REM Sleep and SCN

“When the sense organ has come to rest (ceased to function), the knowledge of the percepts and perceptions arising out of impressions (left by) of the waking state is the dream state.....”

(Paingala Upanishad, Ch.II; v.8)

“There, in sleep, that god (mind), experiences greatness. He sees again whatever object has been seen, he hears again whatever has been heard, he experiences again and again whatever has been experienced in different places and directions. What has been seen and not been seen, what has been heard and what has not been heard, what has been experienced and what has not been experienced, what is existent and what is non-existent, he sees all; being all he sees (all)”

(Prashna Upanishad, Ch. IV; v.5)

The Principal Upanishads by S. Radhakrishnan
1 REVIEW

Sleep, "the gentle tyrant", is a complex amalgam of behavioural and physiological processes; behaviourally, it is a reversible state of perceptual disengagement from, and unresponsiveness to, the environment (Dement and Carskadon, 1989). Sleep is regulated by three basic processes: (a) a homoeostatic process \((\text{process } S)\) mediating a rise in 'sleep pressure' during waking and the dissipation of 'sleep pressure' during sleep; (b) a circadian process \((\text{process } C)\), a clockwork mechanism, morphologically and functionally distinct from the homoeostatic component (Tobler et al., 1983) and independent of prior sleep and waking, defining the alternation of periods with high and low sleep propensity, and (c) an ultradian process occurring within sleep and represented by the alternation between the two basic sleep states- rapid eye movement (REM) sleep and non rapid eye movement (NREM) sleep (Borbely and Achermann, 1992).

1.1. Sleep Regulation

Various models have been proposed to account for sleep regulation and these models have been combined as different modules of a 'combined model of sleep regulation' by Achermann and Borbely (1992). The different modules of this model are sleep homoeostat, sleep inertia, circadian oscillator and REM sleep oscillator [Fig.5.1.]. According to this model, sleep architecture results from the sleep homoeostat in which, due to the input from REM sleep oscillator, ultradian pattern of SWA and REMS is generated. The alertness level \((A)\) results from process \(C\), process \(S\), and sleep inertia \((w)\) which is initiated upon awakening. Involvement of the suprachiasmatic nucleus in this scheme is suggested to be initiation and promotion of waking (Edgar et al., 1993) possibly by modulating the locus coeruleus activity (Chen et al., 1997) via relay
circuits in medial preoptic area (Chen et al., 1998).

The circadian oscillator does not receive any input from other modules except light and therefore is indirectly affected by the sleep-waking timing. C affects sleep homeostat, REMS oscillator and the alertness module. Interaction of S and C in the sleep homeostat determines the sleep-waking timing. Big rectangles = states of the system; Small rectangles = substates of the system; Circles = state derived variables; Arrows = direction of the connections with respect to states (not substates): A = alertness; SWA = slow wave activity; S = process S; x = REM on activity; y = REM off activity; C = basic circadian variable; Cc = complementary circadian variable; w = sleep inertia wake up process (Achermann and Borbely, 1992).

1.2. REM Sleep Oscillator

In many species including man, the time of occurrence of REM sleep and its parameters such as latency and duration are modulated by circadian rhythms. However, the occurrence of REM sleep does not depend on the presence of either a circadian rhythm or an intact circadian oscillator - SCN (Tobler et al., 1983; Eastman and Rechtschaffen, 1984; Eastman et al., 1984; Trachsel et al., 1992) and pineal gland (Mouret, 1982). Instead, this REM sleep oscillator system consists of diverse interacting neuronal populations, and has the characteristics of (producing) relatively constant REM sleep episode duration and median REM
sleep cycle length, and a variable probability of being "on" with a limit cycle organisation (Steriade and McCarley, 1990).  


The revised cycle reciprocal interaction model (LCRIM) of REM cycle control (McCarley and Massaquoi, 1992) originally proposed in 1986 is based on the reciprocal interaction model derived from the simple Lotka-Volterra model (McCarley and Hobson, 1975).

According to this model [Fig.5.2.], the events occurring in the generation of REM sleep cycle are:

1. Cholinergic projections from REM promoting REM-on neurons of laterodorsal tegmental (LDT) and pedunculopontine tegmental (PPT) nuclei depolarise and excite the effector neurons in the pontine, (PRF) and medullary (MRF) reticular formation. The conjoint activation of these effector neurons produces the state of REM sleep, characterised by rapid eye movements, ponto-geniculo-occipital (PGO) waves, muscle atonia and EEG desynchronisation. This positive feed back activation for exponential growth of REM-on activity includes a loop of reticulo-reticular connections, LDT/PPT and PRF.

![Fig.5.2. Schematic of LCRIM (McCarley and Massaquoi, 1992)](image)

1 Limit cycle organisation means, once the oscillator system is turned on, it tends to follow a relatively fixed time course of change whatever be the state of the animal when the oscillator is turned on (Steriade and McCarley, 1990).
(2) The activation of reticular formation effector neurons slowly excites REM-off, REM-suppressive neurons in the dorsal raphe (via excitatory amino acids, EAA) and locus coeruleus (via both EAA and ACh).

(3) When the REM-off neurons become active at the end of a REM sleep period, they terminate the REM sleep because of their inhibition of REM-on neurons. In LDT, cholinergic REM-on neurons firing in bursts, along with some non-burst cholinergic neurons, are inhibited by 5-HT in vitro (Luebke et al., 1992).

(4) Activity of REM-off neurons, maximal just after REM sleep period and decreasing in the following non-REM period, becomes minimal at the onset of REM sleep due to self-inhibitory feedback from recurrent collaterals (i.e., NE inhibition of LC neurons, and 5-HT inhibition of DR neurons).

(5) The decreased activity of REM-off neurons disinhibits REM-on neurons and allows the onset of a REM sleep episode. Then the cycle repeats itself.

The involvement of cholinergic and aminergic systems in the regulation of REM cycle has been verified by Benington and Heller (1995) who showed that serotonergic (from DR) and noradrenergic (from LC) systems are involved in the modulation of REM sleep timing, and cholinergic system in the maintenance of REM sleep.

1.3. REM sleep - Functions

REM sleep, first described by Aserinsky and Kleitman in 1953, is hypothesised to have a variety of important functions both during development (perhaps for sensorimotor programming) and throughout adult life (for information processing) (Jones, 1991). The specific function of REM sleep include modifying learned responses (Crick and Mitchison, 1983; Winson, 1993; Hennevin et al., 1995); stimulation of the nervous system in infancy (Roffwarg et al., 1966), reprogramming of innate behaviour patterns (Jouvet, 1975); regulation of noradrenergic receptor sensitivity (Siegel and Rogawski, 1994); and brain warming (Wehr, 1992).

REM sleep is functionally and homoeostatically related to NREM sleep.

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*In DR, there may also be an unconventional mode of 5-HT release (i.e., not coupled with soma depolarisation) during REM sleep and hence the inhibition (Crespi et al., 1990; Cespuglio et al., 1990).*
rather than to waking; and NREM sleep related process producing REM sleep propensity is closely associated with the process whereby sleep debt accumulated during waking is discharged in NREM sleep (Benington and Heller, 1994).

1.4. REM Sleep - Dysfunctions

The most striking example of all REM sleep disorders is narcolepsy. Narcolepsy is characterized by such symptoms as excessive daytime sleepiness, cataplexy, sleep paralysis, hypnagogic hallucinations and nocturnal sleep onset REM episodes.

More widespread REM sleep disorders are the mood disorders like depression, which show electrographic sleep abnormalities such as reduced REM latency, increased REM density in the first REM sleep period and a tendency to increased duration of the first REM sleep episode (Steriade and McCarley, 1990).

1.4.1. Depression - Theories

Although several theories have been proposed for the links between disorders of sleep and mood, most prominent among them are (a) S-deficiency hypothesis (Borbely and Wirz-Justice, 1982), (b) circadian phase advance hypothesis (Wehr and Wirz-Justice, 1981, 1982) and (c) cholinergic - aminergic hypothesis (McCarley, 1982).

1.4.1.1. S-deficiency hypothesis

This hypothesis postulates that a deficiency in depressives of factor S - which builds up both during waking and other 'non-sleep' states (Borbely and Tobler, 1996) - does not cause deep δ sleep (0.5 - 3 KHz) at sleep onset (Borbely and Wirz-Justice, 1982); instead, this results in a faster onset first REM sleep period (reduced REM latency). This factor S deficiency is postulated to cause the shortening of sleep duration in depressives. However, van den Hoofdakker and Beersma (1985) found that the accumulation curves of δ sleep in depressives with very short and longer REM latencies were similar, in contrast to this theory's prediction of co-variation of REM latency and accumulation of δ sleep.

1.4.1.2. Phase advance theory

This theory states that abnormalities of sleep and mood in depressives are a function of a phase advance in the circadian oscillator controlling both temperature and REM sleep propensity relative to the phase of the rest-activity
V. REM Sleep and SCN

circadian rhythm (Wehr and Wirz-Justice, 1981, 1982). Thus, the REM sleep pattern found in depressives at sleep onset is like that seen near the temperature nadir of normal subjects. This out of phase relationship of the temperature-REM sleep oscillator and oscillator regulating the rest-activity cycle leads to a mood disturbance (Steriade and McCarley, 1990; Duncan, 1996).

Nevertheless, the prediction of the phase advance theory for a longer duration REM latency in depressives during at least some point of the circadian cycle does not hold true in the following observations: (a) depressives show short REM latencies compared with normal subjects when they nap in the daytime (Kupfer et al., 1981) and (b) when they are awakened later in the night (Schulz and Tetzlaff, 1982). Thus, a simple phase change cannot explain the REM sleep abnormalities in depression.

1.4.1.3. Cholinergic-aminergic interaction theory

This theory, derived from the reciprocal interaction model of sleep cycle control, suggests a parallelism between the regulation of sleep cycle and mood, in terms of disturbances of monoaminergic control, cholinergic control and cholinergic–adrenergic balance (Steriade and McCarley, 1990). This theory attributes the shortened REM latency of depression to the increased activity of REM excitatory cholinergic neurons (Gillin et al., 1979) and to the decreased activity of REM inhibitory cholinergic neurons (Schittecatte et al., 1992). This theory hypothesizes that, in depressives, weakened aminergic inhibition produces a faster release from inhibition of cholinergic REM-promoting neurons, and as a result, faster onset of REM (decreased REM latency) and increased intensity of REM sleep episode (increased REM density) (McCarley, 1982).

Evidence is stronger for a monoamine role in depression and for more parallelism between monoamine controls in depression and sleep than for cholinergic system (Steriade and McCarley, 1990): (a) almost all clinically effective antidepressants (tricyclics, monoamine oxidase inhibitors-MAOI- and others) significantly reduce REM sleep and induce a characteristic REM rebound when the treatment is discontinued (Vogel et al., 1980; Sharpley and Cowan, 1995); (b) REM sleep deprivation, known to increase monoamine neuronal

3 However, Mendlewicz et al., (1991) found that REM suppression was not necessary for clinical improvement.
activity, improves depression with about the same efficacy as tricyclic antidepressants (Vogel et al., 1980); (c) both qualitative and quantitative modelling approaches to REM sleep abnormalities in depression have worked on the postulate that the level of monoamine population activity at sleep onset is less in depressives than in normals (Steriade and McCarley, 1990).

However, the aforementioned theories are not mutually incompatible; instead, the factors controlling circadian rhythms, REM sleep and non REM sleep do interact (Steriade and McCarley, 1990).

1.4.2. Depression :- Treatment

The therapeutic intervention of depression is achieved (a) by sleep displacement such as partial sleep deprivation and phase shifting the hours of sleep which activate an antidepressant mechanism and suggests a link between clinical state and the timing of circadian rhythms, and/or (b) by treatment with antidepressants (Duncan, 1996; Dawson and Armstrong, 1996).

1.4.2.1. Antidepressants

The therapeutic properties of antidepressants are linked to their effects on the circadian time keeping. The main classes of antidepressants are tricyclic antidepressants (TCAs), selective monoamine reuptake inhibitors, monoamine oxidase inhibitors (MAOIs) and atypical antidepressants (Sharpley and Cowan, 1995). Antidepressants can influence entrainment, motor activity, sleep-wake cycle, body temperature and/or hormones such as cortisol and growth hormone (Duncan, 1996).

1.4.2.1.1. Antidepressants and light entrainment

The possibility of abnormal light response (and consequently entrainment) in depression has been suggested by the observation of increased light suppression of nocturnal melatonin in bipolar depressives (Lewy et al., 1981). Some antidepressant and psychotropic drugs such as the MAOI, clorgyline, is known to decrease the magnitude of the light induced phase advance (Duncan, 1996). Since MAOIs induce changes in the 5-HT level of SCN (Ozaki et al., 1993), and since 5-HTergic activation decreases the SCN photic responses (Rea et al., 1994; Glass et al., 1995), photic phase shifting effects of MAOI may be mediated by a 5-HT ergic mechanism.
1.4.2.1.2. Antidepressants and sleep - wake cycle

Antidepressants produce striking changes in the sleep EEG (i.e. REM sleep, slow wave sleep - SWS - and sleep continuity) in both the healthy and the depressed subjects. A common feature of the antidepressants (MAOIs, TCAs and SSRIs) is the reduction of REM sleep and prolongation of REM latency; and if the treatment is abruptly stopped, a REM rebound (i.e. increased REM sleep and shortened REM latency) often happens (Sharpley and Cowan, 1995). However, some antidepressants like moclobemide are known to increase REM sleep (Monti, 1989)1 challenging the traditional view that REM sleep suppression is essential for the therapeutic effect of antidepressant (Kupfer et al., 1981). However, since behavioural interruption of REM sleep decreases δ power (Beersma et al., 1990), antidepressants suppressing REM sleep may also decrease δ power. Thus, the antidepressant effect of REM and δ power suppression and NREM arousal could combine to decrease SWA and alleviate depression (Duncan, 1996). Besides, antidepressant treatment is also known to alter diurnal variations in the monoamine levels of SCN and other structures such as dorsal raphe (Ozaki et al., 1993).

1.4.2.2. Sleep Deprivation

Partial sleep deprivation in late night, eliminating REM sleep, has been found to be more effective in elevating mood than partial sleep deprivation earlier in the night (Sharpley and Cowan, 1995). A more effective method to improve endogenous depression is REM sleep deprivation via arousals at sleep onset (Vogel et al., 1980). This phenomenon has been explained by Vogel et al., (1980) in his 'REM generator hypothesis' which states that REM sleep deprivation improves depression by stimulating a weakened NREM-REM sleep cycle oscillator. Thus, REM sleep deprivation acts as a chronobiotic improving depression, and as a chronobiotic, it can possibly act at any of the following seven sites of the circadian system :- (1) input to the pacemaker, (2) master pacemaker (SCN) itself, (3) entrainment mechanism, (4) coupling pathways, (5) slave oscillators, (6) passive systems (eg:- melatonin rhythm of pineal gland), and/or (7) feedback via overt rhythm (Dawson and Armstrong,1996).

1 Nevertheless, a noticeable adverse effect of moclobemide is insomnia (Monti, 1989).
Studies have been made on the effects of sleep deprivation on the electrical discharge rates of dorsal raphe (Lydic et al., 1984), and neurotransmitter receptor rhythms (Wirz-Justice et al., 1981). REM sleep deprivation (and total sleep deprivation) effects on different monoamines were also studied in different brain regions (Bergmann et al., 1984; Farooqui et al., 1996) and whole brain (Asikainen et al., 1995). Influence of REM sleep deprivation on the activity levels of MAO A and B (monoamine metabolising enzymes) in different rat brain regions has been studied as well (Perez and Benedito, 1997).

1.5. In summary:-
* Sleep is a complex phenomenon regulated by interacting homoeostatic, circadian and ultradian processes.
* Combined model of sleep regulation- derived from the amalgamation of different models of sleep regulation - is the most recent attempt to explain the entire cycle of sleep-wakefulness.
* Regulation of REM sleep is explained by LCRIM which is mainly based on the reciprocal interaction of cholinergic and monoaminergic neuronal populations.
* One of the dysfunctions of REM sleep is depression.
* REM disorders in depression are explained by three mutually interacting hypotheses namely, S deficiency hypothesis, phase advance theory and cholinergic- aminergic interaction theory.
* Depression can be therapeutically intercepted by the chronobiotic antidepressants and/or REM sleep deprivation.
* The effects of antidepressants on the photic entrainment and the monoamines of the pacemaker (SCN) is well known.
* The effects of REM sleep deprivation on photic entrainment and monoamines of the pacemaker is not well worked out.

Therefore, the objectives of this study are to find out the effect of REM sleep deprivation on:-

(1) the levels of Glu and SP (RHT neurotransmitters mediating photic entrainment) and VIP (the major target of RHT projection and also an important efferent from SCN) in SCN by ELISA; and
(2) the levels of 5-HT, its precursor 5-HTP, DA and GABA in SCN, dorsal raphe (a REM off nucleus and the main 5-HT source for SCN) and pineal using high performance thin layer chromatography (HPTLC).

2 MATERIALS AND METHODS

For all the experiments, inbred male Wistar rats (225 - 275 gms) kept under 12hL : 12hD cycle and provided with food (Hindustan Levers Ltd.) and water ad libitum were used.

2.1. Materials

2.1.1. Apparatus and accessories

96 well polystyrene ELISA plates (Corning Corp., New York), silical gel coated TLC plates (60 F210; Merck, Darmstadt, Germany), microsyringe (100μ l, Hamilton).

2.1.2. Chemicals

Analytical grade acetic acid, calcium chloride (CaCl2), magnesium chloride (MgCl2), magnesium acetate Mg(CH₃COO)₂ methanol (CH₃OH), phosphoric acid (H₃PO₄), potassium chloride (KCl), sodium chloride (NaCl), sodium carbonate (Na₂CO₃), copper sulphate (CuSO₄), sodium potassium tartrate, sodium-bicarbonate (NaHCO₃) and sodium bi sulfite (NaHSO₃) (Qualigens Fine Chemicals, Glaxo India Ltd.); ethylene diamine tetra-acetic acid (EDTA), Folin's reagent, ascorbic acid, acetone, heptane, potassium bi phosphate, boric acid, butanol, butylacetate, formic acid (BDH, India); 2-Mercaptoethanol and electronmicroscopic grade glutaraldehyde (E.Merck, Germany); DEAE sepharose, sodium borohydride, thimerosal, glutamate, horseradish peroxidase, substance P (SP) and vasoactive intestinal peptide (VIP) and Sigma FAST o-phenylenediamine dihydrochloride (OPD) tablets (Sigma, MO, USA); 5-hydroxy tryptamine (5-HT), 5-hydroxy tryptophan (5-HTP), dopamine, and γ - amino butyric acid (GABA), 1-propanol (HPLC grade) (Sigma, MO., USA); bovine serum albumin -BSA- (Pharmacia Fine chemicals AB, Sweden); monoclonal antibody of substance P (Sera-Lab, UK); monoclonal antibodies for VIP and Glu and secondary antibody - goat anti-rabbit IgG- (Sigma Immuno Chemicals,
St. Louis, MO., U.S.A.). Milli-Q single distilled autoclaved water was used for all the experiments.

2.2. Methods

2.2.1. REM sleep deprivation

2.2.1.1. Principle

Although there are several techniques for REM sleep deprivation (e.g., arousal, treadmill, pendulum, rotating disk, multiple platform etc.,) the one technique which is easy to perform, non-invasive, and does not require any sophisticated instrumentation and, therefore the most widely used is the flower pot (water tank) method developed by Jouvet (1972).

In this method, the animal (rat) is placed on an inverted small platform - flower pot - surrounded by water. This allows the animal to sit and go into NREM but not to REM sleep, because the platform is too small for the animal to maintain its posture for REM sleep due to muscle atonia. At the onset of REM sleep, the animal awakens to avoid falling into water or due to its coming in contact with water. Thus, the animal is selectively deprived of REM sleep.

As a control, a platform large enough permitting the animal to go to both NREM and REM sleep is used, keeping the remaining conditions the same as in the small platform set up.

2.2.1.2. Procedure

Experimental rats were kept for 48 hrs on 6.5 cms diameter inverted beaker (filled with sand) projecting above a pool of water in a polypropylene bucket. To rule out the possibility of non-specific effects, a control group of animals were kept on a large (12.5 - 13.5 cms diameter) sand-filled inverted beaker for an identical period as the experimental rats. All the rats - normal, control and experimental - were kept in the same ambient conditions.

2.2.2. Tissue collection

The rats were killed by cervical dislocation, and pineal was removed and stored in -80°C. Suprachiasmatic nuclei were punched out from 1mm thick sections (located at 5-6 mm posterior to the ventral posterior olfactory lobes) and dorsal raphe (located at 12-13 mm posterior to the anterior ventral cerebral border; ie., 6900 - 7800 µm posterior to bregma) from the frozen brains with a
V. REM Sleep and SCN

needle having an inner diameter of 0.7 mm as described in section IV. Punched out samples were stored in ELISA or TLC buffer and used for the determination of Glu, SP and VIP, or for 5-HT, 5-HTP, DA and GABA. Tissues were punched out from normal, control and REM deprived rats sacrificed at four hour intervals for twenty four hours. Each group (normal, control and REM deprived) consisted of four repeats for both ELISA and HPTLC.

2.2.3. Detection and quantification

2.2.3.1. ELISA (vide Chapter IV)

Punched out SCN were boiled for 10 minutes in 1 N acetic acid containing 0.02 N HCl and sonicated at 25° for 5 minutes. 20 µl of the homogenate from each sample was kept in -20° C for protein estimation (Lowry's method, 1951). After boiling, the samples were chilled on ice and centrifuged at 12,000 rpm for 30 minutes. Aliquots of samples were lyophilised in a Speed Vac (Savant Inc., Mass., USA) at 4° C. The lyophilised samples were reconstituted in 150 µl of assay buffer. SP and VIP were determined by antigen capture assay and glutamate by indirect ELISA as detailed in section IV.

2.2.3.2. High Performance Thin Layer Chromatography (HPTLC)

The punched out samples were analyzed for monoamines and GABA using the method of Arqué et al., (1988) as follows. The samples were kept in 0.05 mM HCl + 5 mM sodium disulfite (10 ml/g of tissue) and were mixed (30 minutes) twice with a mixture of n-pentane and 2-propanol (15:1 v/v) and centrifuged at 3,000 g for 15 minutes. The organic phase was discarded and aqueous phase was ultracentrifuged at 140,000 g for 60 minutes in a Ti-50 or Ti-80 rotor in a Beckman XL-90 ultracentrifuge (Beckman Corp., USA). After ultracentrifugation, the supernatant was lyophilised in a Speed Vac at 4°C. 50 µl of condensation mixture containing 4 M HCl-OPT + 1 M 5% KH₂PO₄ and 0.5 ml of toluene was added to the dried samples. The vials were vortexed for 1 minute and condensation reaction was carried out in a water bath (80° C) for 20 minutes. The tubes were later cooled to room temperature. Aliquots (50 µl) were applied to silica gel TLC plates with Hamilton syringes. To reduce drying time, the samples were placed under a jet of nitrogen gas. After drying, the plates were developed over a distance of 20 cm (for 3 hours) in
closed glass tanks with a mobile phase composed of 1-pentanol:1-propanol:methanol:formic acid:0.5 M HCl (58:30:1:0.5:10 v/v). After development, plates were dried and later analysed on a CAMAG TLC-scanner at 366nm. The standards were prepared in 0.05 M HCl and lyophilised and condensed with 4M OPT-HCl. Concentrations of the neurochemicals in punched out tissue samples were calculated with respect to their protein content determined by Lowry's method (vide section IV).

2.2.4. Statistical Tests

Neurotransmitter levels were plotted every four hour for twenty four hours and the significance of rhythms, if any, of each neurotransmitter in every structure studied (SCN, DR, and pineal) was determined by one way analysis of variance (ANOVA). The significance of difference from normal of a neurotransmitter rhythm (in a given structure) after control and REM deprivation treatments was determined by Friedman repeated measures ANOVA (RM ANOVA) on ranks.

Three markers of a neurotransmitter rhythm, namely mesor, amplitude and acrophase were determined by COSINOR, a simple program for cosinor analysis (Klemfuss and Clopton, 1993). This program fits a multiple regression fit in the form of single cosine curve that approximates the data using the equation

\[ Y = A + B1 \times \cos (2\pi t) + B2 \times \sin (2\pi t) \]

where

- \( Y \) is the actual data values;
- \( A \) is the intercept constant estimated by the regression;
- \( B1 \) is the cosine coefficient estimated by the regression;
- \( B2 \) is the sine coefficient estimated by the regression and
- \( t \) is the time for each \( Y \), expressed as \( t / \text{trial\_tau} \).

Significance of differences between groups in mesor, amplitude and phase of their rhythms, if any, was determined by unpaired Student’s \( t \) test.
3 RESULTS

3.1. REM sleep deprivation and RHT neurotransmitters [Table 5.1; Graph 5.1.1.; 5.1.2.]

3.1.1. Glutamate

Normal glutamate rhythm was significant (p = < 0.05; one way ANOVA) and showed an increase at night and a decrease during day. The acrophase of the rhythm was found at night (5.07 ± 1.52). This rhythm was significantly altered by control as well as REM deprivation treatments (p = < 0.05; Friedman repeated measures ANOVA on ranks).

Both REM sleep deprivation and control reduced the mesor and amplitude of the normal rhythm and also induced a phase shift. Mesor was 3616.67 ± 34.05 in normal, 3148.22 ± 89.56 in control (p = < 0.00005; unpaired Student's t test) and 2203.96 ± 2.39 in REM deprived (p = < 0.000000005 compared to normal; p = <0.04 compared to control; t test).

The amplitude of the rhythm was 283.95 ± 10.14 in normal, 250.30 ± 10.94 in control and 146.39 ± 10.94 in REM deprived (p = < 0.0001; t test).

The acrophase (as an index of the rhythm phase) of the normal rhythm (5.07 ± 1.52) was significantly delayed in the control by 6.25 ± 0.02 hrs (p = < 0.00001; t test) to 11.13 ± 1.34; whereas the acrophase was insignificantly advanced by 0.5 ± 0.01 hrs to 5.13 ± 0.48 in REM deprived.

3.1.2. Substance P (SP)

Normal rhythm of substance P did not show significant day-night variation. This rhythm was appreciably altered by both control (p = < 0.05; RM ANOVA) and REM deprivation (p = < 0.04; RM ANOVA) treatments.

The normal rhythm’s mesor (450.93 ± 2.50) was significantly reduced in both control as well as in REM deprived. The mesor of control was 193.52 ± 24.81 (p = < 0.00005; t test) and of REM deprived was 128.95 ± 6.01 (p = < 0.000000005; t test). Amplitude of the rhythm (20.47 ± 5.48) was not significantly altered in control (19.31 ± 3.12) whereas in REM deprived it was

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5 This test is referred to as RM ANOVA in the following text.
6 Henceforth referred to as I test.
Table 5.1. Effect of REM sleep deprivation on glutamate, substance P and VIP in SCN.

<table>
<thead>
<tr>
<th></th>
<th>Glutamate</th>
<th>Substance P</th>
<th>VIP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Control</td>
<td>REM deprived</td>
</tr>
<tr>
<td><strong>Mesor</strong></td>
<td>3616.67 ± 34.05</td>
<td>3148.22 ± 2.39</td>
<td>2203.96 ± 2.50</td>
</tr>
<tr>
<td><strong>Amplitude</strong></td>
<td>283.95 ± 10.14</td>
<td>250.30 ± 10.94</td>
<td>146.39 ± 10.94</td>
</tr>
<tr>
<td><strong>Acrophase</strong></td>
<td>5.07 ± 1.52</td>
<td>11.13 ± 1.34</td>
<td>5.13 ± 0.48</td>
</tr>
<tr>
<td><strong>Phase Shift</strong></td>
<td>(-)6.25 ± 0.02</td>
<td>0.50 ± 0.01</td>
<td>(+)2.56 ± 1.36</td>
</tr>
</tbody>
</table>

A phase advance is indicated by +ve value and a phase delay by a -ve value. 1= p < 0.000000005; 2=0.00000002; 3=0.00000034; 4=0.000001; 5=0.000002; 6=0.00002; 7= 0.00005; 8= 0.0001; 9=0.0007; 10=0.005; 11= 0.04; 12= 0.05 (Unpaired Student's t test).
Graph 5.1.1. Effect of REM sleep deprivation on the SCN levels of Glu, Sub-P and VIP. Black bars represent D of L/D cycle.
Graph 5.1.2. Effect of REM sleep deprivation on mesor, amplitude and phase of the rhythms of glutamate, SP and VIP. (+) value indicates a phase advance and (-) value represents a phase delay. * represents a minimum significance level of $p < 0.05$ (Unpaired Student's $t$ test)
significantly increased to 73.68 ± 2.95 (p = < 0.0001 compared to normal, and p = <0.00001 compared to control).

The acrophase of the rhythm was advanced by both control and REM deprivation treatments. However, this advance was statistically significant only in REM deprived, where the acrophase was advanced by 4.38 ± 2.43 hrs to 15.06 ± 0.20 (p = < 0.04; t test). Control treatment advanced the acrophase by 2.56 ± 1.36 hrs to 16.58 ± 0.53.

3.1.3. VIP

Normal rhythm of VIP (p = < 0.05; one way ANOVA) with a nocturnal increase and an acrophase at 22.36 ± 0.48 hrs was significantly affected in both control (p = < 0.05; RM ANOVA) and REM deprived (p = < 0.04; RM ANOVA).

Rhythm's mesor (118.39 ± 2.18) was significantly reduced in both control (65.45 ± 1.82; p = < 0.00002; t test) and REM deprived (46.22 ± 2.43; p = < 0.000002 compared to normal and p = < 0.0007 compared to control; t test). The amplitude (21.9 ± 2.61) was significantly reduced in control (13.47 ± 2.88; p = < 0.05; t test), but appreciably increased in REM deprived (26.80 ± 4.23; p = < 0.04; t test).

Although the rhythm's acrophase (22.36 ± 0.48) was advanced in both control and REM deprived, it was significant only after REM deprivation where the advance was by 15.28 ± 1.02 hrs (p = < 0.00000034) to 8.22 ± 0.24 (p = < 0.0000002 compared to normal and 0.000001 compared to control). On the other hand, in control, the phase advance of 1.07 ± 0.02 hrs to 22.13 ± 1.06 was insignificant.

3.2. REM sleep deprivation effects on monoamines and GABA

3.2.1. REM sleep deprivation and GABA [Table 5.2.1.; Graph 5.2.1.1. and 5.2.1.2.]

3.2.1.1. SCN

Normal GABA rhythm showed a diurnal increase (p = < 0.05; one way ANOVA) with an acrophase at 14.43 ± 0.01. This normal rhythm was significantly different from both control and REM deprived (p = < 0.05; RM ANOVA).
Alterations in mesor, amplitude and acrophase (therefore the rhythm phase) were significant only in REM sleep deprived, not in control. The mesor of the normal rhythm (118.29 ± 3.69) was increased in both control (135.65 ± 8.79) and REM deprived (236.76 ± 21.33; p = < 0.03 compared to normal and p = <0.05 compared to control; t test).

Amplitude of the normal rhythm (96.29 ± 3.43) was increased to 111.79 ± 4.83 in control and 183.85 ± 18.10 in REM deprived (p = < 0.04 compared to normal; t test).

The acrophase (14.43 ± 0.01) was delayed by 1.01 ± 0.44 hrs to 15.24 ± 0.25 in control and by 2.56 ± 0.45 hrs (p = < 0.03 compared to normal; t test) to 16.59 ± 0.43.

3.2.1.2. Dorsal Raphe

Normal GABA rhythm showed a diurnal increase with an acrophase at 10.18 ± 0.03 hrs. The individual rhythms in normal, control and REM deprived were significant (p = < 0.05; one way ANOVA) as well as the differences between rhythms under control (p = < 0.05; RM ANOVA on ranks) and REM deprived conditions (p = < 0.04; RM ANOVA on ranks).

REM sleep deprivation as well as large platform control induced a decline in the mesor of the rhythm. Mesor was 539.43 ± 1.57 in normal, 470.84 ± 0.96 in control (p = < 0.02; t test) and 325.56 ± 12.36 in REM deprived (p = < 0.0007; t test).

The amplitude showed a significant reduction only in REM deprived, not in control. Amplitude under different conditions were 511.13 ± 5.94 (normal), 462.47 ± 2.96 (control) and 292.24 ± 13.95 (REM deprived; p = < 0.02; t test).

Although acrophase showed alterations in both control (10.16 ± 0.03) and REM deprived (12.12 ± 1.36) from normal (10.18 ± 0.03), the changes were statistically insignificant. Both control as well as REM deprived showed shifts in phase of the rhythm; an insignificant phase advance of 0.13 ± 0.03 hrs in control and a significant phase delay of 2.34 ± 0.14 hrs (p = < 0.04; t test) in REM deprived.
Graph 5.2.1.1. Effect of REM sleep deprivation on the levels of GABA in SCN, dorsal raphe and pineal. Black bars represent D of L/D cycle.
Graph 5.2.1.2. Effect of REM sleep deprivation on mesor, amplitude and phase of GABA rhythm in SCN, dorsal raphe and pineal. *ve values indicate a phase advance and -ve values indicate phase delay. * represents at least p < 0.05 (Unpaired Student's t test)
### Table 5.2.1. Effect of REM sleep deprivation on GABA in SCN, dorsal raphe and pineal.

<table>
<thead>
<tr>
<th></th>
<th>SCN</th>
<th>Dorsal Raphe</th>
<th>Pineal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Control</td>
<td>REM deprived</td>
</tr>
<tr>
<td>Mesor</td>
<td>118.29 ± 3.69</td>
<td>135.65 ± 8.79</td>
<td>236.76 ± 21.33&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>73.1 ± 0.48</td>
<td>102.69 ± 7.78</td>
<td>174.8 ± 27.68&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Amplitude</td>
<td>96.29 ± 3.43</td>
<td>111.79 ± 4.83</td>
<td>183.85 ± 18.10&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acrophase</td>
<td>14.43 ± 0.01</td>
<td>15.24 ± 0.25</td>
<td>16.59 ± 0.43&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phase Shift</td>
<td>(-)1.01 ± 0.44</td>
<td>(-)2.56 ± 0.45&lt;sup&gt;4&lt;/sup&gt;</td>
<td>(+)0.13±0.03</td>
</tr>
</tbody>
</table>

A phase advance is indicated by +ve value and a phase delay by -ve value. 1 = p < 0.0007; 2 = 0.01; 3 = 0.02; 4 = 0.03; 5 = 0.04; 6 = 0.05 (Unpaired Student's t test).

### Table 5.2.2. Effect of REM sleep deprivation on 5-HTP in SCN, dorsal raphe and pineal.

<table>
<thead>
<tr>
<th></th>
<th>SCN</th>
<th>Dorsal Raphe</th>
<th>Pineal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Control</td>
<td>REM deprived</td>
</tr>
<tr>
<td>Mesor</td>
<td>73.1 ± 0.48</td>
<td>102.69 ± 7.78</td>
<td>174.8 ± 27.68&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>48.19 ± 0.86</td>
<td>50.21 ± 1.93</td>
<td>68.16 ± 18.14</td>
</tr>
<tr>
<td>Amplitude</td>
<td>12.18 ± 0.43</td>
<td>14.36 ± 0.39</td>
<td>14.56 ± 0.47&lt;sup&gt;1,2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acrophase</td>
<td>(-)2.38 ± 1.02</td>
<td>(-)3.18 ± 1.10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>(-)0.09±0.01</td>
</tr>
<tr>
<td>Phase Shift</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A phase advance is indicated by +ve value and a phase delay by -ve value. 1 = p < 0.04; 2 = p < 0.05 (Unpaired Student's t test).
3.2.1.3. Pineal

Normal GABA rhythm \((p = < 0.05; \text{one way ANOVA})\) showed an increase during day with its acrophase at \(12.06 \pm 0.42\) hrs. The changes in GABA rhythm in both control and REM deprived were significant \((p = < 0.05; \text{RM ANOVA})\).

Mesor of the rhythm showed a decline from normal \((85.08 \pm 3.40)\) in both control \((70.51 \pm 0.30)\) as well as REM deprived \((52.20 \pm 1.79)\). However, this reduction was more significant in REM deprived \((p = < 0.01; t \text{ test})\) than in control \((p = < 0.05; t \text{ test})\).

Both the amplitude as well as the acrophase of the rhythm did not show statistically appreciable changes in both control and REM deprived. The amplitude was \(53.37 \pm 4.79\) for normal, \(48.94 \pm 3.81\) for control and \(38.78 \pm 6.36\) for REM deprived. The corresponding acrophases were \(12.06 \pm 0.42\) (normal) \(11.29 \pm 0.03\) (control) and \(13.23 \pm 0.17\) for REM deprived.

Phase shifts were induced by both control and REM deprivation procedures. Control showed an insignificant phase advance of \(0.39 \pm 0.03\) hrs whereas REM deprivation induced a significant phase delay of \(2.18 \pm 0.17\) hrs \((p = < 0.05; t \text{ test})\).

3.2.2. REM sleep deprivation and 5-HTP [Table 5.2.2.; Graph 5.2.2.1. and 5.2.2.2.]

3.2.2.1. SCN

Normal 5-HTP rhythm showed a significant diurnal increase \((p = < 0.05; \text{one way ANOVA})\) with an acrophase at \(12.18 \pm 0.43\). Although both control and REM deprivation induced an increase in 5-HTP level, this was significant only after REM deprivation \((p = < 0.05; \text{RM ANOVA})\), and not after the control treatment.

Both control as well as REM deprived rats showed an increase in mesor; the mesor was \(73.01 \pm 0.48\) in normal, \(102.69 \pm 7.78\) in control \((p = < 0.06; t \text{ test})\) and \(174.76 \pm 27.68\) in REM deprived \((p = < 0.05; t \text{ test})\).

The rhythm amplitude did not show appreciable changes after control \((50.202 \pm 1.93)\) and REM deprivation \((68.16 \pm 18.14)\) compared to normal \((48.19 \pm 0.86)\).
V. REM Sleep and SCN

The acrophase of the rhythm was significantly altered only in REM deprived animals (14.56 ± 0.47; p = < 0.04 as compared to normal animals; p = <0.05 as compared to control; t test), not in control (14.36 ± 0.39). Phase delays induced by control (2.38 ± 1.02 hr) as well as REM deprivation (3.18 ± 1.10 hr) treatments were statistically insignificant.

3.2.2.2. Dorsal Raphe

Normal 5-HTP rhythm was significant (p = < 0.05; one way ANOVA) with a diurnal increase and an acrophase at 13.35 ± 1.19. This normal rhythm was significantly altered in both control and in the REM deprived (p = < 0.05; RM ANOVA).

Although mesor, compared to the normal (151.69 ± 13.23), showed an increase in both control (180.02 ± 1.36) and after REM deprivation (279.24 ± 24.56), this increase was significant only after REM sleep deprivation (p = < 0.04 compared to normal, 0.05 compared to control; t test).

Neither control nor REM deprivation treatment could induce statistically significant changes in amplitude, acrophase and phase. The amplitude was 91.14 ± 7.68 in normal, 91.02 ± 5.34 in control and 124.43 ± 10.51 in REM deprived. The acrophase was 13.35 ± 1.19 for normal, 13.43 ± 0.04 for control, and 14.32 ± 0.39 for REM deprived. Statistically insignificant phase delays were 0.09 ± 0.01 hrs for control and 0.57 ± 0.02 hr for the REM deprived.

3.2.2.3. Pineal

The normal 5-HTP rhythm was statistically significant (p = < 0.05; one way ANOVA) with a nocturnal increase and an acrophase at 23.21 ± 0.33. This rhythm was significantly altered after REM deprivation (p = < 0.05; RM ANOVA), not after control treatment.

However, control as well as REM deprivation treatments did not induce statistically significant changes in amplitude, acrophase and phase. Amplitude of the rhythm was 19.58 ± 0.64 for normal, 21.24 ± 2.59 for control, and 21.60 ± 2.03 for REM deprived. The corresponding values for acrophase were 23.21 ± 0.33 for normal, 23.42 ± 0.02 for control and 23.39 ± 0.24 for REM deprived. Statistically insignificant phase delays were 1.62 ± 0.15 for control and 0.09 ± 0.01 for REM deprived.
Graph.5.2.2.1. Effect of REM sleep deprivation on the levels of 5-hydroxytryptophan (5-HTP) in SCN, pineal and dorsal raphe. Black bars represent D of L/D cycle.
Graph 5.2.2.2  Effect of REM sleep deprivation on mesor, amplitude, and phase of 5-HTP rhythm in SCN, dorsal raphe and pineal. +ve value indicates a phase advance and a -ve value indicates a phase delay. * represents a significant change of at least p < 0.05 (Unpaired Student's t test)
3.2.3. REM sleep deprivation and 5-HT [Table 5.2.3.; Graph 5.2.3.1., 5.2.3.2.]

3.2.3.1. SCN

Normal 5-HT rhythm ($p = < 0.05$; one way ANOVA) showed a diurnal increase with an acrophase at $11.06 \pm 2.04$. This rhythm was significantly altered after REM sleep deprivation ($p = < 0.05$; RM ANOVA), and not after control.

Both control and REM sleep deprived animals showed an increase in mesor and amplitude as compared with normal subjects. Mesor was $50.35 \pm 8.40$ (normal), $69.07 \pm 9.19$ (control) and $117.49 \pm 0.09$ (REM deprived; $p = < 0.02$ compared to normal, and $0.03$ compared to control; $t$ test). The corresponding values for amplitude were $29.26 \pm 6.23$ (normal), $31.85 \pm 9.69$ (control) and $55.31 \pm 0.64$ (REM deprived; $p = < 0.05$; $t$ test).

The acrophase of the rhythm did not significantly differ from normal ($11.06 \pm 2.04$). The acrophase was $12.12 \pm 2.31$ for controls and $15.19 \pm 1.03$ for REM deprived.

Although both REM sleep deprivation and control phase delayed the rhythm, this was significant only in the REM deprived ($5.14 \pm 2.46$ hrs; $p = < 0.05$; $t$ test) not in controls ($2.21 \pm 0.42$ hrs).

3.2.3.2. Dorsal Raphe

Normal rhythm of 5-HT ($p = < 0.05$; one way ANOVA) had a diurnal increase with an acrophase at $14.11 \pm 0.10$, which significantly differed from the REM deprived ($p = < 0.05$; RM ANOVA), but not from the control.

Mesor of the normal rhythm ($107.61 \pm 11.74$) was significantly increased after REM deprivation ($162.67 \pm 29.15$; $p = < 0.05$; $t$ test), not after control treatment ($113.34 \pm 7.09$).

Neither the amplitude nor the acrophase (and hence the phase) did not significantly change in control and REM deprived animals. Amplitude was $62.12 \pm 4.19$ (normal), $55.98 \pm 2.47$ (control) and $72.06 \pm 3.39$ (REM deprived); the corresponding acrophases were $14.11 \pm 0.10$ (normal), $14.28 \pm 0.03$ (control) and $14.16 \pm 1.38$ (REM deprived). The phase delays observed (statistically insignificant) were $0.15 \pm 0.02$ hrs (control) and $0.05 \pm 0.01$ (REM deprived).
Graph 5.2.3.1. Effect of REM sleep deprivation on serotonin (5-HT) levels of SCN, dorsal raphe and pineal. Black bars represent D of L/D cycle.
Graph 5.2.3.2. Effect of REM sleep deprivation on mesor, amplitude and phase of 5-HT in SCN, dorsal raphe and pineal. +ve values indicate a phase advance and -ve values indicate a phase delay.

* represents a minimum significance level of $p = < 0.05$ (Unpaired Student's $t$ test).
Table 5.2.3. Effect of REM sleep deprivation on 5-HT in SCN, dorsal raphe and pineal

<table>
<thead>
<tr>
<th></th>
<th>SCN</th>
<th>Dorsal Raphe</th>
<th>Pineal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Control</td>
<td>REM deprived</td>
</tr>
<tr>
<td>Mesor</td>
<td>50.35 ± 8.4</td>
<td>69.07 ± 9.19</td>
<td>117.5 ± 0.09</td>
</tr>
<tr>
<td>Amplitude</td>
<td>29.26 ± 6.23</td>
<td>31.85 ± 9.69</td>
<td>55.31 ± 0.64</td>
</tr>
<tr>
<td>Acrophase</td>
<td>11.6 ± 2.64</td>
<td>12.12 ± 2.31</td>
<td>15.19 ± 1.03</td>
</tr>
<tr>
<td>Phase Shift</td>
<td>(-)2.21 ± 0.42</td>
<td>(-)5.14 ± 2.46</td>
<td>(-)0.15 ± 0.02</td>
</tr>
</tbody>
</table>

A phase advance is indicated by +ve value and a phase delay by -ve value. 1 = p < 0.0002; 2 = 0.003; 3 = 0.004; 4 = 0.005; 5 = 0.02; 6 = 0.03; 7 = 0.05 (Unpaired Student’s t test).

Table 5.2.4. Effect of REM sleep deprivation on 5-HT/5-HTP ratio in SCN, dorsal raphe and pineal

<table>
<thead>
<tr>
<th></th>
<th>SCN</th>
<th>Dorsal Raphe</th>
<th>Pineal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Control</td>
<td>REM deprived</td>
</tr>
<tr>
<td>Mesor</td>
<td>0.73 ± 0.09</td>
<td>0.70 ± 0.12</td>
<td>0.70 ± 0.13</td>
</tr>
<tr>
<td>Amplitude</td>
<td>0.15 ± 0.02</td>
<td>0.14 ± 0.08</td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td>Acrophase</td>
<td>3.14 ± 0.28</td>
<td>10.20 ± 3.37</td>
<td>9.41 ± 2.15</td>
</tr>
<tr>
<td>Phase Shift</td>
<td>(-)7.26 ± 3.29</td>
<td>(-)7.07 ± 2.37</td>
<td>(-)2.39 ± 0.12</td>
</tr>
</tbody>
</table>

+ve value indicates a phase advance and a -ve value indicates a phase delay; 1 p = <0.02; 2 p = <0.04 (Unpaired Student’s t test).
3.2.3.3. Pineal

Normal 5-HT rhythm (p = < 0.05; one way ANOVA) showed a diurnal increase with an acrophase at 14.26 ± 0.38. This rhythm was significantly altered after REM deprivation (p = < 0.05; RM ANOVA).

Mesor of the normal rhythm (19.13 ± 0.59) was significantly increased after REM deprivation (39.34 ± 0.5; p = < 0.002 compared to normal; p = < 0.003 compared to controls; t test), but not in controls (22.45 ± 0.77).

Although amplitude showed an increase from the normal (9.78 ± 0.88) in both REM deprived (10.83 ± 0.45) and control subjects (10.91 ± 0.76), they were statistically insignificant.

Acrophase of the rhythm remained relatively unperturbed in control (14.25 ± 0.06) from normal (14.26 ± 0.38) with an insignificant phase delay of 0.59 ± 0.02 hrs; but in REM deprived the acrophase was significantly delayed by 8.23 ± 1.09 hrs (p = < 0.02 t test) to 22.29 ± 0.51 (p = < 0.005 compared to normal; p = < 0.004 compared to control).

3.2.4. REM sleep deprivation and 5-HT/5-HTP ratio [Table 5.2.4.; Graph 5.2.4.1.; 5.2.4.2.]

3.2.4.1. SCN

Rhythm of 5-HT/5-HTP ratio in normal as well as in control and REM deprived were not statistically significant. Differences of the control and REM deprived rhythms from normal were also statistically insignificant except for the acrophase.

The mesor of the rhythm was 0.73 ± 0.09 (normal), 0.70 ± 0.12 (control) and 0.70 ± 0.13 (REM deprived). The corresponding amplitudes were 0.145 ± 0.02 (normal), 0.135 ± 0.08 (control) and 0.125 ± 0.03 (REM deprived).

Both the control as well as the REM deprivation treatments significantly delayed the acrophase of the normal subjects (3.14 ± 0.28); delayed by 7.26 ± 3.29 hrs (p = < 0.04; t test) to 10.20 ± 3.37 in normal, and by 7.07 ± 2.37 (p = < 0.04; t test) to 9.41 ± 2.15 in REM deprived subjects

3.2.4.2. Dorsal Raphe

Normal rhythm of the 5-HT/5-HTP ratio was significant (p = < 0.01; one way ANOVA) with a nocturnal increase and an acrophase at 18.15 ± 2.12. This
Graph 5.2.4.1. Effect of REM sleep deprivation on the ratio of 5-HT to 5-HTP in SCN, dorsal raphe and pineal. Black bars represent D of L/D cycle.
Graph.5.2.4.2. Effect of REM sleep deprivation on mesor, amplitude and phase of the rhythm of 5-HT/5-HTP ratio in SCN, dorsal raphe and pineal. +ve values indicate a phase advance and -ve values indicate a phase delay. * represents a minimum significance level of p = < 0.05 (Unpaired Student's t test)
normal rhythm was not significantly altered by either control or REM deprivation treatments.

Except for the acrophase (and consequently the phase shift), the mesor and amplitude of the rhythm remained relatively undisturbed after both control and REM deprivation treatments. Mesor of the rhythm was $0.73 \pm 0.02$ in normal, $0.64 \pm 0.04$ in control and $0.60 \pm 0.06$ in REM deprived. The corresponding amplitudes were $0.04 \pm 0.02$ (normal), $0.06 \pm 0.01$ (control) and $0.05 \pm 0.01$ (REM deprived).

The acrophase of the normal rhythm ($18.15 \pm 2.12$) was delayed by a phase delay of $2.39 \pm 0.12$ hrs to $20.14 \pm 2.45$ in control, and advanced by $4.0 \pm 1.29$ hrs to $13.55 \pm 7.25$ in REM deprived ($p = < 0.04$; $t$ test).

### 3.2.4.3. Pineal

Significant rhythm of 5-HT/5-HTP ratio was found in both normal and control ($p = <0.0001$; one way ANOVA). On the contrary, the rhythm was abolished in REM deprived. REM deprivation significantly reduced the mesor and amplitude of the rhythms, while the control did not. Mesor was $1.00 \pm 0.05$ in normal, $0.84 \pm 0.04$ in control and $0.54 \pm 0.05$ in REM deprived ($p = < 0.02$ compared to normal; $p = < 0.04$ compared to control; $t$ test). Amplitude of the rhythm was $0.49 \pm 0.01$ (normal), $0.71 \pm 0.08$ (control) and $0.08 \pm 0.03$ (REM deprived; $p = < 0.02$ $t$ test).

Acrophase of the normal rhythm ($12.29 \pm 0.10$) remained relatively similar in control ($12.40 \pm 0.03$) with an insignificant phase delay of $0.12 \pm 0.01$ hrs; on the contrary, REM deprivation caused an insignificant advance of the acrophase by $1.12 \pm 0.33$ hrs to $12.17 \pm 0.24$.

### 3.2.5. REM sleep deprivation and dopamine [Table 5.2.5; Graph 5.2.5.1.; 5.2.5.2.]

#### 3.2.5.1. SCN

Normal dopamine rhythm ($p = < 0.05$; one way ANOVA) showed a diurnal increase with its acrophase at $15.15 \pm 0.31$. Both control as well as REM deprivation treatments ($p = < 0.05$; RM ANOVA) significantly varied from the normal.
Mesor of the normal dopamine rhythm (27.68 ± 1.28) was significantly enhanced in both control (32.33 ± 0.93; p = < 0.04 t test) and REM deprived (55.42 ± 3.63; p = < 0.02 compared to normal; p = < 0.03 compared to control; t test).

However, the amplitude of the rhythm remained relatively unaffected. They were 28.39 ± 33.6 (normal), 29.71 ± 4.94 (control) and 31.17 ± 3.85 (REM deprived).

The acrophase of the normal rhythm (15.15 ± 0.31) was unaltered in the control (15.21 ± 0.50) with an insignificant phase delay of 0.06 ± 0.01, whereas it was appreciably advanced by 3.34 ± 1.05 hrs (p = < 0.05; t test) to 12.21 ± 0.33 (p = < 0.02 compared to normal; p = < 0.04 compared to control; t test).

3.2.5.2. Dorsal Raphe

The normal rhythm (p = < 0.05; one way ANOVA) had a diurnal decrease (nocturnal increase) with an acrophase at 1.31 ± 0.48, which was significantly altered by both control and REM deprivation treatments (p = < 0.05; RM ANOVA).

REM sleep deprivation significantly increased the mesor of the normal rhythm (13.38 ± 1.57) to 36.63 ± 3.62 (p = < 0.03; t test), whereas control did not (16.49 ± 1.01).

Similarly, only REM deprivation could significantly increase the amplitude of the rhythm from the normal (5.40 ± 0.47) to 12.91 ± 0.04 (p = < 0.003; t test) not the control (4.47 ± 0.29).

In the REM deprived, acrophase of the normal rhythm (1.31 ± 0.48) was significantly delayed by 23.05 ± 0.47 hrs (p = < 0.001; t test) to 23.17 ± 0.09 (p = < 0.0005 compared to normal; p = < 0.000006 compared to control); on the other hand, control induced an insignificant phase advance of 0.51 ± 0.02 hrs to 1.23 ± 0.05.

3.2.5.3. Pineal

Normal rhythm (p = < 0.04; one way ANOVA) showed a nocturnal increase with its acrophase at 2.12 ± 0.51, which altered significantly after REM deprivation (p = < 0.05; RM ANOVA), but not after control treatment.
Graph 5.2.5.1. Effect of REM sleep deprivation on the levels of dopamine (DA) in SCN, dorsal raphe and pineal. Black bars represent D of the L/D cycle.
Graph 5.2.5.2. Effect of REM sleep deprivation on mesor, amplitude and phase of dopamine rhythm in SCN, dorsal raphe and pineal. + ve values indicate a phase advance and -ve values indicate a phase delay. * represents a minimum significance level of $p = < 0.05$ (Unpaired Student's $t$ test)
Table 5.2.5. Effect of REM sleep deprivation on **Dopamine** in SCN, dorsal raphe and pineal

<table>
<thead>
<tr>
<th></th>
<th>SCN</th>
<th>Dorsal Raphe</th>
<th>Pineal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Control REM deprived</td>
<td>Normal</td>
</tr>
<tr>
<td>Mesor</td>
<td>27.68 ± 1.28</td>
<td>32.33 ± 0.93</td>
<td>55.42 ± 3.63</td>
</tr>
<tr>
<td>Amplitude</td>
<td>28.39 ± 3.36</td>
<td>29.70 ± 4.94</td>
<td>31.17 ± 3.85</td>
</tr>
<tr>
<td>Acrophase</td>
<td>15.15 ± 0.31</td>
<td>15.21 ± 0.50</td>
<td>12.21 ± 0.33</td>
</tr>
<tr>
<td>Phase Shift</td>
<td>(-)0.06 ± 0.01</td>
<td>(+)3.34 ± 1.05</td>
<td>(-)0.51 ± 0.02</td>
</tr>
</tbody>
</table>

A +ve value indicates a phase advance and a -ve value indicates a phase delay; 1= p < 0.000006; 2=0.0004; 3=0.0005; 4=0.001; 5= 0.003; 6=0.02; 7=0.03; 8=0.04; 9=0.05 (Unpaired Student’s t test)
V. REM Sleep and SCN

Mesor of the normal rhythm (5.39 ± 0.12) was significantly increased in REM deprived (20.75 ± 3.64; p = < 0.05 t test), not in control (6.11 ± 0.14).

Amplitude of the normal rhythm (3.85 ± 0.18) remained largely undisturbed in both control (3.31 ± 0.15) and REM deprived (5.57 ± 2.58).

The acrophase of the rhythm (2.12 ± 0.51) was insignificantly delayed by 1.16 ± 0.2 hr to 2.27 ± 0.12 in control; whereas REM deprivation significantly delayed it by 3.11 ± 0.52 hrs (p = < 0.03; t test) to 4.23 ± 1.24.

4 DISCUSSION

The results of this section are discussed under the titles (1) REM sleep deprivation and RHT neurotransmitters and (2) REM sleep deprivation and monoamines.

4.1. REM sleep deprivation and RHT neurotransmitters

In this study, REM sleep deprivation induced a reduction in the SCN levels of glutamate, SP and VIP and phase advanced the acrophases of the rhythms of glutamate and VIP.

4.1.1. Glutamate

Normal diurnal rhythm observed for glutamate in SCN with its peak during dark and trough in light periods is similar to the earlier observations of Glass et al., (1993) in Syrian hamster and of Takeuchi and Takahashi (1994) in rats. However, the glutamate peak in this study is towards end rather than in the middle of the dark phase unlike in the aforementioned studies. This could be due to the difference in the lighting periods: 14h L: 10h D in Glass et al., (1993), and 15.00 - 3.00 light and 3.00-15.00 dark period in Takeuchi and Takahashi (1994).

Although glutamate mesor levels were reduced in both large platform control and REM deprived rats, reduction was more pronounced in REM deprived rats. The reduced glutamate level after REM deprivation could be due to inhibitory effect on extracellular glutamate of 5-HT (Selim et al., 1993; Srkalovic et al., 1994), whose release will be enhanced by the reportedly increased firing of REM -off dorsal raphe neurons during REM sleep deprivation (Lydic et al., 1984) due to the desensitization of 5-HT1A somatodendritic inhibitory autoreceptors (Maudhuit et al., 1996).
Since 5-HT/its agonist is known to phase advance SCN neuronal activity (Prosser et al., 1990), and phase shifting of the SCN is ultimately achieved by alteration in the levels of glutamate or GABA (van den Pol et al., 1996), the phase advanced glutamate level (in relation to the control treatment) of SCN in the REM deprived could be due to the increased 5-HT release (vide 4.2.1.2) brought on by enhanced activity of REM- off DR neurons (Lydic et al., 1984) during REM sleep deprivation. The phase advanced glutamate rhythm can advance the sleep onset time, which is considered to have an antidepressant action (Wehr et al., 1979).

4.1.2. Substance P

Normal circadian rhythm of SP in SCN with no significant peaks or troughs across the circadian cycle corroborate the earlier findings of Otori et al., (1993) and Inouye (1996). Although the SP level is reduced in both large platform control and REM deprived rats, reduction is more significant in REM deprived; more importantly, in REM deprived rats, SP levels during day are much higher than at night in contrast to both normal and control where SP level does not appreciably change diurnally.

Since SP is reported to decrease REM sleep (Riou et al., 1982; Jones, 1991) (therefore SP level and presence and amount of REM sleep considered to be antagonistic to each other), and SP is considered to be a RHT neurotransmitter (Takatsuji et al., 1991; Mikkelsen and Larsen, 1993; Piggins et al., 1995), the daytime increase of SP after REM deprivation should have been due to the REM deprivation induced disinhibition of SP. This increase was not found in normal or control where REM sleep was intact.

The phase advance observed for both glutamate and substance P after REM sleep deprivation (a putative antidepressant therapy) is contrary to the observation that clorgyline (a MAOI used as an antidepressant) decreases the magnitude of the light induced phase advance (Duncan, 1996) suggesting that REM sleep deprivation and (MAOI) antidepressant treatment may not share similar mechanisms in influencing the pacemaker action via RHT (input pathway). The present observations, however, correspond well with the suggestion of Wehr et al., (1979) that shifting the sleep timing earlier can
function as an antidepressant mechanism since phase advance of sleep timing can be possible due to the phase advanced RHT neurotransmitter rhythms.

4.1.3. VIP

The normal diurnal rhythm of VIP of SCN found in this study (i.e., decrease during light and increase in the dark) supports the similar observations of Albers et al., (1990) Shinohara et al., (1993), Morin et al., (1993), and Inouye (1996). The reduced levels of VIP after REM sleep deprivation suggesting that VIP in SCN is essential for REM sleep corroborates the earlier following observations:- (a) intracerebroventricular (i.c.v.) administration of VIP significantly enhances REM sleep in both normal (Drucker-Colin et al., 1984; Obal et al., 1989), and insomniac animals (Pacheco-Cano et al., 1990; Prospero-Garcia et al., 1993); (b) REM sleep or REM rebound is prevented by VIP antagonist (Mirmiran et al., 1988) and VIP-containing CSF obtained after REM sleep deprivation (Jimenez-Anguiano et al., 1993); and (c) REM sleep deprivation increases VIP receptors in SCN possibly as an upregulating mechanism compensating for the decreased VIP levels due to the altered REM distribution (Jimenez-Anguiano et al., 1996).

VIP administration into SCN, especially in combination with GRP and /or PHI induces a phase delay in locomotor activity of the hamster (Albers et al., 1992). This observation, considering that VIP level of SCN can be used as a marker of phase of behavioral rhythms (Albers et al., 1992; Romijn et al., 1996), is indirectly corroborated by the finding of the present study that REM sleep deprivation (which reduced the VIP level) advanced the phase of the VIP acrophase,

Since VIP is known to phase shift behavioral rhythms along with GRP and PHI (Albers et al., 1991; 1992), the phase advance and mesor reduction of VIP found in this study after REM deprivation could possibly exert an action on the rhythm of GRP and PHI as well.

Since only REM sleep deprivation (not the control treatment) caused a significant phase advance of the VIP, the normal action of REM sleep might be to delay the VIP rhythm of SCN. This is contrary to the findings of Murck et al., (1996) who observed a phase advancing effect of VIP on REM-NREM ratio in
man. Thus these two contradictory implications suggest that regulation of sleep-wake cycle by VIP may vary across species, possibly due to differences in the activity pattern of the animal - nocturnal (e.g., rat) or diurnal (e.g., man).

The phase advanced VIP rhythm after REM deprivation can possibly act via medial POA whose only source of VIP innervation is SCN (Watts and Swanson, 1987; Kalsbeek et al., 1993). Medial POA is involved in the regulation of REM sleep (Koyama and Hayaishi, 1994; Lin and Sakai, 1994) and slow wave sleep (Alam et al., 1995) in addition to other functions. Since REM sleep deprivation is known to act as an antidepressant therapy (Vogel, et al., 1980), and an antidepressant mechanism can involve shifting the sleep timing earlier (Wehr et al., 1979), the phase advanced VIP rhythm can probably advance the sleep timing by influencing mPOA, and therefore act as an antidepressant mechanism.

However, the phase advance of the VIP acrophase after REM sleep deprivation does not agree with the hypotheses that depression is due to the phase advanced position of the oscillator controlling REM sleep and body temperature cycles (Wehr and Wirz-Justice, 1981; 1982), and that an antidepressant treatment such as REM sleep deprivation is expected to phase delay the oscillator (Ozaki et al., 1993; Sharpley and Cowan, 1995).

The increased amplitude of the VIP (efferent) rhythm after REM sleep deprivation (as an antidepressant mechanism) is of interest in view of the following findings: (a) reduced circadian amplitude is a major factor responsible for many sleep-wake disturbances in depression (Czeisler et al., 1987) and, (b) an increased circadian amplitude of many physiological rhythms often accompanies remission of depressive symptoms (Souètre et al., 1988).

Therefore, to conclude, the antidepressant mechanism released by REM sleep deprivation is probably different from the mechanism involved in antidepressant therapy by MAO inhibitors (MAOIs), TCAs and SSRIs which normally induce a delay in REM sleep onset. Thus, there are probably at least two different (and opposite) antidepressant mechanisms: one involving REM sleep suppression mediated by 5-HT DR nucleus system which advances the sleep onset time as is suggested by this study, and another DR independent
mechanism triggered by antidepressants such as nefazodone, a selective 5-HT uptake blocker (Vogel et al., 1998).

4.2. REM sleep deprivation and monoamines

Monoamines are important in the regulation of REM sleep (Jouvet, 1974; Mouret, 1982; Farooqui et al., 1996) and their malfunctioning is thought to lead to sleep disturbances in affective disorders such as depression (Meltzer et al., 1990; Duncan, 1996). In this study, the effect of REM sleep deprivation, a treatment for depression, on the monoamine rhythms of three structures important in REM sleep regulation i.e., dorsal raphe, SCN and pineal (Mouret, 1982) was investigated. The results are discussed under the titles: (1) REM sleep deprivation and Dorsal Raphe monoamines, (2) REM sleep deprivation and SCN monoamines, (3) REM sleep deprivation and pineal monoamines and (4) REM sleep deprivation and 5-HT to 5-HTP ratio.

4.2.1. REM sleep deprivation and Dorsal Raphe (DR)

Normal rhythm of three neurotransmitters (i.e., GABA, 5-HTP and 5-HT), of the four monitored in dorsal raphe (DR), showed a rhythm with their respective acrophases during day; however, dopamine showed a decline during day, and its acrophase was at night. The changes in the levels of these neurotransmitters under normal and REM deprived conditions are correlated with the function of DR as a REM-off 5-HTergic nucleus (McGinty and Harper, 1976; Trulson and Jacobs, 1979; Jacobs and Fornal, 1991).

4.2.1.1. GABA

Dorsal raphe is a REM-off nucleus with a maximum firing in awake condition (i.e. night time for a nocturnal animal like rat) and decreased (minimum) firing in sleep (REM sleep) (Jacobs and Fornal, 1991) reportedly due to the increased inhibitory action of GABA during (REM) sleep (Nitz and Siegel, 1997). Since sleep (including REM sleep) in rat predominantly occurs during day, the diurnal variation of GABA (increase during day and decrease at night), reported here for the first time, is not unexpected due to the increased inhibitory GABA action during sleep (day). This diurnal variation of GABA in DR (which might drive rhythms of other neurotransmitters implicated in sleep-wake
regulation) could be responsible for the characteristic distribution of sleep and wakefulness across a circadian cycle.

Since DR cooling increases REM sleep (Cespuglio et al., 1979), and alternatively, forced locomotor activity (which reduce sleep including REM sleep) increases DR neuronal activity (Lydic et al., 1984), reduced GABA levels after REM sleep deprivation or large platform control (possibly due to stress caused by relatively restricted movement) probably has a permissive influence (disinhibition) on DR firing activity. Although GABA levels (mesor) are reduced in both control and REM deprived rats, reduction is more significant in REM deprived; more importantly, only REM sleep deprivation (not the large platform) phase delays the GABA rhythm of DR which can probably influence rhythms of other neurotransmitters in DR as discussed in the following sections.

4.2.1.2. 5-HT

Normal rhythm of 5-HT in DR with a diurnal increase and an acrophase during day supports similar observations of Agren et al., (1986) and Ozaki et al., (1993). The increased extracellular 5-HT is known to inhibit DR firing activity via 5-HT 1A inhibitory autoreceptors - autoinhibition (Aghajanian et al., 1990; Portas et al., 1996) - precipitating more sleep. Thus, the relatively increased extracellular 5-HT levels in DR might be responsible for the predominance of sleep in rat during day compared to night when the 5-HT levels are low (and raphe activity is presumably high) precipitating wakefulness, supporting the finding of increased 5-HT release from the serotonergic neuronal dendrites in the DR of rat during sleep (Cespuglio et al., 1990).

The normal 5-HT rhythm is probably driven by the GABA rhythm of DR since the GABA rhythm has a similar phase and an inhibitory influence on the DR firing activity (vide 4.2.1.1). This is consistent with the finding that GABAergic input to serotonergic DR neurons regulates the state dependent activity of the latter (Levine and Jacobs, 1992).

The significant elevation of 5-HT mesor in REM deprived compared to normal or large platform control could be due to the increased wake and SWS periods after REM sleep deprivation, since these two stages are known to be accompanied by increased extracellular 5-HT release in DR compared to REM
when the 5-HT release is low (Portas and McCarley, 1994). Increase in mesor of 5-HT rhythm in DR was also found after chronic clorgyline (a MAOI) treatment (Ozaki et al., 1993).

Since sleep deprivation reduces the sensitivity of DR neurons to the inhibitory effects of selective serotonin reuptake inhibitors (SSRIs) (Prevot et al., 1996; Maudhuit et al., 1996) mediated by 5-HT₁A somatodendritic autoreceptors, the increased 5-HT level in DR after REM sleep deprivation may not precipitate reduced raphe unit activity (autoinhibition), a characteristic of depression (Yavari et al., 1993). Instead, REM sleep deprivation might cause enhanced DR activity releasing more of 5-HT in forebrain structures such as SCN (vide 4.2.2.2.), similar to the neurophysiological action of some of the antidepressants (Maudhuit et al., 1996), which might alleviate depression (Wauquier and Dugovic, 1990).

Another observation from this study worthy of consideration in the comparative analysis of mechanism of action of REM sleep deprivation and antidepressants in the therapeutic management of depression is that REM sleep deprivation does not cause any significant change in the 5-HT rhythm's phase in DR. This resistance of dorsal raphe 5-HT rhythm's phase to REM deprivation is similar to the inaction of clorgyline, a MAOI on the phase of the 5-HT rhythm of DR (Ozaki et al., 1993).

Therefore, the results of this study, taken together, suggest that antidepressants such as MAOIs and REM sleep deprivation might share common mechanism of action at the 5-HT level rhythm of DR.

4.2.1.3. 5-HTP

5-HTP, being the precursor of 5-HT (Green, 1989), shows a normal circadian rhythm similar to that of the latter.

The significantly increased level of mesor after REM sleep deprivation corroborates the finding that intra peritoneal (i.p.) administration of 5-HTP reduced REM sleep (Ursin, 1976).

Since the effect of REM sleep deprivation on both the mesor (an increase) and phase (no change) of the rhythm were similar for 5-HTP and 5-HT, the
changes induced by REM sleep deprivation in 5-HT rhythm (vide 2.1.2.) could be via 5-HP, the precursor of 5-HT.

4.2.1.4. Dopamine

DR contains serotonergic DA synthesizing neurons which contain aromatic amino acid decarboxylase converting L-DOPA (the precursor of DA) to DA (Arai et al., 1994), and DA fibres originating from ventral tegmental area and A10 and A11 hypothalamic DA cell groups (Peyron et al., 1995).

Normal dopamine (DA) rhythm showed a nocturnal increase with an acrophase past midnight when the nocturnally active rat has the least sleep including REM sleep. Thus, DA level in DR, being in antiphase to the sleep period can be considered as a marker of wakefulness.

The REM sleep deprivation caused a significant increase in the mesor of the DA rhythm. The enhanced mesor of DA can increase the mesor of 5-HT in DR (vide 4.2.1.2.), since systemic apomorphine (a non selective DA agonist) administration increased extracellular 5-HT level in DR (Feera et al., 1994).

However, the phases of the DA and 5-HT rhythms may be independently regulated, since REM sleep deprivation delays the DA rhythm while having no effect on the phase of 5-HT rhythm (vide 4.2.1.2.).

4.2.2. REM sleep deprivation and SCN

Normal rhythm of all four neurotransmitters measured (GABA, 5-HTP, 5-HT and DA) showed a diurnal increase with their respective acrophases during day.

REM sleep deprivation increased the amplitude of rhythms of all four neurotransmitters (significantly for GABA and 5-HT). Since the reduced rhythm amplitude of many circadian body functions is thought to be a major factor responsible for many sleep disorders in depression (Schulz and Lund, 1983; Czeisler et al., 1987), and increased amplitude of physiological rhythms accompanies alleviation of depressive symptoms (Souètre et al., 1989), the increased amplitude of neurotransmitter rhythm after REM sleep deprivation could be the mechanism of non-pharmacological antidepressant treatment (such as REM sleep deprivation) as has been proposed by Czeisler et al., (1987). The increased amplitude has been suggested as the basis of mechanism for
pharmacological antidepressant treatments as well (Souêtre et al., 1989). Therefore, similar effects of antidepressants and REM sleep deprivation on the amplitude of neurotransmitter rhythms measured suggest similar mechanism of action.

4.2.2.1. GABA

GABA, besides being a component of SCN afferents (Moore, 1996), and efferents (Hermes et al., 1996), is considered to be the principal neurotransmitter of the circadian system (Speh and Morre, 1993), endowing the pacemaker with rhythmic function due to the endogenous rhythm of Cl− equilibrium potential (Wagner et al., 1997). Moreover, the importance of GABA in the circadian timing system is evident from the fact that any pharmacological manipulations of GABA neurotransmission induce significant phase shifts (Turek and Losee-Olson, 1986; Turek et al., 1995).

REM sleep deprivation significantly increased the mesor, and phase delayed the GABA rhythm. Considering GABA rhythm of SCN as a state parameter of the pacemaker (due to the central importance of GABA in SCN pace making function as mentioned above), the present finding is similar to the effect on the pacemaker of alprazolam. Alprazolam is a benzodiazepine (BDZ) agonist potentiating GABA action, used as an antidepressant, which reduces REM sleep and increases REM latency (Hubain et al., 1990; Zarcone et al., 1994), and phase delays the nocturnal plasma melatonin level indicating a delay of the pacemaker (McIntyre et al., 1993). This suggests a similar mechanism of antidepressant action for both REM sleep deprivation and BDZ/its agonist administration.

In view of the suggestion that phase shifts induced by 5-HT agonist administration into DR are mediated by GABA of SCN (Mintz et al., 1997), the phase delay observed in GABA rhythm in the absence of an apparent phase shift in 5-HT rhythm of DR after REM sleep deprivation (vide 4.2.1.2.) suggests that GABA can possibly exert its phase shifting action independently, implying a superior role for GABA in the circadian timing system.

The delayed GABA rhythm (representing the pacemaker’s phase delay) observed after REM sleep deprivation is consistent with the prediction of the phase advance theory of depression (Wehr and Wirz-Justice, 1981; 1982) that
V. REM Sleep and SCN

delaying the phase of the central pacemaker can act as an antidepressant mechanism.

By virtue of the colocalisation of GABA with all other neurotransmitters of SCN (Speh and Moore, 1993; Moore, 1996), GABA rhythm and its changes after REM sleep deprivation may influence the rhythm of other neurotransmitters of SCN or alternatively can be modulated by the rhythmic changes of the latter.

4.2.2.2. 5-HT

The normal rhythm of 5-HT with a diurnal increase and an acrophase near noon agrees with similar observations of Cagampang and Inouye (1994).

The increased mesor and phase delay of 5-HT rhythm after REM sleep deprivation is similar to the effects of REM sleep deprivation on GABA rhythm (vide 4.2.2.1.). This observation suggesting a synergistic action of GABA and 5-HT or alternatively a superior influence of GABA over 5-HT is contradictory to the finding that 5-HT inhibits GABA-induced current in SCN neurons (Kawahara et al., 1994). This could be due to the predominance of GABA ergic neurons compared to 5-HT ergic neurons in SCN.

The increase in the mesor and phase delay of 5-HT rhythm after REM sleep deprivation is similar to the effects induced by the MAO inhibitor, clorgyline (Ozaki et al., 1993). This suggests a similar mechanism of action for REM sleep deprivation and clorgyline. However, the major difference between their action is the increased amplitude of 5-HT rhythm of SCN after REM sleep deprivation (this study), an effect not found after clorgyline treatment (Ozaki et al., 1993).

The increased amplitude in 5-HT rhythm of SCN after REM sleep deprivation argues for the superior antidepressant action of REM sleep deprivation since the increased amplitude of physiological rhythms reportedly alleviates depressive symptoms (Souètre et al., 1989).

However, overall similarities in the alteration observed in 5-HT rhythm of SCN after REM sleep deprivation and clorgyline treatment suggests that both these antidepressant therapies might be phase delaying the advanced position of the oscillator (Wehr and Wirz-Justice, 1982).
4.2.2.3. 5-HTP

5-HTP as the precursor of 5-HT (Green, 1989) shows a rhythm similar to that of 5-HT; i.e., diurnal increase and an acrophase near noon.

The increase in 5-HTP mesor induced by REM sleep deprivation (and consequently enhanced wake period) is consistent with the observations that SCN controls the sleep-wakefulness cycle by promoting wakefulness (Edgar et al., 1993) and 5-HTP administration is known to reduce REM sleep (Ursin, 1976).

Similar action of REM sleep deprivation on the phase of the rhythms of 5-HTP and 5-HT suggest that changes in the rhythm of precursor (5-HTP) lead to those of 5-HT.

4.2.2.4. Dopamine (DA)

Normal DA rhythm with a diurnal increase and an acrophase during day is in antiphase to the DA rhythms of DR and pineal (which show a nocturnal increase) and could be considered as an index of sleep or less locomotor activity since a variety of partial and full DA agonists are known to cause dose dependent locomotor depression (Jackson and Westlind-Danielsson, 1994). REM sleep deprivation significantly increased the mesor of DA rhythm consistent with the observations that L-DOPA (DA precursor) administration reduces REM sleep in rat (Gallaraga et al., 1986) and man (Gillin et al., 1973). The phase advanced DA rhythm after REM sleep deprivation indicating a phase advanced locomotor rhythm is different from the action of antidepressants such as TCAs, SSRIs and MAOIs which decrease nocturnal motor activity (Duncan, 1996).

4.2.3. REM sleep deprivation and the pineal gland

The normal rhythm of GABA and 5-HT showed a diurnal increase while 5-HTP and DA showed a nocturnal increase. These neurotransmitters’ rhythms in normal as well as after REM sleep deprivation are discussed with respect to their influence on the pineal gland production of melatonin, a neurohormone that suppresses REM sleep (Goldstein and Pavel, 1981).

4.2.3.1. GABA

The normal rhythm of GABA with an increase during day and an acrophase near noon differs from the GABA rhythm in Syrian hamster where the
acrophase was at night (Kanterewicz et al., 1993), possibly due to the longer photoperiod (14 h L/ 10h D cycle) and a different species in the latter study.

However, normal rhythm of GABA as observed in the present study is consistent with and supplements the finding that diazepam (a GABA agonist) inhibits synthesis of melatonin (Wakabayashi et al., 1991) in the pineal gland, which normally peaks during the night time (Reiter, 1993). This inhibitory action of GABA on melatonin production is mediated by GABA A receptors (Rosenstein et al., 1989). This regulatory action of GABA on melatonin production is probably intrinsic to pineal since the GABA synthesising enzyme (GAD) has been found in pineal gland (Rosenstein et al., 1990), suggesting the semiautonomous nature of the pineal.

REM sleep deprivation reduced the mesor and amplitude of GABA rhythm. The REM sleep induced reduction in mesor of GABA rhythm can promote the melatonin production; similar to the enhanced melatonin synthesis after treatment with the antidepressants clorgyline and moclobemide (Oxenkrug et al., 1991; 1994). This is also consistent with the observation that melatonin suppresses REM sleep (Goldstein and Pavel, 1981).

The phase delay of GABA rhythm after REM sleep deprivation can delay the termination of melatonin production which indirectly can delay the nocturnal activity. A delay in rodent nocturnal activity was observed after treatment with clorgyline, the MAO inhibitor, as well (Wirz-Justice et al., 1982).

Thus, GABA rhythm of pineal seem to be similarly influenced by REM sleep deprivation and treatments with such antidepressants as clorgyline, suggesting similar mechanism of action of these two antidepressant regimens. Alterations induced in GABA rhythm after REM sleep deprivation correspond well the phase advanced theory of depression (Wehr and Wirz-Justice, 1982).

4.2.3.2. 5-HT

5-HT is the precursor for melatonin synthesis (Foulkes et al., 1997). In this pathway, 5-HT is transformed by N- acetyl transferase (NAT) to N-acetyl serotonin which in turn is converted to melatonin by hydroxy indole - O - methyl transferase (HIOMT) (Foulkes et al., 1997). The normal rhythm of 5-HT with a diurnal increase agree with the findings of Hermes et al., (1994) and Miguez et
where 5-HT showed a diurnal increase and a nocturnal decrease respectively. The nocturnal decrease of 5-HT could be due to the increased conversion of 5-HT to melatonin (Hermes et al., 1994). The normal rhythm of 5-HT is similar to that of GABA (vide 4.2.3.1.) and the latter might be driving the 5-HT rhythm since increased GABA level is known to elevate 5-HT level in pineal (Rosenstein et al., 1989) and modulate the melatonin synthesis by regulating the 5-HT release (Chuluyan et al., 1992), although these two studies considerably differed in details.

REM sleep deprivation induced increase in mesor and phase delay of 5-HT rhythm can elevate the melatonin synthesis rate in the pineal, parallel to the effects of clorgyline administration i.e., increased melatonin production and N-acetyl serotonin, but decreased 5-HIAA, the metabolic product of 5-HT (Oxenkrug et al., 1991). The phase delay induced, similar to that for GABA rhythm, could delay the termination of melatonin production acting as an antidepressant. The REM sleep deprivation induced phase delay supports the phase advanced theory of depression (Wehr and Wirz-Justice et al., 1982).

Similar effects of REM sleep deprivation on GABA and 5-HT rhythms suggest that these two neurotransmitter rhythms interact and possibly modulate one another, as suggested by Rosenstein et al.,(1989) and Chuluyan et al.,1992).

4.2.3.3. 5-HTP

The normal rhythm of pineal 5-HTP with a nocturnal increase and an acrophase at night is consistent with the similar observation found in hamster (Miguez et al.,1996). 5-HTP rhythm with a phase opposite to that of 5-HT (vide 2.3.2.) suggests that activity of 5-HT synthetic enzymes in 5-HT pathway is differentially regulated. However, the phase relationship of 5-HTP rhythm is similar to that of melatonin (Reiter, 1993) suggesting that 5-HTP, being the precursor of melatonin and 5-HT, is preferentially converted to melatonin within pineal, accounting for the reduced pineal 5-HT level at night.

Increase in mesor of 5-HTP induced by REM sleep deprivation is similar to the increased synthesis of 5-HTP after clorgyline administration in the rat (Reuss and Oxenkrug, 1989). Since the elevated melatonin synthesis is thought to contribute to the antidepressant action of MAOI (Reuss and Oxenkrug, 1989),
the antidepressant action of REM sleep deprivation also could be by enhanced melatonin synthesis in pineal.

4.2.3.4. Dopamine (DA)

Normal DA rhythm was nocturnal with an acrophase at night, consistent with the findings of Hermes et al., (1994) Miguez et al., (1995) and Moujir et al., (1997). Since DA increase at night has been linked to melatonin increase and 5-HT decrease, DA is suggested to be involved in the induction of melatonin synthesis (Hermes et al., 1994; Miguez et al., 1995).

Increased mesor of the DA rhythm (which presumably will enhance the melatonin synthesis) induced by REM sleep deprivation corresponds to the observation that melatonin suppresses REM sleep (Goldstein and Pavel, 1981), and the consequent expectation that REM sleep deprivation should increase the melatonin synthesis.

The significant phase delaying effect of REM sleep deprivation on the DA rhythm suggests that melatonin synthesis might be delayed which can delay the sleep onset time as has been observed in human depressives after melatonin administration (Wirz-Justice et al., 1990).

4.2.4. REM sleep deprivation and 5-HT to 5-HTP ratio

In this study, 5-HIAA/5-HT ratio normally used as an index of the metabolic turnover of 5-HT (and therefore an indirect measure of 5-HT neuronal activity) was not used due to the following reasons:

1. Extracellular 5-HIAA is not a reliable measure of depolarization induced 5-HT release from nerve terminals (Auerbach et al., 1989; Kalen et al., 1989) and
2. Under specific circumstances, 5-HT neuronal firing, release and metabolism are independent of one another (Crespi et al., 1990).

Therefore, in the present study, 5-HT/5-HTP ratio was used as an index of the synthetic activity of serotonergic neurons.

4.2.4.1. SCN

The normal rhythm of 5-HT/5-HTP ratio was insignificant suggesting that 5-HT synthetic rate does not follow a circadian pattern but possibly occurs at a steady rate. The normal diurnal rhythm of 5-HT in SCN (vide 4.2.2.2.) could be
due to the rhythmic release of 5-HT from serotonergic terminals from DR which shows a rhythm in the 5-HT synthetic rate (vide 4.2.4.2.).

5-HT/5-HTP rhythm did not significantly alter from normal after both control and REM deprivation treatments, suggesting that REM deprivation did not influence the synthetic rate of 5-HT in SCN.

4.2.4.2. Dorsal Raphe

The normal rhythm of 5-HT/5-HTP ratio was significant with a diurnal increase and an acrophase during day.

However, the rhythm of the synthetic rate was insignificant after both control and REM deprivation treatments suggesting a breakdown of the rhythm of synthetic rate possibly due to the increased wake and SWS periods (at the expense of REM) and an overall decrease in the synthetic rate across the circadian cycle.

4.2.4.3. Pineal

Normal 5-HT/5-HTP rhythm was significantly diurnal with an acrophase near noon.

Control treatment, not significantly different from normal, did show a significant synthetic rate (5-HT/5-HTP) rhythm, which was eliminated by REM sleep deprivation.

Thus, in all the three structures studied, REM sleep deprivation significantly disrupted the 5-HT synthetic rhythm. However, this effect of REM sleep deprivation might not be therapeutically important since alterations after REM sleep deprivation in the rhythms of individual neurotransmitters were, in general, similar to those after antidepressant treatments.

5 CONCLUSION

Alterations induced by REM sleep deprivation in the rhythms of neurotransmitters measured were similar in many aspects to those induced by (or expected by) antidepressant treatments, and the observed effects mostly corresponded well with different theories (observations) of antidepressant treatments (Table 5.A).
Table 5.A. Effect of REM sleep deprivation on the neurotransmitters’ rhythms and the corresponding antidepressant (AD) treatment/ theory

(i) Glutamate, Substance P and VIP of SCN

<table>
<thead>
<tr>
<th>Neurotransmitter</th>
<th>Effects</th>
<th>Corresponding AD treatment/ theory</th>
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<tbody>
<tr>
<td>Glutamate</td>
<td>↓Mesor,</td>
<td>Differs from the action of clorgyline which reduces light induced phase advances (Duncan, 1996).</td>
</tr>
<tr>
<td></td>
<td>↓Amplitude,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>[+ Δ ψ]</td>
<td>Sleep onset time advance improves depression (Wehr et al., 1979).</td>
</tr>
<tr>
<td>Substance P</td>
<td>↓Mesor,</td>
<td>Differs from the action of clorgyline which reduces light induced phase advances (Duncan, 1996).</td>
</tr>
<tr>
<td></td>
<td>↑Amplitude,</td>
<td></td>
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<tr>
<td></td>
<td>+ Δ ψ</td>
<td>Sleep onset time advance improves depression (Wehr et al., 1979).</td>
</tr>
<tr>
<td>VIP</td>
<td>↓Mesor,</td>
<td>Advancing the sleep onset time improves depression (Wehr et al. 1979).</td>
</tr>
<tr>
<td></td>
<td>↓Amplitude,</td>
<td></td>
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<tr>
<td></td>
<td>+ Δ ψ</td>
<td></td>
</tr>
</tbody>
</table>

↓ decrease; ↑ increase; + Δ ψ significant phase advance; [ ] insignificant effect.
(ii) Neurotransmitters of SCN, Dorsal Raphe and Pineal

<table>
<thead>
<tr>
<th>Neurotransmitter</th>
<th>SCN</th>
<th>Dorsal Raphe</th>
<th>Pineal</th>
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<tr>
<td></td>
<td>Effects</td>
<td>Corresponding AD treatment/ theory</td>
<td>Effects</td>
</tr>
<tr>
<td>GABA</td>
<td>Mesor, Amplitude, -Δψ</td>
<td>Similar to BDZ action (McIntyre, 1993; Zarcone et al., 1994); Increased amplitude of rhythms alleviates depression (Souètre et al., 1989); Phase advance theory (Wehr and Wirz-Justice, 1982).</td>
<td>Mesor, Amplitude, -Δψ</td>
</tr>
<tr>
<td>5-HT</td>
<td>Mesor, Amplitude, -Δψ</td>
<td>Similar to clorgyline action (Ozaki et al., 1993); Increased amplitude of rhythms alleviates depression (Souètre et al., 1989); Phase advance theory (Wehr and Wirz-Justice, 1982).</td>
<td>Mesor, Amplitude, -Δψ</td>
</tr>
<tr>
<td>5-HTP</td>
<td>Mesor, Amplitude, -Δψ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine</td>
<td>Mesor, Amplitude, +Δψ</td>
<td>Differ from the action of TCAs, SSRIs, MAOIs, (Duncan, 1996).</td>
<td>Mesor, Amplitude, -Δψ</td>
</tr>
</tbody>
</table>

△ decrease; ▲ increase; +Δψ significant phase advance; -Δψ significant phase delay; [ ] insignificant effect.