REM SLEEP
AN OVERVIEW
Mammalian sleep-wakefulness is bistable process consisting of continuous alternation between different stages. The different stages of sleep-wakefulness have been classified on the basis of bioelectrical recordings viz. electroencephalogram (EEG), electromyogram (EMG) and electrooculogram (EOG). These different stages correlated well with some of the observed physiological, biochemical and behavioral features of wakefulness and sleep. The five different stages in the rat are as follows:

**ACTIVE AWAKE**: Active awake corresponds to attentive and/or psychomotor active waking state. This stage is characterized by the presence of desynchronized EEG or waves of low voltage (20-50 Uv) and high frequency (30 to 50 Hz) (Gottesmann, 1992). The EOG shows frequent and irregular pattern of eye movements whereas the EMG shows high muscle activity.

**QUIET AWAKE**: Quiet awake is a representative of nonattentive waking or a stage of non-motivated motor activities. This stage is characterized by the presence of desynchronized EEG along with spindles which may or may not be present and a decrease in the muscle tone (Gottesmann, 1992). The EOG shows few or no eye movements whereas the EMG shows low movement tone.

**SLOW WAVE SLEEP**: This first stage of sleep is characterized by the presence of low frequency (6-18 Hz) synchronized EEG or waves with progressive increase in the amplitude (50-300 Uv) and progressive increase in the appearances of spindles (for 25 to 50% of total time). Muscle tone is further decreased as compared to quiet awake (Gottesmann, 1992).

**DEEP SLEEP**: Deep sleep is a stage in which synchronization (low frequency (6-18 Hz) and high amplitude (50-300 Uv) waves) is
present for more than 50% of the time. There is a near absence of eye movement and the muscle tone is very low.

**RAPID EYE MOVEMENT (REM) SLEEP**: This state has been characterized by desynchronized EEG, with burst of rapid eye movement, along with the loss of muscle tone. PGO waves, or the prominent phasic electrical potentials observed during REM sleep in the dorsal lateral geniculate nucleus (LGN) and visual cortices and from the pons are another important characteristics of REM sleep. It was the discovery of REM sleep as a distinct state (Aserinsky and Kleitman 1953, Dement and Kleitman, 1957) that revolutionized the understanding of sleep and sleep process.

**B) THE ONTOGENY AND PHYLOGENY OF REM SLEEP**:

REM sleep was thought to be phyllogenetically recent as it is non-existent in reptiles and fishes. Ontogenically it is known to be present in ovo and in utero (Jouvet, 1972). In fetus and immature new born, total sleep time is occupied by REM sleep. This lacks the characteristic cortical EEG desynchronization and muscle atonia but is identified by muscle twitches and eye movement that occur in quiescent state. REM sleep as such can only be identified in the embryo only after the development of the inhibitory processes, though it has been suggested that the spontaneous movements occurring in the early embryo represent a precursor state similar to that of REM sleep (Jones, 1991). Across the mammals, duration of REM sleep per bout is the function of the sleep cycle length, increases with the increase in the size of the brain and is negatively correlated with the basal metabolic rate (Jones, 1991).

**C) THE RHYTHMICITY OF REM SLEEP**:

REM sleep occurs in a cyclic manner. In humans a 45-85 min of NREM sleep (progressing from longer to shorter periods
throughout the night) is followed by a 5-65 min period of REM sleep, progressing from shorter to longer periods throughout the night, in a reciprocal manner to the length of NREM sleep (Jones, 1991). The ultradian rhythm of sleep cycle correlates well with the ultradian temperature cycle. In decerebrate cats lacking hypothalamic regulatory mechanisms, REM sleep can be triggered by lowering of the brain temperature (Jones, 1991). Hence it seems that initiation of REM sleep is dependent on the lowering of the body-brain temperature and does not initiate with elevated temperature in normal animals. Sleep cycle represent a basic rest-activity cycle of the brain. This cycle is independent of wakefulness and passes through quiet (NREM sleep) and active (REM sleep) periods. These are indicated by the heat loss, heat gain periods. Wakefulness is imposed upon this rest-activity cycle at the end of REM sleep period (Jones, 1991).

D) METABOLISM DURING REM SLEEP:

Along with temperature changes, respiratory control, cerebral blood flow and glucose metabolism are also altered during sleep cycle. During REM sleep, basal metabolic rate is higher than that of NREM sleep but lower than that of waking (Jones, 1991). Respiratory control, especially sensitivity to CO2 is lost during REM sleep but the O2 sensitivity is maintained. Sawaya and Ingvar (1989) reported increase in oxygen consumption during REM sleep. Reivich (Reivich et al. 1968) found that the cerebral blood flow increases during REM sleep in 25 regions of the brain. In adult and term infants, cerebral blood flow is highest during REM sleep followed by waking and is least in NREM sleep (Sawaya and Ingvar, 1989). Glucose metabolism, in most areas of the brain, is highest during REM sleep whereas it decreases upto 30% during NREM sleep (Ramm & Frost, 1986). The majority of the brain areas which
showed the changes in the glucose metabolism, during REM sleep, are the ones which are proposed to be involved in regulation of REM sleep (Lydic et al. 1991).

E] THE ANATOMICAL SUBSTRATES OF REM SLEEP:

Initial investigation to identify the anatomical substrates involved in REM sleep were carried out by transection, lesion studies and monitoring the effect on REM sleep signs. Jouvet and others (Villablanca, 1966, Jouvet, 1969) using transection studies, identified the areas of the brain which were involved in the generation of REM sleep. These studies, employing complete transections of the brain, at different levels, showed that the diencephalon and the telencephalon did not show any signs of REM sleep when separated from the brain stem, whereas the brain stem and the periphery showed signs of REM sleep. The forebrain of these transected animals showed both the synchronized as well as desynchronized state that alternate spontaneously (Villablanca, 1966). Hence, the transected animals showed wakefulness and NREM signs in both the caudal and rostral structures but REM sleep signs were only seen in the caudal to cut regions of the brain. When transections were made at the junction of spinal cord and the medulla, all the REM sleep signs were seen rostral to the cut (Siegel, 1989). This showed that spinal cord was not essential for the generation of REM sleep. Siegel and his group (Siegel et al. 1984, 1986) transected between the medulla and the pons and found the brain areas caudal to the cut did not show the characteristic signs seen in REM sleep. Hence medulla and spinal cord disconnected from the rostral structures were not sufficient for the generation of REM sleep. Again brain areas rostral to the cut showed the signs of REM sleep. The caudal area fluctuates between the wakefulness state (characterized by high muscle tone and accelerated respiration and heart rate) and the NREM state.
(characterized by low muscle tone and depressed respiration and heart rate). When transections were made right at the midpoint of pons (midpontine transections), the characteristics of REM sleep were lost in both the rostral and the caudal side of the transections. Hence, these isolation studies implicated pons as the critical region of the brain necessary for the generation of REM sleep.

F] THE REGULATORY MECHANISMS OF REM SLEEP:

Communication between the neurons takes place mainly due to the activity of chemical agents known as the neurotransmitters. These substances diffuse across the synaptic cleft and change the activity of the post-synaptic neuron. Alterations between NREM sleep, REM sleep and wakefulness are believed to arise from the activity of neurotransmitters.

Many theories have been proposed to define the physiological and neurochemical mechanisms involved in generation and maintenance of REM sleep. Most of those theories have implicated interaction between two groups of neurons viz. cholinergic group and the aminergic group of neurons. Acetylcholine (ACh) and noradrenaline (NE) are the two neurotransmitters thought to play a major role in the generation and regulation of REM sleep. Importance of the aminergic and cholinergic neurons in REM sleep has its origin from the study of Hess, who had proposed the notion of ergotropic process act through aminergic (sympathetic) systems whereas the trophotropic processes are controlled by the cholinergic (parasympathetic) systems (Hobson et al. 1986)

I] CATECHOLAMINES AND REM SLEEP:

Understanding of the neurochemical mechanisms or the ‘wet physiology’ involved in REM sleep had its thrust from the histochemical demonstration of the presence of noradrenaline (NE) containing neurons within the pontine tegmentum specifically the LC
and sub-coeruleus with widespread projections to the forebrain (Dahlstrom and Fuxe, 1962).

i) **LESION STUDIES**:

Jouvet (1972) reported partial disruption of REM sleep by electrolytic lesioning of NE-containing neurons found in the pontine tegmentum. Destruction of the entire area lead to complete elimination of REM sleep. These studies implicated noradrenaline to have a prime role in the generation of REM sleep. Later, selective electrolytic lesion of NE-containing neurons in the same area did not show the same effect (Jones et al. 1977, Henley and Morrison 1974). Selective destruction of NE-ergic cells of the dorsolateral pontine tegmentum or mesencephalic tegmentum, using 6-hydroxydopamine (a false neurotransmitter and is proposed to destroy the NE-axons and cell bodies by excitotoxicity) and causing depletion of NE has yielded contradictory results. There are reports of either decreased REM sleep, or increased REM sleep or no change in REM sleep (Monti, 1983) after destruction of the NE-containing neurons by 6-hydroxydopamine. Such contradictory results may be due to unequal changes in the neurotransmitter systems (Monti, 1983).

ii) **PHARMACOLOGICAL STUDIES**:

Pharmacological manipulation of neurochemical transmission, by using different drugs as tools, provided an opportunity to understand in depth the chemistry involved in the mechanism of behavioral states. Catecholamine synthesis can be inhibited by alpha-methylparatyrosine ($\alpha$-MPT), an analog of tyrosine, which inhibits tyrosine hydroxylase, the first rate limiting enzyme. In rats, the effect of $\alpha$-MPT on REM sleep has not been very clear. After administration of $\alpha$-MPT, in rats, there are varying reports of either no change in REM sleep (Monti, 1983), or increased (Hartmann et al. 1971), or decreased REM sleep (Monti, 1983).
human studies α- MPT caused an increase both in REM sleep and NREM sleep, when the dose was low whereas with higher doses REM sleep decreased (Gaillard, 1990). Systemic administration of the NE synthesis inhibitors diethyldithiocarbamate to rats and disulfiram or fusaric acid to cats decreased REM sleep (Monti, 1983). Inhibitors of monoamine oxidase (the enzyme involved in the degradation of monoamines) as well as monoamine uptake blockers are known to suppress REM sleep (Vogel et al. 1990). In cats, clonidine (alpha-2 agonist) dose dependently decreased REM sleep (Monti, 1983). In humans, low doses of clonidine inhibited REM sleep, whereas higher doses of clonidine caused an increased wakefulness (Gaillard, 1990). Piperoxan, an antagonist of alpha-2 receptor increased REM sleep and decreased wakefulness, at higher doses. At lower doses the effect was reversed (Gaillard and Kafi, 1982).

iii) UNIT RECORDING STUDIES

Unit recording studies showed that the NE-containing neurons in and around locus coeruleus (LC) and the serotonin containing raphe neurons decreased their firing as REM sleep approaches and stop their firing during REM sleep (Chu and Bloom, 1974, Hobson et al. 1975, McGinty and Harper, 1976, Aston-Jones and Bloom, 1981, Jacobs et al. 1981, Jacobs, 1986). All the above mentioned studies indicate that NE-containing neurons may be essential for the regulation of REM sleep.

II) ACETYLCHOLINE AND REM SLEEP

Involvement of cholinergic neurons in REM sleep as well as in cortical activation during wakefulness has gained ground in the last few years mainly due to the development of antibody against choline acetyl-transferase (ChAT), the enzyme involved in the synthesis of ACh. AChE was not a suitable marker for cholinergic neurons as it was present even in the regions where ACh was not present.
Identification of cholinergic neuron in the brain and within the brain stem of rat (Mesulam et al. 1983, Kimura et al. 1984) and cat (Shiromani et al. 1988) has shown that ChAT-immunopositive neurons was largely restricted to dorsolateral tegmentum in all the mammalian species examined so far. ChAT immunopositivity was also seen scattered in the medial and lateral medullary reticular formation (Jones, 1990). These cell groups also approximately correspond to the REM-ON cells as described by Sakai (1980, 1985a).

i) LESION STUDIES:
Electrolytic lesion, of the ChAT-positive cholinergic neurons at the level of pons has been reported to diminish REM sleep in the rat (Gnadt and Pegram, 1986). Radio frequency lesion of the lateral and the dorsolateral pontine tegmentum were capable of eliminating REM sleep (Jones, 1991), but when the pontine cholinceptive gigantocellular tegmental field (FTG) neurons were specifically lesioned either with radio frequency or with neurotoxic chemicals REM sleep was not affected (Friedman and Jones, 1984). Using kainic acid, Jones and her colleagues caused extensive destruction of ChAT positive cholinergic neurons (75-80%), of the dorsolateral pontine tegmentum and eliminated REM sleep for two to three weeks (Jones, 1991).

ii) PHARMACOLOGICAL STUDIES:
Hemicholinium-3, a choline uptake blocker abolished REM sleep as well as caused a decrease in wakefulness (Gillin et al. 1985). Physostigmine, an AChE inhibitor, has been shown to enhance cortical activation during wakefulness and prolongs REM sleep (Gillin et al. 1985). Direct application of carbachol, a mixed cholinergic agonist, in the pontine-mesencephalic reticular formation
induced REM sleep (George et al. 1964). Further studies have shown that application of carbachol in medulla and midbrain decreased REM sleep where as administration in the pons increased REM sleep (Baghadayon et al. 1984, Shiromani and Fishbein, 1986, Vanni-Mercier et al. 1989). Neostigmine, an AChE inhibitor is also known to enhance REM sleep. The precise site within the pons where the cholinergic agonist acts is not very clear but dorsolateral pontine tegmentum is indicated to be the important area.

iii) UNIT RECORDING STUDIES:

Unit recording studies conducted by Hobson and McCarley (McCarley and Hobson, 1971, Hobson et al. 1975) showed that neurons of the medial/gigantocellular field of the pontine reticular formation increased their firing as REM sleep approaches and these neurons fired maximally during REM sleep. These neurons are presumed to be cholinergic and/or cholinceptive (Jones, 1991). Thus, due to the selectivity in the firings of the these neurons, it was proposed that cholinergic neurons of the pontine reticular formation (mainly neurons of the medial/gigantocellular field (FTG) of the pontine reticular formation) may play an executive role in the generation of REM sleep.

III) OTHER NEUROTRANSMITTERS AND REM SLEEP:

Though acetylcholine and norepinephrine have been implicated to play a major role in the regulation of REM sleep, dopamine, serotonin, neuropeptides and nucleosides are some of the neurotransmitters or neuromodulators proposed to have a modulatory role in the generation and regulation of REM sleep.

i) SEROTONIN AND REM SLEEP:

Studies on the involvement of serotonin as one of the neurotransmitter in the regulation of REM sleep has provided mixed results. It was Jouvet (1972) who after lesioning dorsal raphe nuclei
reported decreased sleep. When para-chloro-phenylalanine was used to inhibit the synthesis of serotonin, it caused insomnia (Jouvet, 1972). Tryptophan is known to cause drowsiness in animals (Jouvet, 1972). In human studies L-5-hydroxy tryptophan increases paradoxical sleep (Jouvet, 1972). Serotonin uptake blockers are known to inhibit REM sleep and this inhibition is reduced by administration of para-chloro-phenylalanine (Jouvet, 1972). Unit recording studies have shown that the serotonin containing cell group of the raphe nuclei were REM-OFF cells (McGinty and Harper, 1976, Jacobs, 1986). Hence, it has been proposed that serotonin like NE may have an inhibitory role in the regulation of REM sleep (Hobson et al. 1986).

ii) DOPAMINE AND REM SLEEP

Most of the evidences implicating dopamine to have a role in the regulation of REM sleep have come from pharmacological manipulation. High doses of apomorphine (an agonist of dopamine) has been shown to decrease REM sleep, but with low doses it increases REM sleep (Monti, 1983). The decrease in REM sleep, at low concentrations, may be due to the activation of presynaptic receptors (inhibitory) of dopamine. However, as the presynaptic receptors of dopamine are located on central NE neurons, apomorphine (low concentration) may also turn off the activity of the NE neurons. At high concentration apomorphine may activate the post synaptic dopamine receptor and this may induce arousal (Monti 1983). Pimozide, a specific dopamine antagonist also shows the biphasic effect, in low doses it decreased REM sleep, whereas in high doses the reverse occurred (Monti, 1983). Dopamine receptor blocker SCH 23390, selective for D1 receptors, increased REM sleep (Trampus and Ongini, 1990). Hence from the above studies, it might be possible that dopamine has a modulatory role in the regulation of REM sleep.
iii) NEUROPEPTIDES AND REM SLEEP:

Neuropeptides or bioactive peptides are a group of small molecular weight proteins, present in the central nervous system, which are known to play a role in neuromodulation. Some of those peptides are proposed to play a role in the modulation of REM sleep. These are:

1) Substance P: Substance P, which is co-localized with acetylcholine in the dorsolateral pontine tegmentum neurons, when injected intracerebroventricularly is known to decrease REM sleep (Jones, 1991).

2) Somatostatin: Somatostatin has been localized in the vicinity of the cholinergic and the NE-ergic cell groups within the central gray and peribrachial region (Jones, 1991). When injected intracerebro-ventricularly, it is known to induce an increase in REM sleep (Danguir, 1986).

3) Vasoactive intestinal peptide (VIP): VIP is one of the peptides which has been found to induce or elicit REM sleep (Drucker-Colin et al. 1984, Obal et al. 1989). This peptide is present within the dorsolateral tegmentum and the peribrachial region. It has been demonstrated to coexist with acetylcholine in the cortical neurons. It has been proposed that VIP could be released along with acetylcholine from a cholinergic neuron or by some other VIP containing neuron under a specific condition and thus contribute to the modulation of REM sleep (Jones, 1991).

iv) NUCLEOSIDES:

Adenosine, one of the nucleosides, is known to be a potent neuromodulator. Radulovacki and his co-worker have extensively studied the effect of adenosine on sleep and wakefulness (Radulovacki et al. 1983, Radulovacki et al. 1985). Inhibition of degradation of adenosine, by deoxycoformycin, a potent inhibitor of
adenosine deaminase, has been reported to produce an increase in REM sleep (Radulovacki et al. 1983). Though it is yet premature to ascribe any significant role of adenosine, better understanding of the role of adenosine and its receptors subtype may be helpful in overall elucidation of the regulatory mechanisms of REM sleep.

IV) INTEGRATION

When the levels of acetylcholine alone were enhanced by inhibition of acetylcholinesterase, wakefulness was observed, but along with the enhancement of acetylcholine, depletion of the monoamine by reserpine produced REM sleep (Karczmar et al. 1970). Unit recording studies have also shown that while the NE-ergic neurons of the LC are the REM-OFF group of neurons, the cholinergic neurons of the dorsolateral tegmentum and the pendunculopontine nuclei are of the REM-ON group. These evidences led McCarley and Hobson to propose their reciprocal interaction theory (Hobson et al. 1975, McCarley and Hobson 1975, Hobson et al. 1986). Sakai (1985b) and later Jones (1991) have proposed an interplay and not a direct interaction between the ponto-medullary cholinergic and the aminergic neurons in the regulation of REM sleep.

Jones (Jones, 1991) has studied the distribution of the aminergic and the cholinergic cell groups, by immunohisto-chemistry, and they have found that both these cell groups are present very close to each other. The dendrites of the aminergic LC and the cholinergic dorsolateral tegmentum nuclei run parallel and close to each other in the periventricular gray (Jones 1989, 1991). The varicose fibers from one area are in the close proximity to the soma or the dendrite of the other. A similar situation is also reported in case of the serotonergic fibers from the dorsal raphe nuclei, the dendrites of which extend far into the cholinergic dorsolateral tegmentum. With
such close proximity of the aminergic and the cholinergic cell groups, there is a potential for these cell groups to have mutual interaction.  

G] REGULATION OF REM SLEEP EVENTS:

For further understanding the importance of the interaction between the aminergic and the cholinergic mechanism involved in the generation and the regulation of REM sleep, it will not be out of place to elaborate the anatomical organization, the chemical substrates and the neurophysiological mechanisms relevant to each of the tonic and phasic events of REM sleep.

I) TONIC EVENTS:

Physiological concomitants observed during this state of sleep are usually divided into two distinct types of events, known as ‘tonic’ (events lasting more than 1 sec) events and ‘phasic’ (less than one sec) events (Hobson et al. 1986). EEG desynchronization, postural atonia and the hippocampal theta waves are the examples of the tonic events.

i) EEG DESYNCHRONIZATION:

One of the most striking characteristics of REM sleep is the desynchronization of cortical EEG and this desynchronization of EEG is very similar to desynchronized EEG pattern seen during wakefulness. It was Dement (1958) who first reported the phenomenon of desynchronization to occur in REM sleep. Jouvet (1972) proposed the importance of the nucleus pontis caudalis in the induction of cortical desynchronization during REM sleep and also suggested that the EEG desynchronization during waking had a separate anatomical substrate. Carli and Zanchetti (1965) and later Zanchetti (1967) showed that lesioning of anterior raphe nucleus and LC and nucleus pontis caudalis did not affect EEG desynchronization during REM sleep, but lesioning of nucleus pontis oralis affected the cortical desynchronization during REM sleep. Hobson (1965)
proposed that the midbrain was more important than pons for the induction of cortical EEG desynchronization during REM sleep. Candia et al. (1967) and later Sastre and his colleagues (1981), after extensive lesioning of the pontine tegmentum found no effect on REM sleep. Hobson et al. (1986) have proposed that the cortical activation during is via the disinhibition associated with the arrest of firing in the diffusely projecting aminergic neurons, whereas during waking the cellular generators of EEG desynchronization must overcome the aminergically mediated inhibitory restraint. Hence, it was believed that desynchronization during REM sleep originates from the brain stem reticular formation with increase in the activity of both the inhibitory as well as the excitatory components. Though the neurochemical mechanisms of these pathways are not very clear, cholinergic neurons are implicated to have the prime role in the generation of cortical EEG desynchronization (Sakai, 1985b).

ii) POSTURAL ATONIA:
Postural atonia or the loss of activity in the antigravity muscle is a tonic event which occurs during REM sleep. In intact animals, there is a powerful postsynaptic inhibition during REM sleep, which restrains all but the oculomotor movements, respiration, and the phasic twitches of the skeletal musculature (Pompeiano, 1976). The pontine neurons, located mainly within the medial part of LCa and the surrounding peri-LCa (Sakai et al. 1979, 1981) exert an excitatory influence on the medullary inhibitory center of Magoun and Rhines (1946), which in turn excite the inhibitory interneurons of the spinal cord, thereby leading to a generalized post synaptic inhibition of the anti-gravity muscles. Small bilateral electrolytic lesions placed in the region in and around the LCa causing destruction of the command neurons leads to the suppression of atonia whereas stimulation of these neurons lead to the synaptic
excitation of the magnocellularis neurons. Lesions placed within the pontine tegmentum caudal and ventral to this region lead to disruption of the motor inhibition during REM sleep (Friedman and Jones, 1984, Sakai, 1980). Knife cuts of the fibers passing at the ventral ponto-medullary junction also eliminates atonia during REM sleep (Webster et al. 1986). The atonia executive neurons of the pontine region may be cholinergic or cholinoceptive though histochemically these are yet to be identified. NE-ergic neurons of the LC complex as well as the serotonergic neurons of the raphe group of neurons may not be involved in the generation of postural atonia because these are known to stop firing during REM sleep (REM off neurons). However these may play an indirect role by defacilitation of those neurons which are involved in postural atonia (a loss of inhibitory effect by the stoppage of firing of these REM-OFF neurons).

iii) **HIPPOCAMPAL THETA ACTIVITY**

Hippocampal theta activity is one of the tonic characteristics of REM sleep. This sinusoidal pattern of electrical activity in the frequency range of 5-10 Hz is present in virtually all mammalian species. Hippocampal theta activity is also present during certain waking condition, but is different for different species for e.g. in rat, theta activity during waking is related to voluntary movements, whereas in cat it is associated with attentional processes (Vertes, 1984) and in rabbit with arousal. Green and Arudini (1954) discovered this phenomenon, in rabbits and reported that it could be evoked by the activation of the brain stem reticular formation. Polc and Monnier (1970) showed that theta activity could be generated by stimulation of medullary reticular formation or the medially adjacent raphe magnus. Klemm (1972a, b) reported that elicitation
of the hippocampal theta waves was not limited to the midbrain but extended up to the medullary reticular formation. Vertes (1984) mapped the brain stem and concluded that the pontis oralis, a nucleus within the pontine reticular formation, which contain the REM-ON neurons, is the site responsible for the generation of hippocampal theta waves. Though the LC innervates the hippocampus heavily, LC and median raphe has no role to play in the generation of the hippocampal synchronization (Robinson and Vanderwolf, 1978, Vertes, 1984).

II) PHASIC EVENTS :

The phasic events occur in sporadic manner. Ponto-geniculate-occipital (PGO) waves, muscle twitches and rapid eye movements are the examples of the phasic events (Jouvet 1972, Hobson et al. 1986).

i) PONTO-GENICULATE-OCCIPITAL (PGO) WAVES:

PGO waves usually refer to the prominent phasic electrical potentials observed during REM in the pons, dorsal lateral geniculate nucleus (LGN) and visual cortices. Initially thought to be a phenomenon unique to REM sleep, PGO like waves, with similar characteristics but smaller amplitude have been recorded from the same structures in close association with the eye movement during waking (Brooks and Gershon, 1971, Hu et al. 1989). These spiky waves are known as eye movement potentials (EMPs). PGO executive neurons are located in the caudal mesencephalic and rostral pontine tegmental area (Area X (Sakai 1980), in and around brachium conjunctivum, the areas just caudal to area X and the area rostral to locus coeruleus) and are known to discharge preceding the onset of LGN PGO waves. These neurons are called the 'PGO burst' on 'PGO on' neurons (McCarley et al. 1978, Saito et al. 1977, Sakai 1980, Sakai & Jouvet 1980). Destruction of the PGO burst neurons leads to suppression of the thalamocortical PGO waves. The PGO
executive neurons are proposed to be cholinergic or cholinoceptive (Sakai 1980). Administration of atropine, a cholinergic antagonist, reduces PGO bursts (Shiromani et al. 1987) whereas physostigmine, an inhibitor of acetylcholinesterase triggers PGO waves in collicular or pontine transacted cat (Shiromani et al. 1987). Microinfusion of carbachol, a cholinergic agonist, in the dorsolateral pontine tegmentum induces PGO activity selectively (Baghdoyan et al. 1984, Shiromani et al. 1987). Mecamylamine, a nicotinic antagonist, selectively suppresses LGN-PGO waves (Hu et al. 1988). Pharmacological evidences have shown that monoamines to have an inhibitory role. Injection of serotonin antagonist, para-chlorophenylalanine, lesions of the raphe nuclei, all lead to the initiation of PGO waves (Sakai et al. 1976).

ii) RAPID EYE MOVEMENT

Eye movement during REM sleep is one of the phasic characteristics of REM sleep which led to the discovery of this state of sleep. Involvement of the brain stem in eye movement control was first demonstrated by Cohen and Komatsuzuki (1972). Unit recording studies from the pontine reticular formation in awake animals revealed an intricate mechanism which was involved in the control of fast eye movements (Henn and Cohen, 1976, Curthoys et al. 1981). Many cell types controlling different aspects of the eye movement have been identified, out of which a group of pontine burst neurons (excitatory burst neurons) are believed to be the immediate pre-motor element generating horizontal saccades (Vertes, 1984). Although it may be that the pontine burst neurons involved in the generation of fast eye movement during waking may do the same during REM sleep (for generating the rapid eye movements), yet there are no reports in which these group of cells have been analyzed systematically during REM sleep, in intact animals. Pompeiano and
his colleagues (Hoshino et al. 1976a) examined the relation between pontine reticular formation discharge and the eye movements in decerebrate cats. Rapid eye movements occur spontaneously in chronic decerebrate animal and can be induced with eserine in the acute decerebrate animal. Two types of the cells were described, one type showed slow rhythmical oscillations in discharge and was proposed to be involved in changing the direction of the eye from side to side, while the second population exhibited phasic burst in discharge correlated with the discrete burst of rapid eye movements which resemble the pattern of the excitatory burst neurons in conscious animals. The oscillatory type were distributed throughout the pontine tegmentum whereas the phasically discharging cells were located on, or slightly lateral, to the midline in the region of abducens nucleus (Hoshino et al. 1976b). Further analyses of the unit activity in a more extensive region of the brain stem, including the core of the pontomedullary tegmentum as well as the surrounding regions of the reticular formation, led to the identification of pontomedullary cells which were found to discharge in association with the eye movement during REM sleep as well as during waking (Siegel and Tomaszewski, 1983). These burst neurons were clustered in and around the peri-abducens region. Hence, cells located in the peri-abducens region of the pontine reticular formation may be involved in controlling the saccadic eye movements of REM sleep as well as during waking.

iii) MUSCLE TWITCHES:

Closely correlated with the phasic burst of the rapid eye movement during REM sleep is the occurrence of another phasic characteristics of REM sleep i.e. muscle twitches especially in the muscle of the face and the distal limbs (Gassel et al. 1964). Early reports identified the reticulo-spinal system as critically involved in
the phasic muscle twitches of REM (Gassel et al. 1964, 1965, Marchiafava and Pompeiano, 1964). Unit recording studies, in behaving animals, have indicated the involvement of the pontomedullary cells which show discharge on with the phasic muscle twitches (Siegel and McGinty, 1977, Vertes, 1984).

RESPIRATORY FLUCTUATIONS:

Significant respiratory changes take place during REM sleep largely associated with the phasically occurring motor events of that site (i.e., rapid EMs and myoclonic twitches). Specifically, during REM sleep breathing becomes shallow, more frequent and irregular correlated with bursts of rapid EMs. Cell groups primarily responsible for both the respiratory and cardiovascular alterations of REM are proposed to be the medial parabrachial nucleus (MPB) of the dorsolateral pons (Vertes, 1984). It has been proposed that these MPB cells are directly involved in modulating the respiratory rhythm during states of sleep (Sieck and Harper, 1980).

CARDIOVASCULAR FLUCTUATIONS:

The most striking cardiovascular changes in REM involve phasically occurring increases in heart rate and blood pressure largely coincident with the other phasic events of REM sleep (Vertes, 1984). Although several cell groups of the brain stem, cerebellum (fastigial nucleus) and forebrain have been implicated in cardiovascular control (Vertes, 1984) probably the parabrachial complex is critically involved in the heart rate and blood pressure changes of REM (Vertes, 1984). The demonstration of both cardiac and respiratory-related units (Vertes, 1984) within the medial parabrachial nucleus suggests that the MPB may be involved in integrating the cardiovascular and respiratory changes of REM sleep and possibly other states as well.
A brief summary of the studies mentioned above suggests that:

1] The pontine reticular formation is the area within the brain stem reticular formation which is essential for REM sleep.

2] There are two group of neurons, the REM-ON neurons (which start or increase their rate of firing as the REM approaches and during REM) and the REM-OFF neurons (which stop or decrease their firing rate) which are proposed to be involved in the generation and regulation of REM sleep.

3] The REM-ON neurons are proposed to be cholinergic whereas REM-OFF neurons are proposed to be aminergic.

4] Neurochemical mechanisms involved in REM sleep implicate an interaction between the cholinergic and the aminergic group of neurons.

5] ACh and NE are the two main neurotransmitters involved in the initiation and the regulation of REM sleep. NE is thought to play the inhibitory role whereas the ACh is thought to play facilitatory role.

6] There is a possibility that neuropeptides like VIP or some REM sleep factor or nucleosides, like adenosine, may have a modulatory role in the regulation of REM sleep.

H) REM SLEEP DEPRIVATION:

To understand the regulatory mechanisms and the functional significance of REM sleep, different methodologies have been used. The methodologies used are either to induce REM sleep or cause deprivation of REM sleep. Injection of chemicals, electric shocks, lesioning, stimulations etc are some of the common techniques which have been used. These techniques are invasive techniques or likely to
cause non-specific effects. To study the biochemical mechanisms involved in the regulation or the function of any behavioral phenomenon the technique used should ideally be noninvasive, nonsurgical and non-chemical so as to nullify the drawbacks of non-specific effects and side effects should be minimum. REM sleep deprivation is one of the ideal ways by which it is possible to understand the biochemical regulation as well as the functions of REM sleep without using any invasive methods. REM sleep deprivation technique has the unique advantage, it can give information about the functional as well as the physiological, biochemical and behavioral aspects of REM sleep. A detailed review of the studies, using REM sleep deprivation is as follows:

1) REM DEPRIVATION AND BEHAVIORAL CHANGES

It was Dement (Dement, 1960) who performed the first REM sleep deprivation experiments using human subjects. He constantly monitored them and as soon as they displayed rapid eye movement or activated EEG, immediately the subject was awakened. One of the major finding was that greater the REM deprivation, greater number of times the subjects went into REM sleep. During the recovery the subjects spent higher percentage of their sleeping time in REM sleep. This showed that the REM sleep was necessary and there was a tendency to recover lost REM sleep. This compensatory trend has been referred as the ‘rebound effect’. Behavioral changes like anxiety, irritability and increase in appetite were also noticed (Dement, 1960). In another study too, the sleep deprived subjects showed similar kind of behavioral changes (Dement and Fisher, 1963). Sampson (1966) deprived six subjects for 3 nights. He also reported increase in appetite, irritability, increase in euphoria and a modification in the sense of reality.
Clemes and Dement (1967) deprived human subjects and conducted the first controlled study to demonstrate psychological changes using psychological tests (e.g., Nowalis-Green Mood Check List, Holtzman Inkblot Test and Welsh Figure Preference Test). Behavioral changes like signs of confusion, suspicion, and withdrawal was also noted in another study (Agnew et al. 1967). There were several other studies where no significant alterations in the behavior were observed (Feldman and Dement, 1968, Ekstrand et al. 1971).

Animal studies have also helped in understanding the behavioral changes due to REM sleep deprivation. Morden et al. (1968) examined the effect of shock induced fighting in rats after REM sleep deprivation by the flower pot method. They showed that after three, five and seven days of REM sleep deprivation there was a significant increase in the shock induced fighting. Even after 16 days of recovery there was a significant increase in the shock induced fighting as compared to normal controls. REM sleep deprivation increased sexual behavior (Morden et al. 1968), increased cortical excitability (Satinoff et al. 1971), decreased threshold for intracranial self stimulation and showed significant higher response rates (Steiner and Ellman, 1972). Albert and his co-workers (1970) observed increased motor activity after REM sleep deprivation. Moore et al. (1979) also reported increased aggressiveness, diminished fear, increased exploration, increased preference for novelties etc. on REM sleep deprivation. Rechtschaffen and his coworkers (1989) observed many behavioral changes including increase aggressiveness, decreased grooming, increased irritability etc. All the above studies show that there is a tremendous alteration in the behavior of the animal after REM sleep deprivation and these changes could only take place due to alteration in the physiology and the biochemistry of the central nervous system.
II) REM SLEEP DEPRIVATION AND CHANGES IN LEARNING AND MEMORY:

Effect of REM sleep deprivation on learning and memory has been a subject of intense research (Fishbein and Gutwein, 1977, McGarth and Cohen, 1978). There are many reports in which there is an increase of REM sleep after a learning task (Fishbein and Gutwein, 1977, McGarth and Cohen, 1978, Portell-Cortes et al. 1989). It has been shown that REM sleep deprivation affects learning and memory in at least two different ways:

1) REM sleep deprivation affects the conversion of short term memory trace into long term memory (Fishbein, 1970) and
2) REM sleep deprivation interferes with the active maintenance of the consolidated memory trace (Fishbein et al. 1971, Fishbein and Gutwein, 1977, McGarth and Cohen, 1978).

Dement (1965) showed that REM sleep deprivation affects performance of a learning task in cats. Pearlman (1971) observed that REM sleep deprivation in rats interfered with the retention of the passive avoidance task. Fishbein (1970) observed that mice, trained for passive avoidance before REM sleep deprivation exhibited impairment in the long term memory trace, whereas short term memory and acquisition of new memory was not affected. Injections of anisomysin (a potent inhibitor of cerebral protein synthesis) in mice, after a learning task, disrupted long term memory (Fishbein and Gutwein, 1977). Sagaales and Domino (1973) reported similar observation using automated shuttle box conditioning. Anisomysin, was later known to inhibit REM sleep with no effect on NREM sleep or wakefulness (Fishbein and Gutwein, 1977). In another study conducted by Fishbein and his coworkers (Fishbein et al. 1971) showed that REM sleep deprivation affects the maintenance of long term memory trace. Shiromani et al. (1979) have reported that 3 h
REM sleep deprivation, after an aversively motivated learning does not affect memory and learning but REM sleep deprivation for a protracted period of time slows down the stabilization of memory.

III) REM SLEEP DEPRIVATION AND BIOCHEMICAL CHANGES:

REM sleep deprivation is known to affect the biochemistry of the brain. Other organs have not been studied in greater detail.

i) IN BRAIN

Hobson (1989) described REM sleep as for the brain, by the brain and of the brain. Hence, it would be imperative for REM sleep deprivation to alter the biochemistry of the brain.

REM sleep deprivation causes variety of changes in the brain. Cerebral free amino acid levels in rats are known to be altered (Micic et al. 1967, Davis et al. 1969). Micic et al. (1967) reported changes in the levels of gama-aminobutyric acid (GABA) in rat brain (Table B), Davis et al. (1969) reported changes in the level of almost all the amino acids in rats (Table A). Mendelson and his colleagues (1974) have reported changes in the levels of lactate, pyruvate, and l-malate in rat brain. Heiner et al. (1968) have observed changes in the decrease in the levels of potassium in the rat brain after 10 days of REM sleep deprivation. Karadzic and Mrsulja (1969) showed that REM sleep deprivation decreases the glycogen content in rat brain and three hours recovery did not bring back the level of glycogen. Ammonia metabolism in the rat brain is also reported to be altered after REM sleep deprivation, activities of glutamine synthetase, glutaminase and AMP deaminase also increased after REM sleep deprivation. Though there was no significant change in protein synthesis after REM sleep deprivation, an increase in the uptake of radio-labeled amino acids in the brain has been reported (Bobillier et al. 1971). Bowers et al. (1966) showed a decrease in the level of
acetylcholine after REM sleep deprivation whereas Cramer et al. (1973) observed that REM sleep deprivation induced an increase in the turnover of serotonin. Hery et al. (1970) have reported an increase in the synthesis of serotonin after REM sleep deprivation. Sinha et al. (1976) have shown that REM sleep

Table A:

Effect of REM sleep deprivation on the levels of Amino acids in the rat brain.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>In Whole Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic Acid</td>
<td>Increase</td>
</tr>
<tr>
<td>Threonine</td>
<td>Increase</td>
</tr>
<tr>
<td>Serine</td>
<td>Increase</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Increase</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>Increase</td>
</tr>
<tr>
<td>Glycine</td>
<td>Increase</td>
</tr>
<tr>
<td>Alanine</td>
<td>Increase</td>
</tr>
<tr>
<td>Valine</td>
<td>Increase</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Increase</td>
</tr>
<tr>
<td>Leucine</td>
<td>Increase</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Increase</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Increase</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Increase</td>
</tr>
<tr>
<td>Lysine</td>
<td>No Change</td>
</tr>
<tr>
<td>Arginine</td>
<td>No Change</td>
</tr>
<tr>
<td>GABA</td>
<td>No Change</td>
</tr>
</tbody>
</table>

Adapted from Davis et al. 1969
Table B: Effect of REM sleep deprivation on the levels of Amino acids in the rat brain.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Reticular Formation</th>
<th>Thalamus</th>
<th>Caudate Nucleus</th>
<th>Colliculi</th>
<th>Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic Acid</td>
<td>Increase</td>
<td>Increase</td>
<td>Decrease</td>
<td>N. S.</td>
<td>Increase</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>N. S.</td>
<td>Increase</td>
<td>N. S.</td>
<td>N. S.</td>
<td>N. S.</td>
</tr>
<tr>
<td>GABA</td>
<td>Increase</td>
<td>Increase</td>
<td>Increase</td>
<td>Decrease</td>
<td>Increase</td>
</tr>
</tbody>
</table>

Adapted from Micic et al. 1967
deprivation caused an increase in the activity of tyrosine hydroxylase whereas recovery after REM sleep deprivation has been shown to increase the turnover of NE (Pujol et al. 1968, Mark et al. 1969, Stern et al. 1971). REM sleep deprivation increased the activity of the enzyme Na-K ATPase (Gulyani and Mallick, 1993). REM sleep deprivation has been found to affect the activity of acetylcholinesterase, monoamine oxidase, hexokinase, glucose-6-phosphatase and 5'-nucleotidase as reported in detail in this thesis (Thakkar and Mallick, 1991, 1992, Mallick and Thakkar, 1991, 1992). All the studies mentioned above have used the flower-pot method for REM sleep deprivation.

ii) IN OTHER ORGANS

Very few studies have reported the effects of REM sleep deprivation on organs other than brain. Potassium levels in the blood have been reported to decrease on REM sleep deprivation.

Using the rotating disc technique Rechtschaffen and coworkers have studied the effect of REM sleep deprivation in rats for a very long period (as long as 54 days) (Kushida et al. 1989). Death occurred after long term REM sleep deprivation (Kushida et al. 1989). Food intake and the water intake increased after REM sleep deprivation whereas the body weight has been reported to decrease (Kushida et al. 1989). Ulcerative and keratotic lesions were observed on the tails and planter surfaces and the fur turned brownish yellow (Kushida et al. 1989). There was no observable body fat whereas there was a reduction in the connective tissues (Kushida et al. 1989). Pinpoint erosions were seen in the stomach (Kushida et al. 1989). All the internal organs appeared normal, except that there was an enlargement of the adrenal glands (Kushida et al. 1989). Hematological studies have shown that white blood cell count and polymorphonuclear cells showed an increase on REM sleep deprivation.
deprivation whereas no change was observed in RBC count (Kushida et al. 1989). Increased plasma urea, indicative of increased protein catabolism was observed after REM sleep deprivation. Urinary pH has been reported to decrease, whereas no change was observed in urobilinogen, ketone bodies, glucose etc. in the urine (Kushida et al. 1989). Long term REM sleep deprivation has been reported to cause an increase in the energy expenditure but the mediating factors have not been elucidated (Bergmann et al. 1989). Plasma level of T3 and T4 has been reported to decrease on REM sleep deprivation whereas epinephrine showed an initial increase but on long term deprivation epinephrine came back to the baseline level. Long term REM sleep deprivation is reported to cause a nonsignificant increase in the levels of plasma adrenocorticotropic hormone (ACTH) and corticosterone (Bergmann et al. 1989).

IV) REM SLEEP DEPRIVATION AND NEURONAL CHANGES:

Dement and co-workers have proposed that deprivation of REM sleep causes a generalized increase in the cortical excitability (Cohen and Dement, 1965). Using the threshold of electro-convulsive shock as a gross measure for the change in neuronal excitability (Cohen and Dement, 1965), these authors observed that the threshold was considerably decreased. They proposed an overall increase in the neuronal excitability which was reversed when the animals were allowed to recover. Dewson et al. (1965) found accelerated auditory recovery in REM sleep deprived cats. REM sleep deprivation has been found to affect the firing rates as well as the auditory stimulation induced inhibitory response of the pontine neurons (Mallick et al. 1989, 1991).

V) THE METHODOLOGY USED FOR REM SLEEP DEPRIVATION:

To perform REM sleep deprivation suitable methodology is
probably the most crucial factor. A brief description about the methodologies used to deprive the animal and human subjects is as follows:

i) **THE AROUSAL TECHNIQUE**:  
   It was Dement (Dement, 1960) who performed the first REM sleep deprivation experiments using this technique on human subjects. He constantly monitored the electrophysiological parameters of sleep in human subjects and as soon as the subject displayed rapid eye movement or activated EEG, the subject was immediately awakened manually. As in case of humans, arousal technique has been tried in animals too. The animal is recorded polygraphically and at the onset of REM period the animal is aroused by external stimulus.

ii) **TREADMILL AROUSAL TECHNIQUE**:  
   In this technique used first by Ferguson and Dement (1967), the experimental rats were kept moving on a moving treadmill for 16 to 22 hrs a day. Theoretically no sleep is possible, but in practice NREM sleep becomes possible as the animal learns to rush to the beginning of the treadmill and sleeps or rests for 15 to 20 seconds depending on the speed of the treadmill. Ferguson and Dement (1967) recorded cats and observed slowing of EEG resembling NREM sleep, which was around 40% of the total time on the treadmill. This technique along with the hand arousal has been used for a few studies.

iii) **THE PENDULUM TECHNIQUE**:  
   Another technique developed for REM sleep deprivation and used by the authors for the first time, is the pendulum technique (Van Hulzen and Coenen 1980). In this technique the animals are prevented from entering into REM sleep by allowing them to sleep for a very short period of time. An apparatus, which moves the
animal cage back and forth, resembling a pendulum is used. At the extremes of the motion postural imbalance compels the animal to walk downward to the other side of the cage. A minimal amount of REM sleep (0-2%) with moderate amount of NREM sleep (19-30%) has been reported. Deprivation by this technique resulted in the REM rebound when recovery of sleep was allowed.

iv) THE ROTATING DISK TECHNIQUE

A very innovative technique for total as well as selective REM sleep deprivation has been developed by Rechtschaffen et al. (1983). This technique deprives the animal of sleep (total or selective) with relatively benign stimulation, with minimum of stress and the same stimulation could be given to the control subjects without any compromise on their sleep. The experimental apparatus for deprivation consisted of a horizontal disk that was suspended on water. On the disk the experimental and the control animals were housed on either side. EEG, EMG and the theta activity of both the animals were continuously monitored and later scored by a microcomputer for wakefulness, high amplitude NREM sleep, low amplitude NREM sleep, REM sleep and total sleep. The microcomputer was programmed to rotate the disk (within 5 seconds) when a forbidden state of sleep was recorded only in the experimental animal. Direction of the rotation was random and the speed was 3.5 rev/min. The rotation of the disk awakened both the animals and both started walking in the direction opposite to the rotation of the disk so as to avoid immersion in water. The control animal was also awakened, but the experimental animal was not sleeping all the time. Therefore when the experimental animal was awake, the control animal could sleep as the disk rotated only when the experimental animal entered the forbidden state of sleep. Thus, the control animal could get as much sleep as it needs.
v) **THE FLOWER POT TECHNIQUE**

The flower pot or the water tank technique has been used to eliminate REM sleep specifically without a significant loss of NREM sleep. It was Jouvet (Jouvet, 1972) who developed this very simple but highly effective technique of REM sleep deprivation. In this technique the animal (cat, rat or mouse) is placed on an inverted flower pot, a small platform surrounded by water. This allows the animal to sit and sleep (only NREM sleep) but no REM sleep is possible because the platform is very small and the animal is unable to maintain the posture necessary for REM sleep due to the postural atonia during REM. At the onset of REM the animal is awakened to avoid falling into the water or due to its coming in contact with water. Hence the animal on the platform can go to NREM sleep but not REM sleep.

Large platform controls are placed on platforms which are large enough for them to procure both NREM as well as REM sleep but the rest of the conditions are the same as the experimental animal on the smaller platform. Many other controls have been used. One of the different controls used is the movement restriction control, in which the animals are kept in very small cages so that their movement is restricted. Another control which has been frequently used is the swimming control to eliminate the effect of increased muscular activity on smaller platforms. Though it may be difficult to equate the stress induced by deprivation and due to swimming. The flower pot technique for REM sleep deprivation is the most extensively used technique for REM sleep deprivation.

vi) **THE MULTIPLE PLATFORM TECHNIQUE**

Further modification of the flower pot technique has been the use of multiple small platforms instead of one single platform, so that the animal can move around and hence the immobility, which
may be a stressful condition, in case of the single platform is eliminated in the multiple platform technique.

**COMPARISON OF DIFFERENT METHODS FOR REM SLEEP DEPRIVATION**:

For REM sleep deprivation study, suitable control experiments and achieving REM sleep deprivation are basic methodological criticisms which can reasonably be raised. The arousal technique is a noninvasive technique of REM sleep deprivation, but it requires tremendous labour and can be used only when the deprivation period is short. This technique becomes impractical for long term deprivation, as the number of arousal becomes inordinately large in a short period and the procedure becomes impossible to perform (Morden et al. 1967). The treadmill technique is no longer used for selective REM sleep deprivation because the treadmill deprives the animal of a very high proportion of NREM sleep. The rotating disk technique requires very sophisticated instrumentation and hence not easily accessible. The pendulum technique is also reported deprive the animal of a high amount of NREM sleep. The flower pot method for REM sleep deprivation, is most widely used (Bowers et al. 1966, Mendelson et al. 1974, Vogel, 1975, Yanick and Radulovacki, 1987, Oniani et al. 1988, Mallick et al. 1989, Thakkar and Mallick 1991). This is due to the fact that this technique is easy to perform, non-invasive and does not require any sophisticated instrumentation. Many studies evaluating the stress induced by this method have been done and all of them have suggested the effect of stress, if any, is negligible (Vogel, 1975, Van Luijtelenaar and Coenen, 1976, Hicks et al. 1977, Oniani et al. 1988, Coll- Andreu et al. 1989). Hence, the flower pot technique has been used in this study.

**I] THE FUNCTION OF REM SLEEP**:

Besides the mechanism of the generation of REM sleep, another
important question relates to the functions of REM sleep. The basic question still remains, what is the function of REM sleep? Even after forty years of intensive research very little is known about its functional significance. Mainly three different approaches have been used to understand the functions of REM sleep. Some investigators have used the phylogenetic or the evolutionary approach (Snyder, 1966), whereas others have used the developmental or the ontogenetic approach (Roffwarg, et al. 1966). The third major approach has been to deprive the animal of REM sleep and observe the physiological, biochemical and/or behavioral changes taking place thereafter. Many hypothesis have been proposed (Ephron and Carrington, 1966, Roffwarg et al. 1966, Snyder, 1966, Berger, 1969, Stern and Morgane, 1974, Fishbein and Gutwein, 1977, Vogel, 1979, Crick and Mitchinson, 1983, Siegel and Rogawaski, 1988, Winson, 1990).

Ephron and Carrington (1966) gave the 'homeostatic' hypothesis. In this hypothesis REM sleep functions as a counterbalance to the fall in the state of vigilance (cortical tonus) which occurred during NREM sleep. A protective role to an otherwise potentially dangerous condition of the sleeper, REM sleep excites the cerebrum so as to restore the tonus. Snyder's sentinel theory (Snyder, 1966) hypothesized that the function of REM sleep was not only to bring the consciousness, but also allow the organism to have a glimpse of the outside world, for quicker response against any danger.

Dement one of the founding fathers of REM sleep, along with his colleagues (Roffwarg, et al. 1966) has also proposed a very interesting 'ontogenetic' hypothesis to explain the importance of REM sleep. Their theory is based on the fact that REM sleep is more in younger individual than in adults. These authors have hypothesized that REM sleep functions as an endogenous stimulant,
providing functional excitation to the higher centers of the brain and helps in the process of structural development, maturation and differentiation of the important sensory and the motor areas. These stimulations are very important in utero and shortly after birth, before the availability of external stimulation to the central nervous system. Hence, the central nervous system is sort of prepared to handle the enormous rush of the external stimulation provided by the postnatal milieu.

Berger (1969), in his oculomotor innervation hypothesis proposed that REM sleep provides a mechanism for the development and establishment of the neuromuscular pathways for the conjugate eye movements. Stern and Morgane (1974) in their hypothesis have suggested that REM sleep maintains the functioning of the aminergic systems in the central nervous system and also may play a significant role in the synthesis /turnover of proteins. This hypothesis is based mainly on the effect of pharmacological agents on REM sleep and the effects of REM sleep deprivation. Fishbein and Gutwein (1977) have also implicated REM sleep to play an important role in the learning processes. This hypothesis is mainly based on the effects of REM sleep deprivation studies on learning and memory and on the observations that pharmacological alterations in brain protein synthesis, cholinergic and aminergic neurotransmitter activity are paralleled with increase or decrease in REM sleep, along with concomitant changes in memory. Vogel, after observing the increase in the excitability after REM sleep deprivation proposed a 'motivational' hypothesis (Vogel, 1979) suggesting that REM sleep keeps the excitability down, in controlled state.

One of the recent attempt to explain the function of REM sleep has been made by the Noble Laureate Prof. Francis Crick and his colleague Prof. Graham Mitchinson (1983). These authors have
proposed that forgetting or 'reverse learning' takes place during REM sleep. Memories are stored by development of new synapses or new neural connections, which may include what is not required or useless memories. These useless memories are removed during REM sleep and hence dreams are difficult to remember. This cleans up the cerebrum so it does not get cluttered up. The primitive egg laying mammals do not have REM sleep due to the fact that those animals have a very big cerebrum. Siegel and Rogawaski (1988) have proposed that REM sleep has the function of maintaining the optimal functional integrity of the NE receptors. The REM-OFF cells of the LC are active throughout the wakeful period and the NREM period and stop/decrease their firing only during REM. Based on this evidence they have proposed that these NE-containing LC cells decrease their firing during REM to prevent the desensitization of NE receptors. These NE receptors which are continuously sensitized by LC discharge have to be desensitized to remain maximally effective. Winson, in his hypothesis has proposed that the reprocessing of the crucial information acquired during the period of waking takes place in REM sleep (Winson, 1991).

J) REM SLEEP AND DISEASED STATES:

Sleep disorders were known to mankind since the early writing of the medical history. Hypersomnolence and insomnia were classified as sleep disorders during early 18th century (Thorpy, 1990). The first REM sleep disorder to be mentioned is narcolepsy, though there has never been a common agreement for considering narcolepsy as an exclusive REM sleep disorder (Broughton, 1990). Narcolepsy is a condition in which the brain stem mechanism of sleep-wakefulness (mainly REM sleep) are affected. Narcolepsy represents a model of dissociated sleep wakefulness, with the intrusion of REM sleep in wakefulness. This disordered state of
REM sleep is characterized by a tetrad of four major symptoms. These are: 1) Sleep attacks, with excessive daytime sleepiness, 2) Cataplexy (similar to REM sleep’s muscle atonia), 3) Sleep paralyses and 4) vivid hypnagogic hallucination. The genetics of narcolepsy suggests that this disease may be due to the presence of a narcolepsy-susceptible gene proposed to be present on chromosome 6, though the exact location is yet to be determined. Pharmacological and pathological studies of humans and canine narcolepsy have showed abnormalities in the cholinergic and aminergic mechanisms which are involved in REM sleep (Mefford et al. 1983, Aldrich, 1990). Cholinergic antagonist as well as serotonin and NE uptake blockers, inhibit cataplexy in dogs (Foutz et al. 1981, Montplaisir and Godbout, 1986), whereas cholinergic agonist and alpha-1 antagonist are known to exacerbate cataplexy (Langdon et al. 1986, Aldrich and Rogers, 1989). Dopamine levels, upregulation of muscarinic as well as dopaminergic D2 receptors have also been observed in pontine reticular formation (Mefford et al. 1983, Bowersox, et al. 1987).

REM SLEEP BEHAVIOR DISORDER:

REM sleep behavior disorder is a recently recognized clinical syndrome, which was predicated twenty years ago in cats with pontine lesions (REM sleep without 1–4Xat(Mahowald and Schenock, 1989). This disorder is evident by the presence of continuous (phasic and tonic) presence of the muscle tone, vigorous jerks, sleep shouting, sleep talking, aggressive behavior, and emergence of variable complex, potentially dangerous, behaviors during REM sleep. This clinical syndrome has been reported in association with multiple types of neurological diseases including patients with degenerated cells in the pendunculo-pontine region (olivo- ponto-cerebellar atrophy) and these patients have decreased ChAT in their brain (Mahowald and Schenock, 1989).
OTHER DISEASE:

PARKINSONISM:

Parkinsonism, Parkinson’s disease and related movement disorders are neurological diseases characterized by bradykinesia, rigidity, resting tremor, and loss of postural reflexes. Degenerative process, behavioral, respiratory, motor dysfunctioning and medication affecting neurophysiological as well as neurochemical systems responsible for the normal regulation of sleep wake cycle, are known to cause disruption in sleep-wake cycle (Aldrich, 1989). Clinical observations have shown significant reduction in REM sleep, duration of REM period have been found to be reduced, presence of EEG alpha activity, slow wave activity during REM sleep in patients with parkinsonism (Aldrich, 1989).

DEMENTIA AND ALZHEIMER’S DISEASE:

Patients with dementia or Alzheimer’s disease are reported to have alteration in sleep-wake cycles as compared to normal aged individual. Cholinergic systems are known to be affected in patients with dementia or Alzheimer’s disease which are characterized by decrease in the levels of ChAT (Bliwise, 1989). Hence, abnormalities in REM sleep are of particular interest as it is known that cholinergic system plays a crucial role in the regulation of REM sleep. REM sleep has been reported to decrease in some cases whereas in one case it has been reported to increase significantly. Increased REM latency or late appearance of REM sleep has also been observed in patients with dementia (Bliwise, 1989).

EPILEPSY:

Epileptic seizures are known to occur predominantly during NREM sleep or during arousal from NREM sleep, whereas REM sleep has been hypothesized as a protective stage of sleep. Although local epileptic surges can persist during REM sleep, desynchronization of
EEG is thought to be incompatible to the spread of EEG. Profound motor inhibition during REM sleep may also have a role to play in restriction of motor seizures (Shouse, 1989).

PSYCHIATRIC DISORDERS:

Dreaming is very similar to psychosis. Dream sleep or REM sleep is a state in which hallucinations, bizarre thinking, distortion of perception and temporary delusions are mixed with normal thinking and perceptual processes (Zarcone, 1989). Hence, abnormalities in REM sleep are often observed in cases of psychiatric disorders.

AFFECTIVE DISORDERS:

Most of the patients suffering from major depressive illness have been reported to show a decreased first NREM period leading to an early appearance of REM sleep (decreased REM latency), altered intra-night temporal distribution of REM sleep, increased REM sleep time and increased rapid eye movements during REM sleep or increased REM density (Reynolds, 1989). REM sleep deprivation via arousal at the onset of REM periods have been reported to improve endogenous depression (Vogel, 1989). Imbalance in the cholinergic and the aminergic systems have been hypothesized to be the cause of endogenous depression and the importance of the cholinergic and the aminergic system in the organization of REM sleep is known (Gillin, 1989). Anti-depressant drugs have been hypothesized to have arousal type of REM sleep deprivation as their mechanism of action (Vogel, 1989).

SCHIZOPHRENIA:

Early studies of schizophrenic patients was aimed at testing the hypothesis to see whether schizophrenic had any intrusion of REM sleep in waking, which may have led to these bizarre state. But schizophrenic patients did not show any electrophysiological sign of REM sleep in waking state. Later studies indicated abnormalities in
REM rebound in schizophrenic patients. Abnormalities in the latency of REM sleep have been observed by some group, but other reports have shown contradictory results (Zarcone, 1989).

I] SUMMARY:
A critical survey of studies carried out, till date, suggests that:

a] REM sleep is essential for life and long term REM sleep deprivation ultimately leads to death.

b] After REM sleep deprivation there is a compulsion to recover the lost bout of REM sleep, which is known as the 'rebound effect'.

c] The pontine reticular formation is the area within the brain stem reticular formation which is essential for REM sleep.

d] There are two groups of neurons, the REM-ON neurons (which start or increase their rate of firing as the REM approaches and during REM) and the REM-OFF neurons (which stop or decrease their firing rate) which are proposed to be involved in the generation and regulation of REM sleep.

e] Neurochemical mechanisms involved in REM sleep implicate an interaction between the cholinergic and the aminergic group of neurons in which the aminergic group are thought to play the inhibitory role whereas the cholinergic mechanisms are thought to play facilitatory role.

f] Acetylcholine and noradrenaline are the two main neurotransmitters involved in the initiation and the regulation of REM sleep, whereas there is a possibility that neuropeptides like VIP or some REM sleep factor or nucleosides, like adenosine, may have a modulatory role in the regulation of REM sleep.

g] REM sleep deprivation is one of the important ways by which the neurochemical, physiological and the functional aspects of REM sleep can be understood.

h] The flower pot technique is the widely used and easy to
perform, non-invasive, non-chemical method for selective deprivation of REM sleep. The effect due to stress, non-specific reasons, etc. is very less in the animals deprived of REM sleep by this method.

i] AChE level showed an increase whereas NE level did not show any change after REM sleep deprivation but there was an increased synthesis of NE.

j] There was an increased glucose metabolism during REM sleep whereas REM sleep deprivation causes a very high increase in the energy expenditure.

k] REM sleep deprivation is known to cause an increase in the activity of the enzyme Na⁺-K⁺ ATPase which probably induces an increase in the neuronal excitability.

[1] LACUNAE :

A critical survey of the studies, summarized above revealed that:

1) Though acetylcholine is implicated to be the major neurotransmitter in the regulation of REM sleep, and the levels of acetylcholine is altered during REM sleep as well as during REM sleep deprivation, the enzymes responsible for the synthesis and degradation have not been studied.

2) Noradrenaline is also known to be involved in the regulation of REM sleep. Effect of REM sleep deprivation on the levels, synthesis and the turnover has been studied, however there are no studies where the effect of REM sleep deprivation has been studied on the degrading enzyme, monoamine oxidase.

3) Studies have been conducted to see the metabolism of glucose during REM sleep and energy expenditure during REM sleep deprivation. There has been no study to see the effect of REM sleep deprivation on the enzymes involved in the metabolism of glucose.

4) REM sleep deprivation causes an increase in the neuronal excitability the changes in the membrane fluidity or membrane
constituents have not been studied.

5) Adenosine levels and the regulation of adenosine receptors after REM sleep deprivation have been studied but the enzymes which alter the adenosine level have not been studied.

6) Effect of REM sleep deprivation on transport of different ions is not yet known. Changes in the activity of Na-K ATPase, if any, have not been studied after REM sleep deprivation.

K) OBJECTIVES:

Therefore the objectives of these study were to investigate the

1) the effect of long term and short term REM sleep deprivation on the activity of acetylcholinesterase (the enzyme involved in the degradation of acetylcholine) and its molecular forms, in the whole brain homogenate and in different areas of the brain viz. the cerebrum, the cerebellum, the brain stem, the medulla, the pons and the midbrain.

2) whether long term and short term REM sleep deprivation has any effect on the activity of monoamine oxidase (an enzyme of the metabolic pathway of catecholamines) and its different forms, in the whole brain homogenate and in different areas of the brain.

3) the alteration in the activity of the key enzymes of glucose metabolism after long term and short term REM sleep deprivation in different regions of the brain.

4) the change in the activity of the enzyme 5'-nucleotidase, (an enzyme of the adenosine metabolism pathway) after REM sleep deprivation in different areas of the brain.

5) the changes in the membrane fluidity after long term and short term REM sleep deprivation in different areas of the brain.

6) the changes in the concentration of phospholipid after REM sleep deprivation in different areas of the brain.