universal phenomenon among mammals and REM sleep is reported in most placental mammals barring cetaceans (whale, dolphin). Allison *et al*[23] reported that echidna (a monotreme mammal) lacks REM sleep. However, Siegel *et al*[24], on the basis of unit activity recorded from the brainstem reticular formation reported the presence of certain characteristic features associated with REM sleep state in echidna. Presence of a REM sleep-like state has also been reported in platypus, which is another monotreme mammal[25]. Among the cetaceans that include dolphin and whale, although there is no definitive evidence of REM sleep-like state, presence of muscle jerks and eyelid movements during the resting phase indicate that some form of a REM sleep-like state might be present in them as well[26]. Significantly, it has been recently shown that whale and its newborn offspring do not sleep for about a month postpartum[27]. Monotremes diverged from the placental and marsupials very early in mammalian evolution. The reported absence of REM sleep-like state in monotremes supported the hypothesis that REM sleep appeared late in the mammalian evolution. However, the recent studies that demonstrated the presence of REM sleep-like state in the monotremes imply that REM sleep has much older evolutionary origin than was previously thought[24,25].

REM sleep – Ontogenetic precursor or companion of slow wave sleep?

The presence of REM sleep in neonates and infants has become a hotly debated issue in recent times. The development and appearance of non-REM and REM sleep is also a matter of intense investigations with no consensus in sight in near future. The altricial mammals (those born immature, *e.g.*, rat, cat, human) have much higher amounts of REM sleep at birth and during the early years of development as compared to that present during adult stages[28]. In precoccial mammals (those born mature, *e.g.*, cow, guinea pig, horse) the REM sleep percentage at the time of birth and during development is low as compared to the altricial mammals[28]. The altricial mammals show a progressive reduction in the amount of REM sleep with age[29]. The precoccial mammals also show a similar decrease in REM sleep but to a considerable lesser extent[28]. Thus, conventionally, it has been argued that in altricial mammals neonatal/immature form of REM sleep (also called as active sleep) and slow wave sleep (also called as quiet sleep) are the "precursors" of REM and non-REM sleep, respectively, in adults[28,29]. Thus, the maturation of REM and non-REM sleep from active and quiet sleep, respectively, is simply an orderly addition of behavioural and neurophysiological components that characterize the mature forms of REM and non-REM sleep found in adults.

The validity of this "precursor theory of sleep" has become a hotly debated issue recently[30-33]. Frank and Heller contended that early neonatal REM sleep (active sleep) is not truly REM sleep but is an undifferentiated state from which both the REM sleep as well as non-REM sleep develope[31]. It was proposed that all mammals exhibit a period of spontaneous, dissociated foetal activity called "pre-sleep" which is extended as behavioural active and quiet sleep in the post-natal period[30,31,34].

The contention of Frank and Heller[31] has been challenged by Vogel and Feng[32] and Blumberg *et al*[33]. Vogel and Feng[32] found no evidence of an undifferentiated state with both REM sleep and slow wave sleep processes [termed as "half activated paradoxical sleep (or REM sleep) state" (Frank *et al*[31])] in neonatal rats studied polysomnographically. According to Blumberg's group[33], sleep is reliably characterized by the presence of tonic (*i.e.*, muscle atonia) as well as phasic (*i.e.*, myoclonic twitching) components in infant rats that characterize different stages of sleep. Also, the neural substrates of infant sleep are strikingly similar to those of adults although the neural circuitry underlying these components is not completely known[35]. Further, as early as day two postnatal, myoclonic twitching can be seen occurring

exclusively in conjunction with muscle atonia, which indicates that electrophysiological parameters can be used to characterize different stages of sleep[35]. Therefore, Blumberg *et al*[33] have contended that "the pre-sleep theory, at least in its present form, does not accurately reflect the phenomenology of infant sleep" and thus, at the moment the ontogenetic development of REM sleep in mammals is a debatable issue.

Architecture of REM sleep

The tonic events associated with REM sleep includes desynchronization of the cortical EEG, theta rhythm in the hippocampus and muscle atonia. These events continue uninterrupted throughout the duration of REM sleep. As long as these events continue, the episode is termed as REM sleep. The phasic events associated with REM sleep appear intermittently within a REM sleep episode every 16-120 sec that last 2-9 sec[1]. It is characterized by bursts of rapid eye movements and middle ear movements, along with twitches in the muscles of the face and that of the extremities. The ponto-geniculo-occipital (PGO) spikes/waves appear in phases usually in bursts, a few seconds prior to the start of REM sleep. The other phasic events are fluctuations in the body temperature and heart as well as respiratory rates.

Tonic components

Cortical EEG desynchronization : The EEG is a record of the temporal and spatial summation of post-synaptic field potentials generated by a large population of cortical neurons, the main contribution for which comes from the large pyramidal neurons in the cortex. The basic substrate for EEG generation is the interactions between the cortical pyramidal, thalamocortical principal relay and the thalamic reticular neurons[36,37]. The thalamic reticular neurons are a group of GABA-ergic neurons interposed between thalamus and cerebral cortex surrounding the antero-dorsolateral thalamus. These thalamic reticular neurons receive excitatory glutamatergic projections from cortex and inhibitory cholinergic projections from laterodorsal/ pedunculopontine tegmentum (LDT/PPT) and basal forebrain[38]. The thalamo-cortical principal relay neurons are glutamatergic and form reciprocal neuronal connections with GABA-ergic thalamic reticular neurons[38].

Depending on the functional state of the brain, thalamocortical principal relay neurons operate in tonic/ relay and burst/oscillatory firing modes[39]. The tonic mode is associated with the processing of sensory information under conditions of active vigilance during which the thalamus relays afferent sensory information to the cortex and discharge with tonic firing. Thus, during waking, thalamic and cortical neurons fire in a tonic or relay mode implying a sustained and high spontaneous activity[40]. This variable discharge pattern with a low synchronization between neurons is recorded as small but irregular and heavily fluctuating waves in EEG. This is the desynchronized EEG pattern.

The burst/oscillatory mode of the thalamocortical principal relay neurons leads to EEG synchronization through the generation of rhythmic discharge pattern that result in the appearance of spindles. The appearance of sleep spindles (12-14 Hz waves with waxing and waning amplitude) on a background of intermediate frequency and low-amplitude EEG signals conventionally marks non-REM sleep onset. The thalamic reticular neurons act as spindle pacemaker and are primarily involved in spindle generation[38]. Berger[41] first described spindle, while Loomis *et al*[42] introduced the term sleep spindle.

The switching between the tonic/relay and burst/ oscillatory mode occurs because of the inactivation and activation of low threshold calcium channels present in thalamocortical principal relay neurons. The low threshold calcium channels (low threshold or low membrane voltage for opening) conduct calcium current when the neuron is in a hyperpolarized state but are inactivated by depolarization of the neuron. The hyperpolarization of thalamocortical principal relay neurons by the thalamic reticular neurons activates these calcium channels, which then conduct a calcium current driving the neuron's membrane potential towards a more depolarized state[43]. In this situation the neurons respond to brief depolarization by producing a burst of action potentials. The firing of neurons in burst mode leads to opening of more calcium channels that raises the intracellular calcium levels, which ultimately trigger a calcium-activated potassium current. The calcium-activated potassium current hyperpolarizes the neuron, which results in a pause after the burst of action potentials. The hyperpolarization by potassium current prepares the neuron for next cycle of burst firing and subsequent pause by activating the low threshold calcium channels. The transition to desynchronized EEG as observed during waking or REM sleep is associated with maintained depolarization of thalamocortical principal relay neurons, which causes inactivation of low threshold calcium channels resulting in production of the single action potentials instead of burst discharges.

The depolarization of thalamocortical principal relay neurons is a result of inhibition of the inhibitory thalamic reticular neurons by cholinergic inputs from LDT/PPT and basal forebrain[43].

The thalamocortical principal relay neurons are also involved in the generation of delta waves, which are slow-frequency (0.5-4 Hz) high amplitude waves that occur during deeper non-REM sleep states[43]. These high voltage low frequency waves become manifest when thalamocortical principal relay neurons undergo hyperpolarization to about –70mV till –90mV. The delta waves have large amplitude, which implies that extended populations of neurons fire rather synchronously, interspersed with prolonged hyperpolarization. In contrast to spindles, these waves are not rhythmical but highly irregular[39,44]. Therefore, while spindle oscillations are generated in the thalamic reticular nucleus, they are synchronized by the glutamatergic thalamocortical neurons[45]. Thus, the progressive hyperpolarization of the membrane potential switches the state of thalamocortical neurons from the 'tonic mode' characteristic of wakefulness and REM sleep into a 'spindle mode' and ultimately into a 'delta mode' of non-REM sleep[36,46].

Moruzzi and Magoun[47] were the first to demonstrate the involvement of medial aspects of the brainstem reticular formation in the induction and maintenance of cortical desynchronization. Later, it was shown that nucleus pontis caudalis (NPC)[48] as well as nucleus pontis oralis (NPO)[49] are involved in cortical activation. The activities of the locus coeruleus (LC) noradrenergic (NA-ergic) neurons have been causally and positively correlated to behavioural and EEG indices of arousal[50-52] as defined by cortical low voltage and fast EEG waves (desynchronization). Also, the PPT neurons through their projections to the intralaminar and midline thalamic nuclei function as part of the nonspecific activating system responsible for cortical desynchronization and regulation of the sleep-wake cycle[53]. EEG desynchronization occurs during wakefulness as well as REM sleep and the NA-ergic neurons in LC and the cholinergic neurons in LDT/PPT are known to be responsible for EEG desynchronization through their projections to thalamus. However, detailed analysis of the EEG desynchronization during wakefulness and REM sleep is needed as it has been reported that there are separate groups of neurons in the pontine region responsible for EEG desynchronization during wakefulness and REM sleep[54]. Significantly, it has also been shown that noradrenaline and acetylcholine (ACh) activate different frequency of waves in the EEG[55].

Hippocampal theta rhythm : The theta rhythm recorded from the hippocampus is the largest signal (1-2 mV) that can be recorded in the normal EEG of mammalian brain[56]. Theta rhythm appears selectively during exploratory and active behavioural wakefulness and during REM sleep[56] but is absent in immobile animal[57]. In behaving rats, it occurs as a sinusoidal oscillation of 3-12 Hz[57]. In addition to the hippocampal formation, theta waves have been recorded from several other structures in the brain, including the subicular complex, entorhinal cortex, perirhinal cortex, cingulate cortex and amygdala[58]. Theta oscillation is most regular in frequency and largest in amplitude in the structure lacunosum-moleculare of the hippocampal CA1 region. Despite similar cytoarchitecture of the CA1 and CA3 regions of the hippocampus, the extracellular theta currents in the CA3 region are considerably smaller than in the CA1. Although these structures are the main current generators of the extracellularly recorded theta waves, none of these cortical structures is spontaneously (autochthonous) active and capable of generating theta rhythm on their own[58].

The theta waves can be triggered by electrical stimulation of the brainstem reticular formation and NPO is supposed to be one of the primary generators for such waves[59,60]. The NPO possesses cholinoceptive neurons[61] and receives cholinergic projections from the PPT[62]. It has been shown that temporary inactivation of PPT results in the suppression of theta rhythm[63] indicating that the functional integrity of PPT is critical for the occurrence of hippocampal synchronization at theta frequency and thus PPT is also one of the important sites in the brainstem reticular formation for theta generation[63]. The serotoninergic neurons of the median raphe have also been shown to be involved in the regulation of hippocampal waves[56]. Presence of neurons in the median raphe showing concurrent increase (theta-on) and decrease (theta-off) in their activity in association with theta waves has been reported. These neurons mutually interact for the regulation of theta frequency in the hippocampal EEG. The activation or inhibition of the theta-on cells has been proposed to generate or block the hippocampal theta activity, respectively[64].

Muscle atonia : Muscle atonia is one of the main characteristic identifying features of REM sleep[1,65]. The pontomedullary neuronal networks are involved in the regulation of muscle tone. Stimulation and lesion studies have shown that there are two muscle tone regulatory centres in the pontomedullary region. The first one is

the pontine inhibitory area (PIA) extending from NPO to the rostral portion of NPC[66-69]. The other inhibitory centre, the medullary inhibitory area that was first identified by Magoun and Rhines[70], is present in the ventromedial medulla and includes the nucleus gigantocellularis (NGC), nucleus magnocellularis (NMC) and nucleus paramedianus (NPM)[69,71-73].

The PIA sends projections to NGC[74], NMC[71,75] and NPM[76] of the medullary region, which in turn project to the spinal cord[72,75,77] to hyperpolarize motoneurons to control the spinal circuits for inducing atonia during REM sleep[78,79]. The PIA excites neurons in ventromedial medulla, which in turn induce glycine-mediated hyperpolarization of the spinal and hypoglossal motoneurons[71,78-80] resulting in muscle tone suppression. In addition, there are direct projections from the pontine region to the spinal cord[81]. The NMC in rostral medulla was found to be activated by glutamate while NPM in the caudal medulla was activated by acetylcholine[71]. Further, it was found that release of glutamate in NMC (rostromedial medulla)[82] and acetylcholine in NPM (caudomedial medulla)[83] increased during REM sleep. The PIA receives cholinergic inputs from LDT/PPT[84] and medulla[85,86] and glutamatergic inputs from the rostral brainstem and medullary inhibitory area[87]. Carbachol injections into these sites induced atonia in decerebrate cats[88] and REM sleep in freely moving rats[89,90]. Further, the acetylcholine levels in the pons increased during REM sleep[91]. Thus, there is an active interaction between neurons in the pons and medulla for the maintenance of muscle tone during waking and suppression of muscle tone during REM sleep. An intact ponto-medullary connection seems to facilitate muscle atonia[92] while there was complete absence of muscle atonia in chronic preparation of cats transected between pons and medulla[93]. A two-way interaction has been proposed between the medulla and pons is crucial for the control of muscle tone[94].

The REM-OFF and the REM-ON neurons in the ponto-medullary region are directly involved in the regulation of muscle tone. The REM-ON neurons in LDT/PPT project to ponto-medullary reticular formation[84,95]. Destruction of the cholinergic neurons in LDT/PPT resulted in loss of both REM sleep and muscle atonia[96], while electrical stimulation of these neurons increased REM sleep[97] and suppressed muscle tone[98,99]. The REM-OFF neurons increase their activity just prior to the return of muscle tone during transition from REM sleep to wakefulness[100]. The activation of these neurons does not allow initiation of REM sleep and it has been shown that these neurons cease firing during cataplexic attacks[101]. The inhibition of NA-ergic neuronal activity in the LC also causes muscle tone suppression[102]. Further, the activation of pontine and medullary inhibitory regions produced a co-ordinated reduction in the activity of the LC neurons and neurons located in the midbrain locomotor region related to muscle tone facilitation[103]. The LC also has strong projections to the spinal cord, which exert facilitatory effect on the motoneurons and the reduction of muscle tone could result from the withdrawal of this facilitatory effect on the α-motoneurons[104]. This view may be supported by the fact that mild continuous activation of LC neurons decreased REM sleep[105]. Thus, the decrease in LC neuronal activity causes an increase in the pontine cholinoceptive neuronal activity, which in turn increases the firing of the medullary inhibitory neurons producing muscle atonia.

Phasic components

Rapid eye movements (REMs) **:** Dement and Kleitman[2] described the eye movements occurring during REM sleep as binocularly synchronous and apparently similar to waking fixational eye movements occurring with similar velocities in all directions. The muscle tone of the extra-ocular muscles was found to be at its lowest levels during non-REM sleep with some of the muscles becoming completely atonic[106]. REM sleep is accompanied by return of the muscle tone similar to that observed during wakefulness. The REMs during REM sleep are generated in the pontine reticular formation and are closely linked to PGO waves[107]. Some studies reported no difference between REMs during wakefulness and REM sleep whereas other studies reported that in monkeys[108] and humans[109], the REMs occurring during REM sleep were slower than those occurring during wakefulness. There is no strict relationship between amplitude and frequency of REMs during REM sleep[110]. The differences in the eye movement during wakefulness and REM sleep suggest that they possibly have different mechanism of generation[111] and furthermore, that REMs and PGO waves may have a common neuronal generator[112].

Ponto–geniculo-occipital (PGO) waves : PGO waves are the field potentials generated in the pontine region from where they propagate to the occipital cortex through lateral geniculate nucleus (LGN). In addition to the pons[113], LGN[114] and occipital cortex[107], these waves have been recorded from thalamus, cortex, oculomotor nuclei, cerebellum, amygdala, cingulate gyrus and hippocampus. For experimental purposes the PGO

waves have mostly been recorded in cats, though a PGO wave–like activity has been reported in other mammalian species including humans[115], non-human primates[116,117] and rodents[118]. These are biphasic waves with duration of 60-120 ms, amplitude of 150-300 μV[118,119] and show spikes having both single as well as burst firing pattern (3-5 waves/burst). The single spikes, known as type-I PGO waves, occur mostly during non-REM sleep and are independent of the occurrence of rapid eye movements[120]. The burst firing mode of PGO waves, also known as type-II PGO waves, are associated with rapid eye movements and occur during REM sleep with the spike density ranging from 30-60 spikes/min[119,120]. The caudolateral peribrachial area (C-PBL) in the cat[121] and the subcoeruleus area in the rat[122] constitute the neuronal machinery required for triggering the PGO waves. After generation, these waves are transferred to higher brain structures by LDT/PPT neurons[122,123]. The generation of PGO waves is modulated by monoaminergic (from LC and dorsal raphe), cholinergic and nitric oxide ergic (both from LDT/PPT) and GABA-ergic (from substantia nigra pars reticulata) neuronal groups. The NA-ergic LC and serotoninergic dorsal raphe neurons are active during wakefulness and non-REM sleep and cease firing during REM sleep, thereby, inhibiting the generation of PGO waves during wakefulness while allowing their appearance during REM sleep[122]. The cholinergic, NO-ergic and GABA-ergic neuronal groups have an excitatory effect on the PGO waves triggering neurons and facilitate the generation of PGO waves[122]. Integrity of PPT is required for the occurrence of phasic events related to REM sleep including PGO[124].

Respiratory rate : The breathing becomes fast and irregular during REM sleep, which persists through hypoxia, hypercapnia and metabolic alkalosis suggesting a strong overriding influence of complex neurogenic mechanism(s) over chemical feedback control[125,126]. The loss of muscle tone during REM sleep significantly reduces the activity of intercostal muscles without affecting the muscle tone of the diaphragm, which may even be increased to compensate for intercostal muscle loss. There is increased resistance in the upper respiratory pathway contributing to a decreased efficiency of the ventilatory effort leading to mild hypoxaemia[126], which when increased to pathological proportions can cause obstructive sleep apnoea syndrome.

Temperature regulation : The increase and decrease in the brain temperature is modulated by circadian rhythm[127]. The body and brain temperatures are causally related to each other as any shift in one brings about homeostatic changes in the other. Parmeggiani and Rabini[128] first described the disturbance or loss of thermoregulatory control during REM sleep. The peripheral thermoregulatory expressions such as sweating, shivering and panting are largely suspended[129] with the body temperature showing a tendency to reach the ambient temperature. The loss in the peripheral sympathetic control leads to an increased heat exchange between the skin and the environment strongly influencing the blood temperature. Therefore, if the ambient temperature during REM sleep is low then the body temperature falls and if it is high, the body temperature rises[127]. However, it has also been reported that the brain temperature rises during REM sleep irrespective of the ambient temperature[130,131]. The rise in brain temperature has been attributed to the increased intracranial blood flow and the occurrence of REM sleep has been positively correlated with the decrease in core temperature during slow wave sleep[127]. However, the body temperature falls during REM sleep deprivation[132,133]. Although the detailed and precise mechanism of such changes in body temperature during REM sleep and its deprivation are unknown, it has been suggested that withdrawal and increase in the levels of NA in the brain during normal REM sleep and its deprivation, respectively, play a critical role for such body temperature modulation[134]. The role of NA in thermoregulation in relation to REM sleep and its deprivation may be supported by the fact that it induces hypothermia by acting on α-1 adrenoceptors in the preoptic area[135] where such receptors have been identified on the thermosensitive neurons[136].

Autonomic nervous system : During tonic REM sleep parasympathetic activity increases, which is primarily because of a decrease in sympathetic activity. The phasic REM sleep exhibits an increased sympathetic as well as parasympathetic tone resulting in dilation of the pupil, increased heart rate and blood flow to most of the brain regions[137].

Mapping the brain areas responsible for the regulation of REM sleep

Initial experimental studies to localize the brain area(s) responsible for the phenomenon of sleep-wakefulness employed transection, electrical as well as chemical lesion and stimulation techniques[138]. A transection is a complete separation of one brain region from another. A lesion is a localized destruction, while stimulation is activation of a group of neurons by

electrical or chemical means. The assumption underlying the transection and lesion experiments is that if a normal manifestation, behavioural or otherwise, of a living organism continues to be expressed unaltered even after the destruction of certain brain area(s), the damaged area of the brain is unlikely to be essential for normal manifestation of the function under consideration, while stimulation of an area in the brain is supposed to exaggerate the specific function of that brain area in particular. These techniques were also employed for the identification of brain areas involved in the regulation of REM sleep.

Spinal cord does not play any role in REM sleep regulation : As has been mentioned earlier, the discovery of REM sleep in humans was followed by the identification of this phenomenon in animals[3]. The discovery of REM sleep in animals (cat) paved the way to design experiments for understanding the mechanism of its regulation. After the discovery of REM sleep, several unexplained and largely ignored observations that were reported during the study of sleep-wakefulness were reinterpreted in retrospect in the light of the discovery of REM sleep[138,139]. Transection studies showed that removal of brain matter rostral to the pons, including the hypothalamo-hypophysis, did not suppress the periodic occurrence of REM sleep in the chronic pontile preparation of cats[48,140-142]. Further, studies in cats with experimental transection of spinal cord and in human patients with spinal injury showed that the spinal cord makes no essential contribution to the brainstem signs associated with REM sleep[48,143]. These observations suggested that the REM sleep generating mechanism(s) was located in the brainstem.

Brainstem regulation of REM sleep : In an attempt to further localize specific site within the brainstem responsible for REM sleep regulation, different pontine nuclei were damaged and the effects on REM sleep observed. Some of such studies identified NPC[48] while others[49] reported NPO to be responsible for REM sleep regulation. Lesions restricted to caudal LC[66] or in the region of NPO adjacent to the LC[67] produced REM sleep without atonia. Further, it was shown that although lesion of LC in kittens did not disrupt REM sleep[144], it produced loss of atonia during REM sleep[145]. Subsequent studies implicated LC to be crucial for REM sleep generation[146], while lesion of both the NPO and NPC did not prevent occurrence of REM sleep[147]. Therefore, it seemed likely that LC played a significant role in REM sleep regulation.

Ponto-medullary area and REM sleep regulation : It was observed that even after isolation of the pons from other brain regions by rostral and caudal transections, the periodic episodes of REMs and PGO spikes persisted, which in a normally behaving animal were seen only during REM sleep[142]. However, a midpontine transection abolished the major defining characteristic signs of REM sleep[148]. Subsequently, by transection of brainstem at different levels in rostro-caudal axis, it was shown that the signs of REM sleep were expressed in the structures with which the pons remained connected. In experiments where the transection was made above the pons *i.e.*, the pons remained attached 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 attached to the mid- and forebrain, the REM sleep signs were expressed in the latter structures. Thus, it was concluded, that the pons is both necessary and sufficient to generate the basic phenomenon of REM sleep[149]. However, this view did not receive universal support. It was observed that while microinjection of cholinergic agonist, carbachol, into the medio-dorsal pontine tegmentum including LC-alpha and peri-LC-alpha in the intact cat induced high amount of REM sleep with short latency of less than 5 min, similar microinjection in the cat where brainstem was transected between the pons and the medulla, failed to induce REM sleep with short latency[150]. Thus, it was contended that the pons alone is insufficient for REM sleep generation; the connections between the pons and the medulla are necessary and cholinergic inputs play a significant role in the regulation of REM sleep.

Neurochemical modulation of ponto-medullary region for REM sleep regulation : Jouvet[50] suggested that NA-ergic neurons in LC were essential for REM sleep generation, whereas others proposed that cholinergic neurons in LC-alpha, peri-LC alpha and LDT/PPT[151,152] are responsible for REM sleep generation. Studies in cats emphasized the central co-ordinating role of NPO for REM sleep generation. In most of these studies latency to the onset of REM sleep following carbachol injection has been used as a marker for the most effective site for REM sleep generation. Although the site, volume and dose of carbachol injection into pontine reticular formation by different research groups[151,153-155] varied, the boundary of the area covered by the injected volume included the ventral part of the nucleus pontis oralis (vNPO)[156]. It has been shown earlier that the lesions of vNPO[157,158] significantly decreased REM sleep whereas injection of small dose of carbachol induced REM sleep

with a shorter latency[159,160]. Further, the latency of REM sleep induction reduced with the distance of site of injection of carbachol from the vNPO[156]. The vNPO receives cholinergic projections from rostral peri-LC alpha, PPT, LDT and parabrachial nuclei as well as GABA-ergic projections from postero-lateral hypothalamus[161]. Also, the oculomotor, facial and masticatory nuclei, the caudal pontine tegmentum and the prepositus hypoglossi nuclei project to vNPO[162]. Recent studies also showed that the cholinergic system in NPO interacts with GABA-ergic and hypocretinergic system for REM sleep regulation[163,164]. In rats also, the sites where carbachol injection increased REM sleep have been identified as in (and around) NPO and NPC[90,165]. However, the induction of REM sleep with short latency following carbachol injections is not seen in rats and thus a significant increase in REM sleep is taken into consideration for deciding the REM sleep enhancing site[166]. The levels of NA[52], ACh[91] and GABA[167] have been reported to change in relation to REM sleep.

REM-OFF and REM-ON neurons : After the technique to record the activity of single neuron in behaving animals was well established, it provided important insight into the functioning of pontine nuclei at the neuronal level, the interplay of which is central to REM sleep physiology. The single neuronal recording studies in the pontine reticular formation showed the presence of REM sleep related neurons. Those neurons that stop firing or significantly decrease their firing rate during REM sleep[168] are called REM-OFF neurons while those neurons that start firing or significantly increase their firing rate during REM sleep[169] are called as REM-ON neurons. The REM-OFF neurons are monoaminergic, distributed in the LC as the NA-ergic[170-172], in the dorsal raphe as the serotoninergic neurons[173] and in tuberomammilary nucleus in hypothalamic region as the histaminergic neurons[174]. The activity of dopaminergic neurons is not related to REM sleep[175]; however, possibility of some non-monoaminergic REM-OFF neurons in the medulla has also been suggested[176]. The REM-ON neurons are primarily cholinergic present in the LDT[177,178] and PPT[179]; while some non-cholinergic REM-ON neurons have also been suggested[176,180]. Interplay between REM-ON and REM-OFF neurons leads to the generation and regulation of REM sleep.

Evolving concepts of neural regulation of REM sleep

Reciprocal interaction model : The reciprocal interaction hypothesis was put forward by Hobson *et al*[170] on the basis of reciprocal activity profile of the neurons in gigantocellular tegmental field (FTG) and neurons in LC. According to this model, the FTG neurons rarely discharge during waking because they are under tonic inhibition of the LC neurons. During sleep the LC activity decreases and consequently inhibition on FTG neurons begins to wane. The progressive decrease in the LC neuronal activity causes a progressive increase in the activity of the FTG neurons, which along with a possible self-excitation, increase its excitation to such a level that the FTG neurons escape the inhibition from the LC neurons. The firing of FTG neurons beyond this critical point leads to an exponential rise in FTG neuronal activity, which results in the initiation of REM sleep. The increase in FTG neuronal activity causes a progressive increase in the excitation of LC neurons, which then start firing, exert a tonic inhibition on the FTG neurons resulting in the termination of REM sleep episode. Thus, the REM-OFF neurons in LC are inhibitory to REM-ON neurons, while the REM-ON neurons are excitatory to the REM-OFF neurons. Hence, the cessation of activity of REM-OFF neurons dis-inhibits REM-ON neurons resulting in the initiation of REM sleep episode.

Mutual inhibitory model : The basic assumption underlying this model[178] was also the reciprocal activity profile of the neurons (REM-ON neurons) that increase firing during REM sleep and those (REM-OFF neurons) which decrease their firing rate during REM sleep. Thus, it was postulated that the putative monoaminergic REM-OFF neurons are inhibitory to the putative cholinergic/ cholinoceptive REM-ON neurons and the latter are inhibitory to the former. Therefore, the REM sleep may be initiated either by direct excitation of REM-ON neurons or by inhibition of REM-OFF neurons.

Although the reciprocal interaction[170] and mutual inhibitory[178] models provided a plausible explanation for the generation of REM sleep on the basis of the unitary activity of REM-ON and REM-OFF neurons, neither of these models considered the neurochemical nature of the interactions between REM-OFF and REM-ON neurons. Further, the assumption that the cholinergic REM-ON neurons are inhibitory to REM-OFF neurons[178] could not be sustained because it was subsequently reported by *in vitro* study that acetylcholine depolarized the NA-ergic presumably REM-OFF neurons in the LC[181]. Although, the basic scaffold provided by Hobson *et al*[170] still holds good, certain pertinent questions came up. The foremost among these were *(i)* is the cessation of LC neurons a pre-requisite for REM sleep regulation?*(ii)* how do REM-OFF

neurons in LC stop firing?; and *(iii)* how do REM-ON neurons increase firing to allow the generation of REM sleep?

Cessation of REM-OFF neurons is a pre-requisite for REM sleep generation : It was hypothesized[105] that if cessation of REM-OFF neurons is a pre-requisite for REM sleep generation, their continuous activation would cause a reduction of REM sleep. It was shown in chronic preparation of rats that continuous low frequency mild electrical stimulation of LC neurons prevented initiation of REM sleep and after the stimulation was stopped there was rebound increase in REM sleep as was reported after instrumental REM sleep deprivation[105]. This confirmed earlier report that REM-OFF neurons continue firing during REM sleep deprivation[182]. To understand temporal relation between REM-ON and REM-OFF neurons, those two types of neurons were recorded simultaneously[54] and a reasonable temporal relationship was observed. Hence, it was reasonable to expect that increased firing of REM-ON neurons inhibits REM-OFF neurons resulting in the initiation of REM sleep. Thus, it was proposed that the inhibition of REM-OFF neurons is a pre-requisite for generation of REM sleep[183].

How do REM-OFF neurons cease firing ?: It was shown that microinjection of acetylcholine agonist into LC increased REM sleep[151,184,185] and acetylcholine levels increased around LC during spontaneous REM sleep[91]. Since acetylcholine did not hyperpolarize the LC neurons[181], it was proposed[186] that the actual inhibition of REM-OFF neurons in the LC might be caused by an inhibitory neurotransmitter, which might be triggered by acetylcholine, leading to the generation and regulation of REM sleep. GABA was thought to be the probable candidate for inhibition of REM-OFF neurons[187] because of the *(i)* presence of GABA-ergic interneurons and terminals in LC[84,95,188]; *(ii)* the presence of GABA-ergic receptors [189,190] on the neurons in LC; *(iii)* increased GABA levels in LC during REM sleep[167]; *(iv)* reduction of REM sleep by microinjection of picrotoxin, a GABA-A receptor antagonist, in LC[191]; *(v)* activation of GABA-ergic neurons in LC during REM sleep[192] ; and *(vi)* inhibition of NA-ergic neurons in LC by GABA[193].

GABA-ergic interneuron based model : In a series of studies it was found that blocking of GABA-ergic as well as cholinergic transmission in LC by picrotoxin and scopolamine, respectively decreased REM sleep while microinjection of GABA[194] and acetylcholine agonist[151,153,185,194] into the LC increased REM sleep. The increase in the number of REM sleep episodes after carbachol microinjection into LC and an increase in the duration of REM sleep per episode after GABA microinjection into LC showed that whereas acetylcholine in LC regulated the frequency of generation (triggering) of REM sleep, GABA regulated the maintenance (duration per episode) of REM sleep[194].

The next question was whether the GABA and acetylcholine in LC act in parallel or in a sequence (series) to regulate REM sleep? To confirm, a double injection study in rats using the agonist and antagonists of acetylcholine and GABA in sequential combinations was done[194]. The injection of picrotoxin followed by carbachol into the LC decreased REM sleep whereas injection of scopolamine followed by GABA increased REM sleep. The decrease in REM sleep observed after the injection of picrotoxin followed by carbachol was due to a reduction in the duration of REM sleep per episode. The increase in REM sleep observed after sequential injection of scopolamine followed by GABA was due to an increase in the duration of REM sleep per episode. Therefore, the effect of GABA was found to be overriding the effect of cholinergic antagonist or agonist in the double injection study, which is possible only if the GABA was forming the final output path. These findings suggested that acetylcholine is acting on GABA-ergic neurons, which in turn project onto the LC-NA-ergic neurons[194].

Based on the above results, a "GABA-ergic interneuron based model" was proposed[186,187] that envisaged GABA mediating the neurotransmission between cholinergic LDT/PPT and the NA-ergic LC neurons. According to this model, the cholinergic inputs from REM-ON neurons excite the GABA-ergic neurons in LC, which in turn inhibit the NA-ergic REM-OFF neurons in LC facilitating the generation of REM sleep[194]. Subsequently it was shown that some GABA-ergic inputs might be reaching LC from prepositus hypoglossus nucleus[195], which in turn receives cholinergic projection from the PPT[196].

How do REM-ON neurons get activated ? : The "GABA-ergic interneuron based model" also hypothesized that during non-REM sleep and awake states the NA-ergic inputs from LC excite GABA-ergic neurons, which in turn inhibit the cholinergic REM-ON neurons in LDT/PPT, thus preventing REM sleep generation during wakefulness. However, blocking of GABA-ergic transmission in PPT by picrotoxin did not increase but decreased REM sleep significantly by decreasing the

number of REM sleep episodes[197] whereas microinjection of muscimol, $GABA_A$ agonist, into the PPT increased REM sleep by increasing the number of REM sleep episodes[198]. The above findings indicated that blocking GABA transmission decreased whereas facilitating GABA transmission increased REM sleep, possibly by action of GABA on REM-ON neurons. This suggested an excitatory role for GABA in REM sleep regulation; although GABA is normally known to be an inhibitory neurotransmitter. A likely explanation for an excitatory role of GABA is that it acts pre-synaptically on an inhibitory input on the target neurons (REM-ON neurons in this case), causing withdrawal of inhibition resulting in facilitation of excitation of the postsynaptic REM-ON neurons. In a combined study involving systemic injection of NA-ergic agonists/antagonists along with the bilateral stimulation of LC (that result in elevated level of NA in brain), it was proposed that NA acts through beta and alpha adrenoceptors in PPT to regulate REM sleep[199]. In order to further validate the results, NA-ergic agonists and antagonists were microinjected into PPT in freely moving rats. The results showed that whereas alpha and beta adrenoceptors are involved in the generation of REM sleep, a-2 adrenergic receptors are involved in the maintenance of REM sleep[200]. The co-injection of $GABA_A$ antagonist (picrotoxin) and a-2 agonist (clonidine) into PPT caused a simultaneous decrease in the frequency of generation of REM sleep and increase in the duration per episode of REM sleep[200], thereby, further validating the role of $GABA_A$ receptors in the generation of REM sleep and that of a-2 adrenoceptors in the maintenance of REM sleep. It was concluded that GABA acts pre-synaptically on inhibitory NA-ergic terminals to dis-inhibit REM-ON neurons, which then start firing initiating REM sleep. Based on these results a model has been proposed to explain the generation and maintenance of REM sleep (Fig.).

Although the focus of this review was to understand the regulation of REM sleep with emphasis on REM-OFF neurons in the LC, it must be mentioned that isolated independent studies have shown that other areas in the brain also play complex role in such regulation, however, detailed systematic studies to decipher mechanism of such regulation need to be carried out. Some of the interactions between neurons in different areas in the brain for the regulation of REM sleep have been dealt with by different groups[201-203], which compliment the content and the model presented in this review.

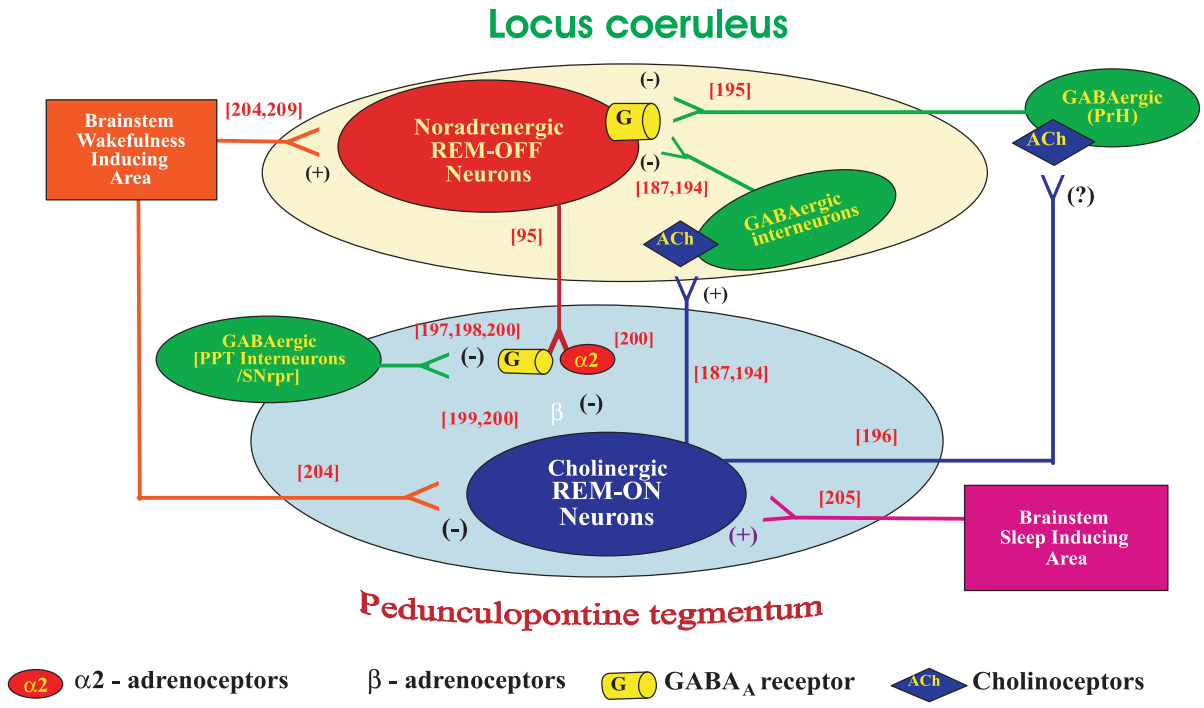

Fig. Proposed connections to and from REM-ON and REM-OFF neurons particularly in locus coeruleus and pedunculopontine tegmental area, involved in the regulation of REM sleep. The numbers in the parentheses indicate the reference number. PPT- Pedunculopontine tegmentum; SNrpr - Substantia nigra par reticulata; PrH- Prepositus hypoglossus; (+) - Excitatory; (-) - Inhibitory.

Why does not REM sleep appear during wakefulness or at sleep onset? Role of sleep and wakefulness inducing areas : The generation of REM sleep is coupled to the expression of non-REM sleep as well as the exclusion of wakefulness during the period immediately preceding its occurrence. The REM-OFF neurons are maximally active during wakefulness but cease firing during REM sleep whereas REM-ON neurons do not fire during wakefulness but are maximally active during REM sleep. Thus, the brain circuitry responsible for non-REM sleep and wakefulness seems to be interlocked with that involved in REM sleep generation. As REM sleep normally does not occur during wakefulness, it was proposed that wakefulness inducing area must have inhibitory influence on REM-ON and excitatory influence on REM-OFF neurons. Subsequently, it was shown that the wakefulness-inducing area, the midbrain reticular formation (MRF), excited the REM-OFF and inhibited the REM-ON neurons[204]. Also, the sleep inducing area in the caudal brainstem was shown to excite the REM-ON neurons[205]. Based on the above results, it was hypothesized that during wakefulness the wake related neurons in the MRF[206-208] excite the NA-ergic REM-OFF neurons in LC, which then remain active throughout waking period and therefore REM sleep does not appear during wakefulness[209]. This view may be supported by the fact that activation of REM-OFF neurons prevented REM sleep[105] and is likely to increase the level of NA in the brain[210] causing cortical activation and desynchronization of the EEG[211]. Further, the NA mediated cortical activation and desynchronization of the EEG may contribute to EEG desynchronization associated with wakefulness but not to that of REM sleep, as has been suggested earlier[54].

Hence, on the basis of the available knowledge, it may be hypothesized that the MRF wake related neurons exert independent inhibitory and excitatory effects on the REM-ON and the REM-OFF neurons, respectively. At the onset of sleep the activity of MRF neurons is reduced[207] that gradually withdraws the excitatory and the inhibitory effects from the REM-OFF and the REM-ON neurons, respectively. Gradually sleep is induced when the sleep inducing neurons further increase firing and the wake active neurons further reduce or cease firing. The reduction or cessation of the wake active neurons and the activation or increased firing of the neurons in sleep inducing area facilitates excitation of REM-ON neurons in the following manner: *(i)* the reduction or cessation of the wake active neurons withdraws the direct inhibition from REM-ON neurons; *(ii)* the reduction or cessation of the wake active neurons withdraws the excitation from the REM-OFF neurons allowing those neurons to withdraw the NA-ergic inhibition from the REM-ON neurons; and *(iii)* the activation or increased firing of the neurons in the sleep inducing area excites the REM-ON neurons. Thus, a combination of the above mentioned phenomena causes the REM-ON neurons to start and continue firing resulting in the initiation of REM sleep (Fig.).

Physiological validation

Based on the studies mentioned above there seem to be enough reasons to accept that there exists interplay of GABA and acetylcholine in LC while that of GABA and NA in the PPT for the regulation of REM sleep. Increased GABA in LC inhibits the NA-ergic REM-OFF neurons that in turn excite the cholinergic REM-ON neurons for initiation of REM sleep. During loss of REM sleep the REM-OFF neurons do not cease firing[182] causing increased levels of NA in the brain[212,213]. Therefore, it was hypothesized that blocking of GABA in LC should induce effects similar to that of REM sleep loss. Also, REM sleep loss and REM sleep deprivation induced effects should be prevented by NA-ergic antagonists.

Behavioural studies on humans and animals have shown that loss of REM sleep causes increased aggressiveness, heightened pain sensitivity, hypersexuality, and has a detrimental effect on memory consolidation as well as brain maturation. At the cellular level, neuronal responsiveness is known to be affected after REM sleep deprivation[132,214]. Based on these observations Mallick *et al*[215] hypothesized that one of the functions of REM sleep is to maintain brain excitability. The basic assumption behind this hypothesis was that electrical excitability is an intrinsic and characterizing property of neurons and the neuronal functions would be affected if the excitable state of neurons is altered. Since Na^+-K^+ ATPase is one of the key factors that maintains neuronal excitability, it was hypothesized[216] that REM sleep deprivation would affect the functioning of Na^+-K^+-ATPase. It was shown that REM sleep deprivation increased Na^+-K^+ - ATPase activity in the brain[216,217]. The increase in the enzyme activity was found to be mediated by NA and it is known that NA levels increase in brain after REM sleep deprivation[212,213,218]. Subsequently, it was shown that NA acts through α-1 adrenoceptors to increase the enzyme activity; an effect that can be prevented by adrenoceptor antagonist[219,220]. Similarly, REM sleep deprivation

induced alteration in neuronal size was prevented by α-1 adrenoceptor antagonist[221]. Finally, it was contended if the GABA mediated cessation of the REM-OFF neurons in LC is necessary for the generation of REM sleep, then the GABA-antagonist in LC should decrease REM sleep and exhibit some of the REM sleep deprivation induced effects. Indeed, it was found that microinjection of picrotoxin, $GABA_A$ blocker, into the LC reduced REM sleep and increased NA^+-K^+ ATPase[222] as was observed after instrumental REM sleep deprivation[216], thus validating our model for REM sleep generation.

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Reprint requests: Dr B.N. Mallick, School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, India
e-mail: remsbnm@yahoo.com