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**CIRCADIAN RHYTHMS IN OBSTRUCTIVE SLEEP APNEA SYNDROME:
AUGMENTATION OF THE EFFECT OF CPAP THERAPY WITH ADJUVANT
MELATONIN ADMINISTRATION**

by

ROCHELLE ZOZULA

**A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of
the requirements for the degree of Doctor of Philosophy,
The City University of New York**

1997

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Abstract

**CIRCADIAN RHYTHMS IN OBSTRUCTIVE SLEEP APNEA SYNDROME:
AUGMENTATION OF THE EFFECT OF CPAP THERAPY WITH ADJUVANT
MELATONIN ADMINISTRATION**

by

Rochelle Zozula

Advisor: Professor Arthur J. Spielman

Obstructive sleep apnea syndrome (OSAS) is associated with chronic fragmentation of sleep due to the repetitive arousals which occur in response to upper airway occlusion. The sleep disruption resulting from recurrent arousals and nocturnal hypoxemia play a role in the pathogenesis in many of the consequences of OSAS, which include excessive daytime sleepiness (EDS), cognitive impairment, changes in cardiovascular function, sexual dysfunction and personality changes. Nocturnal temperature rhythm disturbances are evidenced in OSAS and appear to be related to chronic sleep fragmentation and disrupted sleep architecture.

Nasal continuous positive airway pressure (CPAP) is the non-surgical treatment of choice for OSAS. CPAP administration is associated with significant improvement in sleep-related respiration, oxygenation and sleep fragmentation. However, many patients evidence residual sleepiness and persistent difficulty with daytime functioning. Proposed explanations for persistent daytime deficits have included the following: inadequate sleep duration, continued sleep fragmentation due to sub-optimal CPAP treatment, or changes due to irreversible

anoxic brain damage. It is hypothesized that chronic sleep fragmentation in OSAS contributes to a disturbance in the nocturnal temperature rhythm, which does not resolve with acute CPAP treatment, and is reflected by continued daytime dysfunction. Therefore, it was hypothesized that lowering body temperature at sleep onset by administration of melatonin at bedtime, in addition to CPAP therapy, would facilitate more stable sleep patterns due to a further reduction in arousal which would lead to enhanced daytime performance.

This study utilized a pre/post randomized treatment design. OSAS subjects were age- and sex-matched to a group of non-complaining sleepers. Melatonin or Placebo was given as an adjuvant for CPAP treatment in a double-blind fashion. Body temperature, sleep and respiratory parameters, and objective and subjective measures of daytime functioning were assessed before and after two weeks of continuous treatment. Measures of sleep fragmentation, respiratory disturbance and derived estimates of the temperature rhythm were used to predict the severity of the apneic disorder and subsequent response to treatment.

Decreased amplitude of the nocturnal temperature rhythm was evidenced before treatment in the OSAS group compared to the controls and significant improvement was noted following treatment. Measures of sleep fragmentation, respiration and daytime sleepiness/alertness were markedly altered in the OSAS group prior to treatment. Contrary to expectation, Melatonin did not impact the amplitude of the nocturnal temperature rhythm beyond the effect of CPAP alone. However, a modest (though non-significant) phase-advancing effect of Melatonin was noted post-treatment. Melatonin, in conjunction with ongoing CPAP, had no significant effects on measures of sleep architecture or sleep fragmentation. There was a trend towards a reduction in the nocturnal sleep latency in the

OSAS subjects who received combined melatonin and CPAP therapy. While improvement was noted on sleep latencies on both the Multiple Sleep Latency Test (MSLT) and the Maintenance of Wakefulness Test (MWT), these measures did not completely "normalize" post-treatment. Performance on the Four-Choice Reaction Time Task (i.e., number of gaps and reaction time), reached control group values after treatment. Mean sleep latencies on the MWT were reduced in the Melatonin group compared to the Placebo group. While sleep fragmentation and respiratory parameters had prognostic value in our study subjects, temperature measures bore little to no relationship with the daytime performance measures.

The lack of the effect of melatonin on body temperature and sleep parameters may have been due to the already high circulating levels of endogenous melatonin present at night in the study group. The level of melatonin production/synthesis was not assessed prior to treatment; clearly, this is important with regard to timing and dosage of melatonin administration. Given the fact that the OSAS subjects demonstrated a high propensity to sleep, further reductions in the nocturnal sleep latency in the sub-group who received melatonin was not expected. The finding of a daytime carry-over effect of melatonin (as evidenced on the MWT) was quite surprising, given the documented lack of such effects in other studies with nocturnal melatonin administration. Perhaps these subjects experienced the behavioral sedating properties associated with the substance, without evidencing significant effects on their sleep. The lack of improvement in daytime sleep propensity in the OSAS group, as indicated by persistently lower sleep latency values on the MSLT and the MWT, conflicts with previous studies. This may indicate the relative insensitivity of these tests as behavioral measures of treatment outcome, as compared with the measures

derived from the FCRTT. Evaluation over a longer period of treatment with a greater number of subjects may yield more significant findings.

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DEDICATION

I would like to take this opportunity to thank my family, friends and colleagues for their encouragement and support throughout the entire process.

First, I would like to dedicate this dissertation to the memory of my parents, Leon and Bella Zozula, for teaching me the value of the pursuit of knowledge and for always encouraging me with their love and pride.

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I. INTRODUCTION:

Obstructive sleep apnea syndrome (OSAS) is characterized by recurrent episodes of cessation of respiratory airflow during sleep, which lead to the fragmentation of sleep and decreases in oxyhemoglobin saturation (hypoxemia). In addition to the stated respiratory abnormality, there is also a constellation of symptoms associated with OSAS (thereby, classifying it as a true "syndrome"). One major clinical consequence of OSAS is excessive daytime sleepiness (EDS) thought to result from a combination of the following factors: sleep fragmentation caused by repetitive arousals from sleep; the loss of the deeper stages of sleep; and the effects of hypoxemia on cerebral function. In addition, patients or their families often report cognitive dysfunction and memory loss (Yesavage et al., 1985; Findley et al., 1986; Greenberg et al., 1987; Telakivi et al., 1988; Bedard et al., 1991), as well as personality changes (Viot-Blanc and Peyrical, 1993). These changes, coupled with severe daytime sleepiness, result in difficulty maintaining employment, fulfilling social obligations and meeting other demands of daily life. Other symptoms associated with OSAS include choking or gagging sensations during the night, hypnagogic hallucinations, decrease in sexual drive or impotency (in males), morning headaches, and automatic behavior (Guilleminault, 1994). While it is doubtful that all symptoms will be present in any one individual, frequently several will be observed, and systematic questioning about the overall constellation of symptoms will help confirm the diagnosis of OSAS and the severity of the problem.

Nasal continuous positive airway pressure (CPAP) is the non-surgical treatment of choice for OSAS (Sullivan and Grunstein, 1989).. While CPAP administration is associated with significant improvement in sleep-related respiration, oxygenation and sleep fragmentation (Rajagopal et al., 1986; Lamphere et al., 1989; Phillips et al., 1990), many patients evidence residual sleepiness and persistent difficulty with daytime functioning (Lamphere et al., 1989; Montplaisir et al., 1992; Engleman et al., 1993; Munt et al., 1994). A central issue in current investigations of the consequences of OSAS involves the variability in time course necessary to observe the reversal of symptoms of EDS and cognitive dysfunction in OSAS patients, and whether there is complete normalization of daytime functioning. Some studies have demonstrated improvement in EDS after a single night of CPAP treatment according to self-ratings of sleepiness (Rajagopal et al., 1986; Baker et al., 1988), and by objective measures of daytime sleepiness (Lamphere et al., 1989; Kribbs et al., 1992; Sforza and Lugaresi, 1994). Continued improvement in objective measures of EDS have been reported after two weeks of continuous CPAP usage (Lamphere et al., 1989). Complete normalization of EDS has been shown to occur after six weeks of CPAP treatment (Wittig et al., 1986). Similarly, measures of cognitive functioning have demonstrated that certain abilities related to vigilance (i.e., memory and attentional tasks) were restored in OSAS patients to a level which was comparable to a group of control subjects, while deficits persisted in tasks that involved planning and sequencing or psychomotor skills even after 6-10 months of CPAP therapy (Montplaisir et al., 1992; 1993). These contradictory findings will be examined in greater detail following a discussion of the types of objective and subjective measures that have been employed to assess/quantify daytime sleepiness, mood and other performance

indices.

The reason for this prolonged time course for improvement in OSAS has just begun to be explored. Possible explanations for the persistence of daytime symptoms include inadequate sleep duration (Kribbs et al., 1993a) and continued sleep fragmentation due to suboptimal CPAP treatment (Kribbs et al., 1993b) or changes due to irreversible anoxic brain damage (Montplaisir et al., 1993; Kribbs et al., 1993a). Another possible explanation for persistently short sleep latencies on MSLT following chronic CPAP therapy is that OSAS patients become accustomed to falling asleep rapidly and that this ability remains intact after treatment (Engleman et al., 1994). Gaddy and Doghramji (1991) have proposed that CPAP itself may be a potential source for causing sleep disruption which perpetuates the problem of EDS. I hypothesize that the persistent daytime deficits observed in OSAS patients are due to an acquired temperature rhythm disturbance, as evidenced by the failure of OSAS patients to demonstrate a normal decline in body temperature during the nocturnal (sleep) period.

To date, no data exist which systematically examine the role of sleep fragmentation or hypoxemia in the development of changes in the nocturnal temperature rhythm in individuals with OSAS. There is some evidence which suggests that the intrusion of wakefulness into sleep (i.e., fragmented sleep of "poor" sleepers) results in higher nocturnal body temperature values (Monroe, 1967). In Monroe's study, the entire nocturnal curve of rectal temperature was shifted up to a higher absolute temperature level in the "poor" sleepers as compared to the "good" sleepers. Aschoff et al. (1972) have also demonstrated elevation of the nighttime

values in body temperature in subjects who underwent 48 hours of sleep deprivation. Elevation of the nocturnal temperature values (i.e., higher nocturnal core temperature minima) has been associated with a decrease in the overall amplitude of the circadian (24-hour) rhythm of temperature (Waldhauser and Steger, 1986; Vitiello et al., 1986). It has been suggested that a decline in the amplitude of the temperature rhythm contributes to changes in the distribution of sleep-wakefulness during the course of a 24-hour day in the elderly (i.e., fragmented nocturnal sleep and daytime napping) (Richardson et al., 1982). Nevertheless, it remains unclear as to whether sleep-wake disturbances are primary or secondary to alterations in the regulation of body temperature. It is a distinct possibility that as the integrity of sleep is disrupted, so is the normal expression of circadian rhythmicity. Therefore, in the case of OSAS, it is hypothesized that chronic sleep fragmentation leads to a disruption of normal sleep structure which, in turn, does not allow for the optimal expression of circadian mechanisms.

There have been limited studies examining the circadian temperature rhythms in individuals with OSAS, in both untreated and post-treatment conditions. It has been demonstrated that core body temperature tends to remain elevated at sleep onset in OSAS subjects compared to control subjects (Sforza et al., 1993). Preliminary data in individuals with confirmed diagnoses of moderate to severe OSAS have shown a significant reduction in the amplitude of their temperature rhythms as compared to an age-matched group of non-complaining sleepers, with a non-significant trend towards a higher mean temperature in the OSAS group (Zozula et al., 1993). This is consistent with the finding of a reduction

in amplitude of circadian temperature rhythms among normal subjects who have been kept under sustained wakefulness conditions overnight (i.e, constant routine) (Barrett et al., 1993). In effect, individuals with severe OSAS are subjected to a form of "constant routine", with respect to the fact that they are fairly inactive during the day due to excessive sleepiness and experience chronic sleep disruption at night due to the repetitive arousals which result from apneic episodes. Furthermore, recent evidence using a modified constant routine technique in OSAS patients has shown a blunting of their circadian temperature amplitude prior to treatment, which partially corrects after acute treatment (3 nights) with continuous positive airway pressure (Vogel et al., 1993). The modification of the constant routine technique refers to the fact that subjects' activity was restricted to reading in bed, with regular snacks at 3 hour intervals, but they were permitted to sleep at will the rest of the 24 hour period. Therefore, the masking effect of increased activity on core body temperature patterns during the daytime hours was eliminated. Again, the main difference noted in this study was the lack of the normal decline in nocturnal body temperature in the OSAS group prior to CPAP treatment. However, no attempt was made to link the observed changes in nocturnal temperature rhythms with improvement in daytime symptomatology, nor were there any reported objective (polysomnographic) measurements of their subjects' sleep. The fact that the temperature rhythm corrects so quickly in the study by Vogel et al. suggests that the circadian "clock" mechanism is intact and operational. However, the observed decrease in the amplitude of the temperature rhythm in these studies may suggest that the intrinsic circadian mechanism is not manifested as clearly in the OSAS patient group prior to treatment. Moreover, circadian mechanisms in individuals with OSAS may be more fully

expressed after sleep disruption is eliminated with an appropriate course of treatment (i.e., CPAP therapy).

Since the prominent circadian aberration in OSAS patients is the lack of a drop in nocturnal body temperature (BT) (Sforza et al, 1993; Vogel et al., 1993), resulting in a flattening of the amplitude of the circadian temperature pattern, an intervention that would facilitate the decline in nocturnal temperature may help to restore the expression of normal circadian rhythmicity. Once a more prominent circadian temperature pattern is achieved, this may be reflected by improvement in daytime symptoms, since many psychological rhythms (i.e., performance, mood, subjective activation) have been linked to body temperature rhythms (Colquhoun, 1971). Exogenous melatonin administration (2.5 mg, orally) has been shown to decrease core body temperature in humans (Cagnacci et al., 1992). It has also been reported that melatonin has a general sleep inducing effect on both animals and humans when given in pharmacological doses (Anton-Tay et al., 1971). Recently, it has been suggested that melatonin exerts a hypnotic effect by creating a subtle hypothermic effect within the normal circadian variation in temperature (36-37.5° C) (Dawson et al., 1993). It has been suggested that diurnal variation in core body temperature may be partly mediated by the diurnal variation in plasma melatonin levels (Dawson et al., 1993). This would entail a change in the standard mode of viewing the circadian control mechanisms which regulate body temperature: instead of one circadian pacemaker (located in the suprachiasmatic nucleus of the hypothalamus), it is possible that the pineal gland, which controls the production/synthesis of melatonin, is also responsible for driving the circadian mechanism

which controls temperature. Indirect evidence from studies in older human subjects has associated a decline in the amplitude and duration of the nocturnal melatonin rhythm and a higher nocturnal core temperature with increased nighttime arousal and decreased sleep propensity (Waldhauser and Steger, 1986). More direct evidence of a causal link between melatonin secretion and sleep-propensity rhythms comes from studies in blind subjects (Nakagawa et al., 1992) and normal subjects (Lavie, 1992). Both of these studies demonstrated a phase-locking between the timing of the melatonin secretion and sleep-propensity rhythms. Melatonin has been shown to have a generalized effect on reducing behavioral arousal: in addition to lowering core temperature, it also slows heart rate, increases simple reaction times (Wynn and Arendt, 1988), and reduces performance on complex divided-attention cognitive tasks (Dawson et al., 1992). Therefore, by exerting its effect through thermoregulatory mechanisms, it is proposed that melatonin reduces arousal and increases sleep-propensity. Further evidence in support of the thermoregulatory role of melatonin is based on the finding that specific melatonin binding sites are expressed in the vasculature of the rat: in the arteries that form the circle of Willis at the base of the brain and the caudal (tail) artery, both of which are believed to have a role in thermoregulation in the rat (Viswanathan et al., 1990). Moreover, the authors found that these sites are also functional melatonin receptors. These factors support the usage of exogenous melatonin in OSAS subjects who evidence aberrant nocturnal temperature rhythms, as well as disturbed sleep which results in significant daytime deficits. Furthermore, due partly to its behavioral arousal reducing properties, exogenous melatonin administration may eliminate some of the sleep onset respiratory instability which is associated with OSAS, as well as increasing the

level of sleep continuity, as compared to CPAP treatment alone. This, in turn, may result in better compliance with nasal CPAP treatment, especially in the initial stages of therapy.

It is hypothesized that chronic sleep fragmentation in OSAS patients contributes to a disturbance in their nocturnal temperature rhythms, which does not abate completely with short-term CPAP therapy. It is further hypothesized that with ongoing CPAP treatment and adjuvant melatonin administration, the nocturnal temperature rhythms will resume a pattern that more closely resembles that of normal (non-fragmented) sleepers. Furthermore, the correction of the temperature rhythm disturbance with combined treatment (CPAP and exogenous melatonin) should be accompanied by improvement in the level of daytime functioning, which would not be evidenced by subjects receiving CPAP treatment alone. These predicted outcomes could result from either 1) a direct effect of melatonin on the circadian system, which in turn, influences the behavioral expression of sleepiness/alertness and related functions, or 2) a direct sleep-inducing effect of melatonin, without altering circadian temperature parameters.

Therefore, the specific questions addressed by the study are the following:

- 1) Do OSAS patients demonstrate a disturbance in their nocturnal temperature rhythmicity (as evidenced by decreased amplitude and increased mesor of their temperature rhythm), compared to a group of sex and age-matched non-complaining sleepers?
- 2) Does short-term CPAP treatment alone in OSAS subjects result in normalization of temperature rhythms (as evidenced by greater amplitude and lower mesor values of

temperature rhythm), compared to pre-treatment values?

3) Does adjuvant exogenous melatonin administration augment the effects of acute CPAP treatment in OSAS patients as evidenced by decreased levels of daytime sleepiness (i.e., improved mean sleep latency values on the Multiple Sleep Latency Test) and cognitive impairment?

4) Can the severity of the apneic disorder as measured by the daytime vigilance deficits and subsequent response to treatment be predicted by the degree of the nocturnal temperature rhythm disturbance, apnea-hypopnea index (AHI), sleep fragmentation, or nocturnal hypoxemia indices?

This work will examine the relationship between chronic sleep fragmentation and nocturnal hypoxemia with changes in the nocturnal temperature rhythms in OSAS. It will also explore the relative contribution of each of these factors to the daytime consequences observed in OSAS patients prior to treatment and following a two-week course of CPAP treatment, with and without adjuvant melatonin administration. In order to fully understand the level of sleep disruption, respiratory impairment and daytime sequelae in OSAS, I will first discuss normal sleep structure and architecture and outline the basic neuroanatomic and neurobiologic factors controlling states of sleep and wakefulness. I will then introduce the homeostatic and circadian control mechanisms which regulate the sleep/wake cycle, with particular consideration for the role of the hormone melatonin in maintaining circadian rhythmicity. Since the behavioral outcome measures of daytime sleepiness and cognitive performance are multifarious, I will delineate the objective and subjective measures

employed in the study. Furthermore, I will outline respiratory control mechanisms during sleep and wakefulness and contrast normal respiratory patterns to those observed in OSAS. An explanation of the pathophysiologic mechanisms involved in OSAS will be included to provide the reader with an understanding for the basis of CPAP treatment. Lastly, issues regarding the efficacy of CPAP treatment will be discussed within the context of achieving resolution of daytime symptoms.

A) Sleep and Wakefulness

1) Normal Sleep Structure/Architecture

Sleep and wakefulness are complex behavioral and physiologic states with a variety of subjective and objective correlates. Sleep is defined as a reversible behavioral state of perceptual disconnection from, and unresponsiveness to, the external environment (Carskadon and Dement, 1994). Sleep is usually associated with postural recumbency, quiescence, closed eyes and other indicators which one commonly associates with sleeping. Based upon several electrophysiologic parameters, researchers have identified three basic physiologic states: wakefulness and two distinct sleep states, non-rapid eye movement (NREM) and rapid eye movement (REM) (Dement and Kleitman, 1957). The parameters used for the identification and categorization of sleep into its various stages include electroencephalographic measures from the central and occipital scalp areas (EEG), electro-oculogram (EOG) measures from the right and left outer canthi of the eyes, and the

electromyogram (EMG) recorded from the submental region of the chin. These measures are incorporated into a technique that is used for the objective assessment of sleep which is referred to as nocturnal polysomnography (NPSG).

According to the classification system of Rechtschaffen and Kales (1968), NREM sleep is subdivided into four stages: stage 1 is a transitional stage of sleep and is associated with a low arousal threshold to external stimulation; stage 2 is a slightly deeper stage of sleep which requires a more intense stimulus to produce an arousal compared to stage 1; stage 3 and stage 4 comprise what is referred to as slow wave sleep (SWS), or delta sleep, and reflect a further depth of sleep. An even more intense stimulus is generally required to produce an arousal from stage 3 or 4 sleep than from stage 1 or 2 sleep. The EEG pattern in NREM sleep is commonly described as synchronous, with characteristic waveforms such as sleep spindles, K complexes, and high-voltage slow (delta) waves. In contrast, REM sleep is defined by EEG activation (desynchrony), muscle atonia, and episodic bursts of rapid eye movements. Although REM sleep is generally not divided into stages, it may be classified as having "tonic" and "phasic" periods; the latter being based on short-lived physiologic bursts of activity that tend to occur in clusters separated by episodes of relative quiescence. NREM sleep and REM sleep continue to alternate throughout the night in cyclic fashion. REM sleep episodes tend to become longer across the night and may last from 1-20 minutes per cycle. Across the night, the average period of the NREM-REM cycle is about 90-110 minutes.

Normative data based on polysomnographic studies in young adults have demonstrated a particular pattern, or architecture, to the cyclic interactions of the different stages of sleep. Slow wave sleep (SWS) tends to dominate the NREM portion of the sleep cycle toward the beginning of the night (the first one third); REM sleep tends to be greatest in the last third of the night. Brief periods of wakefulness tend to occur later in the night and are generally associated with REM sleep transitions. In terms of the relative distribution of sleep stages across the night, in a normal young adult (i.e., living on a conventional sleep-wake schedule and without sleep complaints), NREM sleep constitutes 75-80% of the total sleep time; REM sleep typically accounts for 20-25% of total sleep time. Wakefulness within sleep usually accounts for less than 5% of the night. Within NREM sleep, Stage 1 generally comprises 2-5% of sleep; Stage 2 comprises 45-55% of sleep; Stage 3 comprises 3-8% of total sleep; and Stage 4 typically accounts for 10-15% of sleep. (Williams et al., 1974). These values have direct bearing on the interpretation of the NPSG documented sleep in the apneic patient group prior to treatment and subsequent to treatment, to assess if their sleep was “normalized” with respect to sleep stage distribution.

The most robust and consistent factor affecting sleep architecture is age. Slow wave sleep is maximal in young children and decreases markedly as a result of aging (Prinz et al., 1982). In addition, arousals during sleep increase dramatically as a function of age (Carskadon et al., 1982). Other factors that effect sleep architecture include prior sleep history (i.e., total or partial sleep loss) (Bonnet and Rosa, 1987), ambient temperature (Parmeggiani, 1980), drug ingestion, and circadian rhythms (Strogatz, 1986). In terms of

circadian influence, sleep onset is most likely to occur on the descending part of the circadian temperature curve, although a secondary peak in sleep onset is often noted in mid-afternoon (when napping tends to occur). The offset of sleep is most likely to occur on the ascending portion of the body temperature curve (Strogatz, 1986). Furthermore, changes in the overall structure and distribution of sleep may indicate underlying disorders (i.e., primary sleep disorders or other medical disorders) which have an impact upon a variety of psychological and biological functions.

2) Physiological Changes in NREM and REM Sleep

Many organ systems of the body are affected by physiological changes that occur in NREM and REM sleep. In terms of respiration, the control of breathing is somewhat decreased during NREM sleep compared to wakefulness. Breathing patterns are normal and regular during NREM sleep (Bulow, 1963). During sleep onset there may be some periodicity, as short episodes of light NREM sleep may alternate with bouts of wakefulness. In contrast, in REM sleep, the control of breathing is markedly decreased compared to wakefulness. Periodic breathing is sometimes observed in REM, where a sudden change in both respiratory amplitude and frequency may occur (Bulow, 1963). Muscle tone is usually present during NREM sleep. During REM sleep, most skeletal muscles, including the intercostals (accessory muscles for breathing) and pharyngeal muscles, show reduced or absent tonus (which further predisposes the individual to experience apneic episodes during REM). Blood pressure is slightly lower than during wakefulness in NREM sleep. In REM

sleep, blood pressure may be erratic and may increase during phasic events (Guilleminault and Suzuki, 1992). Heart rate and rhythm are fairly regular in NREM sleep; it may vary during REM sleep and may increase during phasic REM episodes. There is no sexual organ activation during NREM sleep; in REM, males usually display erections and females have increased vaginal blood flow (Ware and Hirshkowitz, 1992). In terms of metabolism, NREM sleep is associated with decreased metabolic rate compared to wakefulness (Brebbia and Altshuler, 1965). Depending on the study, metabolic rate in humans in REM sleep is associated either with no change in rate or increased metabolism compared to NREM sleep (White et al., 1985; Palca et al., 1986). This is in direct contrast to metabolic findings in animal studies which shows a decrease in metabolic rate in REM (Roussel and Bittel, 1979). One possible explanation for the differences observed in metabolic rate in REM versus NREM sleep is that there are species difference with regard to the contribution of brain metabolism to total metabolism in humans (Ingvar et al., 1979). Therefore, the increase in cerebral blood flow and brain metabolism in humans during REM sleep would account for higher overall metabolic rate in REM compared to NREM sleep. In addition, while thermoregulatory mechanisms remain intact during NREM sleep (i.e., responding to changes in ambient temperature), in REM sleep, active thermoregulation is suspended (Haskell et al., 1991).

3) Brain Mechanisms Which Regulate Sleep and Wakefulness

The identification of brain regions that are involved in the generation and maintenance

of sleep and wakefulness were accomplished through studies during the early part of this century. Those studies typically involved lesions and brain stimulation to assess the neuroanatomical substrates of sleep and wake states. Prior to the 1930s, physiologists asserted that wakeful/conscious experience was maintained by continuous sensory input to the brain (Kleitman and Camille, 1932). The basic brain mechanisms involved in the regulation of sleep and wakefulness have been outlined by neurophysiological studies conducted after 1930 (Bremer, 1935; Moruzzi et al., 1949; Moruzzi, 1972). One of the earliest experiments was conducted by Bremer (1935) which demonstrated that total transection of the brainstem, which resulted in separation of the cerebrum from the brainstem and spinal cord ("cerveau isole" preparation), produced a state which was remarkably similar to physiological sleep (i.e., cortical synchronous slow waves and miotic pupils). Studies which employed electrical stimulation of the brainstem reticular formation, but not the sensory pathways, resulted in prolonged and generalized activation of cortical structures (Moruzzi and Magoun, 1949). Conversely, lesions of the reticular formation, but not the sensory pathways, resulted in a loss of cortical activation, accompanied by cortical slow wave activity and behavioral immobility (Lindsley et al., 1950). Lesions of the rostral end of the brainstem reticular formation (i.e., oral pontine and midbrain tegmentum) and caudal diencephalon (i.e., posterior hypothalamus, subthalamus, and ventral thalamus) and in the basal forebrain were associated with a chronic decrease or loss of cortical activation and behavioral activity and responsivity. While lesions of the central midbrain tegmentum primarily impact upon cortical activation, lesions of the ventral midbrain primarily alter behavioral arousal (Jones, 1994). Electrophysiological and neuroanatomical techniques

confirmed that the neurons of the reticular formation receive collateral input from visceral, somatic, and special sensory systems and send ascending projections into the forebrain via two major routes: a dorsal pathway which projects to nonspecific thalamic nuclei, which then broadly projects to the cerebral cortex; the ventral pathway passes through and terminates in the subthalamus and hypothalamus and continues into the basal forebrain and septum, where neurons project in a widespread manner to the cortex and hippocampus (Dempsey et al., 1941; Nauta and Kuypers, 1958). Therefore, the neuronal system referred to as the "ascending reticular activating system" was defined, which was located in the core of the brainstem with long ascending projections to the forebrain. This system was deemed both necessary and sufficient for the ongoing maintenance of cortical activation and behavioral arousal that is associated with wakefulness (see Jones, 1994).

Once the idea of a neuronal activating system was accepted in the 1950s, physiologists concurred that sleep was largely the result of fatigue and decrease in the level of activation of this system (i.e., passive deactivation of reticular system) (Moruzzi, 1972). However, Batini et al. (1959) demonstrated that certain transections of the brainstem, below the level of the oral pontine tegmentum, resulted in complete insomnia. This finding confirmed the fact that sleep-generating centers were located in lower regions of the brainstem. Specifically, in humans, lesions of the brainstem (raphe nuclei) and pons have been shown to be associated with EEG activity of wakefulness (usually with dominant alpha activity), despite a lack of behavioral alertness or responsivity (Markand and Dyken, 1976). Low-frequency electrical stimulation of the medullary reticular formation (i.e., dorsal reticular

formation and solitary tract nucleus), was found to produce cortical synchronization of EEG activity indicative of slow wave sleep in a behaviorally awake animal preparation (Magnes et al., 1961). On the other hand, lesions of the dorsal reticular formation and the solitary tract nucleus produced EEG desynchronization in a sleeping animal preparation (Bonvallet and Allen, 1963). These findings suggested that neurons in the dorsal medullary reticular formation and the nucleus of the solitary tract were sleep-promoting. The proposed mechanism of action was thought to involve inhibition of the rostral neurons of the ascending activating system, although a direct influence on forebrain systems which promoted synchronous EEG activity was also hypothesized.

The caudal solitary tract nucleus receives afferent fibers from the vagus and glossopharyngeal nerves. Original anatomical studies traced ascending projections from the nucleus of the solitary tract and dorsolateral reticular formation through the lateral and dorsal tegmentum and periventricular gray area up to the rostral pons and midbrain (Norgren 1978). Norgren demonstrated that projections terminated primarily in the region of the parabrachial nucleus, which projected rostrally to the thalamus, hypothalamus, preoptic regions, bed nucleus of the stria terminalis, amygdala, and frontal cortex (regions of the visceral-limbic forebrain). Studies by Ricardo and Koh (1978) have shown direct projection from the solitary tract nucleus to all of these forebrain structures. The data from these anatomical studies supports the idea that the major impact of the nucleus of the solitary tract is via limbic forebrain structures, and not through projections to the reticular activating system.

As a result of the advances made in biochemical techniques in the 1950s and 1960s, chemically specific neuronal systems within the brain were identified as the generators for sleep-wake states (Jouvet, 1972). Dahlstrom and Fuxe performed some of the earliest histochemical work which revealed that catecholamine-containing (norepinephrine) neurons were located in the brainstem reticular formation and the dorsolateral pontine tegmentum (locus coeruleus) and were important for maintaining wakefulness. Dopamine-containing neurons were found to be localized in the substantia nigra and ventral tegmental area of the midbrain and also scattered through the posterior hypothalamus and subthalamus (Jouvet, 1972). The norepinephrine neurons are mainly involved in processes of cortical activation and project in a diffuse manner to the entire forebrain and all areas of the cortex, as well as to subcortical way stations that overlap with those of the reticular formation (Jones, 1994). The dopamine neurons are mainly implicated in processes of behavioral activity and responsiveness and project heavily into the basal ganglia and frontal cortex (Jones, 1994). The dopamine neurons of the substantia nigra project through the lateral hypothalamus and internal capsule into the striatum. They also innervate basal forebrain structures, nucleus accumbens, septum, amygdala and frontal regions of the cortex. Therefore, midbrain dopamine-containing neurons exert a modulating effect on motor and limbic systems via these efferent projections (Nauta and Domesick, 1978). Cholinergic (acetylcholine-containing) neurons are also implicated in cortical activation and consist of two major groups which project to the forebrain and cortex. One branch is composed of cholinergic neurons located in the caudal mesencephalic-oral pontine reticular formation (i.e., laterodorsal and pedunculopontine tegmental nuclei), which have projections to the forebrain, especially to

the medial-intralaminar nuclei, but also into the lateral hypothalamus and basal forebrain and up to the frontal regions of the cortex (Jones, 1994). The other group of neurons is located within the basal forebrain (nucleus basalis, substantia innominata, nuclei of the horizontal and vertical limbs of the diagonal band, and septum) which have broad projections to the entire cortex. Other neuronal systems implicated in wakefulness include histamine-containing neurons located in the posterior hypothalamus, which have direct projections to the cerebral cortex. Glutamate neurons which are located throughout subcortical structures and in the cortex also play a role in cortical activation and wakefulness. In addition, there are other peptides (i.e., substance P, corticotropin-releasing factor, vasoactive intestinal polypeptide) that have been implicated in wakefulness and are often co-existing with one of the other primary neurotransmitters (i.e., norepinephrine). In general, the neuronal systems implicated in the maintenance of wakefulness may be active in the process of sensory transmission or attention, motor response or activity, and hormonal responses and regulation (which may serve to modulate states of vigilance and arousal).

In terms of the neuronal control of sleep-promoting regions of the brain, serotonin-containing neurons of the brainstem raphe nuclei have been identified, which project forward into the rostral tegmentum, thalamus, subthalamus, hypothalamus, and basal forebrain, and also from midbrain directly to the cerebral cortex and hippocampus. (Jones, 1994). These neurons have been implicated in the facilitation of the onset of slow wave sleep. Other neurons which are implicated in slow wave sleep are gamma-aminobutyric acid (GABA) neurons which are located in the hypothalamus and basal forebrain-septum, which project

to the cerebral cortex and hippocampus. GABA neurons are also directly located in the cortex and they are maximally active during slow wave sleep. In addition, another SWS-promoting set of neurons have been found in the hypothalamus which contain adenosine deaminase, which raises the possibility that adenosine may be implicated in the functional role of these neurons (Nagy et al., 1984). Other peptides have been implicated in the process of slow wave sleep generation (i.e., opiates, alpha-melanocyte-stimulating hormone, somatostatin), and are often co-localized with one of the other primary neurotransmitters (i.e., serotonin or GABA) (Jones, 1994). In general, neuronal systems implicated in the maintenance of slow wave sleep may be active in the processes of sensory inhibition and analgesia, behavioral inhibition, and parasympathetic and hormonal responses and regulation, which facilitate the onset and maintenance of slow wave sleep.

In summary, the neuronal systems outlined above govern the cyclical alternation between sleep and wakeful states over the course of a 24 hour period. As previously discussed, lesions which occur in any of these brain centers (as a result of direct brain injury or disease states) can cause either dysregulation of the control mechanisms which maintain wakefulness or the facilitation of slow wave sleep. Since these patterns of neuronal activation act in concert with other mechanisms that regulate the sleep-wake cycle (i.e., homeostatic and circadian mechanisms), it is possible that imbalance in either of these systems would have an impact on the transmission of neural impulses and chemical messenger systems along these pathways.

B) Regulation of the Sleep/Wake Cycle

1) Homeostatic control mechanisms

Homeostatic influences on sleep-wake regulation refer to the interdependent relationship between the two states of sleep and wakefulness. The homeostatic process, with regard to sleep, attempts to act as a balancing mechanism when sleep is not permitted to occur to its full extent (i.e., total or partial sleep deprivation) or when sleep accrual is in excess of an individual's mean "reference level" (Borbely, 1994). Therefore, an individual's drive to sleep is increased when sleep is not of adequate duration; conversely, when the amount of sleep is in excess of an individual's average (nightly) requirement, the propensity to sleep is reduced.

The intensity of sleep is reflected by the amount of slow wave activity present in the nocturnal EEG activity. Blake and Gerard (1937) were among the first to document that slow wave activity is predominant in the first hours of nocturnal sleep and shows a decline through the rest of the sleep period. Williams et al. (1964) further demonstrated that with increasing sleep loss, behavioral responsiveness to auditory stimuli was reduced and slow wave activity was increased. Partial sleep deprivation studies (ranging from 2-20 hours) demonstrated that as the duration of prior waking episodes increased, slow wave activity increased within the first 30 minutes of each nap opportunity (Dijk et al., 1987). Furthermore, auditory stimulation studies which reduced slow wave sleep (but did not

produce full awakening) in the first third of the night resulted in rebound of slow wave activity later in the sleep period (Dijk et al., 1987). These studies lend support for the regulation of slow wave activity by sleep homeostatic influences. Additionally, slow wave activity could be considered as a useful marker for depth of sleep. This finding has also been replicated in studies in rats, which demonstrated that as the number of brief awakenings from sleep increased, slow wave activity was enhanced (Franken et al., 1991). This also accounts for the fact that the arousal threshold and slow wave activity is higher in the initial part of sleep, when the drive to sleep is greater, and as sleep accrues over the nocturnal period, slow wave activity progressively declined (Blake and Gerard, 1937; Dement and Kleitman, 1957).

Two Process Model of Sleep Regulation

In general, slow wave activity is regulated by a homeostatic process that depends on the prior history of wakefulness and sleep. According to a two process model of sleep regulation proposed by Borbely (1982), sleep propensity in humans is determined by two distinct processes: process "S", which suggests that a hypnogenic factor(s) accumulates in the brain during wakefulness which induces sleep and then declines during the sleep period, and process "C", which is a circadian clock-like mechanism which determines the oscillation of periods with higher and lower propensity for sleep. Borbely proposed that the interaction between these two processes governs the timing of periods of sleep and wakefulness. The time course of process "S" was originally derived from EEG slow wave activity, which was first accomplished with visual analysis of the waveforms, and later with the application of

spectral analysis techniques of the EEG. As early as 1971, sleep deprivation studies performed by Webb and Agnew demonstrated that slow wave sleep qualitatively increased in response to the length of prior wakefulness. Quantitative analysis of slow wave activity using spectral analysis revealed that sleep deprivation (40.5 hours of wakefulness) resulted in enhanced slow waves during recovery sleep (Borbely et al., 1981). Moreover, power density changes in slow wave activity were noted on two recovery nights as compared to baseline values. On the first recovery night, the greatest increase was in the low delta ranges (0.75-4.5 Hz band). In terms of slow wave activity across the successive NREM-REM cycles, there is a decline in this EEG activity across three to four complete cycles, which then reaches a stable low level. Furthermore, daytime nap studies following varying amounts of prior waking have borne out that naps which occurred later in the day contained more slow wave activity than naps earlier in the day (Knowles et al., 1990). Dijk et al. (1987) have also shown that regularly scheduled daytime naps (every two hours) following a period of sleep deprivation resulted in a monotonic rise in slow wave activity over the course of the day. Borbely further speculated that if daytime naps have the ability to reverse the buildup of process "S", then slow wave sleep would be expected to be reduced in the subsequent nighttime sleep episode. This has been demonstrated by studies by Karacan et al. (1970) and later by work by Feinberg et al. (1985), which showed diminished slow wave activity during the nocturnal sleep period.

Evidence to support the idea that the time course of process "S" is independent of the circadian phase at which wake or sleep occurs comes from studies of extended sleep in

normal sleepers. Dijk et al. (1991) studied a group of normal sleepers during recovery sleep, starting at 0700 hour, following 24 hours of sleep deprivation. The decline in slow wave activity over the span of the sleep episode was maintained, despite the fact that recovery sleep was occurring on the rising limb of the body temperature curve. Further evidence to support the independence of process “S” from circadian clock mechanisms comes from extended sleep studies (0000-1500 hour) in normal young adult sleepers which demonstrated that while slow wave activity was highest in the first 3 hours of sleep, occasional bouts of SWS were found in the late morning and early afternoon hours (Dijk et al., 1991). Therefore, while declining body temperature was initially associated with the occurrence of slow wave activity, the reappearance of SWS in the afternoon coincided with body temperature values that were even higher than the original pre-sleep temperatures. This proved that the time course of the circadian rhythm of body temperature was able to be dissociated from the time course of slow wave activity.

NREM versus REM Sleep Homeostasis

The preferential rebound of slow wave sleep over REM sleep during recovery periods from sleep deprivation has been documented in many studies (Berger and Oswald, 1962; Bonnet and Rosa; Bonnet and Arand, 1989). The first recovery night after total sleep loss is associated with an increase in SWS over baseline levels; however older subjects may not display the same degree of SWS rebound (Bonnet and Arand, 1989). The second recovery night is associated with a return to baseline levels of SWS and an increased level of REM

sleep has been found in young adults. By the third recovery night, sleep EEG values returned to baseline levels. In the case of REM rebound occurring on the first night of recovery sleep, other sleep EEG values may normalize by the second recovery period.

Studies of selective REM sleep deprivation have been conducted which result in a buildup of “REM sleep pressure” (Borbely, 1982; Brunner et al., 1990). Early studies determined that selective REM sleep deprivation was accomplished by awakening the subject from REM which resulted in more frequent attempts by the subject to enter into REM sleep (Dement, 1960). Subsequent periods of recovery sleep had higher amounts of REM sleep (REM rebound). However, REM sleep rebound was typically evidenced at a time when SWS pressure was lower (i.e., last third of sleep) or was not increased to a level that could be predicted by the number of REM awakenings needed to keep the individual out of REM (Borbely, 1994). The data suggest that REM sleep homeostasis may not be as robust a process as slow wave sleep homeostasis and that any residual slow wave pressure will override the pressure for REM sleep.

At present, there is no clear electrophysiological indicator of REM sleep pressure. Some investigators have viewed the density of rapid eye movements during REM sleep (“REM density”) as an indication of REM sleep pressure that is evidenced with REM sleep rebound, as has been observed in OSAS patients during the initial period of CPAP treatment (Issa and Sullivan, 1986; Aldrich et al., 1989). The actual measurement of REM density refers to the sum of eye movement scores during REM sleep for the entire night (“REM index”, which

is based on a score of 0 to 8 movements per minute of REM) divided by the time spent in REM sleep (Kupfer and Foster, 1975; Gillin et al., 1979). Work by Kupfer and Foster showed an increase in REM density measures and early timing of REM onset in a group of major depressives. Other researchers believe that REM density is not associated with REM pressure, but is inversely related to the propensity to slow wave sleep (Borbely and Wirz-Justice, 1982). According to Borbely and Wirz-Justice, they suggested that process "S" is deficient in depressive patients. Since process "S" is considered to be an inhibitor of REM sleep, depressives with reduced delta activity will display shorter REM latencies, longer first REM periods, and higher REM density measures. Animal models have suggested that the increase in theta activity in REM sleep following a 24-hour period of sleep deprivation could be an indicator for REM sleep rebound and intensity (Borbely, 1994). Regardless, there is general agreement that there is a reciprocal interaction between slow wave pressure buildup and REM pressure (Borbely et al., 1982), and that each state can affect the other (i.e., REM deprivation results in recovery sleep with reduced slow wave activity; curtailment of slow wave sleep results in recovery sleep with reduced amounts of REM).

These findings have important implications for the expected patterns of recovery sleep in OSAS patients. Since many OSAS patients have both severe SWS and REM suppression, based on the model, it would be expected that SWS should demonstrate a significant rebound on the first few nights of treatment, followed by a significant REM rebound the next few nights. However, many patients exhibit selective REM rebound early in the treatment period and much lower levels of SWS during the recovery period. This could be due to a number

of factors, including residual abnormalities of the homeostatic control mechanism regulating body temperature which will be discussed in the next section.

Slow Wave Sleep and Thermoregulation

Slow wave sleep and temperature regulation are thought to be closely related (Berger et al., 1988). The thermoregulatory aspects of SWS have been recognized by many investigators. Work by Gillberg and Akerstedt (1982) has demonstrated that a rapid fall in rectal temperature is associated with amount of slow wave sleep in humans. They suggest that the slope of the decline in the core body temperature, not the absolute level of temperature, was the important factor in determining the course of SWS. Sewitch et al. have shown that artificially driving body temperature down using a mild cold stress in a group of young women resulted in a specific increase in stage 4 sleep and a lengthening of the first NREM-REM cycle. Other studies have shown that body heating during the late afternoon, either by a hot bath technique or exercise, results in a significant drop in rectal temperature following sleep onset which persists for the first few hours of sleep. In addition, this exaggerated drop in temperature is accompanied by a significant increase in stage 4 sleep. However, other investigators (Hauri, 1968, as cited by Sewitch, 1987) have failed to find a compensatory increase in SWS following evening exercise. Sewitch has raised the possibility that Hauri's exercise condition ended too close to the time of the sleep period, which did not allow for appropriate down regulation of body temperature to occur prior to sleep. Sewitch (1987) has suggested that the down-regulation of body temperature at sleep

onset is critical for the presence of SWS and that specific sub-populations of patients (i.e., chronic insomniacs) who do not display a drop in body temperature at sleep onset would also be predicted to have significant SWS deficiencies. This gives further credence to the idea that adjuvant melatonin administration in OSAS patients may play a role in re-instituting down-regulation of body temperature at sleep onset and thereby, facilitates an increase in stage 4 sleep.

Obal (1984) has suggested that a primary mechanism of sleep is to provide an efficient means to conserve energy through a reduction in metabolism and that heat loss during SWS provides this function. There is recent summary of the evidence suggests that SWS is controlled, in part, by thermoregulatory mechanisms, and provides brain and body cooling as a primary homeostatic feedback process (McGinty and Szymusiak, 1990). According to these researchers, SWS can be described as an integrated thermoregulatory cooling process in that sleep onset evokes a reduction in metabolism and heat loss through vasodilation and increased heat dissipation processes. In addition, SWS can be an immediate or delayed response to an increase in brain or whole body temperature. Studies in humans and rats has shown that sleep onset is associated with decreases in body and/or brain temperature and can often be independent of the circadian rhythm of temperature (Gillberg and Akerstedt, 1982; Obal, 1984). Studies which involved cooling of the thermoregulatory center in the preoptic area of the hypothalamus (POAH) in the rat examined temperature regulation during sleep and wake states (Glotzbach and Heller, 1975). They measured the threshold and rate of change of heat production induced by the cooling technique and found a decrease in the slope

of the metabolic response during SWS as compared to when the animal was awake. Therefore, this was an indication that the decrease in body temperature that is associated with SWS is controlled by changes in the sensitivity of hypothalamic thermoregulatory mechanisms. Furthermore, multiple studies involving the POAH (i.e., lesion, electrical stimulation, and neuronal recording) have corroborated the idea that the POAH is implicated in both sleep-onset and tonic-sleep regulation (McGinty and Szymusiak, 1990). These functions are integrated with thermoregulatory mechanisms that further activation of warm-sensitive neurons in the POAH will induce SWS.

Therefore, with respect to OSAS, while a cumulative sleep debt often accrues in patients with this disorder (particular SWS and REM sleep suppression), homeostatic forces which normally would attempt to increase the amount of those sleep stages, are not able to reach their full expression due to the constant disruption of sleep which results from recurrent apneic episodes. The manifestation of SWS and REM rebound observed in the first few nights following successful CPAP treatment (Dowdell and Javaheri, 1992; Series et al., 1992) could be explained by the reestablishment of sleep-wake homeostasis, which eventually results in stable and more regular sleep architecture. Nevertheless, some OSAS patients still maintain lower levels of SWS even after continuous usage of CPAP for at least one month (Gaddy and Doghramji, 1991). There have been a few innovative studies which attempted to directly drive the process of SWS in OSAS patients (prior to CPAP therapy) by using the pharmacological compound gamma-hydroxybutyrate, which promotes SWS (Series et al., 1992) or by passive body heating via a warm bath prior to sleep (Dowdell and

Javaheri, 1992). While gamma-hydroxybutyrate was found to increase SWS, there was no impact on apnea severity. This finding is contradictory to their prediction that by enhancing SWS, the severity of apnea may be reduced, based on the observation that OSA is often less severe in delta sleep (Series et al., 1994). The finding that external warming via bathing did not have a significant effect on sleep architecture was also unexpected since SWS enhancement and reduction of REM sleep has been shown to occur in response to passive heating prior to sleep in normal sleepers (Horne and Reid, 1985; Horne and Shackell, 1987). The authors concluded that further augmentation of an already high pressure for SWS may have had no additional impact on the sleep architecture in the OSAS patients (Dowdell and Javaheri, 1992). However, in keeping with these ideas, it is hypothesized that melatonin administration, in conjunction with ongoing CPAP therapy (to correct the chronic sleep fragmentation), may achieve an effect in OSAS patients through thermoregulatory mechanisms and will result in a return of homeostatic balance between sleep and wake states (i.e., enhanced sleep continuity and increased daytime performance). This may not necessarily result in an increase in the amount of SWS above and beyond the level observed with CPAP treatment, due to the fact that the OSAS patient group has been predisposed to high levels of SWS pressure due to a chronic SWS deficit.

2) Circadian control mechanisms

Human and other mammalian circadian rhythms

Most human behavioral and physiological processes are characterized by a nearly 24-hour day-night cycle. This cyclicity is clearly evident by the alternating patterns of sleep and wakefulness. Other physiological functions that adhere to this cyclic variation include endocrine secretions (i.e., cortisol, melatonin), body temperature regulation, sensory processing, subjective alertness, and cognitive performance (Moore-Ede et al., 1982). Moreover, all mammals studied have displayed daily rhythms that persist in the absence of periodic external/environmental stimuli. Each rhythm has its own characteristic "phase relationship" (relative position in time) with respect to the environmental day-night cycle as well as with respect to each other. This internal counterpoint of rhythmic structure is remarkably stable and consistent for each species. It appears that a major function of the circadian timing system is the internal sequencing of physiologic events and metabolic processes so that interdependent functions are coordinated and that incompatible processes occur at different times (Moore-Ede and Sulzman, 1981).

Under conditions of temporal isolation (absence of environmental time cues), organisms tend to gain or lose time with respect to the environment and are said to be "free-running from the 24-hour clock" (Halberg et al., 1959). Free-running rhythms have a periodicty (the time required for one complete rhythm cycle) which is approximately, but not precisely, 24 hours. Therefore, these near-24-hour cycles have been termed "circadian rhythms" (derived from Latin *circa* meaning "approximately" and *dies* meaning "day"). Furthermore, it appears that the non-24-hour period is necessary to achieve a stable phase relation with the environmental cycle (Pittendrigh and Daan, 1976) and it may permit certain animals to adapt

more readily to seasonal variations in day length.

Many rhythms are manifested as preceding periodic environmental events, such as dawn or dusk. For example, in humans, body temperature begins to rise before the lights are even turned on or the subject's awakening in the morning. Similarly, cortisol secretion peaks in the early morning hours in the last third of the sleep episode and decreases over the course of the day, reaching its lowest level in the late evening (Moore-Ede et al., 1982). Cortisol secretion in humans seems to be closely regulated to the intrinsic circadian pacemaker, since the secretory pattern is minimally effected by changes in the sleep-wake pattern. Studies in animals have shown that food-seeking behaviors (Boulos et al., 1980) and the activity of gastrointestinal enzymes (Suda and Saito, 1979) are activated in anticipation of a regularly timed meal. The anticipation of environmental events that have a nearly 24-hour periodicity enables animals to predict when regularly recurring daily events will take place and to initiate appropriate behavioral and physiologic responses sufficiently in advance of the event that the maximal response is achieved with a minimal time lag. Ultimately, the correct timing of behavioral responding would have a clear advantage for promoting the survival of the individual animal and the species.

One of the first demonstrations of the persistence of circadian rhythms in the absence of environmental time cues comes from work by de Mairan (1729). He observed that the daily periodicity in the movements of the leaves of heliotropic plants persisted in conditions of constant darkness. Furthermore, de Mairan recognized that similar persistent biological

rhythms were related to the sleeping patterns of bedridden patients, which continued to adhere to a 24-hour schedule even when the patients were unaware of day or night. Evidence to support the existence of internally generated (endogenous) rhythms in humans is derived from temporal isolation experiments conducted in caves (Siffre, 1964) or highly controlled laboratory settings (Aschoff, 1965, Wever, 1979), where persistent daily sleep-cycles were found despite the absence of a clock, other environmental time cues, and a 24-hour light-dark (LD) cycle. However, spontaneous daily sleep onsets became separated by about 25 hours instead of 24 hours. Therefore, the "free-running" human goes to sleep a little later each perceived day. The actual period of the free-running sleep-wake cycle varies among species, ranging from 23 hours to 26 hours and can be modified by factors such as ambient light intensity and hormonal factors. The fact that certain species have a periodicity of less than 24 hours, and others have periods of greater than 24 hours under constant environmental conditions, is a general property of circadian mechanisms. Once again, the fact that the period is not exactly 24 hours provides evidence for endogenous mediation of circadian rhythms and not passive responses to the environmental solar day. Further support for the internal generation of circadian rhythms includes the demonstration that rhythms can be modified by selective breeding (Bunning, 1973), gene mutations (Konopka, 1980) and that they develop normally in successive generations of organisms kept in constant conditions of light or dark (Aschoff, 1960). Therefore, circadian rhythms are considered innate, rather than learned, phenomena.

In addition, temporal isolation experiments (Wever, 1979) demonstrated that, on

occasion, internal desynchronization occurred between the rest-activity cycle and the rhythm of body temperature, so that the rest-activity cycle may display a period of about 33 hours, whereas the body-temperature rhythm had a 24.5-hour period. When internal desynchronization of circadian rhythms develops in a subject, the various physiologic and behavioral indices cluster into two groups: those parameters which track with the rest-activity cycle or those which follow the body-temperature rhythm (Czeisler et al, 1980. Moore-Ede, 1983). Another phenomenon which may occur under constant conditions is referred to as "splitting" of the free-running activity rhythms. This has been observed in hamsters maintained for many weeks in constant light conditions (Davis and Menaker, 1980). During splitting, the daily activity rhythm dissociates into two components that free-run with different periodicities before stabilizing 180 degrees out of phase.

In summary, evidence supporting the endogenous nature of any observed rhythm include the following: 1) it persists in the absence of all rhythmic influences likely to affect it (i.e., conditions of temporal isolation); 2) it retains a roughly 24-hour period when a different period is adopted for the rhythm of habit (i.e., feeding or sleeping schedules) and environment; 3) it does not immediately change its phase after an abrupt shift in the phase of the rhythm of habit and environment; 4) if it has become entrained to a new period or phase (as in the case of #2 or #3), it does not immediately revert to its former period or phase when the former habits and environment are restored; 5) when environmental cues are sufficiently excluded (as in the instance of #1), its period drifts somewhat away from 24 hours.

Mathematical Modeling of Circadian Rhythms

Pittendrigh and Bruce (1957) were the first to propose that circadian rhythms be modeled after physical oscillators. This model provided a pre-existing set of terminology and mathematics for elucidating the properties of biological rhythms and a theoretical framework for interpreting cyclic phenomena. Several groups have since attempted to mathematically model the human circadian timing system (Bunning, 1973; Strogatz, 1986). There has been controversy in the literature as to whether the system is best represented by mathematical models consisting of one or two oscillators. In fact, nearly all of the proposed models consist of two oscillatory processes. This is necessary in order for the models to simulate the data from human studies (data from free-running studies reveal the presence of two periodic processes underlying the human circadian timing system).

The principal components of an oscillatory system are its "period", "phase", "amplitude", and "phase resetting responsiveness". If the quantity studied is a continuous variable, these oscillations may ideally conform to the shape of a sine (or cosine) curve, which is the conventional shape for mathematical treatment. One way to view the data to assess the shape of the waveform, its resemblance to a sine function, and the extent of variation is to construct a simple graph (a time plot), referred to as a "chronogram" (Reinberg and Smolensky, 1990). Using a chronogram, data on a given variable gathered over a specific time period under specific experimental conditions can be examined for temporal features. If the data collected are fairly sinusoidal in nature, one of the standard techniques employed

to ascertain the underlying circadian rhythm is to curve fit the data with a cosinor technique using the method of least squares (Halberg et al., 1972). Using this analysis, circadian parameters (i.e., period, phase, amplitude) can be estimated. However, observed biological rhythms often deviate from this form in general shape (i.e., non-sinusoidal) or in symmetry, which usually results in the peak and trough not being equidistantly spaced. In fact, the typical core body temperature rhythm has been found to have a more square-wave shape than a sinusoidal one, which is the result of the circadian pattern of temperature being obscured by variable patterns of activity (Gander et al., 1985). [This "masking" effect of rest-activity patterns on the assessment of circadian parameters will be discussed in more detail in a later section on chronobiologic techniques]. Therefore, other techniques have been instituted which do not impose the restrictions on the shape (sinusoidal) or symmetry of the data being analyzed (as does the cosinor technique). One such technique is using a polynomial regression algorithm to curve fit a set of data using the method of least squares. Moreover, it has been demonstrated that the polynomial regression technique more closely approximates visual analysis of body temperature data and accounts for more variance in the data than the cosinor technique (Broughton, 1993). The polynomial regression approach has also been applied to fit ultradian extended sleep and circadian temperature data (Campbell and Broughton, 1992).

Regardless of the technique employed, certain circadian measures can be derived. In general, a circadian "cycle" is defined as the shortest part of a rhythm which repeats itself indefinitely. The time occupied by a cycle is the "period", or tau (τ). The period refers to

the temporal duration between consecutive occurrences of any given point or "phase" (Φ) of the cycle of the observed rhythm. The period of a circadian cycle is usually measured as the time between consecutive onsets of physiologic or behavioral activity. The periodicity of a cycle can also simply be determined by measuring the temporal distance from crest to crest, or from trough to trough. The "amplitude" (A) is defined as the distance from the mean value to either extreme, the peak or the trough. However, if a rhythm is not strictly sinusoidal, the more convenient definition of amplitude as being the distance from trough to peak is usually adopted. The "acrophase" of the rhythm is the time of the peak on the best-fitting sine curve. When such a curve has not been fitted, the word "peak" is used. The "mesor" (M) represents the 24-hour adjusted mean and is defined as the level around which rhythmic change occurs.

When all physiologic and behavioral parameters are oscillating in synchrony, the free-running period of the human circadian timing system averages slightly longer than 24 hours. Classical free-run studies, where subjects could select their exposure to light and darkness, resulted in observed circadian periods of approximately 25 hours (Wever, 1979). More recent studies (Klerman et al., 1996), which implement controlled lighting during wake episodes, have shown that the observed period is closer to 24.3 hours. Nevertheless, the observed period appears to be a compromise between the intrinsic period of the endogenous circadian oscillator and that of the periodic patterns of behavior (i.e., rest-activity cycle). Kronauer and colleagues have termed the period of the rhythms of internally synchronized subjects living in temporal isolation the "compromise period" of the synchronized

free-running system (Czeisler et al., 1986). Since the free-running period in humans tends to be 25 hours, there must be a resetting mechanism to keep the body externally synchronized or "entrained" with the earth's 24-hour environmental and social schedules. Therefore, for entrainment to operate, the circadian system must be receptive to external time cues, referred to as "zeitgebers" (from German meaning "time giver"). The zeitgeber can also be defined by its period (T) (i.e., photoperiod duration) and its phase (ϕ). The relationship between the rhythm cycle and the zeitgeber cycle is referred to as a "phase angle difference" (ψ). The light-dark cycle is a potent zeitgeber in all mammalian species, both nocturnal and diurnal. In mammals, entrainment of circadian rhythms by light-dark cycles appears to be mediated via the retinohypothalamic tract. The manipulation of the light-dark cycle is not only a valuable technique in assessing the phase resetting responsiveness of a rhythm, but also has clinical application in the treatment of certain biologic rhythm disorders (i.e., delayed or advanced sleep phase syndrome).

In the literature, the oscillatory processes are referred to by many different terms. In order to clarify these distinctions, the oscillatory processes which reflects the output of an "endogenous oscillator", has also been called the "X oscillator" (Kronauer, 1987), the "stronger (Type I) oscillator" (Wever, 1979), the "C process" (for circadian) (Borbely, 1982) and the "endogenous rhythm" (Mills, 1964). This endogenous circadian oscillator is most commonly referred to as a "pacemaker". The pacemaker has a remarkably stable intrinsic period that dominates the compromise period of the synchronized free-running system and drives the endogenous components of the daily cycles in a variety of physiologic functions

including core body temperature, cortisol release, REM sleep propensity, urinary potassium excretion, alertness, and cognitive and psychomotor performance (Moore-Ede et al., 1982).

The other oscillatory process consists of the behavioral rest-activity cycle and the many physiologic responses that are a direct and passive result of changes in posture, meal-timing, and sleep-wake state that accompany the rest-activity cycle. The second oscillatory process has been called the "Y oscillator" (Kronauer, 1987), the "weaker (Type II) oscillator" (Wever, 1979), the "S process" (for sleep dependent) (Borbely, 1982), and the "exogenous rhythm" (Mills, 1964). The rest-activity cycle is a periodic process whose timing is ultimately driven by the brain. This process, however, has a fairly labile period, with marked cycle-to-cycle deviations, a broad range of entrainment, and a weak influence on the compromise period of the free-running synchronized system (Wever, 1979). The physiologic variables that track with the rest-activity cycle include slow-wave sleep and growth hormone release.

Under normal (entrained) conditions, physiologic systems respond to signals from both pacemakers. The net result of signals from both oscillators are the observed circadian patterns for each variable. Nevertheless, these two oscillatory processes can dissociate from each during constant conditions (forced or spontaneous desynchronization), and the physiologic variables tied to each system assume the respective period for that single oscillator (i.e., slow-wave sleep will be tied to the period of the rest-activity cycle; REM sleep will assume the period of the body temperature cycle). Another possibility is that the

observed rhythm (i.e., activity) may exhibit the phenomenon of splitting. This finding is interpreted to mean that the activity rhythm is generated by at least two primary oscillators with two stable coupling modes 180 degrees apart. This would suggest that the primary pacemaker is itself composed of at least two dissociable oscillatory units.

Identification of the pacemakers

There is considerable evidence to support the existence of a circadian pacemaker in the hypothalamus of mammals. Richter (1965) provided the first body of evidence demonstrating that specific neuroanatomic sites act as circadian pacemakers with a series of lesion and dissection experiments in rats. Richter used the free-running activity rhythm of blinded rats as his marker of the circadian system. Circadian rhythms were found to persist after removal of various endocrine organs (i.e., adrenal, pituitary, thyroid or pineal glands) or focal destruction of many brain areas but were abolished by large lesions placed ventromedially in the hypothalamus. The relevant cell group within the hypothalamus was later identified through a series of experiments which were designed to locate the visual pathways that mediate entrainment. Using the technique of autoradiography, Moore and Lenn (1972) injected tritiated amino acids into the vitreous humor of the eye which was taken up by the retinal ganglion cells and then transported through the neural projections from the retina. A unique retinal projection exiting the optic chiasm and terminating in the suprachiasmatic nuclei (SCN), located ventromedially in the hypothalamus immediately above the chiasm, was identified through this procedure. Soon after this discovery, Moore

and Eichler (1972) demonstrated that destruction of the SCN resulted in the apparent loss of the circadian rhythm of adrenal corticosterone in rats, and Stephan and Zucker (1972) showed a similar loss of circadian rhythmicity in drinking and locomotor activity in hamsters.

Nevertheless, lesion studies alone cannot determine the presence of a "master circadian clock" within the SCN. However, additional evidence from a variety of sources indicates that the major function of the SCN is the coordination of circadian rhythms (Moore, 1979). The firing rate of neurons in the SCN exhibits a circadian pattern in rodents whether they are maintained under light-dark cycles or constant environmental conditions. This rhythmic neural activity appears to be an inherent property of suprachiasmatic neurons, since it persists after isolation of the SCN from adjacent hypothalamic tissue by Halasz knife cuts (Inouye and Kawamura, 1979). Studies have also shown persisting circadian rhythms of neural activity in vitro in slices of hypothalamic tissue containing the SCN (Groos and Meijer, 1985). In addition, studies of the metabolic uptake of SCN neurons in vivo using the 2-deoxyglucose method indicate that, unlike other hypothalamic tissue, the SCN neurons have a circadian pattern of glucose uptake in rodents housed under either cyclic or constant lighting conditions (Schwartz et al., 1980). Furthermore, stimulation of SCN neurons, either electrically (Rusak and Groos, 1982) or chemically (Zatz and Brownstein, 1979), can induce phase shifts of circadian rhythms that are similar to the phase shifts produced by light pulses. Perhaps the strongest evidence that the SCN function as a circadian pacemaker is provided by studies which showed the recovery of circadian rhythms in arrhythmic rodents with SCN

lesions by intracerebral transplants of fetal neural tissue containing the SCN (DeCoursey and Buggy, 1986).

As stated previously, there appear to be two pacemakers which control the organization of the circadian timing system in humans. The question still remains whether the SCN contains one or both pacemakers. In order to address this question, the SCN were destroyed in squirrel monkeys. The behavioral rhythms of activity, feeding, and drinking were profoundly disrupted, but the body-temperature rhythm was found to persist (Fuller et al., 1981). This finding suggested that the endogenous pacemaker may be located outside the SCN, whereas the pacemaker controlling rest-activity rhythms is within it. While it has been argued that some suprachiasmatic tissue may have remained, subsequent neuroanatomic studies have indicated that an area much larger than that of the SCN, including the preoptic area and lateral hypothalamus, was destroyed in these experiments. Reppert and colleagues (1981) have also found a persistent circadian rhythm of cortisol concentration (an endogenously driven variable) in cerebrospinal fluid in rhesus monkeys after total ablation of the SCN. Therefore, gaining further support for the conclusion that the endogenous pacemaker lies outside the region of the SCN, although its exact location has not yet been specified.

Alternative explanations of SCN functioning include the existence of multiple oscillators outside the SCN that are normally synchronized by efferent signals from the dominant pacemaker in the SCN. In the absence of SCN or another external synchronizing agent, the

oscillators fail to couple or achieve only weak and unstable coupling, resulting in ultradian (shorter than 24 hour) or arrhythmic activity patterns (Rusak and Groos, 1982). While these oscillators are mainly considered to be neural, other sources to be considered include isolated visceral organs (i.e., intestines, heart, kidney, liver and adrenal gland) which have displayed sustained circadian rhythms (Moore-Ede et al., 1982). Another view is that the SCN may function as a gating pacemaker that prevents the overt expression of a population of "bout oscillators" (multiple free-running circadian activity components) during phases of rest. Therefore, destruction of the SCN can be interpreted as eliminating this restrictive gating process, so that subordinate oscillators achieve behavioral expression resulting in an ultradian or arrhythmic activity pattern.

Anatomic basis of entrainment

The most important route by which environmental time cues reach the SCN are the optic pathways. Within these pathways, the major component seems to be the retinohypothalamic tract (RHT). The RHT is a monosynaptic direct path to the SCN from specialized photoreceptors in the retina that are uniquely suited to sensing the ambient level of light. Photic stimulation of the retina excites or inhibits neural activity in about one third of spontaneously active SCN neurons recorded in anesthetized rats and hamsters (Groos and Mason, 1980). The neurons of the SCN code for the level of light intensity in a monotonic fashion and appear to transduce the day-night cycle, with its gradual transitions of light at dusk and dawn, into a neural square wave signal. As a result of the small operating range

between threshold and saturation point, SCN responding is well protected from random changes in luminence due to weather.

The functional role of the RHT has been outlined partly by a series of ablation experiments in animals (Rusak, 1977). If the optic nerves in rats are cut bilaterally (cutting the RHT as well), the animal will be blinded, entrainment to light-dark cycles will no longer be possible, and the circadian rhythms will free-run in both a light-dark cycle and constant darkness. When the pacemaker (SCN) is destroyed, leaving visual connections intact, some persistent clustering of activity is seen in the dark phase of the light-dark cycle. This direct effect of light on activity is known as "masking" (to be discussed in detail later on). However, in constant darkness there is virtually complete loss of circadian rhythmicity. When the optic tracts are severed behind the optic chiasm, entrainment to the light-dark cycle persists with an abnormally advanced phase relation (animal is behaviorally blind). When the animal is placed in constant darkness, the rhythm free-runs normally, demonstrating that the pacemaker is still intact.

In addition to the RHT, other neural pathways may play some role in entrainment to light-dark cycles. Autoradiographic techniques have shown projections from the ventral portion of the lateral geniculate nucleus (intrageniculate leaflet - IGL) back to the SCN (Ribak and Peters, 1975). A clue to the geniculohypothalamic tract (GHT) connection is suggested by studies in which the accessory and posterior optic tracts are severed, cutting off the lateral geniculate nuclei from their visual stimuli, while leaving the direct retinal

projections to the SCN. As stated previously, entrainment to the light-dark cycle continues, but the activity rhythm is abnormally advanced with respect to the light-dark cycle (phase shifts of the entraining cycle are tracked by the activity rhythm but at an abnormally slow rate). Furthermore, electrical stimulation of the IGL in hamsters produces phase-dependent phase shifts of free-running rhythms similar to those produced by dark pulses presented during constant light (Meijer et al., 1984). Neuropeptide Y, which is released from GHT terminals in the SCN, has been found to activate SCN neurons recorded in vitro (Harrington et al., 1987), and, when infused through cannulas implanted near the SCN, will mimic the phase-shifting effects of dark pulses in hamsters (Albers and Ferris, 1984).

At present, there is insufficient evidence to definitively map out the routes of communication between the SCN and other oscillators. Neural isolation of the SCN with knife cuts has been shown to eliminate circadian rhythmicity, indicating that timing signals from the SCN are conveyed by neural efferent fibers. Axonal projections from the SCN are primarily concentrated within the hypothalamus, although other projections can be found throughout the brain (Stephan et al., 1981). Projections to the paraventricular hypothalamus seem to be critical for maintaining the circadian rhythm of pineal N-acetyltransferase (NAT) (Klein et al., 1983). Nevertheless, the functional significance of other specific projections is largely unexplored.

The neural pathways mediating the entrainment of nonphotic stimuli on circadian rhythms are not clearly established. While it has been shown that daily feeding schedules

are a potent zeitgeber for circadian rhythms of anticipatory behavior in rodents, it is not clear which of the sensory stimuli associated with feeding act as the zeitgeber. Restriction of water and salt do not entrain anticipatory rhythms (Mistleberger and Rechtschaffen, 1985, Rosenwasser et al., 1985), suggesting that the critical stimuli are related to some other ingested nutrients. Restricted access to palatable food entrains anticipatory rhythms only if the food contains nutrients (Mistleberger and Rusak, 1987). Although human evidence is lacking, the identification of critical nutrients that act as zeitgebers in animals may have further implications for the role of dietary factors in the manipulation of human circadian rhythms.

There is also increasing evidence that hormones have an influence on the regulation of circadian rhythms that may be due to a modulating effect on coupling relations between oscillators and between oscillators and other driven processes. Although studies have shown that endocrine glands and their hormones are not essential for circadian timekeeping (Richter, 1967), other evidence is supporting subtle hormonal interactions with the organization of circadian rhythms. Daily running activity in female rodents has been shown to vary with the phase of the estrous cycle. During the day of proestrus, when estradiol secretion is maximal, running activity starts earlier and is more intense (Zucker, 1979). If the animal is ovariectomized, this effect is abolished and activity onset is delayed during photic entrainment. Furthermore, chronic estradiol exposure produces an even earlier onset of running activity and a shorter free-running period in constant conditions (Morin et al., 1977). Shortening of the circadian period in response to estradiol treatment is not observed

in male or neonatally androgenized female rodents (Zucker et al., 1980). Therefore, estradiol appears to regulate the timing of activity onset in female rodents by modulating the periodicity of a sexually differentiated circadian system.

Steroid hormones have also been found to modulate the circadian timing system in male rodents. Chronic testosterone treatment in male mice leads to a shortening of the period of free-running rhythms and castration results in a lengthening of the period (Daan et al., 1975). Castration has also been shown to increase diurnal and crepuscular (dawn-dusk) activity while decreasing nocturnal activity. Conversely, testosterone implants reduce crepuscular activity and increase nocturnal activity. Similar phase-modulating effects of testosterone have been observed in starlings (Gwinner, 1974). In the starling, testosterone administration can cause splitting of activity rhythms. Thus, gonadal hormones may control coupling relations between circadian oscillators.

Other hormones have been identified as having prominent circadian effects. In humans, the circadian variation of the adrenal corticosteroids has been extensively studied. Pincus (1943) was the first to demonstrate a daily pattern of 17-ketosteroid excretion in the urine of healthy adults, with significantly higher amounts present in the morning and lower amounts in the evening. Once 17-hydroxycorticosteroids could be directly measured, a circadian rhythm of plasma corticosteroids was soon recognized (Migeon et al., 1956). Derived from 24-hour blood sampling in a group of healthy subjects, the lowest mean plasma corticosteroid values were observed between 22:00 and 2:00; at 4:00 values began to increase

with highest mean values at 6:00. With the advent of radioimmunoassay techniques, the specific corticosteroids such as cortisol and corticosterone could be directly assayed. In humans, there are prominent circadian rhythms of plasma cortisol concentration. Although the levels of corticosterone are substantially lower, the rhythm is similarly timed, so that a 13:1 ratio of cortisol to corticosterone is maintained throughout each 24 hour period (Peterson, 1957). In rats, corticosterone is the predominant adrenal steroid. Since rats are nocturnal, the phase relationship with the light-dark cycle is 180 degrees out of phase with humans, so that peak levels are found at the end of the light portion of the daily photoperiod. Therefore, in all species the peak of the corticosteroid rhythm occurs just before or at the onset of activity.

Another hormone which has gained widespread attention is melatonin. Melatonin is a methoxyindole (N-acetyl-5-methoxytryptamine) secreted primarily by the pineal gland and secondarily, by the retina. Under normal environmental conditions it is secreted at night (Arendt, 1985). The (usual) high amplitude rhythm of secretion is endogenously mediated, is generated by the SCN, and is entrained by the light-dark cycle (Moore and Klein, 1974). Light serves to suppress and entrain melatonin production (Reppert et al., 1981) and there are species differences as to whether dawn or dusk is critical for this entrainment. The final common pathway controlling pineal function (as far as indoleamine secretion is concerned) is sympathetic innervation from the superior cervical ganglion (SCG). Noradrenaline released at the pinealocyte stimulates melatonin production with both beta-1 and alpha-1 receptor components involved in the mechanism. There is considerable evidence to support

that melatonin secretion is mediated by the light-dark cycle. Early work in rodents (Tamarkin et al., 1980) and sheep (Rollag and Niswender, 1976) demonstrated that suppression and entrainment of melatonin secretion was possible with normal artificial lighting (<500 lux). However, in early studies in humans, low-level room lighting did not prove to be sufficient to suppress melatonin secretion at night (Arendt, 1978). Lewy et al. (1980) observed that either natural sunlight exposure during a phase-shifted night or bright artificial light (>2500 lux) during a normal night would suppress melatonin secretion in humans. More recent studies have demonstrated that even lower levels of illumination (1260 lux) are sufficient to elicit phase shifts in the endogenous rhythm of core body temperature in humans (Boivin et al., 1994). Since circadian rhythms of temperature, urinary volume, subjective alertness and performance (mental arithmetic) remained synchronized throughout the experimental lighting condition, it was suggested that light exerts an effect through direct biological action on a central pacemaker (Boivin et al., 1994). Reiter et al. (1983) proposed that the lighting history of an animal may be an essential determinant of its sensitivity to light at night in terms of the capacity to suppress melatonin. In addition, constant routine protocols in human subjects have revealed that the characteristic circadian patterns for melatonin secretion and body temperature are inversely related (i.e., increasing melatonin levels and descending body temperature values tend to occur at a similar time during the evening hours) (Akerstedt et al., 1979).

Clinical significance

There are several clinically significant implications of these results:

- 1) The rest-activity cycle, because of its wide range of entrainment and presumed behavioral influence, can be easily manipulated or overridden by a number of factors. For example, the voluntary decision to stay awake all night for work or entrainment is made possible by the lability of this process, in contrast to the oscillator controlling the endogenous circadian temperature cycle.
- 2) Because the intrinsic period of the endogenous circadian oscillator is the dominant influence on the outputs of the human circadian timing system, perturbations in the intrinsic period of that oscillator can lead to abnormalities in the phase relationship between the many neurohumoral and physiologic functions under circadian control.
- 3) The pathophysiological mechanism underlying circadian rhythm disorders is presumed to be tied to the endogenous circadian pacemakers, their coupling processes, or the synchronization of the separate biological rhythms (Roehrs and Roth, 1994). While the intrinsic period can now be reliably assessed using a forced desynchrony protocol (Czeisler et al., 1986), this requires a 3-4 week study. However, abnormalities of either the intrinsic period or the phase resetting responsiveness may result in abnormal entrained endogenous circadian phase or amplitude (Moore-Ede et al., 1982). Therefore, the status of the circadian timing system can be determined in a more practical fashion from a single assessment of the endogenous circadian phase and amplitude after 2 to 3 weeks of stable entrainment to a 24-hour day.

Chronobiologic techniques used to assess endogenous circadian components

In order to diagnose circadian rhythm disturbances or assess the response to treatments of circadian dysfunction, it is necessary to assess the output of the endogenous circadian pacemaker, as distinct from the physiologic responses evoked by the rest-activity cycle. Physiologic measures thought to reflect the output of the endogenous circadian pacemaker include urinary volume and electrolyte excretion, plasma cortisol levels and urinary free cortisol excretion, computation speed, subjective alertness, REM sleep propensity, and core body temperature (Wever, 1979; Czeisler et al., 1980).

Since overt circadian rhythms are considered to be a mixture of endogenous components induced by the oscillator(s) and exogenous components resulting from a direct effect of the environment (i.e., light, darkness), it was deemed necessary to try remove as many extraneous environmental influences as possible. As described previously, many of the early studies in humans employed temporal isolation which took place over a period of several weeks (Wever, 1979). This process resulted in the internal desynchronization of rhythms which permitted the assessment of certain endogenous circadian components. However, there were certain experimental conditions that continued to obscure the real zeitgeber mechanism that are referred to as "masking conditions" (Aschoff, 1960).

Minors and Waterhouse (1989) categorized various types of rhythm-disturbing ("masking") influences. Disturbing influences from the environment were described as "Type 1" if a direct effect of the normal environment was being considered (i.e., suppression of melatonin secretion by light). "Type 2a" masking referred to that produced in the

experimental plant or animal in response to behavioral changes produced by normal environmental changes. "Type 2b" was masking produced by changes in behavior that occur in response to abnormal changes of the environment (i.e., diurnal feeding in nocturnal animals imposed by an environment in which light alternated with absolute, not natural, darkness). "Type 3" masking was introduced to stress the fact that between an oscillator and the overt, measured rhythm that is controlled by the oscillator, was an output pathway. Any of the various stages along the pathway could be modified for example, by behavioral changes.

Since both masking and entraining stimuli can originate externally and both alter the expression of circadian rhythms, it became necessary to distinguish the two processes. Aschoff (1988) proposed the following set of rules to clarify the two processes: 1) a circadian rhythm can be masked by an environmental signal to which the organism is sensitive, independent of whether or not entrainment might be produced by that signal; 2) masking signals affect the overt rhythm directly; they do not work via the pacemaker but may reach it simultaneously; 3) masking lasts for the duration of the masking signal (tonic response); a masking effect cannot lead to the overt rhythm anticipating the masking signal.

Czeisler and colleagues (1985) have proposed the use of a constant routine technique for the assessment of endogenous rhythms in order to eliminate many masking influences. Human subjects are studied in a constant environment and maintain a constant life-style (i.e., subjects are kept awake, sedentary, semi-recumbent, taking regular snacks). During this

time, body temperature is continuously monitored (either using a rectal thermistor probe, oral thermometer, or tympanic thermistor) in order to unmask the trough (nadir) of the temperature cycle, which is used to estimate the endogenous circadian phase. While maintaining a constant environment is technically feasible, many objections have been raised regarding this procedure (Rietveld et al., 1993). First, the constant routine procedure is limited in its application since it is only suitable for humans, due to the fact that only in humans can activities be constantly maintained. Second, objections have been raised with the protocol itself. For instance, if sleep is not permitted, then the accumulation of sleep loss will certainly affect rhythms in mental performance and might also affect other rhythms (i.e., body temperature). In addition, more social input will be required from the experimenters to keep a subject awake during his/her peak of tiredness. This introduces the problem of masking. In particular, the unusual environment associated with the constant routine protocol may lead to changes in behavior, which may be regarded as Type 2b masking. In addition, frequent food intake can present a problem due to the fact that it can produce nausea and discomfort in some subjects, especially during the hours when the subject is normally asleep. This feeling of discomfort is possibly related to poorer absorption from the gut during these hours. Constant intravenous glucose infusion can overcome the problem of gut function (Van Cauter et al., 1991), but it does not ensure that the plasma concentration of glucose is constant. Furthermore, if urinary constituents (i.e., sodium, potassium, chloride) are being used as markers of the internal oscillatory system, similar problems are raised with regard to water and electrolyte intake. Therefore, while constant routines control for Type 1 and Type 2a masking only, they introduce another form

of masking - Type 2b masking.

Another technique for the assessment of endogenous circadian rhythms utilizes the onset of melatonin production under dim light conditions as a "marker rhythm" (Lewy et al., 1980). This is referred to as the "Dim Light Melatonin Onset" (DLMO) testing procedure. As stated previously, melatonin is secreted at high levels at night and light serves both to suppress and entrain the rhythm of its secretion. For this reason, subjects are kept in dim light, in a semi-recumbent position after 16:00 hours and frequent blood samples are drawn for several hours thereafter. The time of the increase in melatonin levels above their low daytime basal values is an indication of when the pineal gland receives its nightly sympathetic stimulation. Stress and acute changes in the rest-activity cycle do not seem to affect levels of melatonin. Therefore, like the constant routine, the DLMO avoids Type 1 and Type 2a masking effects. Melatonin onset acts as a "marker" for the endogenous phase of the secretory rhythm and provides an index of the phase resetting responsiveness of the system (i.e., marker for phase-shifting capability in response to bright light).

Nevertheless, with both the constant routine and DLMO techniques, the output from the internal oscillator has not been directly measured, but rather, the output has been inferred through the use of "marker rhythms". Possibly, the lack of parallelism between the actual oscillator and the marker rhythm led to the concept of Type 3 masking. In general, when marker rhythms are considered, assessing which is best is difficult because it is impacted on by factors of cost, convenience, and the subject's acceptability of the method. Furthermore,

it would seem fair to index the reliability of a marker rhythm based upon its daily variability in phase and amplitude. Even when day-to-day variability in a marker is considered, it is not clear how much of this fluctuation can be accounted for by methodological error or to the properties of the internal oscillator or those of its output pathways (Minors and Waterhouse, 1992).

Another area of interpretive difficulty stems from the use of other hormonal secretory rhythms (i.e., cortisol, ACTH) as marker rhythms. While the concentration in plasma or at some other site may provide convenience for measurement, it is not the best indicator of clock-mediated secretory activity. This is due to the fact that plasma concentration derives from a combination of pulsatile release and exponential removal processes. Velduis and Johnson (1988) has proposed a method to distinguish these two components, allowing for a focus on the timing of secretory pulses.

From the chronobiologic point of view, masking is seen as a dynamic process of the organism whereby the reflex response to a disturbance (i.e., environmental change) is not constant during the course of 24 hours. This necessitates timed controls or periodic sampling over the 24 hours by the organism. While the role of masking influences are not agreed upon by all scientists, an interpretation of masking based upon principles of homeostasis has been proposed by Moore-Ede (1986). One of the ongoing processes of an organism is referred to as "reactive homeostasis". This is defined as deviations of a variable from its set point that is induced by the environment, by behavioral changes, or from within the body, that initiate

reflex changes that correct the error by negative feedback loops. The other process is "predictive homeostasis" which refers to the mechanism by which the internal timing system of the organism can produce circadian changes in the set point of the variable to be changed in advance of, and in preparation for, errors likely to be induced (directly or indirectly) by the environment. In order for an organism to be fully integrated into its rhythmic environment, it is necessary for these two types of processes to act in concert.

An organism is equally disadvantaged if it does not possess an internal timing system that responds to the rhythms of its surrounding environment, but also, if it does not have the ability to temporarily overrule this timing system in response to environmental (masking) influences. According to this theory, when the integration of these two processes occur, those effects directly stemming from the environment increase the amplitude of the rhythms produced by the internal timing system and help stabilize their overt phase. Moreover, either process alone is less effective than the combination of the two. Therefore, while masking can represent an inconvenience when one is trying to measure marker rhythms to assess the endogenous oscillator, in the broader sense, masking may be seen as a process which enables the organism to act immediately and appropriately to sudden changes in the environment.

Clinical Application of Chronobiologic Techniques

The manipulation of endogenous circadian rhythms via light or melatonin administration are based on the principle that circadian pacemakers display a varying responsiveness to

environmental time cues, depending on the circadian time of day (Pittendrigh, 1960). The predicted phase-shifting effect is based on a hypothetical "phase response curve" (PRC). Studies in mice (Pittendrigh and Daan, 1976) have revealed that a light pulse falling early in the animal's subjective night caused a phase delay of the circadian system (i.e., transient lengthening of the cycle). If the same light pulse fell late in the subjective night, it caused a phase advance. However, during the middle of the day, light pulses caused no phase shift at all ("dead zone"). Therefore, the magnitude of the phase shift that is caused by a light pulse of a given intensity and duration depends on the exact timing of that light pulse. In most species, this type of entrainment can be achieved by a single pulse of light every 24 hours, but only when the light pulse falls at the appropriate position on the PRC to effect a shift that bridges the gap between the organism's intrinsic period and the 24-hour day.

Similar studies have been conducted in humans with bright light exposure. Using core body temperature as a marker rhythm, Kronauer (1987) has developed a model which suggests that bright light administration during the daytime in an individual entrained to a normal 24-hour day would have only a modest effect on steady state entrained phase, but would potentially have a robust amplitude enhancing effect. The effect of this light exposure on phase would depend on whether the light was delivered during the morning or evening, inducing a phase advance or phase delay, respectively. Nevertheless, bright light at both times of day has an amplitude enhancing effect. Moreover, Lewy et al. (1983) have proposed a hypothetical PRC for the response of the human melatonin rhythm to bright light pulses. Similar to Kronauer's findings, early morning bright light exposure phase advanced

the melatonin secretory rhythm; evening light phase delayed the rhythm. In addition, exogenous melatonin administration in the late afternoon in humans can also cause a phase-advance of the melatonin rhythm, which suggests a possible direct effect on the endogenous oscillatory system in the SCN. One explanation of this finding is that melatonin administration during the late afternoon is interpreted as a "dark pulse" by the organism and the observed phase advance of the endogenous rhythm is consistent with the known effects of timed dark pulses in animals (Wright et al., 1986). Hence, melatonin may be considered a circadian zeitgeber for "darkness".

The principle of the phase response curve is essential to the understanding of circadian abnormalities and the limits within which humans (and other animals) can adapt to imposed environmental light-dark cycles. In many species, there is a "limited range of entrainment" which defines the maximal shift of the endogenous free-running period that can be achieved in one day. For instance, if the maximal phase delay observed in an organism is approximately two hours, and the maximal phase advance is also about two hours, the maximal length for which the circadian system could be reset in one day is plus or minus two hours. In synchronized human subjects, the typical range of entrainment is about 23.5 to 26.5 hours which easily accommodates normal synchronization to the 24-hour period of the day-night cycle, but allows in any one cycle a phase advance with respect to environmental time of only an additional half hour, while permitting a phase delay of 2.5 hours. This may account for the more rapid adaptation for most people after westbound travel (which requires a phase delay) than after eastbound travel (which requires a phase advance) (Arendt et al.,

1986). If the environmental phase shift is larger than the maximum achievable according to the PRC, it will take several days to resynchronize the circadian system. During such a process of readjustment, the endogenous circadian rhythms of sleep, alertness, mood, gastrointestinal function are not synchronized with the new environmental time and this one of the primary contribution to the symptoms collectively known as "jet lag" occur.

Circadian Modulation of the Sleep/Wake Cycle

It has been demonstrated that the circadian rhythm of core body temperature tends to act as a synchronizing force on the sleep-wake cycle (Aschoff, 1965). In non-complaining ("normal") sleepers, stable phase relationships are found between the physiological circadian patterns of body temperature, hormone production, and brain metabolism, that are organized in synchrony with sleep-wake patterns and the light-dark cycle (Moore-Ede et al., 1982; Richardson et al., 1982). Moreover, intrinsic circadian mechanisms that control body temperature also have an impact on the infrastructure of sleep across the night. The preferential distribution of REM sleep toward the latter part of the night in normal human adults is thought to be linked to a circadian oscillator and can be tied to the oscillation of body temperature (Czeisler et al., 1980; Zulley, 1980). Unlike REM sleep, the preferential distribution of slow wave sleep at the beginning of the night is thought to be linked to sleep initiation, the length of prior wakefulness, and the time course of sleep (Weitzman et al. 1980; Borbely, 1982) and is independent of circadian processes (Dijk et al., 1991).

However, when an individual's intrinsic circadian sleep-wake pattern is no longer in phase with the conventional (environmental) sleep-wake schedule, misalignment between the phases of various biological rhythms (i.e., core body temperature, hormone production) and the sleep-wake cycle tends to occur. A persistent pattern of circadian rhythm misalignment is often observed in the case of delayed sleep phase syndrome (Czeisler et al., 1981). The loss of environmental/social zeitgebers which normally entrain these rhythms results in a desynchronization of the circadian rhythms controlling sleep-wake behavior and underlying biological rhythms. This often results in the development of delayed or advanced sleep phase syndrome. A similar shift in phase relationships is also observed in shift workers or transmeridian travelers (Arendt et al., 1986). As a result of circadian misalignment either due to shift work or jet lag, an individual may suffer from mood disturbances, poor performance, or general lack of well-being. Most studies of individuals suffering from jet lag, or subjects who have experimentally-induced jet lag, have documented shifts in the performance rhythms on a variety of tasks which involve sustained attention (Colquhoun, 1971) and changes in their peak level of subjective alertness (Arendt et al., 1986; Claustat et al., 1992), which can account for their feelings of fatigue, performance decrements, and mood changes.

In addition, some studies have yielded data on possible age-related alterations in phase relationships among various physiological functions resulting in desynchronization of circadian rhythms (Richardson et al., 1982; Weitzman et al., 1982; Campbell et al., 1989). For example, the observed phase advancement of the body temperature and cortisol rhythm

may account for the general changes in the timing of the sleep period and sleep quality in the elderly (i.e., phase advancement of the nocturnal sleep period relative to desired schedule, fragmented nocturnal sleep and daytime napping) (Richardson et al., 1982; Weitzman et al., 1982). Other studies have emphasized gender differences in the core body temperature rhythm in humans. The acrophase in elderly women is advanced approximately one hour compared to age-matched males, and the amplitude of the temperature rhythm is higher in the females (Campbell et al., 1989). Specifically, in older women, the phase advancement of the temperature rhythm may account for their report of earlier rising times compared to older males (Campbell et al., 1989). In addition, the core body temperature rhythm in patients with primary depression is often manifested by an elevation of the core body temperature minima during the night and a suggested phase advancement of several sleep-related features (i.e., shortened latency to REM sleep and early morning awakening) (Kupfer and Reynolds, 1983).

With regard to documenting circadian rhythms in OSAS, very little work has been done. In addition to the studies looking at core body temperature (Sforza et al., 1993; Vogel et al., 1993; Zozula et al., 1993), only one study has systematically looked at a complex set of hormonal rhythms in OSAS patients before and after three months of CPAP therapy. One investigation conducted by Entzian et al. (1996) examined the circadian variations in several different cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6), γ -interferon (γ -IFN), tumor necrosis factor- α (TNF- α), as well as the hormones melatonin and cortisol. Entzian et al. were primarily interested to see if the rhythms of cytokine release were altered in OSAS

patients prior to treatment, since many of the cytokines have high somnogenic properties (therefore, possibly linking them to symptoms of sleepiness in OSAS) and clear temporal patterns (i.e., IL-1 and TNF- α) (Krueger and Majde, 1995). Only the rhythm of TNF- α was significantly different in the OSAS group before treatment compared to a group of normal sleepers. The usual nocturnal peak of this cytokine had virtually disappeared and an additional daytime peak had developed. There were no significant differences in either melatonin or cortisol secretion in the OSAS group. TNF- α rhythms were not normalized following CPAP treatment. Therefore, the authors concluded that TNF- α may play a role in the pathophysiology of OSAS. This remains to be seen, since this work has not been replicated by any other group of investigators. Furthermore, the OSAS group in this study was not well matched to the control group, since the OSAS group consisted of nine males and one female, ranging in age from 42-65 years, with a mean age of 53.5 ± 9.7 years; the controls consisted of five males and five females, ranging in age from 19-29 years. Perhaps with a more stringent control group, the differences between the groups regarding TNF- α rhythm may not be as salient.

Exogenous Melatonin Administration: Effects on Body Temperature, Sleep and Performance

Many studies have documented the effect of melatonin on core body temperature. In non-human species, hypothermia that was induced by melatonin which was associated by extended sleep duration (Barchas et al., 1967, as cited by Dawson and Encel, 1993). Studies

in humans using large oral doses (200-120 mg) have shown reductions in oral temperature (Carman et al., 1976). More recent research has shown that oral doses of melatonin, as low as 0.1-5.0 mg, can significantly lower daytime body temperature (Cagnacci et al., 1992). However, it remains unclear as to whether the reduction in body temperature associated with melatonin ingestion is the result of a direct effect of melatonin or an indirect effect due to changes in secondary systems (i.e., changes in noradrenergic tone or vasodilatory changes).

The phase-resetting properties of melatonin have been applied in several clinical investigations of the alleviation of symptoms associated with jet lag (Arendt et al., 1986; Claustrat, et al., 1992). The majority of studies on jet lag have focused on subjective measures of improvement (i.e., improvement in mood, increased subjective alertness) but have not objectively examined sleep parameters or cognitive performance before or after the implementation of melatonin (Arendt et al., 1986). In the study by Arendt et al. (1986) subjects who received melatonin over the 14 day period following a westward time-zone change also reported shorter sleep onset latencies and significantly improved sleep quality. In addition, there is evidence that melatonin may also act as a phase-setter for sleep-wake cycles in subjects with delayed sleep phase syndrome (Dahlitz et al., 1991). In this study, a double-blind, placebo controlled trial of melatonin versus placebo was used over a 4 week period (1 week washout period). The 5 mg oral dose of melatonin was found to significantly advance the sleep onset time and wake-up time compared to the placebo. Again, these findings were based on subjective sleep-wake diaries which were logged by the subjects.

Early studies conducted by Anton-Tay et al. (1971) have demonstrated significant hypnotic effects following intravenous infusion of 100 mg of melatonin during the day. Lerner and Nordlund (1975) found increased sedation across the daytime waking hours following a one month period of daily ingestion of 1 gram. Once again, subjective reports of sleepiness/alertness were used without objective measures of sleep or daytime performance. One of the first studies to incorporate objective (NPSG) sleep measures involved intravenous infusion of melatonin (50 mg) to normal sleepers prior to bedtime (Cramer et al., 1974). This study demonstrated decreased sleep-onset latency as well as increased sedation and reduced psychomotor activity.. without further alterations in sleep architecture or report of significant side-effects. However, significant changes in sleep architecture were found in a study where normal subjects received a 1 gram oral dose of melatonin in the morning for six days (Anton-Tay, 1974). This resulted in an increase in REM density and the amount of stage 2 sleep, with a reduction in stage 4 sleep. Dawson and Encel (1993) have suggested that since it takes several days of melatonin ingestion to alter sleep architecture, it may be achieving its effect via phase advancement of the circadian clock mechanism. Other studies have failed to consistently observe these hypnotic effects or changes in sleep architecture (James et al., 1987). These studies implemented 1 and 5 mg doses of oral melatonin given to young normal sleepers prior to bedtime. While both dose levels failed to elicit a hypnotic effect, only the 5 mg dose had an effect on sleep architecture (increased REM onset latency). More recent evidence has shown that oral administration of 5 mg of melatonin to normal sleepers resulted in increased duration of REM sleep (Tzichinsky and Lavie, 1994). The lack of hypnotic effect of nocturnal melatonin

administration in otherwise normal sleepers may be explained by the fact that at night, these individuals already have fairly high endogenous melatonin levels; therefore, it is hard to evoke increases in their sleep-propensity rhythm, which is already quite high. However, exogenous nocturnal melatonin administration may still be useful in individuals with insomnias related to circadian disruption or chronic sleep fragmentation (in this case, experimentally induced with external auditory stimulation) (Waldhauser et al., 1990).

Therefore, based on these findings, exogenous melatonin has been investigated as a possible treatment for sleep onset and sleep maintenance forms of insomnia. Exogenous melatonin administered in supraphysiologic doses (75 mg, orally) prior to bedtime has been reported to significantly increase the subjective assessment of total sleep time and daytime alertness in insomniacs (MacFarlane et al., 1991). A study by James et al. (1990) examined the effects of 1 and 5 mg melatonin administration at 2100 hours on NPSG sleep measures in chronic insomniacs. They found no consistent effect on NREM measures, or on mood or alertness measures the next day. However, REM latency was increased with the 1 mg dose, but not the 5 mg dose (opposite effect was found in normal sleepers). Two milligram (oral) doses have been shown to improve objective measures of sleep efficiency in elderly insomniacs (Haimov et al., 1993). However, the objective measures used in the study were wrist activity measures (which infers sleep-wake activity) and direct polysomnographic measures of sleep were not conducted. More recent work has shown that even a lower dose (0.5 mg), sustained-release, physiologic replacement of melatonin achieves the same desired effect as higher pharmacologic dosages (Armstrong and Redman, 1993). Furthermore, low

oral doses of melatonin (0.3-1.0 mg) which were previously shown to increase serum melatonin concentrations during the day to levels normally occurring at night, produce acute hypnotic effects when administered in the evening (Zhdanova et al., 1995). Garfinkel et al. (1995) also demonstrated increased sleep efficiency in elderly insomniacs using a 2 mg sustained-release dose of melatonin after nightly administration for three weeks. Again, sleep measures were inferred from wrist actigraphy measures of rest-activity.

C) Measurements of Sleep and Wakefulness

Since there are many procedures that can be used to assess sleep and wakefulness (objective and subjective), it is useful to outline the measures that I intend to implement in this study. This will also serve to clarify what types of measures other investigators have typically used.

Objective Measures of Sleep and Wakefulness

As stated previously, the standard objective assessment of sleep involves a technique known as nocturnal polysomnography (NPSG). This involves recording of electrophysiological signals from 2 monopolar electroencephalographic (EEG) leads, 2 monopolar electro-oculograms (EOG), bipolar submental (chin) electromyogram to assess sleep parameters. Sleep is scored in 30 second epochs according to standard criteria of Rechtschaffen & Kales (1968). Estimates of total sleep time (TST), time in bed (TIB), sleep

onset latency and REM latency, sleep efficiency (defined as $TST/TIB \times 100\%$), as well as percentage of time spent in each sleep stage can be derived. Indices of sleep fragmentation, which include time spent awake after sleep onset (WASO) and a frequency analysis of consecutive epochs of sleep (FACE) (Rothenberg, 1987), can also be assessed. Additional measures of phasic REM activity can be inferred (REM index and REM density) (Kupfer and Foster, 1975; Gillin et al., 1979).

Actigraphic monitoring is another objective technique used to infer sleep-wake activity patterns (Aubert-Tulkens et al., 1987). Typically, the subject will wear the monitor on their non-dominant wrist. The actigraph unit can be programmed to record/detect activity at varying intervals. Studies which have looked at automatic detection algorithms to assess sleep-wake activity have used 30 second epochs, in order to emulate the scoring epochs used by Rechtschaffen and Kales (Cole et al., 1992). However, when looking at physiological signals that may display a greater frequency of activity (i.e., sleep disruption in severe obstructive apnea), the method of collection may entail sampling at a greater frequency to assess activity within a shorter duration. For example, OSAS patients may exhibit 2 or 3 episodes of "microsleep" (< 15 seconds of sleep) during a minute interval. Therefore, sampling in the present study will be conducted at 10 second intervals. However, actigraphic monitoring can tend to overestimate sleep in patients who are sedentary, but otherwise awake. This is typically a problem when monitoring subjects who are lying quietly in bed, but are still wide awake, as is observed in insomniac patients (Cole et al., 1992). Cole et al. (1992) have suggested that actigraphic monitoring produces fairly reliable estimates for

sleep onset latency scores as compared to NPSG sleep latency measures (especially with sleep onset periods of at least 15 minutes in duration).

Assessment of Daytime Sleepiness/Alertness

There are four general classes of tests that are used to evaluate daytime functioning and the presence of sleepiness following NPSG. These include the following: 1) objective assessment of sleepiness and sleep tendency using the Multiple Sleep Latency Test; 2) objective assessment of behavioral alertness/wakefulness using the Maintenance of Wakefulness Test and the Four-Choice Reaction Time Task; 3) subjective measures of sleepiness using the Stanford Sleepiness Scale and the Epworth Sleepiness Scale; and 4) objective assessment of cognitive functioning. These four classes of daytime tests have been shown to be impacted by sleep deprivation and untreated OSAS (Dinges, 1992).

1) Objective Measures of Daytime Sleepiness

The Multiple Sleep Latency Test (MSLT) is an objective test of physiological sleepiness or sleep tendency (Carskadon et al., 1986). Following the NPSG study in the laboratory, subjects are given 4 or 5 opportunities to fall asleep, starting at approximately 2 hours after they have awakened, and occurring at regular 2 hour intervals. The monitoring is identical to that in the NPSG. Each sleep session ("nap") is terminated with the polygraphic determination of sleep onset (one 30 second epoch of any stage of sleep) or after 20 minutes,

if sleep did not occur. This is a slight modification of the standard MSLT termination procedure, where the test is ended after 3 epochs of stage 1 sleep or the first epoch of any other stage. This is instituted to limit the possibility of accruing any sleep during these testing sessions. Each nap is scored for the latency (time in minutes) to onset of sleep and for the presence of REM and NREM sleep. Mean sleep latency is then determined across the separate nap sessions to get a mean daily value. Some older investigations which have incorporated MSLT sleep latency measures have used the definition of sleep onset as 3-epochs of stage 1, or one epoch of any other stage of sleep (which is the standard definition of nocturnal sleep latency by Rechtschaffen and Kales). This could lead to difficulty in the interpretation of findings of different research investigations. Adult normative values on the MSLT are an average sleep latency value between 10-20 minutes (Richardson et al., 1982). A pathological level of daytime sleepiness is considered an average sleep latency of less than 5 minutes. Scores that fall between the normal and pathological ranges are often viewed as a diagnostic gray area, where clinical judgment must be factor into the interpretation of such results.

In addition, in the present study, post-treatment MSLT evaluation in the OSAS group will be conducted with the CPAP device, where the pressure was set at the subject's recommended titration level. This is done to rule-out the presence of concurrent sleep disorders that could account for persistent sleepiness in this patient group (as recommended by the Standards of Practice Committee of the American Sleep Disorders Association) (Thorpy, 1992).

2) Objective Measures of Daytime Wakefulness

The Maintenance of Wakefulness Test (MWT) is an objective assessment of the ability of an individual to maintain a state of wakefulness (Mitler et al., 1982). Subjects are given 5, 40-minute sessions and instructed to "try to stay awake" while semi-recumbent in a quiet, darkened room. The session is terminated with the polygraphic determination of sleep onset (one epoch of any stage of sleep). Similar to the MSLT, each session is scored for sleep latency and the presence of REM and NREM. In the case of the present study, only 4 MWT sessions will be implemented due to time constraints of the other testing sessions. Normative values for the MWT have been reported for adult normal sleepers (Doghramji et al., submitted for publication). Using a standard one epoch criterion for sleep onset, the mean sleep latency in normals is 32.6 minutes. A stricter definition of sleep onset (3 epochs of stage 1, or one epoch of any other stage of sleep), yielded a mean sleep latency of 35.2 minutes. The MWT has been a useful technique to differentiate groups with normal levels of daytime alertness from those with significant impairment (Poceta et al., 1990; Sangal et al., 1992). However, similar to the MSLT, given the relative monotony of the testing conditions, the results of the test can be heavily impacted upon by motivational factors and on the part of the subject.

The MWT has been shown to be useful in assessing daytime wakefulness in OSAS, is sensitive to the treatment effects of CPAP (Baker et al., 1988; Poceta et al., 1990a) and is

significantly correlated with respiratory disturbance indices (Poceta et al., 1990a). Furthermore, the MWT has a greater range of measurement than the MSLT (40 minutes versus 20 minutes) and the effect of the instruction to stay awake tends to eliminate the "floor effect" of the MSLT (Poceta et al., 1990b). It is important to note that patients with diagnosable disorders of excessive somnolence have proven to be discordant on the MWT and MSLT (i.e., having low sleep latency values on the MSLT but high sleep latency on the MWT (Sangal et al., 1992). Therefore, it is important to emphasize that the MWT and the MSLT are measuring distinctly different abilities (Sangal et al., 1992). While the MSLT measures the subject's ability to sleep, the MWT assesses the ability to remain awake during soporific circumstances (Mitler et al., 1982) and has been used to examine treatment efficacy in a variety of sleep disorders (Sangal et al., 1992).

Four-Choice Reaction Time Test (FCRTT): The FCRTT is a sensitive and validated psychomotor measure of objective alertness (Wilkinson, 1975). The FCRTT is delivered on a PC-based computer system and requires approximately 10 to 15 minutes to complete. It is carried out by subjects once every 2 hours during the daytime. The visual stimuli consists of a pattern of 4 green-outlined squares, with one square displaying a red square inset. This pattern is randomly alternated for each stimulus presentation. The keyboard control consists of a corresponding square of 4 push-buttons. Subjects are required to press the corresponding button whenever a red square is detected. Subjects are given immediate (auditory) feedback of results during the testing session. Subjects' responses are downloaded to the computer and stored for future analysis. As recommended by Glenville

& Wilkinson (1979), three practice sessions of the test are performed the previous evening in order to minimize the practice effect that can appear when practice and testing sessions occur on the same day. For each of the 5 trials administered over the course of the day, the following variables are obtained: mean reaction time (RT), number of gaps (or "lapses", long latency responses with $RT > 1000$ ms), accuracy and throughput (speed x accuracy product). Daily means are then computed for each of these values.

The FCRTT has been used to demonstrate deficits in attention related to impairments in vigilance in OSAS patients before treatment and significant improvement was noted on measures of the FCRTT following a course of CPAP treatment (Bedard et al., 1991; Montplaisir et al., 1992). The main principle behind using functional probes like the FCRTT to reveal performance decrements which result from sleep loss or sleep disruption, is the "contextual dependence hypothesis" (Dinges, 1989, as cited by Dinges, 1992). This states that the greater the physiological sleepiness brought on by sleep disruption, the more dependent the brain becomes on the environment (contextual factors) to maintain wakefulness and the more susceptible it becomes to environmental monotony. The subject's level of motivation and incentive can sometimes override this contextual effect, but only for short periods of time. According to Dinges, tasks that draw upon the brain's vulnerability to drift toward a state of sleep will reveal significant performance decrements early in the testing period. Brief performance tasks (10-30 minutes) that require sustained attention, like the FCRTT, have these test characteristics.

3) Subjective Measures of Sleepiness and Alertness

Stanford Sleepiness Scale (SSS): The scale measures a subject's feeling of sleepiness, or tiredness, at a particular time. Subjective ratings of sleepiness are assessed using a Likert-type, 7-point, self-rating scale (Hoddes, 1973). The SSS presents a series of 7 statements to the subject, who is required to check the statement that best describes their current state. The scale is administered over the course of the entire testing period and attempts to assess fluctuations in the level of subjective sleepiness over time.

The scores on the SSS have been used in clinical as well as in research settings which involve sleep disorder patients. The SSS has demonstrated a fairly high correlation in normal subjects with performance and polygraphically measured sleep onset (Hoddes et al., 1973). However, in patients with OSAS, it has not proven to be reliable with other objective measures of sleepiness (i.e., MSLT) (Roth et al., 1980). This may be partly due to denial on the part of the sleepy patient, who may rate themselves as being fairly alert, while they may be literally falling asleep while filling out the questionnaire (Roth et al., 1980). Significant improvement on the SSS following CPAP treatment is not always observed in OSAS patients with severe EDS, even though they report feeling better and less sleepy during the day. This may be due to the limited response categories on the SSS which creates a "ceiling effect". Thus, following treatment, the OSAS patient cannot find a value which is high enough to indicate their subjective improvement. Another criticism of the SSS lies in the fact that the correlation between measures on the SSS and objective measurements of

daytime sleep propensity (MSLT) have been fairly poor (Roth et al., 1980). In this study, a group of 5 OSAS patients was compared before and after 2-5 months of CPAP therapy, where subjective improvement on the SSS was noted, without evidence of significant changes on the MSLT.

Epworth Sleepiness Scale (ESS): The scale is a simple technique for measuring the general subjective level of persistent daytime sleepiness or sleep propensity in adults (Johns, 1991). The subject rates his/her likelihood of falling asleep during 8 specific activities (Johns, 1991; 1992; 1993). Each question is given a 0 ("least likely") to 3 ("most likely") rating to combine to give a maximum score of 24. Total score is reported for each completed form. Subjects complete this simple, self-administered questionnaire at the beginning of each phase of the experiment. The ESS has also been demonstrated as having a fairly high degree of test-retest reliability and internal consistency of the eight items of the questionnaire (determined by using Cronbach's alpha statistic) (Johns, 1992).

Total ESS scores have been shown to significantly distinguish normal subjects from sleep disorder patient groups including OSAS, narcolepsy and idiopathic hypersomnia (Johns, 1991). Furthermore, ESS scores demonstrated significant differences between a group of primary snorers and three subcategories of OSAS patients (i.e., mild, moderate and severe). Johns (1992) has also observed a reduction in ESS scores in OSAS patients following 3-9 months of CPAP therapy compared to pre-treatment values. However, these scores still were not within completely normal range when compared to age-matched normal

sleepers.

4) Objective Assessment of Cognitive Functioning

Neuropsychological tests that are typically selected cover a broad range of functions known to be affected in OSAS (Bedard et al., 1989; Findley et al., 1986). The battery employed in the present study is a replication of that used by Bedard et al (1991) and is outlined more fully in the "Protocol Schedule" (SEE APPENDIX I). The full scaled score of the WAIS-R will be used to determine general cognitive functioning. Attention will be assessed by the Digit Symbol test of the WAIS-R. Memory (verbal and nonverbal) is assessed by the stories of the Wechsler Memory Scale (WMS) and by the Rey-Osterreith Complex Figure (both immediate and delayed recall condition). Other tests will be given to assess manual dexterity (Purdue Pegboard) and executive functions (Trail Making Test - Part B of the Halstead-Reitan Battery; Verbal Fluency Test; Rey-Osterreith copy condition; Mazes of the WISC-R; Picture Arrangement of the WAIS-R; Block Design of the WAIS-R).

D) Effect of Sleep Disruption on Daytime Functioning

Sleep has a profound impact on waking functions. Disrupted nocturnal sleep results in impaired alertness during the day, which often has a negative impact upon performance ability and mood (Bonnet, 1985; 1986a). While most objective (NPSG) measures of sleep emphasize the quantity of sleep (i.e., total sleep time) or quality (i.e., percent of time spent

in SWS or REM sleep; sleep efficiency), few measures address the issue of continuity, or fragmentation, of sleep. It has been demonstrated that continuous sleep (sleep without interruptions), is critically related to normal daytime functioning (Carskadon & Dement, 1979; Bonnet, 1986a).

Experimental studies in healthy normal sleepers have demonstrated that sleep fragmentation alone, without any decrement in total sleep time, causes increased daytime sleepiness (Stepanski et al., 1987). One study by Bonnet (1986) attempted to model an arousal pattern in normal sleepers using auditory stimulation which was similar to the sleep fragmentation observed in severe OSAS (i.e., one arousal per minute, which is equivalent to an OSAS patient with an AHI = 60 events/hour). Performance in the morning following sleep disruption showed significant impairment on tests of reaction time and the Wilkinson addition task. Magee et al. (1987) also experimentally induced sleep fragmentation in normal control subjects and assessed subsequent daytime sleepiness using the MSLT. They found that objective daytime sleepiness was significantly increased by the 1-minute arousal condition, but not in the condition of fragmenting sleep every 4 minutes (which would be equivalent to an OSAS patient with an AHI = 15). Both these results support the contributing role of sleep fragmentation to daytime sleepiness and performance decrements.

Individuals with specific sleep disorders, such as obstructive sleep apnea, are often deprived of SWS or REM sleep. However, it is not possible to account for the performance loss in these groups based solely on selective sleep stage deprivation because sleep stage

deprivation studies have generally concluded that total sleep time rather than sleep stage distribution is the primary correlate of daytime performance (Johnson et al., 1974; Lubin et al., 1974). However, it is noted that OSAS is a good paradigm in which to investigate sleepiness resulting from sleep fragmentation, without the element of sleep loss (Roth et al., 1980), since total sleep time is not significantly different in untreated OSAS patients compared to a group of age-matched normal sleepers.

One major explanation of the daytime somnolence and performance decrements that accompany significant sleep apnea is that the periodic arousals that accompany the apneas disrupt sleep and make it nonrestorative; thereby, supporting the sleep continuity hypothesis. It is even suggested that the similar changes observed in sleep architecture (i.e., increased percentage of stage 1, decreased stages 3, 4 and REM) in both naturally occurring cases of OSAS and studies of experimentally-induced sleep fragmentation are a result of frequent sleep disruption which contributes to the suppression of those sleep stages (Magee et al., 1987; Philip et al., 1987). Furthermore, it has been demonstrated that the number, type and duration of nocturnal arousals play an important role in subsequent daytime sleepiness, and this may be different in different clinical sleep populations (i.e., sleep apnea, periodic limb movement disorder, insomnia) (Stepanski et al., 1984). Therefore, the level of sleep disruption that is associated with each of these disorders may not be identical and will be expressed with varying degrees of daytime symptomatology. Stepanski et al. (1984) classified arousals in 3 clinical groups (OSAS, myoclonus and insomnia) and a group of normal controls, on the following basis: Type 1 arousal = increase in EEG frequency and

chin EMG amplitude; Type 2A = alpha burst lasting 0-5 seconds; Type 2B = alpha burst 6-29 seconds; Type 3 = stage shift to lower stage; Type 4A = awakening (> 30 sec.) from stage 1 or REM; Type 4B = awakening from NREM (Stage 2,3, or 4). They found a significant positive correlation between the number of arousals and increased sleepiness on the MSLT for groups with EDS. The highest correlation with daytime sleepiness on the MSLT was with the shorter types of arousals (types 1 and 2), as opposed to the longer, and less frequent types of arousals. Therefore, the frequency and the type of arousal pattern were the chief determinants in the development of daytime sleepiness.

E) Respiration: Physiology and Changes during Sleep

1) Respiration in Wakefulness

The process of respiration is an integral function of the human organism: meeting the physiological demands for oxygen (O_2) as well as eliminating metabolic by-products (CO_2). Unlike the heart, the respiratory muscles do not have a built-in pacemaker. These muscles receive input from a region of the medulla referred to as the respiratory center (or respiratory oscillator). In order to adapt to changing physiological conditions, the respiratory center receives and responds to three general types of information: chemical information from chemoreceptors responding to the arterial tensions of oxygen (PaO_2), the arterial tensions of carbon dioxide ($PaCO_2$), and pH; mechanical information from receptors in the lung and chest wall; and behavioral information from higher cortical centers (Douglas, 1994).

Chemical information (i.e., changes in PaO_2 , PaCO_2 and pH) is detected by the carotid body which is a fast responding sensor and sends impulses to the medulla via the ninth cranial nerve. When PaO_2 drops below 60 mmHg, ventilation usually increases. When there is an acute drop in PaO_2 (<30-40 mm Hg), the medulla may be depressed by the drop in oxyhemoglobin saturation (hypoxemia), leading to decreased respiration. PaCO_2 is also sensed in a central receptor in the medulla via information sent by the afferent vagus fibers. When an increase in PaCO_2 is detected (called hypercapnia), there is a rapid increase in ventilation. The increase in ventilation helps the body to eliminate excess CO_2 and produces a normal (eucapnic), or even lower than normal (hypocapnic), level of CO_2 in the blood. In addition, hydrogen ion concentration (pH) is sensed in both sites. This comprises the metabolic (automatic) system of respiratory control which is hypothesized to be the major pattern generator for breathing during wakefulness.

In addition, mechanical information is processed by stretch receptors in the lung and chest wall which also send impulses to the medulla. This information may result from changes in the load of the respiratory system or dysfunction secondary to lung disease. The respiratory system is also utilized for many functions, other than breathing (i.e., speaking, laughing, etc.) that are directed by higher brain centers located in the forebrain. This is referred to as the behavioral system of respiratory control (Phillipson and Sullivan, 1978). The efferent pathways involved may actually circumvent the respiratory center in the medulla and thereby, override the metabolic homeostatic function of the respiratory control system.

Both the metabolic and behavioral systems modify the muscular activity of the upper airway (Douglas, 1994). Each of these systems interacts with the reticular activating system (RAS) of the brainstem, sending impulses from brainstem respiratory neurons directly to the RAS, as well as receiving input from the RAS which subserves the general function of behavioral arousal, as well as that of respiratory control. The brainstem respiratory neurons consist of the following: the dorsal respiratory group (ventrolateral nucleus of the solitary tract) which respond to early-onset inspiratory activity and increase their discharge rate progressively during inspiration; the ventral respiratory group (ventral medulla) which contain both inspiratory and expiratory cell types; the ventral medullary surface which has been found to have three chemosensitive zones; and the pontine respiratory group (pneumotaxic center of Lumsden, located in the rostral dorsolateral pons) which exhibit inspiratory, expiratory and phase-spanning activity. Several models of respiratory pattern generation have implicated the cells of the dorsal respiratory group which integrate afferent information related to breathing and the ventral medullary surface neurons which may initially activate early-onset inspiration neurons (Orem, 1994).

2) Respiration in Sleep

Sleep changes both the breathing pattern and the respiratory responses to many external stimuli. In the adult human, the hypoxic and hypercapnic ventilatory responses are reduced during sleep and are at their lowest during REM sleep (Douglas, 1994). Furthermore, sleep modifies the ventilatory response to added inspiratory resistance (Sullivan and Issa, 1980).

In NREM sleep, ventilation appears to be regulated by the automatic respiratory control system and breathing movements are mainly involved in maintaining metabolic homeostasis. During the early stages of NREM sleep, in particular, at sleep onset, a regular waxing and waning of breathing amplitude is often observed which is referred to as "periodic breathing" (Mosso, 1886). Periodic breathing lasts as long as sleep oscillates between arousal and light stages of sleep (stage 1 or 2) and it disappears when stable stage 2 or deeper sleep is achieved (Bulow, 1963). Once in deeper NREM sleep, respiration becomes fairly stable, but ventilatory responding is definitely diminished compared to wake time values due to the lack of input from the reticular activating system.

In contrast to NREM sleep, during REM sleep breathing does not appear to be primarily regulated by the chemical automatic control system. Instead, breathing movements appear to subserve non-respiratory needs and are relatively unresponsive to classical respiratory stimuli (i.e., hypoxia or hypercapnia). Therefore, REM sleep respiration appears to be driven by inputs that are unrelated to the automatic control system due to the fact that respiratory movements do not seem to be primarily "ventilatory" in nature (i.e., not responding to chemical stimulation). Due to the muscle atonia associated with REM sleep, most major muscle groups are paralyzed, including those of the upper airway, and to some degree, the intercostals. Therefore, respiration is dependent on diaphragmatic muscular activity. The rate of breathing during REM sleep is irregular and the degree of respiratory irregularity during REM increases when rapid eye and other phasic muscular movements are increased (Phillipson and Sullivan, 1978). In normal males, it has been demonstrated that phasic REM

sleep is associated with reduced minute ventilation (Gould et al., 1988), and in patients with chronic obstructive pulmonary disease, phasic REM sleep is associated with frequent oxygen desaturations (George et al., 1987). It is hypothesized that the voluntary (behavioral) system is involved in respiratory control during REM sleep, but there is little data available at the cellular level to support this idea. However, there is some evidence in cats that a learned respiratory response may be performed in REM sleep, which indicates that behavioral control in that state may be possible (Trotter and Orem, 1991).

It appears that the major cause for the decrease in the ventilatory responses during NREM sleep is absence of the wakefulness (behavioral) drive to breathe coupled with the decrease in metabolic rate and increase in airflow resistance (Douglas, 1994). In terms of metabolic rate, oxygen consumption and carbon dioxide production decrease in the range of 10 to 25 percent during sleep (Bulow, 1963). The further reduction in ventilatory response during REM sleep is likely to result from changes in central nervous system function during REM sleep: due to presynaptic and postsynaptic inhibition of afferent neurons, sensory arousal thresholds to external stimuli are raised and postsynaptic inhibition of motoneurons results in postural hypotonia which is associated with REM sleep.

These changes in ventilatory response during sleep may contribute to the pathogenesis of apneas in patients with the sleep apnea syndrome and in sleep-related hypoxemia in individuals with respiratory disease (Douglas, 1994). It is hypothesized that the impaired ventilatory responses during sleep may be accompanied by a reduction in the responsivity

of the dilating muscles of the upper airway to chemostimulation during sleep. Therefore, both factors may play a role in the development and continuation of apneas. Furthermore, patients who are already compromised by respiratory disease may develop clinically significant hypoxia and hypercapnia before arousal occurs due to the increased arousal threshold to external stimuli during sleep.

F) Obstructive Sleep Apnea Syndrome: Definition, Etiology, Symptoms, and Treatment

1) Definition of Apnea

Obstructive sleep apnea is the cessation of airflow at the nose and mouth which results in an apneic episode during sleep. Events are defined as lasting 10 seconds or longer and are usually associated with disruption/fragmentation of sleep and reductions in blood oxygen saturation (SaO_2). Three types of sleep apnea have been described: 1) obstructive (upper airway) apnea, characterized by cessation of air flow despite persistent respiratory efforts; 2) central apnea, characterized by cessation of air flow resulting from termination of respiratory effort; and 3) mixed central and obstructive apnea, defined by the occurrence of a central phase (no air flow and no respiratory effort) followed by an obstructive phase in the latter part of the episode (Guilleminault et al., 1978).

Based upon polysomnographic findings, patients with sleep-disordered breathing may display hundreds of apneic episodes per night, with all three types of apnea present.

However, the obstructive type predominates in the vast majority of patients. The entire physiologic spectrum of sleep-disordered breathing ranges from partial airway occlusion and increased upper-airway resistance, manifested as loud snoring and episodes of reduced airflow (hypopnea), to complete cessation of airflow due to total airway collapse (apnea). The severity of the sleep-related breathing disturbance can be determined by the patient's overall apnea-hypopnea index (AHI) which is the number of apneic or hypopneic events per hour of sleep.

During these apneic periods, futile respiratory efforts continue and severe arterial hypoxemia often develops, until the apnea is terminated by a brief awakening (usually accompanied by loud snoring), and airway patency is restored. Over the course of a night, apneic events tend to increase in duration and severity. Frequent apneic episodes may result in a drop of oxygen saturation to as low as 40%, from a baseline value of 90-95%. Repetitive events may result in transient decreases in the oxygen (O_2) level in the blood and an increase in carbon dioxide (CO_2) levels. The resultant hypoxemia creates vasoconstriction of the pulmonary blood vessels, which has been postulated to lead to systemic and pulmonary arterial hypertension and right-sided heart failure (Guilleminault, 1982). It is hypothesized that many of these hemodynamic changes are mediated through cholinergic mechanisms as a consequence of repeated arousal (Guilleminault and Suzuki, 1992). Further evidence in support of the role of arousal in the mediation of blood pressure comes from studies of non-respiratory induced arousals in normal sleepers (Belt et al., 1993) or periodic limb movement related arousal (Ali et al., 1991). It is further postulated that the mechanical

effort of breathing against a partially or completely obstructed airway may also play a role in the development of increased pulmonary arterial pressures during sleep (Guilleminault and Suzuki, 1992). Therefore, hypoxemia is not the only factor involved in sleep-related hemodynamic changes; sleep disruption appears to play a significant role in modulating changes in blood pressure during the nocturnal sleep period (Ringler et al., 1990). Other cardiovascular manifestations of sleep apnea include cardiac arrhythmias (i.e., bradycardia; tachycardia) and heart block (Guilleminault, 1994). According to a report of the National Commission on Sleep Disorders Research (1993), it was estimated that patients with OSAS have twice the prevalence of hypertension, three times greater incidence of heart disease, and four times as much cerebrovascular disease than is found in the normal population. Moreover, the Commission estimated that 38,000 cardiovascular deaths occur annually as a result of sleep apnea.

Central apnea results from a failure in the neural mechanisms controlling respiration. This lack of regulation of breathing may be sleep induced (occurring at sleep onset or during transitions in sleep stage) and may result from poor integration of metabolic and behavioral control systems. It is believed that the CO_2 response, and indirect response to changes in pH level, may play a key role in central apnea (Douglas, 1994). Central apnea may also result from brain injury or illness. Although there is controversy regarding the existence of mixed apnea, it was previously thought that the pattern was initiated by decreased central respiratory drive which permitted the muscles in the oropharynx to collapse. Once collapsed, the tissues in the upper airway would occlude the airway and require a stronger force to

reopen them; thereby producing the obstructive component of apnea.

2) Pathophysiology of Obstructive Sleep Apnea Syndrome

There are many theories regarding the pathophysiological mechanisms involved in obstructive sleep apnea. However, three fundamental features have become apparent regarding the closure of the upper airway in patients with obstructive sleep apnea. First, the site of the upper airway obstruction usually lies in the pharyngeal region, rather than the larynx, mouth or nose (Weitzman et al., 1978; Hudgel, 1992; Isono and Remmers, 1994). The typical site of obstruction lies either in the nasopharynx at the level of the posterior soft palate or in the oropharynx at the level of the base of the tongue (Rapoport, 1996). Several studies have reported an increased incidence of abnormal flow-volume curves upon pulmonary function testing in patients with sleep apnea, showing rapid flow oscillations and/or variable extrathoracic obstruction (Tammelin et al., 1983). These abnormalities were thought to be caused by redundant mucosal tissue in the pharynx. Both radiographic and acoustic imaging techniques have provided additional evidence in support of the pharyngeal narrowing hypothesis in patients with obstructive apnea (Isono and Remmers, 1994). Second, the size of the pharyngeal lumen (opening) depends on the balance between intrapharyngeal suction pressure and outward forces generated primarily by pharyngeal muscles. During a normal inspiration, negative pressure is generated in the lumen of the upper airway by contraction of the diaphragm acting through the resistance of the nose. Since the luminal walls are flexible, this increase in negative pressure favors collapse of the

upper airway. Therefore, the physiology of the upper airway during sleep can be likened to the behavior of a collapsible tube (Rapoport, 1996). Studies by Sullivan et al. (1980) have demonstrated that when the muscles of the pharynx and the genioglossus muscle of the tongue become atonic during sleep, the pharyngeal lumen is sucked closed by increased negative intra-airway pressure which is created by the inspiratory pull of the diaphragm (Sullivan and Issa, 1980). Thirdly, anatomic abnormalities of the pharynx and its associated structures are often present in patients with obstructive sleep apnea. For example, enlargement of the tonsils or adenoids, deviated nasal septum, or a skeletal abnormality of the face (i.e., micrognathia, retrognathia) can all contribute to reduced upper airway patency. Furthermore, obesity is a major factor in the development of apnea, due to the increased fatty tissue which, in particular, places an increased burden on the neck muscles to maintain a patent airway (Guilleminault, 1982).

The increased inspiratory pull that develops in response to the airway narrowing can be highly variable. If the suction force is great enough, it will suck the airway closed, resulting in the occurrence of frank apneic episodes. In other instances, partial airway closure will lead to increased upper airway resistance, resulting in “flow limitation” or hypopnea. Flow limitation refers to the behavior observed when increasing effort applied to a system produces no measurable increase in flow (Rapoport, 1996). With normal inspiration, the collapsing pressure usually exhibits a sinusoidal contour pattern. When the airway is relatively rigid (patent), the inspiratory contour also exhibits a sinusoidal contour because flow is proportional to the driving pressure. However, if the airway becomes more

collapsible, as is evident during sleep in OSAS patients, flow limitation occurs and the contour of the inspiratory pattern develops a flattened shape, or plateau (Condos et al., 1994). Rapoport et al. (1995) have demonstrated that the presence of this characteristic plateau of the flow tracing is also likely to lead to subsequent arousal.

3) Arousal and Sleep Fragmentation in Obstructive Sleep Apnea Syndrome (OSAS)

The key factor in the termination of an apneic event is arousal. The level of arousal increases the overall muscle tone of the upper airway helping to return to a rhythmic pattern of breathing. Direct evidence that arousal from sleep is the key factor which terminates upper airway occlusion comes from the invariable occurrence of increased EMG activity in nonrespiratory postural muscles (i.e., submental muscle of the chin; nuchal muscle of the neck) just prior to the relief of airway occlusion and the frequent occurrence of gross body movements (Sullivan and Issa, 1980). The arousal response is thought to alleviate obstruction either by the activation of postural muscle activity in the tongue and pharyngeal muscles or by providing additional drive to the muscles of inspiration (Sullivan and Issa, 1980). It has been postulated that arousal, induced by asphyxia/hypoxia, is the crucial event which terminates obstruction in patients with severe apnea. Indirect evidence for this comes from the fact that supplemental oxygen administration lengthens the episodes of obstructive sleep apnea (Motta and Guilleminault, 1978).

However, controversy still remains regarding the exact mechanisms and neural pathways

responsible for provoking end-apneic arousal (Kimoff, 1996). The activation of chemoreceptors in response to either hypercapnia or hypoxia could lead to arousal either via direct projections to arousal centers in the brain (of which there is limited evidence). Conversely, the chemoreceptor activity could be mediated indirectly through an influence on respiratory drive and/or ventilatory effort (Bowes et al., 1984). Recent evidence by Kimoff et al. (1994) have provided support for the latter mechanism. They have shown that inhibition of respiratory muscle activity using inspiratory pressure support during a hypercapnic challenge significantly delayed the arousal response to CO₂ (Kim et al., 1995). Alternatively, they have demonstrated that increasing the amount of respiratory effort for a given degree of chemical stimulation (i.e., imposing a flow-resistive load during a hypoxic rebreathing challenge), significantly lowered the time to arousal (Yasuma et al., 1991). Furthermore, in studies of human subjects with OSAS, Kimoff et al. (1994) have demonstrated that changing the degree of chemoreceptor stimulation during apneas (i.e., lowering O₂ desaturation with oxygen administration or increasing hypercapnia with CO₂ administration), produced changes in apnea duration, but it did not change the level of inspiratory effort at arousal. Therefore, they concluded that arousal and apnea termination appeared to be most closely tied to the level of inspiratory effort and end-apnea, rather than the degree or nature of the chemical stimulation. This suggests that the mechanoreceptor impulses generated in the respiratory system during obstructed inspiratory efforts play a major role in mediating end-apneic arousal and termination of the apnea. Further support for this hypothesis is provided by physiologic data from patients with upper airway resistance syndrome (UARS). This syndrome typically involves snoring, upper airway flow

limitation, and repeated arousals that occur in temporal association with increased inspiratory effort, in the absence of significant oxyhemoglobin desaturation (Guilleminault et al., 1993). Thus, arousal appears to result from increased inspiratory effort and is independent of measurable fluctuations in blood gas exchange.

There are several key factors which modulate one's arousal mechanism. First, based upon evidence in sleeping dogs, sleep fragmentation will elevate the arousal thresholds to hypercapnia and hypoxia (Phillipson et al., 1980; Bowes and Phillipson, 1984). In addition, studies of sleep-deprived normal subjects showed decreased ventilatory response to hypoxia and hypercapnia (White et al., 1983). Second, chronic recurrent hypoxemia likely causes depression in neurotransmitter synthesis (i.e., acetylcholine) and thus, can result in dysfunction in arousal mechanisms (Sullivan and Issa, 1980), which will lead to changes in the arousal response to chemical stimulation. There is also evidence of an impaired arousal response to airway occlusion in OSAS patients. This is demonstrated by an increased time to arousal and inspiratory effort at arousal compared with compared with normal subjects undergoing induced airway occlusion during sleep (Kimoff et al., 1994). Furthermore, there is evidence that apnea duration is increased over the course of a single night, characterized by an increase in inspiratory effort and end-apnea (Charbonneau et al., 1994). This also suggests the development of an impaired arousal response to airway occlusion over the course of the night.

Sleep fragmentation which results from repetitive end-apneic arousal may also have an

impact on apnea severity. The frequency of apnea is determined by factors related to apnea initiation (i.e., upper airway collapse during sleep); whereas the degree of hypoxemia is influenced by the duration of the apneic episode, which is a factor of end-apneic arousal and apnea termination (Kimoff, 1996). Sleep fragmentation may influence both of these factors. It has been shown that sleep deprivation selectively decreases upper airway muscle activity in normal subjects (Leiter, 1985) and it increases the number of apneas and prolongs their duration in snorers and patients with mild OSAS (Guilleminault and Rosekind, 1981).

In obstructive sleep apnea in humans, the pattern of chronic sleep fragmentation and combined hypoxemia is often evidenced. As a result of upper airway occlusion, sleeping and breathing become mutually exclusive events and set up a cycle of arousals from sleep which may occur on the order of hundreds of times per night, which leads to elevation of arousal thresholds in sleep. It is highly possible that in the early stages of the disease, sleep fragmentation is the dominant factor depressing the arousal response to chemical stimulation; but as the individual develops greater levels of desaturation, hypoxemia becomes the critical factor. In addition, there is evidence in non-apneic (human) subjects that sleep fragmentation plays a role in increasing upper airway collapsibility, which could certainly contribute to the pathogenesis of the apneic disorder (Series et al., 1994). Furthermore, it has been demonstrated that central nervous system depressants (i.e., alcohol, sedatives, hypnotic drugs) are aggravating factors for OSAS which may be explained by two mechanisms: selective depression in hypoglossal muscle activity and an "anti-arousal" action which tends to prolong apneic episodes and leads to more severe desaturation (Berry et al., 1992;

Langevin et al., 1992).

4) Cognitive Dysfunction and Daytime Sleepiness in Obstructive Sleep Apnea Syndrome

Neuropsychological assessment has proven to be useful in localizing brain dysfunction and quantifying cognitive deficits in a wide variety of neurological and psychiatric conditions (Lezak, 1979). Neuropsychological abnormalities have also been documented in subjects with a variety of sleep-related disorders (Derderian et al., 1988). In particular, there is a body of evidence which documents cognitive impairments in OSAS (Berry et al., 1986; Findley et al., 1986; Greenberg et al., 1987; Bedard et al., 1991). Greenberg et al. (1987) compared cognitive functioning in a group of apneics, a sleepy control group (individuals with other sleep disorders associated with EDS), and a group of normal sleepers. The groups were fairly well matched on the demographic features of age, education and premorbid intelligence. They found that hypoxemic OSAS patients demonstrated cognitive dysfunction beyond what can be attributed to the effects of either EDS or aging. In particular, the OSAS group showed deficits on measures of attention, motor efficiency, and graphomotor ability compared to the controls. They also noted that the motor and perceptual-organizational deficits were related to the severity of nocturnal hypoxemia. Other neuropsychological test measures (i.e., sub-scales of the Wechsler Adult Intelligence Scale) and overall neuropsychological status (a clinical rating given by an independent experienced neuropsychologist) were minimally related to hypoxemia. They claimed that the observed

deficits in the OSAS group could not be attributed to sleepiness alone, since subjects in the groups were said to be comparable in their level of sleepiness. However, objective measures of sleepiness and sleep fragmentation were not adequately assessed in these groups. Instead, subjective ratings of sleepiness (SSS) and clinical judgment was used. Therefore, on the basis of this study, it is hard to discern the true nature of the cognitive impairment seen in the OSAS group.

Other investigators have since replicated these findings in OSAS patients with hypoxemia (Bedard et al., 1989, 1991; Engleman et al., 1993; Naegelé et al., 1995). Findley et al. (1986) examined the relative effects of hypoxia and nocturnal arousals upon cognitive function in a group of 26 OSAS patients. The apneics were divided into two groups on the basis of hypoxia (i.e., nighttime $\text{SaO}_2 < 90$; daytime $\text{PaO}_2 \leq 75$ mm Hg). They found that those patients with hypoxia had greater levels of cognitive impairment than those with apnea alone. Both waking and sleeping hypoxia levels were positively correlated with cognitive impairment. A major limitation in the Findley et al. study is that hypoxic patients also demonstrated daytime hypoxia (i.e., awake PaO_2 values ranging from 63-78 mm Hg and slight hypercapnia, with mean PaCO_2 value of 44 ± 2 mm Hg). Therefore, these patients do not represent the “typical” OSAS patient, evidencing only nocturnal hypoxemia. In addition, patients in this study were reported to have Respiratory Disturbance Indices (RDI) of 5 or greater per hour, without further breakdown of the number of observed apneas and hypopneas seen in each group. This severely limits the ability to determine the relative contribution of hypoxia and sleep fragmentation in the development of cognitive deficits.

Bedard et al. (1991) found that compared to a group of age- and sex-matched normal sleepers, moderate and severe OSAS patients were different on many tests of cognitive function, with the severe apneics demonstrating deficits on certain tests that were considered to be within normal limits in the moderate apneic group (i.e., deficits in general intellectual functioning, as in overall IQ, and tests of planning and shifting organizational set and constructive ability). They attributed this to a discontinuity in the appearance of cognitive deficits as OSAS progresses. The authors of these studies regard hypoxia as the primary cause of cognitive impairment, but do not rule out a continued role of sleep fragmentation in the development of cognitive deficits. Nevertheless, based on the results of these neuropsychological procedures, the extent, exact nature and cause of the cognitive impairment in OSAS is still not completely understood.

Neuropsychological deficits in other medical conditions with specific etiological factors can serve as a frame of reference for the impairments seen in patients with OSAS. One such condition is chronic obstructive pulmonary disease (COPD), which is associated with nocturnal and daytime episodes of hypoxia (Grant et al., 1982). The cognitive impairment observed in these patients was primarily related to abilities of abstract reasoning and complex perceptual-motor integration, but there were also decrements noted in motor speed, strength and coordination. Grant et al. (1982) noted that the level of dysfunction was systematically related to the degree of waking hypoxemia. However, it should be emphasized unlike the continuous waking hypoxemia of COPD, most OSAS patients experience only periodic nocturnal episodes of hypoxemia. In addition, it should be noted

that patients with COPD also evidence significant sleep disruption and report poor sleep quality (Cormick et al., 1986), which could also lead to changes in daytime functioning. Other studies have examined the effects of mild to moderate hypoxia on brain functions in normal subjects and found that abilities of concentration, short-term memory, and critical judgment were significantly impaired (Gibson et al., 1981). Investigators have also looked at hypoxemia induced by high altitude climbing (West, 1984, as cited by Berry et al., 1986) and found decreased motor speed (i.e., slower reaction times), declines in verbal learning, short-term memory and verbal fluency.

Current evidence suggests that apnea-related sleep fragmentation is largely responsible for symptoms of EDS associated with OSAS. Evidence in support of the contributing role of sleep fragmentation to daytime somnolence, leading to deficits in cognitive function, has been shown by studies in normal sleepers (Bonnet, 1986; Stepanski et al., 1987). As stated previously, Stepanski et al. (1987) found the strongest correlation with sleepiness was with brief nocturnal arousals. To further elucidate the role of chronic sleep fragmentation in the development of daytime deficits in OSAS, Roehrs et al. (1989) looked at objective measures of daytime sleepiness, nocturnal oxygen desaturation, and respiratory arousal index (RAI) in a group of 466 patients with OSAS. By using multiple regression analyses, they found that the greatest predictor of physiological sleepiness (based on MSLT scores) was the RAI. They did not totally rule out the contribution of hypoxia to daytime sleepiness, but concluded that hypoxia did not contribute any more information to the correlation compared with sleep fragmentation alone. In addition, improvement in sleepiness as a result of CPAP treatment

correlates more closely with improvement in sleep fragmentation measures than with improvement in hypoxemia (Sforza and Krieger, 1992). In addition, treatment of OSAS by oxygen administration relieved apnea-related periods of oxygen desaturation, but did not lead to improvements in daytime sleepiness (Gold et al., 1986). This finding implicated the ongoing contribution of sleep fragmentation to EDS. On the other hand, CPAP treatment which permitted the occurrence of repeated hypoxemic events (versus standard CPAP therapy which eliminates hypoxemia) also led to improvement in symptoms of EDS (Colt et al., 1991). Furthermore, studies employing experimentally-induced awakenings in otherwise normal sleepers have repeatedly demonstrated that sleep continuity may be more integral to restoration of performance than total sleep time or specific sleep stage amounts (Bonnet, 1985; 1986a; Downey and Bonnet, 1987; Levine et al., 1987; Stepanski et al., 1987). These data support the contributory role of sleep fragmentation to symptoms of daytime sleepiness.

In order to partial out the contribution of sleep fragmentation and hypoxemia on the cognitive impairment observed in OSAS, several investigators have tried to establish neuropsychological test batteries that are sensitive to hypoxemia-related impairments, as well as tests sensitive to sleepiness-related impairments (Roehrs et al., 1995). In general, hypoxemia-sensitive tests are those assessing complex and abstract reasoning and perceptual-motor skills (i.e., Trailmaking test; Digit Symbol test; Purdue Pegboard); sleepiness-sensitive tests involve functions of attention, tracking, memory and learning (i.e., Digit Span; Four-Choice Reaction Time Task) (Bedard et al., 1993). More recent work by

Naegele et al. (1995) has implemented a battery of cognitive tests which directly target frontal lobe functioning in 17 patients with OSAS. These frontal lobe, "executive function" tasks assessed planning and programming capacities, categorizing activities, and verbal fluency. The tests of planning and categorical organization were The Wisconsin Card Sorting Test (modified version), the Tower of "Toronto", and The 20 Questions Procedure. Other tests assessed attention, short-term memory spans, and learning abilities. In general, their findings replicated the results of Bedard et al. (1991) by confirming deficits in tasks related to frontal lobe functioning. Naegele et al. concluded that OSAS patients had a significantly decreased ability to initiate new mental processes and to inhibit automatic processes, and displayed a tendency to for perseverative errors.

OSAS patients have consistently demonstrated a variety of cognitive impairments: deficits in attention and visuo-motor abilities (Greenberg et al., 1987; Bedard et al., 1991); long-term memory (Bedard et al., 1991); and intellectual efficiency (Telakivi et al., 1988; Montplaisir et al., 1992). In addition, decrements in alertness/wakefulness, as assessed by the Multiple Sleep Latency Test, have been observed in untreated OSAS patients in many studies (Wittig et al., 1986; Lamphere et al., 1989; Montplaisir et al., 1992; Roehrs et al., 1995). It is thought that a subset of the cognitive impairments observed in OSAS, particularly attentional and memory deficits, are primarily related to the decreased wakefulness in these patients before treatment. Data from most studies have confirmed an impairment of the "executive functions", which refer to the mental capacities necessary for formulating goals, planning how to achieve them, and carrying out the plans effectively

(Luria, 1966; Lezak, 1982). Evidence from both human and animal studies have implicated the prefrontal cortex as the neuroanatomical substrate for these executive functions (Lezak, 1982). However, there are no findings which have documented specific neurological impairment in prefrontal areas in patients with OSAS. This does not rule out the possibility of the development of cognitive deficits which are related to imbalances in neurotransmitter systems. For example, studies by Gibson et al. (1980; 1981) have implicated the role of hypoxemia in the impairment of central cholinergic metabolism. Acetylcholine is involved in higher cognitive brain functions and changes in cholinergic transmission can contribute to deficits in arousal, learning and memory (Gibson et al., 1981).

Therefore, the overriding feeling to date is that the cognitive deficits observed in OSAS are related to problems of attention, short- and long-term memory, and executive tasks (Bedard et al., 1993; Engleman et al., 1994; Naegele et al., 1995). These studies are fairly controversial with regard to the relative contribution of sleep fragmentation and hypoxemia in the development of these deficits. Nevertheless, it appears that sleepiness and sleep fragmentation have a greater impact on tests of sustained-attention, memory or learning, whereas nocturnal hypoxemia is more closely related to performance on tests of complex or abstract reasoning, or psychomotor performance. Furthermore, it has not been determined as to whether these cognitive functions fully normalize following an appropriate course of treatment for OSAS. Following CPAP treatment, it appears that sleep fragmentation-related impairments tend to revert to relatively normal values (Bedard et al., 1993; Engleman et al., 1994); impairment of planning abilities and manual

dexterity/psychomotor skills may not revert completely following treatment (Bedard et al., 1993). In general, it is believed that both sleep fragmentation and hypoxemia play a role in the development of cognitive deficits witnessed in OSAS, with hypoxia becoming a more significant factor when it is particularly severe, or when there are prolonged hypoxic periods.

5) Treatment of OSAS: Nasal Continuous Positive Airway Pressure (CPAP)

Nasal continuous positive airway pressure (CPAP) has been considered as the non-surgical treatment of choice for OSAS (Sullivan and Grunstein, 1989; Series et al., 1992). The CPAP unit consists of a small compressor which is attached to flexible plastic tubing which connects to a fitted nasal mask (or nasal pillows which are inserted into the nares) which the patient wears to bed. Continuous positive pressure is applied through a tight fitting nasal mask. The pressure acts as a pneumatic splint and prevents airway collapse. When there is airway obstruction, the positive pressure from the unit builds against the obstruction, forcing the throat open and splinting the airway until normal breathing resumes. Once breathing is adequate, the air simply blows past the nostrils.

The main goal of treatment with CPAP is to minimize the occurrence of the arousal-provoking stimuli (apneas) that produce sleep disruption and associated daytime consequences (Kimoff, 1996). To that end, CPAP is effective in maintaining upper airway patency, normalizing blood gases, and inspiratory effort during sleep. Many studies have noted that therapeutic CPAP administration is associated with significant improvement in

sleep-related respiration, oxygenation, and sleep fragmentation (Rajagopal et al., 1986; Lamphere et al., 1989; Phillips et al., 1990). Since many patients with OSAS experience chronic REM sleep and slow wave sleep deprivation and disruption (Dowdell and Javaheri, 1992; Series et al., 1992), the first night of CPAP is often associated with an increase in REM sleep and/or slow wave sleep time (REM and SWS rebound). REM rebound is frequently accompanied by increased density of eye movements (REM density) (Issa and Sullivan, 1986; Aldrich et al., 1989). Of particular note, when patients with OSAS display SWS episodes prior to treatment, the apneic episodes tend to be less severe than in stage 1, stage 2, or REM sleep (Kimoff, 1996). This may be related to the homeostatic component of SWS which attempts to preserve some minimal balance between sleep and wake regulation in these patients (in this case, by maintaining a lower degree of respiratory instability during SWS, the occurrence of SWS is permitted). This finding also has implications for determining adequate CPAP pressure levels during the initial night of therapy. Early in the sleep period the patient may require lower pressure settings to maintain upper airway patency, until they reach their first REM period, which may necessitate an increase in pressure settings due to the muscle atonia and changes in response to chemical stimulation associated with REM sleep.

Initial improvement in EDS has been reported after a single CPAP treatment night as measured subjectively by self-ratings of sleepiness (Rajagopal et al., 1986; Baker et al., 1988) and by objective measures of daytime sleepiness (mean sleep latency on the Multiple Sleep Latency Test - MSLT) (Lamphere et al., 1989; Sforza and Lugaresi, 1994; Kribbs et

al., 1992). However, the degree of objective improvement in EDS based on MSLT scores often reveals a level of sleepiness that is still clinically significant (mean sleep latency < 10 minutes) and not within the range of alert, non-sleepy adults (Engleman et al., 1992; Kribbs et al., 1992; Sforza and Lugaresi, 1994). Continued improvement in objective measures of EDS has been demonstrated after 2 weeks of continuous CPAP usage (Lamphere et al., 1989). In the study by Lamphere et al. (1989) continued CPAP usage up to 6 weeks did not result in any further improvement over the 2 week period on MSLT testing (which at 2 weeks had reached a functionally alert range of 9.6 minutes). They site the possible lack of compliance with CPAP therapy as a source of their failure to achieve continued improvement in daytime symptoms. Gaddy and Doghramji (1991) examined objective and subjective sleepiness in a group of 63 OSAS patients who used CPAP for a minimum of 26 days. They found that 60% of the treated group remained measurably sleepy (as defined by mean sleep latency values on MSLT < 10 minutes). The residual sleepiness post-treatment was not related to any NPSG evidence of residual apnea or sleep fragmentation (as measured by WASO or SE). They speculated that CPAP itself may serve as a source of sleep disturbance which perpetuates EDS. However, the data did not include estimates of nightly CPAP usage, which could have contributed to the failure of some of the subjects to revert to more normal levels of daytime sleepiness.

Complete normalization of EDS (as measured by the MSLT) has been demonstrated after 6 weeks of CPAP treatment by one group of investigators (Wittig et al., 1986). On the whole, the severity of the disorder was quite high in this group of OSAS patients, with a

mean "respiratory events index" of 79 events per hour, which was associated with a fairly high degree of hypoxemia (mean of 7.3 minutes/hr. spent at $\text{SaO}_2 < 85\%$). However, other investigations by Sforza et al. (1995) and Engleman et al. (1994) have shown that while long-term CPAP therapy (> 1 year) leads to a significant reduction in daytime sleepiness in OSAS patients according to MSLT values, many patients still do not completely "normalize" (mean values remained < 10 minutes on MSLT). Sforza et al. (1995) found that patients who became "normal" tended to have a higher AHI at baseline, a greater improvement in nocturnal hypoxemia, and greater reported compliance with CPAP treatment at follow-up. This is consistent with the improvement noted in the patients in the study of Wittig et al. (1986). Therefore, these studies suggest that compliance may be a factor of the patient's initial perception of improvement after instituting CPAP; the greater the pre-treatment level of daytime impairment, the higher the motivation to continue to use CPAP. This statement is also supported by findings of Kribbs et al. (1993b) which demonstrated that both frequency and duration of CPAP use in the first month reliably predicted use in the third month.

Similarly, measures of cognitive functioning have demonstrated that certain abilities related to vigilance (i.e., memory and attentional tasks) were restored to a level comparable to that of control subjects, while deficits persisted in executive and psychomotor-related tasks even after 6-10 months of CPAP therapy (Montplaisir et al., 1992; 1993). Additional evidence has shown improvements in both objective daytime sleepiness (MSLT) and mood in a group of OSAS patients after 3 months of CPAP treatment, but no evidence of major

improvements in cognitive function (Engleman et al., 1993). Other studies have also demonstrated generalized improvement in mood in OSAS patients following one to two months of CPAP therapy (Derderian et al., 1988; Charbonneau et al., 1992).

The major obstacle to the accurate interpretation of these discrepant findings on tests of cognitive ability and mood is the lack of information regarding CPAP compliance in most of these studies. In general, the studies do not provide any information regarding nightly CPAP usage. There is abundant evidence to suggest that even subjective reports of usage may be relatively inaccurate, as they can often differ significantly from actual usage (Kribbs et al., 1993). A few studies have obtained more direct information regarding nightly CPAP usage through the use of covert monitors that are built into the CPAP devices to detect actual pressure at the mask for every minute over a 24-hour period (Kribbs et al., 1993). Kribbs et al. have found that when CPAP was used, the mean duration of use was 4.88 ± 1.97 hrs. Furthermore, patients' subjective reports of CPAP use overestimated actual use by over 1 hour. Engleman et al. (1993) conducted one of the few studies that combined measures of nightly CPAP usage, objective sleepiness (MSLT), and cognitive performance. They found that the average usage for a group of 21 OSAS patients was 5.9 hours per night. This is slightly higher than the value found in the Kribbs et al. study and could be attributed to the fact that they monitored "machine on" time and not the time spent at effective pressure. Therefore, an important aspect of the present study is to incorporate measures of compliance, which include the following: a report of the hours of CPAP usage (determined by a counter on the back of patient's CPAP unit, which gives the actual number of hours that machine

was "on"); telephone call-in information which provides a subjective report of CPAP usage and to document any difficulty with using CPAP or adverse experience; written sleep-wake diaries which also confirm the hours in bed and sleep time (with or without CPAP). These measures will be further correlated with daytime improvement measures (i.e., MSLT and MWT values and FCRTT scores).

G) Summary of Issues

The present study will attempt to address these specific issues:

- 1) Do OSAS patients demonstrate a disturbance in their nocturnal temperature rhythmicity compared to a group of sex and age-matched non-complaining sleepers?
- 2) Does short-term CPAP treatment alone in OSAS subjects result in normalization of temperature rhythms compared to pre-treatment values?
- 3) Does adjuvant exogenous melatonin administration augment the effects of acute CPAP treatment in OSAS patients as evidenced by decreased daytime deficits?
- 4) Can the severity of the apneic disorder as measured by the daytime deficits and subsequent response to treatment be predicted by the degree of the nocturnal temperature rhythm disturbance, apnea-hypopnea index (AHI), sleep fragmentation, or nocturnal hypoxemia indices?

II. METHODS:

A) Subjects

Sixteen patients with polysomnographic confirmed diagnoses of moderate to severe OSAS were recruited from the New York University Medical Center Sleep Disorders Center. Subjects were between 21-65 years of age. Due to the nature of OSAS, subjects were predominantly middle-aged males; however, there were no restrictions for sex or ethnic background. OSAS subjects were randomly assigned to either a CPAP/Melatonin or CPAP/Placebo condition so that eight OSAS subjects were in each treatment cell. Eight healthy individuals without sleep complaints were recruited to serve as matched control subjects. These non-complaining sleepers were matched to the OSAS group on the basis of gender, age, and whenever possible, body mass index (BMI). These individuals were screened with polysomnography to ensure that they did not have apnea or another sleep disorder that produces excessive daytime sleepiness (i.e., periodic limb movement disorder).

All subjects received a complete medical history and physical examination by the medical staff of the Sleep Disorders Center. All subjects received a full explanation of the research protocol and were informed of the experimental nature of this work. Written informed consent was obtained from all research participants (in accordance with the Human Subjects Institutional Review Board). All data collected were kept strictly confidential and information was released to their physicians upon request. At the termination of the study,

subjects were debriefed as to the nature of the experiment and were informed as to their treatment condition (CPAP/ Melatonin, or CPAP/Placebo condition).

A total of 26 subjects entered the protocol. Three subjects in the OSAS group failed to complete the study. One individual was excluded due to the presence of a secondary sleep disorder which was discovered during the initial NPSG; two others were lost to follow-up as a result of their decision to withdraw from the study. None of the subjects failed to complete the study as a result of complaints related to treatment condition (adverse reaction to melatonin or CPAP).

Inclusion Criteria

Criteria for inclusion in the OSAS group was an apnea-hypopnea index (AHI) greater than 25 events per hour of sleep. Inclusion criteria for the matched control group was an AHI of less than 10 events per hour of sleep.

Exclusion Criteria

Subjects with any of the following were excluded from further participation:

1. Medical disorders as follows:

- Endocrine abnormalities (i.e., uncontrolled diabetes, thyroid disorder, pregnancy)
- Neurological disorder (i.e., epilepsy, head trauma, mental retardation, coma, stroke,

other neurological disease)

-Cardiovascular disease (i.e., congestive heart failure, coronary artery disease, vasculitis of extremities, uncontrolled hypertension)

-Pulmonary disease (i.e., COPD, emphysema, TB)

-Rheumatoid disorder (i.e., injury, neuromuscular disorders which have the propensity to cause pain)

-Psychiatric illness (i.e., current major depression; schizophrenia; manic-depression)

-Evidence of any other primary sleep disturbance on initial NPSG (i.e., narcolepsy, periodic leg movements)

2. Toxic exposure: current or history of extensive drug/alcohol abuse; exposure to chemotherapy or radiation; exposure to occupational or other environmental toxins such as pesticides or solvents

3. Medication: current or prolonged use of psychotropic or other sleep-altering drugs (i.e., benzodiazapenes, corticosteroids) during the last 6 months; birth control pills or hormone replacement drugs; chronic use of anti-inflammatory drugs (i.e., ibuprofen, salicylates); current use of B-adrenergic blocking drugs (i.e., atenolol, propranolol)

B) Protocol

In order to standardize NPSG collection times, potential subjects were asked to adhere

to a regular bedtime schedule for a minimum of 2 weeks prior to their scheduled date for NPSG evaluation. This was confirmed through the use of written sleep-wake logs that subjects filled out for a 2 week period prior to coming into the laboratory. NPSG evaluation was conducted primarily between the hours of 11pm and 7am, with some exceptions made for subjects with slightly later in-bed times and later rise times.

The protocol consisted of the following phases and assessment measures:

<u>Weeks 1-2</u>	<u>Week 2</u>	<u>Weeks 3-5</u>	<u>Weeks 5-6</u>	<u>End of Study</u>
Baseline	CPAP Titration	Treatment (short-term)	Post-Treatment	Unblinding/Debriefing
HOME Sleep-wake logs (2 weeks at home prior to lab testing + 2-3 days with home monitoring) ----- LAB NPSG MSLT MWT FCRTT Neuropsych. Testing ESS SSS BT/UT Questions ----- HOME Actigraphy (2-3 days) Body Temperature (")	LAB NPSG with CPAP titration to therapeutic levels BT/UT Quest.	HOME CPAP used nightly + Melatonin or Placebo (x 14 nts) Sleep-wake logs CPAP usage from counter (hrs) Telephone call-in	LAB NPSG MSLT MWT FCRTT Neuropsych. Testing ESS SSS BT/UT Quest. ----- HOME Actigraphy (2-3 days) Body Temperature (")	Break blinded code for tx. Debrief S's

C) Procedures

The study implemented a randomized, matched block design where subjects in each group were matched on the basis of gender and age (by decade). OSAS subjects were randomly assigned to either a CPAP/Melatonin or CPAP/Placebo condition by the staff of the Drug and Formulary Department of Bellevue Hospital. Melatonin was administered in a double-blind fashion, beginning on the first night of home treatment. Therefore, the main experimental intervention was to compare the OSAS subjects' response to exogenous melatonin or placebo administration on measures of body temperature, sleep and daytime performance.

Testing procedures in the laboratory were identical for all groups. However, due to laboratory scheduling and staff constraints, control subjects did not undergo a repeat of the NPSG and daytime laboratory testing at weeks 5-6. In addition, following the Baseline assessment phase and again at the end of the Post-Treatment phase, all subjects were required to undergo 2-3 days of temperature and actigraphy monitoring at home to ascertain circadian patterns of temperature and rest-activity. Four matched control subjects were studied 5-6 weeks after their initial study to assess the stability of their temperature and sleep-wake patterns via actigraphy and temperature monitoring at home. Due to equipment limitations, it was possible to run only four subjects in the repeat assessment phase. This was performed to rule out regression to the mean.

Matched normal control subjects completed the Baseline assessment phase of the study to obtain normative sleep and daytime performance measures. OSAS subjects underwent one additional night of NPSG testing with therapeutic titration of nasal CPAP which was their first treatment night. During this night, CPAP pressure was titrated for each subject so that apneic/hypopneic and desaturation events were abolished. In addition, episodes of flow limitation were obliterated. The determined CPAP pressure level was maintained throughout all phases of the study. Most importantly, after 2 weeks of continuous CPAP usage, only those OSAS subjects demonstrating compliance with CPAP treatment (as confirmed through daily logs, telephone call-in, CPAP usage monitor) were considered for further participation in the study. Based upon Kribbs et al's. (1993) study on patterns of CPAP use in patients with OSAS, an acceptable level of compliance with CPAP treatment was defined as usage of at least 4 hours per day on at least 70% of all days monitored.

OSAS subjects in the short-term treatment phase were given capsules which contained either 3 mg of "Melatone" (an over-the-counter, non-prescription preparation manufactured by Cardiovascular Ecological Formulas), or placebo (cornstarch in an unmarked gelatin capsule, which looked identical to melatonin capsules), as prepared by the Drug and Formulary Department of Bellevue Hospital. Subjects were instructed to take 2 capsules at their scheduled bedtime (as determined by sleep-wake logs) for 14 consecutive nights at home. The Melatone preparation delivered a total equivalent of 6 mg of synthetic melatonin. Based on preliminary information from an independent laboratory, radioimmunoassay (RIA) analysis of the formulation demonstrated that each capsule contained the equivalent of 3 mg

of melatonin. In addition, in order to better enhance the data, the Clinical Research Center at Oregon Health Sciences University conducted similar tests of the sample being used in the present study at two separate times to quantify the amount of melatonin in each capsule and to assess the stability of the compound (Time 1 and Time 2, which was approximately 6 months later). Based upon gas chromatography mass spectrometry (GCMS) and RIA analyses, it was found that the capsules in the sample lot consisted of 3mg of melatonin at Time 1 and Time 2. Therefore, the Melatone formulation contained the appropriate amount of melatonin and was considered a stable compound.

OSAS subjects underwent a repeat of the Baseline testing procedures in the laboratory after 2 weeks of continuous CPAP/melatonin or CPAP/placebo administration (see Protocol for details of testing procedures). This included a repeat NPSG, neuropsychological testing, MSLT, MWT and daytime performance on the FCRTT. In addition, several questionnaires were re-administered (SSS, ESS, Bedtime and Up-time Questionnaires). Exact specifications for each of the tests are as follows:

a. Body Temperature: Body temperature was monitored continuously using a rectal probe which was connected to a multi-channel temperature logger (Mini-Logger, Mini-Matter). Subjects were instructed how to insert/remove temperature probes and on general care of the equipment. Average collection time was 2-3 days at Baseline (Week 1-2) and at the Post-Treatment phase (Week 5-6). The data were downloaded onto a PC computer for further analysis.

b. Nocturnal Polysomnography (NPSG): All subjects were studied with standard nocturnal polysomnographic procedures which consisted of recording 2 monopolar electroencephalographic (EEG) leads (C3-A2 and O1-A2), 2 monopolar electro-oculograms (EOG), bipolar submental (chin) and tibialis (leg) electromyograms (EMG), and electrocardiogram (ECG). Respiratory airflow was monitored through the use of a nasal cannula attached to a 2 cm H₂O pressure transducer. This airflow recording technique is more sensitive with regard to the detection of flow limitation than the standard nasal/oral airflow thermistor recording technique (Rapoport, 1996). Respiratory effort was measured by plethysmography using piezoelectric sensors (Resp-EZ, EPM Systems). Oxygen saturation was measured with a finger clip lead and pulse oximeter (Microspan, Biochem, or Oxy-Shuttle, SensorMedics). All the sleep parameters were collected simultaneously by a Grass Model 78 polygraph and by a computerized acquisition system (Sleepscan, Bio-logic). Sleep was scored in 30 second epochs according to standard criteria of Rechtschaffen & Kales (1968).

c. Assessment of Daytime Function: There were four classes of tests that were used to evaluate daytime functioning and the presence of sleepiness following NPSG. These included: 1) objective assessment of sleepiness and sleep tendency using the Multiple Sleep Latency Test; 2) objective assessment of behavioral wakefulness/vigilance using the Maintenance of Wakefulness Test and the Four-Choice Reaction Time Test, respectively; 3) objective assessment of neuropsychological functioning; and 4) subjective measures of sleepiness using the Stanford Sleepiness Scale and the Epworth Sleepiness Scale. The four

classes of daytime functions employed in the study have been shown to be impacted by sleep deprivation and untreated OSAS (Dinges, 1992).

1) Multiple Sleep Latency Test (MSLT): To assess objective, physiological sleepiness, subjects underwent the MSLT (Carskadon et al., 1986) during the daytime testing sessions in the laboratory. Subjects were given four opportunities to fall asleep, starting at approximately 1000 hours and occurring at regular 2 hour intervals. Due to the addition of several other daytime performance measures, it was deemed necessary to curtail the number of nap opportunities from the standard five-nap MSLT procedure. The monitoring was identical to the recording montage used during the NPSG (EEG, EOG, chin EMG and ECG). Each sleep session ("nap") was terminated with the polygraphic determination of sleep onset (one 30 second epoch of any stage of sleep) or after 20 minutes, if sleep did not occur. This was done in order to eliminate the possible accrual of sleep during each session. Each nap was scored for the latency (time in minutes) to onset of sleep and for the presence of REM and NREM sleep.

In addition, post-treatment MSLT evaluation in the OSAS group was conducted with the CPAP device, where the pressure was set at the subject's prescribed therapeutic titration level. During these sessions, CPAP pressure and airflow was monitored in addition to the standard MSLT recording montage (according to recommended practice parameters outlined by Thorpy, 1992). This was done since OSAS subjects were now accustomed to sleeping with CPAP, even during nap sessions at home.

2) Maintenance of Wakefulness Test (MWT): In order to objectively assess the

ability to stay awake, subjects underwent the MWT (Mitler et al., 1982). Subjects were given four, 40-minute sessions beginning at 0900 hours and occurring every two hours. Subjects were instructed to "try to stay awake" while semi-recumbent in a quiet, darkened room. The recording montage was identical to that used in the MSLT. The session was terminated with the polygraphic determination of sleep onset (one epoch of any stage of sleep), or after 40 minutes if sleep did not occur. Similar to the MSLT, each session was scored for sleep latency and the presence of REM vs. NREM.

3) Four-Choice Reaction Time Test (FCRTT): The FCRTT is a sensitive and validated psychomotor measure of alertness (Wilkinson & Houghton, 1975). The FCRTT is delivered on a PC-based computer system and requires 10-15 minutes to complete. It was carried out by subjects once every 2 hours during the daytime, beginning at approximately at 0840 hours. A total of five testing sessions were administered. The visual stimuli consisted of a pattern of 4 green-outlined squares, with one square displaying a red square inset. This pattern randomly alternated for each stimulus presentation. The keyboard control consisted of a corresponding square of 4 push-buttons. Subjects were required to press the corresponding button whenever a red square was detected. Subjects' responses were downloaded to the computer and stored for future analysis. As recommended by Glenville & Wilkinson (1979), three practice sessions of the test were performed the previous evening in order to minimize practice effects that can occur when practice and testing sessions take place on the same day.

4) Assessment of Neuropsychological Functioning: Neuropsychological tests were selected to cover a broad range of functions known to be affected in OSAS (Bedard et

al., 1989; Findley et al., 1986). The battery employed is a replication of that used by Bedard et al. (1991) and is outlined more fully in the "Protocol Schedule" (SEE APPENDIX I). The full scaled score of the WAIS-R was implemented to determine general cognitive functioning. Attention was assessed by the Digit Symbol test of the WAIS-R. Memory (verbal and nonverbal) was assessed by the stories of the Wechsler Memory Scale (WMS) and by the Rey-Osterreith Complex Figure (both immediate and delayed recall condition). Other tests were given to assess manual dexterity (Purdue Pegboard) and executive functions (Trail Making Test - Part B of the Halstead-Reitan Battery; Verbal Fluency Test; Rey-Osterreith copy condition; Mazes of the WISC-R; Picture Arrangement of the WAIS-R; Block Design of the WAIS-R).

5) Stanford Sleepiness Scale (SSS): Subjective sleepiness was assessed using this Likert-type, 7-point, self-rating scale (Hoddes et al., 1973). This was administered before the lights-out period on nights of NPSG testing in the lab and upon awakening. In addition, the SSS was given before each MSLT and MWT testing session to assess subjective sleepiness across the entire day.

6) Epworth Sleepiness Scale (ESS): Subjects completed this simple, self-administered questionnaire at the beginning of the Baseline and Post-Treatment phase of the experiment. The subject rates the likelihood of falling asleep during eight specific sedentary activities (Johns, 1991; 1992; 1993). The ESS assesses the subjective level of persistent daytime sleepiness.

7) Bed-time and Up-time Questionnaires: Subjects completed self-rating scales regarding their sleepiness/alertness prior to their scheduled bed-time and upon arising in the

morning during the NPSG phases of the study. In addition, questions regarding daytime level of activity, caffeine or medication intake, nap opportunities are incorporated into the BT Questionnaire. UT Questionnaire also inquires about the subjective level of refreshment regarding the prior nocturnal sleep episode.

d. Home Assessment of Sleep-Wake Activity and Treatment Compliance: The information from diary and call-in techniques ensured that we had complete data on sleep-wake behavior in the week prior to NPSG assessment for each phase of the experiment. In addition, the actigraphy data provided us with information on rest-activity cycles, as well as sleep fragmentation, during the Baseline and Post-Treatment phases of the experiment. The home assessment data also permitted us to assess whether subjects were sleeping longer than indicated by the counter on the CPAP machine.

1) Actigraphic Monitoring: Objective sleep-wake activity monitoring was carried out using actigraphs worn on the subject's non-dominant wrist. The Mini-Motionlogger actigraph unit (Ambulatory Monitoring, Inc.) was worn by the subject for a minimum of 2-3 days following the baseline phase and at the end of the post-treatment phase of the study. The unit was programmed to record movement in 10 second epochs and data were downloaded and analyzed by a desktop PC computer.

2) Daily Diaries: The sleep-wake diary was completed by each subject for 2 weeks prior to the Baseline phase of the study. In addition, subjects were asked to continue log-keeping during home monitoring for temperature and sleep-wake activity in order to correlate bedtimes and rise-times to better interpret the data (Week 1-2 and Week 5-6).

Subjects were asked to complete sleep-wake logs during the Treatment phase CPAP trial (Weeks 3-5) to assess compliance with CPAP and to ensure regular sleep-wake times. The sleep-wake diary was completed twice each day: once in the morning upon arising to report their nocturnal sleep and once in the evening before retiring to bed to document their daytime activities.

3) Telephone Call-In Data: Subjects were asked to call into a telephone answering machine once a day to report in-bed time, up-time, total sleep time, and estimated hours of CPAP usage for that night. Any other sleep-related difficulties were also reported (i.e., illness, new medication, difficulties with CPAP usage or adverse effects of melatonin/placebo). All subjects were given a written script to follow to facilitate their understanding of the information being requested. Telephone call-in data was used to assess subject compliance and to document the presence of adverse effects.

4) Monitoring of Nasal CPAP Use: CPAP treatment compliance was estimated from the counter on the back of the home CPAP delivery system which displayed the hours of machine "on-time". When subjects returned for their post-treatment assessments, they were asked to bring their CPAP machines so this counter could be read by the technical staff of the NYU Sleep Disorders Center.

D) Study Parameters

The investigational procedures incorporated in the study provided the following variables:

a. Body Temperature: Temperature data was sampled at 2 minute intervals with an average collection time of 2-3 days. Raw data was smoothed by using a 5-point moving average. As stated previously, the polynomial regression approach more closely approximates visual analysis of temperature data and accounts for more of the variance in the data than the cosinor technique (Broughton et al., 1993). Therefore, curve fitting of the data was accomplished by employing the following polynomial regression algorithm (up to ninth order) using the method of least squares:

$$Y = m_0 + m_1 * x + m_2 * x^2 + m_3 * x^3 + \dots + m_9 * x^9$$

The phase (Φ) of the temperature nadir of the fitted curve was calculated as clock time (in hours) with reference to local midnight. The amplitude (A) of the fitted curve was defined as the value from peak to trough. The mean of the smoothed temperature data was used as the "mesor" equivalent, since the polynomial intercept value tended to overestimate the average temperature for each subject. In addition, the integrity of the temperature rhythm was ascertained by a "goodness of fit" index (R^2 = coefficient of determination), which reflects the proportion of the total variance in the raw data accounted for by the polynomial regression function. An acceptable level for the "goodness of fit" of the fitted curve was greater than 0.50. In addition to the derived amplitude, "goodness of fit" was used as a measure of improvement in circadian rhythm strength in the Baseline phase versus the Post-Treatment phase. Time spent below the mean (the mesor equivalent) was examined over a 24 hour period to assess the magnitude of the change in the temperature rhythm as a

function of treatment in the OSAS group.

b. Nocturnal Polysomnography: Sleep parameters included the following: Total Sleep Time (TST); Total Time in Bed (TIB); Sleep Latency (SLAT); Latency to REM Onset (REMLAT); Sleep Efficiency (SE) - defined as $TST/TIB \times 100\%$; and percent time spent in each stage of sleep (% Stage 1, 2, 3, 4, REM, Awake). Indices of sleep fragmentation included time spent awake after sleep onset (WASO) and a frequency analysis of consecutive epochs of sleep (FACE) (Rothenberg, 1987), which represents the number of transitions to Stage 1 or Awake for the entire night. Phasic REM activity was estimated using the measures of REM index (REMI) and REM density (REMD) (Kupfer and Foster, 1975; Gillin et al., 1979). REM index was defined as the sum of eye movement scores during REM sleep for the entire night, based on a score of 0 to 8 per minute of REM; REM density was defined as REM Index/REM Time.

Respiratory parameters included the following: episodes of cessation of nasal airflow for greater than 10 seconds were classified as an apneic event. It was called "central" when there was no associated respiratory effort, "obstructive" when there was continuing respiratory effort during the event as indicated by movements of the chest and abdomen, and "mixed" when there was initially no respiratory effort but effort later in the apneic episode. A hypopneic event was defined as at least a 50% decrement in airflow, for greater than 10 seconds, and was associated with one or more of the following: a fall in oxygen saturation (at least a 4% drop from baseline SaO_2 values), frequent arousals from sleep, or

bradycardia. Respiratory events were examined for number, length and type. The cumulative number of respiratory events per hour of sleep yielded an overall apnea-hypopnea index (AHI). The AHI was calculated separately for supine, lateral and prone body positions. O₂ saturation was plotted across time of night. These data were used to obtain a plot of cumulative time at different levels of O₂ saturation (Slutsky & Strohl, 1980). From this plot the following variables were extracted: O₂ nadir (MINDESAT) and percentage of time spent at oxygen saturation levels of < 80% (PCT<80). In addition, number of desaturation events were calculated per hour of sleep ("desaturation index" = DESATIND).

c. Assessment of Daytime Function: Assessment of daytime function and its relationship to improvement in circadian and sleep fragmentation parameters were the key outcome variables.

1) MSLT: An average sleep latency score (SLAT) was calculated based on the individual "nap" sessions. The number of REM onset periods was also noted. If REM was present, an average REM latency score (REMLAT) was calculated for the 4 naps.

2) MWT: An average sleep latency score (SLAT) was calculated based on the 4 "stay awake" sessions. Similar to the MSLT, REM onset periods were noted and REMLAT was calculated.

3) FCRTT: The following variables were derived based on the subjects' responses: mean reaction time (RT), number of gaps (long latency responses with RT>1000ms), accuracy (%), and throughput (speed-accuracy product). For each of these scores, means were computed for each of the five testing sessions. Daily mean values were

also calculated for each of the variables. In addition, the value for the number of gaps per trial underwent a square root transformation in the final analysis. This was due to the fact that the group variances were proportional to the means.

4) Neuropsychological Measures: Standard scores for the WAIS-R scales and subtests were used. Raw scores were used for the other test measures. Additionally, the Mazes of the WISC-R was scored for the total number of impulsive errors (i.e., backtracking into a blind alley; crossing lines of maze- not attributed to poor dexterity). Purdue Pegboard score was the total number of pegs introduced with the dominant hand. In the Trail Making Test (Part B) both the time of execution and the presence of shifting errors were noted.

5) SSS: A score of 1 ("most alert") to 7 ("sleepy") was obtained for each administration of the SSS.

6) ESS: Each question was given a 0 ("least likely") to 3 ("most likely") rating to combine to give a total possible score of 24. Total score was reported for each completed form.

7) Bed-time and Up-time Questionnaires: Subjects provided their estimates of the following variables: in-bed time, total sleep time, latency to fall asleep, quality of their sleep, level of daytime activity, and ingestion of caffeine, alcohol and medication.

d. Home Assessment of Sleep-Wake Activity and Treatment Compliance:

1) Actigraphic Monitoring: Analyses included the calculation of movement and fragmentation indices during sleep and wake periods. The frequency and distribution of motor activity during sleep (sleep disruptions) were analyzed using the Frequency Analysis

of Consecutive Epochs (FACE technique) (Rothenberg, 1987; Rapoport et al., 1995). Using the FACE technique, durations of continuous runs of inactivity were tabulated. Onset of the run was defined by a fall in movement intensity below a set threshold for more than one 10 second epoch. Termination of the run was defined by movement intensity above the set threshold. A curve was then constructed in which run length was plotted against the cumulative percent of time spent in runs of that length or less. The position of the curve was described by the weighted median run length (ACTIFACE), where 50% of the time spent in all inactivity runs was spent in runs of this duration or shorter. This provided an index of continuity of inactivity, which was an estimated measure of sleep fragmentation. Fragmented sleep, characterized by short runs of inactivity, was depicted by a left-shifted curve. Continuous sleep, characterized by long runs of inactivity, showed a right-shifted curve.

2) Daily Diaries: The diary consisted of general questions regarding sleep onset and offset, quality of sleep, and level of alertness upon awakening. Sleep quality was scored on a 5 point subjective rating scale, where 1 = "the best" and 5 = "the worst". There were also questions about daytime level of activity, daytime alertness, daytime sleepiness, number of naps that day, and medication, alcohol, caffeine, or medication ingestion.

3) Telephone Call-in Data: Similar to the daily diaries, the data provided subjective estimates of in-bed time, up-time, quality of sleep, and other qualitative information regarding CPAP usage.

4) Monitoring of Nasal CPAP Use: Total number of hours registered on the CPAP unit were logged for each phase of the experiment. The number of hours logged was

divided by the actual number of elapsed days to give an estimate of the average of nightly CPAP usage.

E) Data Analyses

The following analyses addressed the specific issues of the study:

1) Do OSAS patients demonstrate a disturbance in their nocturnal temperature rhythm compared to matched controls? This was assessed by examining the differences in derived amplitude, mean and phase of the temperature rhythm for the OSAS group before treatment (Baseline phase) and the matched control group using a multivariate analysis of variance procedure (MANOVA) and post-hoc Scheffé tests.

2) Does short-term CPAP treatment alone result in normalization of the nocturnal temperature rhythm in OSAS subjects? This was determined by first comparing the derived amplitude, mean and phase of the temperature rhythm for the OSAS group following 2 weeks of treatment and the matched control group at Baseline using a MANOVA and post-hoc Scheffé tests. After this step, a repeated measures ANOVA was employed to assess differences in temperature values in the CPAP/Placebo and CPAP/Melatonin conditions before and after treatment.

3) Does adjuvant exogenous melatonin administration augment the effects of acute

CPAP treatment in OSAS patients as evidenced by decreased levels of daytime sleepiness and cognitive impairment? This was ascertained by comparing daytime performance measures for each of the experimental conditions (CPAP/Melatonin and CPAP/Placebo) before and after treatment using a repeated measures ANOVA. Successful treatment outcome for all subjects was determined by the following criteria: Sleep Efficiency >85% and AHI < 5 events per hour. In order to control for differences in the level of CPAP usage, only those OSAS subjects meeting the criteria for CPAP compliance (Kribbs et al., 1993b) throughout the 2 week treatment phase underwent post-treatment assessment in the laboratory. Because it was hypothesized that exogenous melatonin administration would result in a significant decrease in nocturnal body temperature, it was expected that the sub-group of OSAS subjects receiving CPAP and melatonin treatment would have a larger drop in body temperature during the night as compared to the group receiving CPAP and placebo treatment. This, in turn, would be reflected by overall enhancement of the derived temperature amplitudes in the CPAP/Melatonin group versus the CPAP/Placebo group. It was predicted that these changes in nocturnal temperature regulation would be evidenced by concurrent improvement in the level of daytime functioning.

4) Can the severity of the apneic disorder as measured by the daytime vigilance deficits and subsequent response to treatment be predicted by the degree of the nocturnal temperature disturbance, respiratory events index, sleep fragmentation, or nocturnal hypoxemia indices? Because it was hypothesized that the prominent aberration in the temperature rhythm in the OSAS group prior to treatment is a lack of a drop in

nocturnal body temperature, which leads to a blunting of the overall amplitude of the temperature rhythm, the degree of nocturnal temperature disruption was defined by using the estimated values for the Amplitude and Mean. The values for the derived estimates of the temperature rhythm (Amplitude and Mean), respiratory events index (AHI), sleep fragmentation parameters (NPSG derived mean FACE values and actigraphy derived ACTIFACE values), hypoxemia indices (DESATIND and MINDESAT), and objective daytime measures of wakefulness/vigilance (mean sleep latency on the MWT and number of gaps on the FCRTT) were subjected to a series of correlations. Due to the large group variance observed in the OSAS subjects on the number of gaps on the FCRTT, the raw number of gaps in responding underwent a square root transformation prior to conducting further statistical analyses. In addition, due to the non-normal distribution of MWT data, Spearman rank-order correlations were considered more appropriate tests than multiple regression analyses. The first set of correlations examined the relationship between temperature, sleep, respiratory and daytime performance measures in the OSAS group prior to treatment. The second group of correlations examined the interrelationship of the change scores (post-treatment minus pre-treatment values) across temperature, sleep and respiratory parameters ("physiological parameters") and change scores on daytime performance measures to assess treatment response. A third set of correlations examined the relationship of the physiological parameters prior to treatment and change scores on daytime performance measures to predict response to treatment with regards to initial severity of the disorder.

In order to verify if the improvement with treatment "normalized" functioning in the

OSAS group, OSAS subjects were compared to the matched control subjects both prior to treatment and after 2 weeks of continuous treatment. This was done using multivariate analyses of variance (MANOVAs) and post-hoc tests using Scheffé's criterion. Due to the large number of dependent measures, study parameters were classified into three major sets of variables: Sociodemographic, Physiological and Neuropsychological. Each of the three sets of variables were submitted for MANOVA testing. Since each set of variables contained multiple measures, inferential statistics were performed on a sub-set of all measures collected; descriptive statistics were reported for all other measures. Since it was anticipated that the standard measures of sleep architecture (i.e., sleep stage percentages, WASO) and respiratory parameters (i.e., AHI, minimum oxygen desaturation) would correct following continuous CPAP therapy, these parameters were not considered for further analysis in the final MANOVA. The inferential measures consisted of the following: 1) Sociodemographic and subjective data - Education (EDUC), Epworth Sleepiness Scale (ESS), Self-rating of Scale of Memory Function (MEM), Beck Depression Inventory (BDI); 2) Physiological variables - Temperature measures of Amplitude (AMPL), Mean, and Phase; Sleep measures of Mean FACE (FACE), SE, SLAT, REMLAT, REMI, and REMD; Actigraphy measures of sleep fragmentation (ACTIFACE); 3) Neuropsychological and Daytime performance measures - Mean SLAT values of the MSLT and MWT; number of gaps on the FCRTT (using square root transformation of the data); Performance IQ (PERFIQ) and Picture Arrangement (PICTARR) on the WAIS-R; time of execution on Trail Making Test - Part B (TRAILS B).

In addition, all other neuropsychological parameters were further evaluated using a blocked design analysis where subjects were matched by age and gender to the control group. Paired-sample *t* tests were used to examine differences between the OSAS group, both before and after two weeks of treatment, with the matched control group.

Subjective measures of sleepiness (SSS) were evaluated for all phases of the study using two factor repeated measures analyses of variance (where "Group" and "Time" were the factors).

In addition to the standard parametric analyses using the overall mean values for the daytime MSLT and MWT sessions, non-parametric analyses (Kruskal-Wallis ANOVA by ranks) were employed using the median values of these tests. This was done due to the fact that the nature of both of these tests lead to the censoring of data and for non-normal distribution of data.

In order to control for regression to the mean, paired-sample *t* tests were employed to assess body temperature and actigraphy measures in four of the subjects in the matched control group. Derived temperature values for Amplitude, Mean, and Phase and sleep fragmentation (ACTIFACE) measures were compared at Baseline (Time 1) and 5-6 weeks later (Time 2). This was done to evaluate the stability of these measures in the matched control subjects over time.

III. RESULTS:

For each class of dependent measures, values for the matched control group are presented first, followed by the results for the OSAS group before and after two weeks of continuous treatment. The results of the OSAS group following treatment are then further sub-divided into CPAP/Melatonin and CPAP/Placebo groups. Mean scores (and their standard deviations) for each of the measures are reported. F values and the results of post-hoc tests are reported for the sub-set of parameters that were submitted to MANOVA testing. The effect of treatment in the OSAS group was further evaluated by comparing Baseline (pre-treatment) and Post-Treatment values using paired-sample t tests. In addition, repeated measures analyses of variance were conducted to assess pre-post treatment differences in the two experimental conditions (CPAP/Melatonin and CPAP/Placebo). After the presentation of these basic findings, correlational analyses are described across the classes of dependent measures.

Results are presented separately for the following: sociodemographic and subjective data; physiological parameters (Body temperature, NPSG measures, Actigraphy measures); and daytime performance (MSLT, MWT, FCRTT) and neuropsychological measures.

1) Sociodemographic and Subjective Data: Means and standard deviations for all demographic measures are presented in Table 1. Since subjects were matched on the basis of their age and gender, these variables were not part of the multivariate testing procedure.

As would be predicted, the OSAS patients were heavier (according to their BMI - Body Mass Index) than the matched control subjects. The OSAS group and control group were comparable in their age and level of education.

A significant multivariate group effect was detected using MANOVA ($p < 0.01$). Univariate F values are included in Table 2. The groups differed on the Epworth Sleepiness Scale ($p < 0.0001$) and the Beck Depression Inventory ($p < 0.01$). Post-hoc testing (with Bonferroni correction factors for multiple comparisons) revealed significant differences between the OSAS group before treatment and the matched controls, as well as between the OSAS group following treatment and the matched controls, for both measures. Therefore, despite adequate correction of their sleep fragmentation and sleep-related respiratory disturbance, the OSAS group continued to report greater levels of (subjective) sleepiness and higher ratings of depression following treatment, as compared to the matched control group. Post-treatment values for the Epworth and Beck remained virtually unchanged from levels observed prior to treatment. This persistent difference is suggestive of a lack of normalization on both these subjective measures in the treated apneic group.

Subsequent paired-sample t tests were employed to look for differences between the Baseline and Post-Treatment phases in the OSAS group (Table 2). This revealed significant improvement following treatment on the Self-Rating Scale of Memory Function (MEM) ($p < 0.05$), but only a trend towards improvement post-treatment on the BDI ($p = 0.10$). No significant changes were observed in ESS values following the course of treatment. Scores

on the ESS remained significantly elevated in the OSAS group following two weeks of treatment relative to the matched controls (12.0 ± 5.0 versus 4.1 ± 2.2).

Repeated measures ANOVAs were performed to assess pre- and post-treatment (Time) differences between the two experimental conditions (CPAP/Melatonin and CPAP/Placebo). There were no significant effects in terms of treatment Condition on any of the measures; only MEM showed a significant Time effect ($F(1,12) = 4.98$, $p < 0.05$), with improvement in subjective ratings of memory observed in both conditions (see [Table 3](#)). Therefore, no further changes could be seen on these measures that could be attributed to adjuvant melatonin administration.

2) Physiological Parameters: Since certain sleep measures and respiratory parameters were expected to change as a result of treatment (i.e., Stage 1%, REM%, WASO, AHI, DESATIND, MINDESAT), these variables are reported by descriptive statistics and treatment effects were evaluated with paired-sample t tests (Baseline OSAS group compared to Post-Treatment OSAS group). These traditional measures of sleep and respiration were not part of the initial multivariate analysis (see [Tables 4 and 5](#)). A main effect of group ($p < 0.0001$) was detected using MANOVA on a sub-set of physiological parameters (as outlined in the Methods section). Univariate F values for the physiological measures and post-hoc Scheffé tests are presented in [Table 6](#).

a. Body Temperature Measures: The derived estimates of Amplitude, Mean

and Phase were submitted for MANOVA testing as a sub-set of the physiological parameters being investigated (see Methods section). Only Amplitude (AMPL) demonstrated a significant group difference ($p < 0.05$), such that the Amplitude was blunted in the apneic group before treatment compared to the matched controls. The average fitted Amplitude for the matched control group was $1.40 \pm .15$ degrees; $1.11 \pm .32$ degrees for the OSAS group before treatment (Baseline); $1.36 \pm .28$ degrees for the OSAS group following two weeks of treatment. Mean temperature values were comparable for the three groups (see [Table 6](#)). While OSAS subjects displayed later times for their temperature Phase both before and after treatment (05:05 and 05:11, respectively) compared to matched controls (03:40), this failed to reach significance due to the large variability displayed by subjects within each group. Post-hoc testing displayed a trend towards significance in Amplitude both between the matched control group and the OSAS group prior to treatment ($p = 0.076$) and between the OSAS group before and after treatment ($p = 0.075$). The differences between the average fitted curves for temperature for the three groups (matched controls, OSAS groups before and after 2 weeks of treatment) are displayed in [Figure 1](#).

Subsequent paired-sample t tests revealed a significant effect of treatment on Amplitude ($p < 0.05$) in the OSAS group (see [Table 6](#)). The average increase in the Amplitude of the fitted temperature curve was 0.25 ± 0.28 degrees, for the OSAS group as a whole. Examined separately in terms of condition, the CPAP/Melatonin Amplitude increased by 0.31 ± 0.50 degrees; the CPAP/Placebo group increased by 0.20 ± 0.25 degrees (see [Table 7](#)). However, the difference between the two conditions was not statistically significant. No significant

differences were detected in the other two temperature measures. All groups displayed comparable values for the "goodness of fit measure" (R^2) - all subjects having greater than .50 of the variance accounted for by the fitted polynomial function. Independent t tests did not show any significant differences for mean R^2 values between the matched controls and the OSAS group before treatment ($.85 \pm .10$ and $.83 \pm .12$, respectively). There was a non-significant increase in R^2 values following treatment in the OSAS group as a whole ($.86 \pm .12$). Change scores (post- minus pre-treatment values) for R^2 measures in the CPAP/Melatonin and CPAP/Placebo conditions showed a trend towards a higher "goodness of fit" of the polynomial curve in the CPAP/Melatonin condition. Mean R^2 values increased by .10 in the CPAP/Melatonin condition, and decreased by .03 in the CPAP/Placebo condition. Therefore, circadian temperature rhythm strength, as indicated by R^2 , was relatively unchanged in the two Post-Treatment conditions.

In order to assess the magnitude of the temperature change as a result of treatment, an additional measure of time spent below the mean (the mesor equivalent) over a 24 hour period was examined. Paired-sample t tests were employed to examine the values for the OSAS group at Baseline and following two weeks of continuous treatment. There were no significant differences detected, despite a slight increase in time spent below the mean indicated in the apneic group after treatment (46.8% versus 44.9% at Baseline). Moreover, independent t tests did not reveal any significant differences between the matched control group (44.5%) with either the OSAS group before, or after, treatment. An interesting, but non-significant, finding was that a 5.4% increase in time spent below the mean temperature

was detected in the CPAP/Melatonin condition, while a .2% decrease was noted in the CPAP/Placebo condition following treatment.

In addition, paired-sample t tests were used to examine body temperature measures in four of the matched controls at Baseline (Time 1) and 5-6 weeks later (Time 2) to assess the stability of their temperature rhythms over time. Temperature measures remained fairly constant in the matched controls over time. Derived measures of Amplitude, Mean and Phase were virtually unchanged at follow-up. A non-significant reduction in mean Amplitude was noted at Time 2 (1.33 ± 0.11 versus Baseline values of 1.40 ± 0.15 degrees). Non-significant increases were noted on repeat assessment for measures of Mean and Phase ($37.11 \pm .22$ degrees versus Baseline value of 37.08 ± 0.34 degrees and 240 ± 50.1 minutes versus 220 ± 88.0 minutes, respectively).

Repeated measures ANOVAS revealed a significant Time effect for Amplitude ($F(1,12) = 6.37, p < 0.05$), but there were no differences attributed to the CPAP/Melatonin or CPAP/Placebo condition. (see Table 7). There was, however, an interaction effect (Condition X Time) noted for Phase ($F(1,12) = 7.35, p < 0.02$). Prior to treatment, subjects in the CPAP/Melatonin condition had an average Phase of 06:43 hours ± 157.3 minutes. Those in the CPAP/Placebo condition had their temperature minima at 03:53 hours ± 119.2 minutes. Following treatment, the subjects in the CPAP/Melatonin condition showed an average phase advance of 143.7 minutes, while those in the CPAP/Placebo condition had an average delay of 117.3 minutes. Post-hoc testing revealed only a trend for a Condition effect

before treatment ($p = 0.15$) due to the large individual differences in the values for Phase in each group. Prior to treatment, phase values had a range of approximately 6.5 hours in each condition; following treatment, values for the fitted temperature minima had a range of 4 hours in the CPAP/Melatonin condition, but a wider distribution of 8.5 hours was observed in the CPAP/Placebo group.

b. Sleep Measures: Mean values and standard deviations for traditional sleep measures (NPSG and respiratory parameters) are reported for the matched controls and the OSAS groups before and after treatment in Table 4. Examination of the table clearly shows that the respiratory indices of the apneic group were markedly improved as a result of CPAP treatment. Initial (Baseline) mean AHI for the OSAS group was 66.6 events per hour, with a range of 25.0 to 143.8; post-treatment mean values fell to 3.6, with a range of 0 - 15.1. The individual who displayed the higher than normal AHI had primarily hypopneic events which were not clearly associated with significant nocturnal oxygen desaturation events. The mean AHI for males ($N = 11$) was 68.1; for females in the present study ($N = 5$), the mean AHI was 63.3. Paired-sample t tests reveal significant improvement following two weeks of treatment on measures of AHI ($p < 0.0001$), desaturation index (DESATIND) ($p < 0.001$), and minimum nocturnal oxygen desaturation (MINDESAT) ($p < 0.0001$). Furthermore, sleep architecture measures reflect that the OSAS group significantly improved following treatment compared to Baseline. Paired-sample t tests show a significant reduction in time spent awake after sleep onset (WASO) ($p < 0.01$), which reflects a 51% decline in WASO following 2 weeks of continuous treatment. The standard measure of TST also displayed a

significant increase in the OSAS group following treatment ($p < 0.05$). There was also a significant decrease in the latency to REM sleep (REMLAT) ($p < 0.01$) and the percentage of Stage 1 sleep ($p < 0.01$) following treatment. Significant increases were noted in percentage of REM sleep ($p < 0.001$), as well as non-significant increases in slow wave sleep (Stages 3 and 4). Sleep stage percentages for the matched controls and the apneics before and after treatment are graphically presented in [Figure 2](#). In addition, the degree and direction of change in sleep stage percentages following treatment for the CPAP/Melatonin and CPAP/Placebo sub-groups are presented in [Figure 3](#). Due to the large degree of subject variability observed in both treatment conditions for each of the sleep measures, no significant differences emerged between the CPAP/Melatonin and CPAP/Placebo conditions (independent t tests). Group means and standard deviations for the sleep measures for each Condition at Baseline and Post-Treatment are presented in [Table 5](#).

Sleep fragmentation measures derived from nocturnal polysomnography (mean FACE and SE), fragmentation measures derived from Actigraphy (ACTIFACE), as well as other sleep measures (SLAT, REMLAT, REMI, REMD) were submitted for MANOVA testing. Mean FACE, ACTIFACE, REMLAT, and REMI demonstrated significant group differences. Univariate F values for these measures and post-hoc testing results are given in [Table 6](#). Post-hoc Scheffé tests revealed significant differences between the OSAS group prior to treatment and matched controls ($p < 0.01$), such that the apneic group before treatment had lower levels of sleep continuity as indicated by NPSG-derived measures of mean FACE compared to the controls. In addition, a significant increase in mean FACE was noted in the

OSAS group after two weeks of treatment ($p < 0.001$). Initial values for FACE in the OSAS group were 3.8 ± 2.3 minutes; post-treatment values increased to 10.2 ± 4.4 minutes, which was comparable to the matched control values of 9.2 ± 3.7 minutes. Similarly, significant group differences were noted post-hoc on actigraphic-derived measures of ACTIFACE, which is also an inferred measure of sleep fragmentation, based on continuous runs of inactivity. Prior to treatment, the OSAS group differed significantly from the controls ($p < 0.01$), such that the apneics had lower values on ACTIFACE. Following treatment, the OSAS group showed significant improvement on measures of ACTIFACE ($p < 0.001$). These values reflect changes that are comparable to those found with the NPSG-derived measures of FACE (see [Table 6](#)).

Significant group differences were also found on REMLAT between the OSAS group at Baseline and Post-Treatment ($p < 0.01$), such that REMLAT decreased following treatment (see [Table 6](#)). Only a trend was noted between the OSAS group prior to treatment and the matched normals ($p = 0.09$). This could be due to the large individual differences observed in REMLAT in the OSAS subjects before treatment (range of 52.0 to 274.5 minutes). In addition, the measure of REM Index (REMI), a measure of phasic REM activity, revealed significantly lower values for the OSAS group at Baseline compared to the matched controls ($p < 0.01$). Following treatment, the OSAS group had significantly higher REM Indices compared to Baseline values ($p < 0.01$), which was comparable to levels observed in the matched controls. However, measures of REM Density (REMD) did not demonstrate significant group differences. This is due to the fact that this measure is

based upon the amount of time spent in REM sleep (REMI/REM Time), which increased in proportion to the increases found in REMI in the OSAS group following treatment (see Table 6).

A non-significant trend was found on standard measures of Sleep Efficiency (TST/TIB x 100%) both between the OSAS group before treatment and the matched control group ($p = 0.07$), and between the OSAS group before and after two weeks of treatment ($p = 0.05$). Prior to treatment, the OSAS group had lower values of SE compared to the control group. Following two weeks of treatment, the OSAS group demonstrated increased SE values. However, the lack of a significant finding on measures of SE in the OSAS group compared to the controls may be due to the fact that two of the matched control subjects displayed an extended period of wakefulness which preceded the final wake-up time (approximately 30 to 45 minutes). This led to substantial reductions in overall sleep efficiency. Therefore, an additional measure of Sleep Period Time (SPT) was utilized in place of the standard measure of Time in Bed to re-calculate Sleep Efficiency (SE). Since SPT is defined as the time from sleep onset to the final epoch of sleep, this eliminates any significant time spent awake in bed following the terminal awakening time. When Sleep Efficiency was calculated using SPT in the denominator (as opposed to the standard Time in Bed value), this revealed a significant difference between the matched control group and OSAS group before treatment (independent t test, $p < 0.05$), as well as a significant increase in the apneic group following two weeks of continuous treatment over Baseline values (paired-sample t test, $p < 0.01$). Therefore, within the major sleep period (sleep onset to terminal awakening time), sleep

continuity (as assessed by SE) was significantly improved in the OSAS group following treatment. This value for SE (TST/SPT) is reported in Tables 4, 5 and 6.

Subsequent paired-sample *t* tests were employed to look for differences due to treatment on the measures of REMD and SLAT. No significant changes in either measure were noted as a result of treatment. Nocturnal sleep latency remained virtually unchanged following treatment in the OSAS group (12.0 ± 8.3 minutes, versus 12.0 ± 8.9 minutes post-treatment). Nevertheless, SLAT values in the matched controls were not too disparate from these measures (14.1 ± 20.4 minutes) (see Table 6). This also demonstrates greater variability in nocturnal sleep onset latency in the matched control group of sleepers. In addition, paired-sample *t* tests were used to examine the actigraphic data at Baseline and 5-6 weeks later in four of the matched controls. Mean ACTIFACE values were unchanged at follow-up in the matched controls, with values of 14.5 ± 11.2 minutes at Baseline and 14.9 ± 9.1 minutes at follow-up. Therefore, the control group remained significantly different from the OSAS group before treatment ($p < 0.01$).

Repeated measures ANOVAs were performed to assess Time and Condition effects on these sleep measures (see Table 7). A significant Time effect was noted for many of the variables: FACE, SE, REMLAT ($p < 0.01$); ACTIFACE and REMI ($p < 0.001$). A trend towards an interaction effect was noted for SLAT ($p = 0.10$). Initially, the CPAP/Melatonin group had higher mean SLAT values than the CPAP/Placebo group prior to treatment (15.8 versus 10.6 minutes). Following treatment, mean SLAT fell to 9.8 minutes in the

CPAP/Melatonin group and increased to 13.8 minutes in the CPAP/Placebo condition. Both the CPAP/Melatonin and CPAP/Placebo groups displayed improved values on FACE following treatment (9.9 ± 3.2 minutes, and 10.3 ± 5.4 minutes, respectively); pre-treatment levels were between 3-4 minutes in each group. Post-treatment values were comparable to those observed in the matched control group (9.2 ± 3.7 minutes). This demonstrates similar levels of sleep continuity in the both treatment conditions, as well as the normal sleepers. In addition, a trend towards a Condition effect was seen on the ACTIFACE measure. While standard FACE measures, derived from the NPSGs, show comparable levels of improvement post-treatment, the actigraphy-based measure shows higher levels of sleep continuity in the CPAP/Placebo condition (29.5 ± 14.9 minutes) than the CPAP/Melatonin condition (18.4 ± 7.1 minutes). Nevertheless, these values were improved from pre-treatment mean ACTIFACE levels of 4-5 minutes. Comparable values for Sleep Efficiency were noted following treatment in both the CPAP/Melatonin and CPAP/Placebo groups ($91.4\% \pm 5.3\%$ and $91.3\% \pm 5.7\%$, respectively), which were nearly identical to the SE values observed in the normals ($91.5\% \pm 4.0\%$). While average REMLAT appeared to be longer in the pre-treated OSAS group as a whole (152.5 ± 76.1 minutes), compared to the matched normals (99.1 ± 36.4 minutes), this did not reach statistical significance as stated previously. However, when examined as separate groups, both the CPAP/Melatonin and CPAP/Placebo groups demonstrated significant reductions in REMLAT following treatment (68.0 ± 38.1 minutes and 85.06 ± 14.0 minutes). This resulted in approximately a 1 to 1.5 hour advance of the timing of REM sleep in these groups following treatment. There was no notable difference in terms of the degree of this advance between the two conditions. One subject

in the CPAP/Melatonin group exhibited an unusually short REM latency following treatment (9.5 minutes), although there was no further suggestion of early REM onset during any of the daytime testing sessions. REMI also displayed significant increases in both treatment conditions following treatment (127.8 ± 54.1 for the CPAP/Melatonin group; 163.3 ± 68.5 for CPAP/Placebo). These values were similar to the REMI observed in the matched controls (177.0 ± 77.7). Post-treatment values for REMLAT and REMI in both conditions reflect a normalization of REM processes, with more appropriate timing of the REM sleep cycle, as well as intensity of the REM activity (as indicated by the phasic eye movement count used to determine REMI).

3) Daytime Performance and Neuropsychological Measures: Since many classes of measures were used in the assessment of daytime functioning, descriptive statistics for each of the measures will be given in tabular form. Separate analyses for each of the individual testing sessions of the MSLT and MWT are reported in Table 8. This depicts the mean sleep latencies and their standard deviations for each session for the matched controls and the OSAS group before and after treatment. Table 9 shows the breakdown for the CPAP/Melatonin versus the CPAP/Placebo condition. Overall group means for the MSLT and MWT are included in the MANOVA test results (see Table 12). FCRTT data (number of gaps, mean reaction time, accuracy and throughput) are presented as mean values for each group on each of the five trials (Table 10). The results of 16 neuropsychological test measures are presented in Table 11. Scores from the WAIS-R are presented as scaled scores. Subsequent paired-sample t tests, incorporating a blocked design analysis for age and gender,

were utilized to examine treatment effects (see [Table 11](#)). Therefore, the mean scores reported are representative of a subset of the OSAS group ($n = 8$) who were matched to the control group.

Six variables were submitted for MANOVA testing. Two of those measures were used to assess changes in the ability to maintain wakefulness/vigilance (MWT and number of gaps on FCRTT); one was a measure of sleep propensity (MSLT); the other three measures assessed neuropsychological functioning, particularly in the domain which has been associated with "executive functions" (PERFIQ, TRAILS-B, PICTARR). A significant multivariate group effect was detected ($p < 0.001$). Univariate F values and post-hoc t test results are presented in [Table 12](#). The groups differed on the MSLT ($p < 0.01$), the MWT ($p < 0.0001$) and the gaps on the FCRTT ($p < 0.05$). There was a trend towards a group effect on the TRAILS-B ($p = 0.07$). Prior to treatment, the apneic group appeared to be sleepier than the matched controls on daytime testing as indicated by lower sleep latency values on the MSLT and MWT, and daytime performance was impaired as indicated by the greater number of gaps/lapses in responding on the FCRTT. Post-hoc Scheffé testing revealed significant differences between the OSAS group prior to treatment and matched controls on the MSLT ($p < 0.01$). Mean sleep latency values on the MSLT were 4.6 ± 2.1 minutes for the OSAS group before treatment and 10.1 ± 4.6 minutes for the matched controls. Only a trend towards significance was observed between the OSAS group after treatment and the matched controls. While significant improvement was noted in the OSAS group at Baseline compared to Post-Treatment ($p < 0.01$), average sleep latency values on

the MSLT remained fairly low (6.78 ± 3.7 minutes). This reflects a persistent decrement in MSLT scores in the OSAS group following treatment, with mean sleep latency values remaining in the borderline pathological range (< 8 minutes). Figure 4 graphically displays the MSLT mean sleep latency values across the four testing sessions in the matched controls and the OSAS group prior to, and following, treatment.

Post-hoc testing of MWT values indicated significant differences between the matched controls and the OSAS group at Baseline ($p < 0.0001$), such that the OSAS group had significantly lower sleep latency values prior to treatment. However, an unexpected finding was that the OSAS group continued to display significantly lower mean MWT sleep latencies even after two weeks of continuous treatment as compared to the matched controls ($p < 0.0001$). There was no significant improvement detected between the OSAS group at Baseline and Post-Treatment. The matched controls mean latency on the MWT was 33.3 ± 12.9 minutes, which differed significantly from the OSAS group both at the Baseline phase and Post-Treatment phase (8.4 ± 4.0 minutes and 13.1 ± 7.5 minutes, respectively). While this shows some degree of improvement in the ability of the OSAS subjects to maintain wakefulness following treatment, this is still not comparable to normative values. Figure 5 presents the MWT data for each of the 4 daytime sessions in each of the groups (matched controls and OSAS group before and after treatment). FCRTT values for the number of gaps (square root transformed) was significantly higher in the OSAS group prior to treatment compared to the matched controls ($p < 0.05$). Following treatment, gap measures on the FCRTT significantly improved in the apneic group compared to Baseline values (p

< 0.01), such that the number of gaps were comparable to the level displayed in the control group.

Subsequent paired-sample *t* tests revealed significant improvement in the OSAS group following treatment on the MSLT and FCRTT (both $p < 0.01$), but only a trend towards improvement following treatment on the MWT ($p = 0.02$, with Bonferroni correction factors applied) (see [Table 12](#)). No significant changes were observed in the neuropsychological measures of PERFIQ, PICTARR or TRAILS-B.

Repeated measures ANOVA revealed a significant Time effect for the overall mean sleep latency values of the MSLT ($F(1,12) = 1.22$, $p < 0.01$) (see [Table 13](#)). There was no effect of Condition, although the CPAP/Melatonin group had a lower overall mean MSLT score compared to the CPAP/Placebo group following treatment (6.2 ± 3.0 minutes versus 7.2 ± 4.2 minutes). Both the CPAP/Melatonin and CPAP/Placebo groups had comparable pre-treatment values on the MSLT (4.4 ± 1.5 versus 4.9 ± 2.5 minutes; unpaired *t* test, not significant). An effect of Time ($F(1,12) = 5.97$, $p < 0.05$) and Condition ($F(1,12) = 5.05$, $p < 0.05$) was noted for the overall scores of the MWT ([Table 13](#)). Prior to treatment, the CPAP/Melatonin and CPAP/Placebo groups had comparable mean sleep latencies on the MWT (6.9 ± 2.7 and 9.4 ± 4.6 minutes, respectively, $p = \text{n.s.}$). While both the CPAP/Melatonin and CPAP/Placebo groups improved following treatment, the CPAP/Placebo group increased by 7.12 minutes, on average, the CPAP/Melatonin group only increased by an average of 1.73 minutes. Therefore, these findings demonstrate a

differential effect of the two treatment conditions on this objective behavioral measure of sleepiness/wakefulness. In terms of examining change scores (Post-Treatment minus Baseline) for the CPAP/Melatonin and CPAP/Placebo conditions, repeated measures ANOVA revealed only a trend for a Condition effect for the MWT ($F(1,3) = 2.22, p = 0.16$), whereby greater improvement on the MWT was found in the CPAP/Melatonin condition (see Table 9a). The FCRTT also displayed a significant Time effect ($F(1,12) = 13.23, p < 0.01$), with both treatment conditions resulting in a decrease in the presence of gaps during performance testing. The actual number of gaps decreased by 13.4 in the CPAP/Melatonin condition and by 8.2 in the CPAP/Placebo condition, but levels still remained higher (12.7 ± 16.5 in the CPAP/Melatonin condition; 9.8 ± 19.4 in the CPAP/Placebo condition) than the matched normals (3.4 ± 5.5). No significant Time or Condition effects were observed for any of the neuropsychological measures.

Neuropsychological functioning was evaluated by employing paired-sample *t* tests using a blocked design analysis (age) on a subset of 8 OSAS subjects. The OSAS group prior to treatment was compared to the matched control group and was compared to themselves following a two week course of treatment. The blocked design analysis was chosen to counter any differential effects in ability/performance due to age differences between the subjects. Prior to treatment, OSAS subjects displayed significantly poorer performance than the matched controls in terms of overall IQ measures (full scaled score of the WAIS-R) ($p < 0.05$), Trail Making - Part B of the Halstead-Reitan Battery (time to complete TRAILS-B) ($p < 0.01$), and the delayed recall condition of the Rey-Osterreith Complex Figure Test

(number of items recalled in the delayed condition - REYDEL) ($p < 0.05$) (see [Table 11](#) for group means and standard deviations). All these measures reflected decreased levels of performance and psychomotor slowing (TRAILS-B) in the OSAS group compared to the controls. The difference in overall IQ measures could be a function of the groups not being exactly matched in terms of level of education, although the groups were not significantly different from one another (control group mean level of education was $15.75 \pm .71$ years; OSAS group was 15.0 ± 1.60 years). Non-significant trends were observed for VERBIQ ($p = 0.057$), STORYIMM ($p = 0.072$), STORYDEL ($p = 0.069$), and PURDUE ($p = 0.071$). Differences between the OSAS group before and after treatment were significant for the measures of BLOCKDES, DIGSYMB and TRAILS-B (all p levels < 0.05). A trend towards significance was observed for overall IQ ($p = 0.055$). These measures all reflected enhanced performance on these tests following the course of treatment (see [Table 11](#)). Differential effects on neuropsychological measures as a result of treatment condition (post- minus pre-treatment differences in CPAP/Melatonin versus CPAP/Placebo) were assessed using independent t tests. Only STORYDEL demonstrated a significant difference as a result of Condition ($p < 0.05$). The CPAP/Melatonin condition showed enhanced recall in the delayed condition as compared to the CPAP/Placebo group (mean increase of 2.8 units versus .63). There was also a non-significant increase ($p = 0.082$) detected on the Digit Symbol Substitution test on the WAIS-R, with the CPAP/Melatonin group increasing by 1.8 scaled scores, and CPAP/Placebo rising by 0.63.

In order to assess subjective levels of sleepiness throughout the testing sessions, the

Stanford Sleepiness Scale (SSS) was analyzed using a two factor repeated measures analysis of variance, with Group and Time as the major factors. Pre-treated OSAS subjects were compared to the matched controls, using eleven different measures of SSS (repeated measures over the course of the night and day testing sessions) to ascertain if there was any significant Time effect. Only a significant Group effect was detected between the OSAS group before treatment and the matched controls ($F(1,10) = 17.22$, $p < 0.001$), such that the apneic group indicated greater subjective levels of sleepiness compared to the control group. The Time effect was not significant ($p = 0.15$). Subsequent to treatment, repeated measures ANOVA was performed to assess whether there was an effect of treatment Condition (CPAP/Melatonin versus CPAP/Placebo) over Time. There was a significant Time effect ($F(1,10) = 2.49$, $p < 0.01$), but there was no longer a significant Group effect. Post-hoc testing using Bonferroni's Multiple Comparison Procedures revealed a significant effect of Time ($p < 0.05$), with regards to increased levels of sleepiness at 23:00 (prior to bedtime) for both treatment conditions. Significant differences emerged for the comparisons between 23:00 hours and three particular times of day - 09:00, 13:00 and 14:00 hours. During these three time periods, subjective sleepiness was markedly reduced in both OSAS groups. On average, the overall level of sleepiness was similar during the day for all groups. Following treatment, OSAS subjects in both conditions appeared to be sleepier at 23:00 hours, which is also consistent with the timing of subjective reports of sleepiness in the matched control group.

Similar data collection to the SSS was achieved with the subjective Bed-time and

Up-time questionnaires which was administered during the subjects' NPSG testing sessions in the laboratory. This set of data also contains a Likert-type 7 point scale which can be used to assess the individual's sense of "feeling refreshed" (= 1) or "unrefreshed" (= 7). Questionnaire responses pertain to how the subjects perceived how they felt at three particular times: 1) immediately upon awakening at home the morning prior to their NPSG; 2) immediately prior to lights-out in the lab during NPSG testing; 3) immediately following awakening in the lab after NPSG testing. These measures were used to corroborate the findings of the SSS. Repeated measures ANOVA was performed to assess Time (up-time at home vs. bed-time in lab vs. up-time in lab) and Group (OSAS group before treatment versus matched controls). A significant Group effect was detected ($p < 0.01$), as well as a significant Time effect ($p < 0.01$). The interaction effect was also significant ($p < 0.001$). Post-hoc testing revealed that, not surprisingly, the OSAS subjects prior to treatment were significantly sleepier by subjective reports compared to the control group ($p < 0.01$). Furthermore, significant differences were found between the value reported at bed-time in the lab vs. up-time at home ($p < 0.001$) and between bed-time in the lab vs. up-time in the lab ($p < 0.01$). Paired-sample *t* tests were employed to examine differences in the OSAS group before and after two weeks of treatment. There was a significant difference noted in the up-time in lab report of "feeling refreshed" ($p < 0.01$) which showed marked improvement in subjective morning sleepiness. There was a trend towards significance in improved subjective quality of sleep according to the up-time home assessment ($p = 0.086$), as indicated by improved levels of "feeling refreshed". Unpaired *t* tests were used to assess pre-post differences in the CPAP/Melatonin and CPAP/Placebo conditions. No significant

differences were found between the two conditions on any of the subjective reports. This could be due to the large within group variance observed on these rating scales. These findings are consistent with those obtained with the SSS, which showed a reduction in subjective sleepiness throughout the day following treatment.

Non-parametric analyses (Kruskal-Wallis ANOVA by Ranks) were employed to look at pre- and post-treatment differences on the MSLT and MWT. Due to the fact that both these measures tend to truncate the range of data and have non-normal distributions, it was deemed appropriate to use the median values on these tests, as a supplement to the parametric analyses reported above. A significant Group effect was detected on the MSLT ($p < 0.01$) and on the MWT ($p < 0.01$) between the OSAS group at Baseline (before treatment) and the matched controls, such that the OSAS group displayed significantly shorter sleep latency values on both of these tests compared to the control group. These findings were consistent with the results of the MANOVA of the daytime performance measures, which utilized mean sleep latency values on these tests, rather than median values. Examining the groups in terms of their treatment condition, ranked data showed a significant Condition effect between the CPAP/Melatonin, CPAP/Placebo, and matched controls on the MSLT prior to treatment ($p < 0.05$), and on the MWT before treatment, as well ($p < 0.01$). A non-significant trend was found Post-Treatment on both the MSLT ($p = 0.083$) and the MWT ($p = 0.071$) between the CPAP/Melatonin and CPAP/Placebo conditions. Mann-Whitney U Tests were employed to look at pre- and post-treatment differences in the two different conditions. No significant differences were found between the CPAP/Melatonin and the CPAP/Placebo condition

following two weeks of continuous treatment on sleep latency measures of the MSLT. However, a trend towards significance was found on the Post-Treatment measures of the MWT between the CPAP/Melatonin and CPAP/Placebo condition ($p = 0.081$). This is also consistent with findings on the MANOVA using group mean values, as opposed to the median ranked values used in this analysis.

In order to more fully explore time of day effects on the MSLT and MWT in each of the groups, the mean values for each testing session were compared between the OSAS group at Baseline and the matched controls, as well as in the OSAS group following treatment and the matched control group. Unpaired t tests revealed significant differences between the OSAS subjects before treatment and the matched controls on the first three testing sessions of the MSLT (10:00, 12:00 and 14:00 hours) (see [Figure 4](#)). At Baseline, the OSAS group was significantly sleepier on these sessions ($p < 0.01$), but did not differ significantly from the controls on the last nap session at 16:00 hours ($p = 0.12$). Before treatment, the OSAS group significantly differed on all MWT sessions compared to the matched controls ($p < 0.001$) (see [Figure 5](#)). Following treatment, the OSAS group differed significantly from the controls only on the first MSLT session at 10:00 hours (mean SLAT 5.7 ± 3.3 versus 12.6 ± 6.9 minutes, respectively; $p < 0.01$) (see [Figure 4](#)). Following the course of treatment, the OSAS group still demonstrated a reduced ability to maintain wakefulness across the entire day (over all the MWT sessions) as compared to the matched controls ($p < 0.01$ for first three MWT sessions) (see [Figure 5](#)). The last session at 15:00 hours revealed the most significant difference between the two groups with the Post-Treatment OSAS group displaying a group

mean MWT sleep latency value of 8.2 ± 9.5 minutes ($p < 0.001$). The effect of treatment condition was also examined with unpaired t tests. No significant differences were found between the CPAP/Melatonin or CPAP/Placebo conditions prior to treatment on any of the individual MSLT or MWT sessions. Following treatment, the CPAP/Melatonin group appeared to be less able to maintain wakefulness across all MWT sessions compared to the CPAP/Placebo group, with a non-significant trend emerging at 09:00 hours ($p = 0.071$) (see [Figure 6](#)). There were no significant differences between the two conditions on any of the MSLT sessions. Both treatment groups still demonstrated persistently shorter latencies on all MSLT naps as compared to the matched controls. Overall, the CPAP/Melatonin group appeared to be sleepier across the entire day compared to the CPAP/Placebo group, as evidenced by their lower MWT scores. This may suggest a residual behavioral sedating effect of the melatonin formulation, that may persist well into the daytime (waking) hours.

4) Treatment Compliance Measures: In addition to the measures outlined above, three measures were employed to assess the compliance of the OSAS subjects during the entire treatment period. As described in the Methods section, CPAP usage was determined by the number of hours displayed on the counter on each of the OSAS subject's CPAP machines. This value was determined by the technical staff of The Sleep Disorders Center who read the CPAP counter on two occasions: before the first night of CPAP use at home and after a minimum of two weeks of continuous treatment. The total of number of hours was divided by the number of days between the two separate readings to obtain average nightly CPAP usage. The overall group mean was 5.49 ± 0.85 hours per night, which is in accordance with

findings of Engleman et al. (1993) which demonstrated mean CPAP usage of 5.9 ± 1.4 hours per night (this study also used the CPAP counter technique). All of the subjects in the present study demonstrated adequate compliance in terms of the Kribbs et al. (1993b) standards of a least 4 hours per day on at least 70% of all days monitored.

In addition, OSAS subjects were required to call-in their subjective reports of sleep time, sleep latency and sleep quality every morning for two weeks during their home treatment period. Sleep quality was reported on a scale of 1 to 5, with 1 being "the best" and 5 being "the worst". Independent t tests were employed to examine differences on the telephone call-in data between the CPAP/Melatonin and CPAP/Placebo condition. Although there were no significant differences between the conditions on the subjective estimates of Total Sleep Time, Sleep Latency, or Sleep Quality, the CPAP/Melatonin group reported slightly higher estimates of TST compared to the CPAP/Placebo group (7.0 ± 1.0 hours versus 6.3 ± 1.2 hours). Also, the CPAP/Melatonin group reported slightly reduced SLAT values compared to the CPAP/Placebo group (11.1 ± 6.6 minutes versus 15.0 ± 16.3 minutes). This failed to reach significance due to the large variability reported by the subjects in the CPAP/Placebo condition. There were virtually no differences in the reported values for "Sleep Quality" (2.37 ± 0.58 in the CPAP/Melatonin group; 2.48 ± 0.84 in the CPAP/Placebo group). Nevertheless, the subjective differences found between the two conditions were not directly paralleled by any of the objective measures of SLAT or TST derived from NPSG testing in the laboratory. However, there was a trend noted for an interaction effect between the CPAP/Melatonin and CPAP/Placebo conditions on measures of objective SLAT on Post-

Treatment assessment: the CPAP/Melatonin group had a non-significant decrease in SLAT following treatment, while the CPAP/Placebo group displayed an increase in nocturnal SLAT on NPSG testing.

Further examination of sleep-wake diaries provided validation of compliance with CPAP treatment and an assessment of the regularity of sleep-wake schedules. Diary information regarding sleep latency, bedtime, up-time, total sleep time was consistent with telephone call-in data. In addition, measures of overall daytime alertness (reported on a scale of 1 to 7, with 1 being the "most alert" and 7 being "not at all") indicated significant improvement in the OSAS group following a minimum of two weeks of continuous treatment (paired-sample t test, $p < 0.05$).

5) Correlational Analyses: Three separate groups of correlations were performed to assess the interrelationships across various categories of measures. Spearman Rank Order Correlations (with corrections for ties and multiple tests) were employed for the examination of these relationships. The physiological parameters which underwent correlational analyses included measures of respiration (AHI, DESATIND, MINDESAT); sleep continuity measures (FACE and ACTIFACE); and temperature measures (AMPL and MEAN). The two dependent measures were daytime performance measures of mean MWT sleep latency values and FCRTT (mean number of gaps, square root transformed).

The first group of correlations was performed across various physiological and daytime

performance measures in the OSAS group prior to treatment to study the relationship between them (see [Table 14](#)). The only physiological parameter that bore a significant relationship to the number of gaps on the FCRTT was the minimum nocturnal oxygen desaturation value (MINDESAT). As the minimum oxygen desaturation level decreased, the number of gaps on the FCRTT increased ($r = -0.58$, $p < 0.01$). The mean value for ACTIFACE (which is an inferred measure of sleep continuity derived from actigraphy data) demonstrated a significant positive relationship with mean sleep latency values on the MWT ($r = 0.74$, $p < 0.0001$). There were also significant positive correlations found between NPSG sleep fragmentation measures of FACE and the mean sleep latency measures on the MWT ($r = 0.71$, $p < 0.001$) and between MINDESAT and mean sleep latencies on the MWT ($r = 0.62$, $p < 0.01$). Measures of AHI in the OSAS group before treatment and mean latencies on the MWT were also found to have a significant inverse relationship ($r = -0.71$, $p < 0.001$).

The second group of correlations was performed to examine the interrelationship of the change scores in the OSAS group (post- minus pre-treatment) across physiological parameters and daytime performance measures ([Table 15](#)). Change scores of the DESATIND were significantly correlated with MWT change scores ($r = -0.60$, $p < 0.05$), which shows that as the Desaturation Index decreases as a result of treatment, the MWT scores are becoming higher. MINDESAT change scores were negatively correlated with FCRTT change scores ($r = -0.56$, $p < 0.05$). Therefore, as the level of oxygen desaturation became improved due to CPAP treatment, the number of gaps on the FCRTT was

significantly reduced. A significant positive correlation was found between change scores on ACTIFACE and MWT scores ($r = 0.74$, $p < 0.01$). This was interesting to note, since no significant correlations were found between the NPSG measure of sleep fragmentation (FACE) and the daytime performance measures.

Baseline (pre-treatment) values in the OSAS group were also correlated with change scores across measures to further examine predictive patterns, in terms of treatment response (Table 16). Physiological measures in the OSAS group before treatment were correlated with the change scores of daytime performance measures. A significant positive correlation was found between the measure of DESATIND in the OSAS group prior to treatment and change scores of the MWT ($r = 0.58$, $p < 0.05$). This reflects the fact that subjects with higher initial oxygen desaturation indices (more frequent desaturation events) evidenced more dramatic improvement on the MWT following treatment. A significant correlation was also noted for MINDESAT prior to treatment and change scores on the number of gaps on the FCRTT ($r = 0.72$, $p < 0.01$). This signifies that OSAS subjects with the higher oxygen desaturation values before CPAP treatment evidenced a higher degree of improvement on the FCRTT subsequent to treatment. Therefore, OSAS subjects with lower desaturation minima did not show the same degree of improvement on the performance task following two weeks of continuous treatment. In addition, a significant positive relationship was found for the actigraphic measure of sleep continuity (ACTIFACE) in the OSAS group prior to treatment and change scores on the FCRTT ($r = 0.58$, $p < 0.05$). Therefore, OSAS subjects with more continuous periods of inactivity (sleep) prior to treatment, displayed

greater improvement on performance on the FCRTT. Furthermore, temperature measures bore no statistically significant correlations with any of the daytime performance measures.

IV. DISCUSSION:

The principal findings of the present study support the hypothesis that chronic sleep fragmentation and disrupted sleep architecture in untreated obstructive sleep apnea syndrome contributes to a disturbance in the nocturnal temperature rhythm, as evidenced by decreased amplitude of the derived temperature rhythm compared to a group of age- and sex-matched non-complaining sleepers. Other temperature measures (i.e., phase or mean temperature values) were not significantly different from the control group prior to treatment. The lack of a significant difference in mean temperature measures between a group of moderate-severe apneics and age-matched controls in the present study was not in accordance with earlier findings (Zozula et al., 1993), which noted a significant trend. However, the previous study employed a slightly different temperature acquisition technique (tympanic temperature rather than rectal). Due to a greater degree of variability in observed temperature values with tympanic recording compared to rectal monitoring, estimates of mean temperature could have been less reliable than rectal temperature. While the untreated OSAS group and matched control group demonstrated clear differences in the average value of the timing of their temperature minima (phase), this did not reach significance due to the large individual differences observed within each group.

Significant improvement was noted in the amplitude of the temperature rhythm following two weeks of continuous CPAP treatment in the OSAS group. However, contrary to expectation, melatonin did not enhance the effect of CPAP therapy with regard to the observed change in nocturnal temperature amplitude. However, a modest, though non-significant phase-advancing effect of melatonin was noted following treatment. This failed to reach significance due to the wide range of variability in the timing of the temperature nadir in OSAS subjects in both treatment conditions. Prior to treatment, the OSAS group displayed a range of 6-7 hours in the timing of their temperature minima. Following treatment, the CPAP/Melatonin group evidenced a more restricted range in the timing of the temperature nadir (4 hours) as compared to the CPAP/Placebo group, which displayed a wider range in phase values (8 hours). This could possibly reflect a higher degree of consolidation in the timing of the nadir of nocturnal temperature rhythm in the CPAP/Melatonin group. This finding is consistent with other studies which have reported phase-resetting (advancing) properties associated with melatonin administration (Arendt et al., 1986; Dahlitz et al., 1991). An alternate explanation may involve the temperature reducing properties associated with exogenous melatonin administration (Cagnacci et al., 1992). Therefore, in the present analysis, the acute fall in body temperature in the beginning of the nocturnal sleep period in the OSAS subjects, is appreciated as a phase advance in the timing of temperature nadir.

In addition, a surprising interaction effect was noted. Prior to treatment, there was a non-significant degree of disparity between the timing of the temperature minima in the

CPAP/Melatonin and CPAP/Placebo groups (403 minutes after local midnight, and 232 minutes, respectively). Following treatment, the CPAP/Melatonin group evidenced a phase advance of approximately 2.5 hours; the CPAP/Placebo group evidenced a phase delay of 2 hours. This is suggestive of an effect of melatonin on the clock mechanism of the temperature rhythm. This change in temperature phase could not be accounted for by differences in the timing of the individual subjects' sleep/wake cycles, since they were compliant in terms of keeping to their regularly scheduled bed-times and wake-times during the study period. This is supported by evidence from studies of jet-lag which have demonstrated the phase-shifting properties of melatonin (Arendt et al., 1986).

Since body temperature was assessed against a background of sleep/wake activity, it can be inferred that the intrinsic circadian mechanism was not manifested as clearly in the OSAS group prior to treatment as a result of chronic sleep disruption. This was further supported by the difference in the temperature amplitude between the OSAS and control groups at Baseline, without any significant differences observed in the timing of their respective rhythms. In addition, there was no further suggestion that there was a difference in the strength of their respective clock mechanisms, as inferred by the "goodness of fit" measure (R^2) of the temperature rhythm. All groups exhibited average values for R^2 of greater than 80%. No significant changes were noted in R^2 values following treatment in the OSAS group or upon repeat testing in the control group.

Furthermore, there is a suggestion that with appropriate (CPAP) therapy to correct the

chronic sleep fragmentation, the temperature rhythm in apneics more closely resembles that of normal sleepers. The change witnessed in amplitude, alone, is suggestive of a change in the thermoregulatory process in OSAS subjects, particularly at night. This is consistent with findings of Sewitch (1987), which showed that slow wave sleep (SWS) deficiency is associated with the failure in the thermoregulatory system to down-regulate (reduce body temperature) at sleep onset. This could, similarly, be applied to the group of OSAS patients who had very low levels of SWS prior to treatment. Subsequent to treatment, SWS returned to more normal levels; as did the amplitude component of the temperature rhythm. Alternatively, Aschoff et al. (1972) have reported that recovery sleep after sleep deprivation results in an exaggerated drop in sleep (nocturnal) temperature. It is possible that the group of moderate-severe apneics are still displaying a modicum of "recovery" sleep, even after 2 weeks of continuous CPAP treatment. This is also in keeping with Wittig et al's. (1986) finding that continued improvement in daytime sleepiness is observed in OSAS patients over a six week period of CPAP treatment. Therefore, this continuation of "recovery" sleep could also account for the significant differences observed in the amplitude of the derived temperature rhythm before and after treatment in the OSAS group. Overall, the present findings suggest that the temperature disturbance was primarily in the ability of the OSAS subjects at Baseline to lower their body temperature prior to sleep onset (thermal down regulation), and was not clearly associated with an actual defect in the circadian timing mechanism of the clock (no differences in phase in the OSAS group compared to controls).

In general, as has been previously reported (Rajagopal et al., 1986; Phillips et al., 1990;

Series et al., 1992), nasal CPAP therapy was associated with significant improvement in measures of sleep-related respiration, nocturnal oxygenation, and sleep fragmentation in the OSAS subjects. Following two weeks of continuous treatment, all OSAS subjects demonstrated significant improvement in their overall sleep organization and sleep-related indices of respiratory disturbance. Sleep architecture also reflected marked improvement in most OSAS subjects after two weeks of treatment. Wake time after sleep onset significantly declined following treatment in all but two OSAS subjects, where it remained virtually unchanged. The percentage of Stage 1 sleep was dramatically reduced in all but three OSAS subjects after treatment; these subjects had very low baseline percentages of Stage 1 sleep, and Post-Treatment levels remained relatively the same. Furthermore, the percentage of Stage 3 sleep increased in all but three OSAS subjects. Overall, there was a marked difference noted in Stage 3 in the OSAS group following treatment. REM sleep significantly increased in all but two subjects, who displayed minor decrements in REM percentage. Respiratory disturbance (apnea-hypopnea index, or AHI) measures also reflected significantly decreased levels following treatment in all OSAS subjects. Only one subject had AHI values of greater than 10 events per hour following two weeks of continuous CPAP treatment. This subject had an initial apnea-hypopnea index of 107 events/hour. The residual finding of >10 events/per hour following treatment, primarily consisted of hypopneic events, which were associated with EEG arousal, but not with significant oxygen desaturation events. Therefore, this finding was not deemed clinically significant. In addition, the number of nocturnal desaturations per hour of sleep decreased significantly in all but two OSAS subjects (their initial Baseline indices were both less than 3.0 events per hour). All

OSAS subjects displayed significant increases in their nocturnal oxygen desaturation minima after treatment. Therefore, a base of treatment effect existed against which temperature measures, neuropsychological variables, daytime performance measures, and other subjective parameters could be fully examined.

In addition, measures of sleep fragmentation demonstrated significant improvement in the OSAS group following two weeks of treatment. Sleep continuity measures (FACE and ACTIFACE) showed significant improvement in all but one OSAS subject, who happened to have the highest initial Baseline level of the NPSG-derived measure of sleep continuity (8.1 minutes) compared to all other OSAS subjects before treatment. Both NPSG-derived (FACE) and actigraphy-derived (ACTIFACE) measures of sleep continuity/fragmentation reflected improvement in the OSAS group after treatment to levels comparable to that of the controls (mean FACE = 10.2 minutes versus 9.2 minutes in the matched controls; ACTIFACE = 24.8 minutes versus 24.1 minutes). The average values for NPSG-derived measures of sleep continuity are remarkably consistent with the minimum amount of time thought to be required for sleep to be "restorative" - greater than 10 minutes (Bonnet, 1986; Downey & Bonnet, 1987). While similar levels of improvement were noted in both the CPAP/Melatonin and CPAP/Placebo conditions on NPSG-derived sleep continuity measures, a trend towards a condition effect was noted on actigraphy-derived measures. The actigraphic measure actually demonstrated a higher level of sleep continuity in the CPAP/Placebo condition (29.5 minutes) than the CPAP/Melatonin condition (18.4 minutes). This finding was counter to the original prediction of evidencing increased sleep stability

following melatonin ingestion at bedtime. Nevertheless, OSAS subjects in both treatment conditions had comparable levels of NPSG-derived measures of Sleep Efficiency following treatment (91.4% in the CPAP/Melatonin condition and 91.3% in the CPAP/Placebo condition), which were comparable to values observed in the control group at Baseline (91.5%).

In terms of the effect on sleep parameters, melatonin administration at bedtime, in conjunction with ongoing CPAP therapy, had no significant effects on measures of sleep architecture or sleep fragmentation in the OSAS group. There was a trend towards a reduction in the nocturnal sleep latency in the group of OSAS subjects who received combined melatonin and CPAP therapy. A subtle difference was noted between the sleep latencies in the CPAP/Melatonin condition (9.8 minutes) and the CPAP/Placebo condition (13.8 minutes) following treatment. However, this failed to reach significance due to a high degree of variability in sleep latency values in subjects in both conditions. In terms of slow wave sleep, the CPAP/Melatonin and CPAP/Placebo groups did not significantly differ from each other following treatment. There were slightly higher values of slow wave sleep, particularly Stage 3 sleep, observed in the CPAP/Melatonin group compared to the CPAP/Placebo group Post-Treatment (20.5% versus 15.6% slow wave sleep).

Due to the large deficit in REM sleep observed in untreated OSAS patients in previous studies (Series et al., 1992), it was hypothesized that the pressure for REM sleep would be so great in these individuals that they would evidence signs of this additional "REM

pressure" (i.e., shortened REM latencies; higher levels of phasic REM activity) following CPAP treatment. In fact, the degree of REM-related respiratory disturbance was so great in this group of OSAS subjects that their Baseline level of phasic REM activity (REM Index) was less than 50% of the control group level. REM sleep latency was also longer in the Baseline OSAS group than the controls (140.6 minutes and 99.1 minutes, respectively). However, this failed to reach statistical significance. Baseline levels of phasic REM activity were comparable to levels found by Aldrich et al. (1989) in a study of OSAS patients (REM Index = 74.9 and 76.7, respectively). However, once successfully treated, higher levels of phasic REM activity were found (REM Index = 148.7) and nocturnal REM sleep latency was significantly shortened (decreased by almost 70 minutes). However, our findings were not consistent with Aldrich et al. with respect to REM density measures (REM Index/Time Spent in REM). Whereas Aldrich et al. found an increase in REM density following CPAP treatment, REM density measures decreased in the OSAS group following treatment in the present study. This could be accounted for by the fact that our patient group had proportional increases in REM sleep time, while the group of OSAS patients in the Aldrich et al's. study did not have the same level of increase in REM time. This could be attributed to the fact that the Aldrich et al. study examined the acute effects of one night of CPAP treatment, rather than several nights, as in the present study. Therefore, REM sleep levels were probably offset by the significant pressure for slow wave sleep. The present finding is also inconsistent with the findings of Benoit et al. (1975), which have shown that phasic REM events are recovered to a greater extent than is the REM sleep time itself following selective REM sleep deprivation.

When examined in terms of treatment condition, both the CPAP/Melatonin and CPAP/Placebo groups demonstrated significant reductions in REM sleep latency (68.0 ± 38.1 minutes and 85.1 ± 14.0 minutes). This resulted in approximately a 1 to 1.5 hour advance of the timing of REM sleep in these groups following treatment. There was no significant difference in terms of the degree of this advance between the two conditions following treatment. One subject in the CPAP/Melatonin group exhibited an unusually short nocturnal REM sleep latency following treatment (9.5 minutes), although there was no further suggestion of early REM onset during any of the daytime testing sessions. Phasic REM activity (REM Index) also displayed significant increases in both treatment conditions following treatment (127.8 ± 54.1 for the CPAP/Melatonin group; 163.3 ± 68.5 for CPAP/Placebo). These values were similar to the amount of phasic REM activity observed in the matched controls (177.0 ± 77.7). Post-Treatment values for REM sleep latency and phasic REM activity in both conditions reflect a normalization of REM processes, with more appropriate timing of the REM sleep cycle, as well as intensity of the REM activity (as indicated by the phasic eye movement count used to determine REM Index). This is not consistent with findings of James et al. (1987), who observed increased REM sleep latencies in a group of non-complaining sleepers after administering 5 mg of melatonin before bedtime. In addition, Anton-Tay et al. (1974) observed increased REM density in a group of normal sleepers following 1 mg melatonin dosage in the morning. The observed differences in the present study could be attributed to the fact that OSAS subjects had acquired a sufficient REM sleep debt, so that the pressure for REM sleep remained fairly

high even after two weeks of continuous CPAP treatment. Therefore, the effects of adjuvant melatonin treatment on sleep measures, may have been obscured by the continuing process of sleep recovery in the OSAS subjects.

It was further hypothesized that performance on daytime measures of functioning should parallel these improvements in sleep fragmentation in the OSAS group. The finding of significantly reduced mean sleep latencies on the MSLT in the OSAS group at Baseline compared to the control group are consistent with the findings of Roth et al. (1980). Since the MSLT has been shown to be sensitive to sleep disruption (Stepanski et al, 1985), it was initially thought that alleviation of the chronic sleep fragmentation with appropriate CPAP therapy in the OSAS group would result in improved MSLT values. However, unlike the findings of Lamphere et al. (1989), we failed to find significant differences following 2-3 weeks of continuous treatment. While sleep latency increased by approximately 2 minutes (from Baseline values of 4.6 minutes to Post-Treatment values of 6.8 minutes), on average, values were still appreciably less than the matched controls (10.1 minutes). In part, this may reflect differences in the criteria used to define sleep onset during the nap sessions in the various protocols. In the present study, sleep onset was defined as the first epoch of any stage of sleep, rather than the first 1.5 minutes (3 epochs) of Stage 1 sleep, or one epoch of any other stage of sleep. This was in order to detect short sleep episodes that are quickly disrupted by apnea-related arousals. Although the criteria for sleep onset might change the absolute value of the mean sleep latency value on the MSLT in comparison with other studies, it is still not clear that this would alter the ability to detect improvement following

treatment. This indicates that the OSAS group had retained their sleep propensity, or "sleepability", despite adequate treatment. This is consistent with other studies which also demonstrated little to no improvement on MSLT sleep latency values following treatment (Kribbs et al., 1992; Engleman et al., 1992; Sangal et al., 1992). It would be interesting to see if a higher degree of normalization would result with a longer treatment duration (as evidenced by Wittig et al., 1986, after a period of 6 weeks) or more accurate measures of compliance. Furthermore, our lack of improvement on the MSLT cannot be adequately explained by lack of response to treatment (as evidenced by dramatic improvement in sleep and respiratory measures) or poor compliance in our group of OSAS subjects. The average rate of daily/nightly CPAP usage of 5.5 ± 0.9 hours is similar to levels found by Engleman et al. (1993) of 5.9 ± 1.4 hours, and by Krieger's finding (1992) of mean daily rate of use of 5.6 ± 0.1 hours. In addition, the present study implemented redundant measures to ensure compliance on the part of the subjects using CPAP (i.e., sleep-wake diaries, telephone call-in, and hours of use on the counter on the CPAP unit).

The finding of significant improvement in MWT sleep latency values in all but two OSAS subjects (1 subject in the CPAP/Melatonin condition; 1 in the CPAP/Placebo condition) following treatment were consistent with the idea that patients may display discordance on these two measures (MSLT mean sleep latency values were not significantly changed after treatment). While initial Baseline MWT values were almost two standard deviations from the matched controls (8.41 minutes versus 33.31 minutes), latencies following treatment improved to 13.14 minutes, on average. A significant difference was

also noted between MWT mean sleep latency values in the CPAP/Melatonin versus the CPAP/Placebo group Post-Treatment (8.6 ± 3.5 minutes versus 16.5 ± 8.1 minutes). While this indicates that there was a failure for our group of OSAS subjects to completely “normalize”, this increased ability to maintain wakefulness was reflected in enhanced performance measures (i.e., number of gaps in responding on the FCRTT; faster reaction times on the FCRTT), which will be discussed in more detail later.

In summary, the lack of improvement in daytime sleep propensity in the OSAS group, as indicated by persistently lower sleep latency values on the MSLT and the MWT, conflicts with previous studies (Wittig et al., 1986; Lamphere et al., 1989). However, more recent studies have also failed to demonstrate significant improvement on MSLT measure (Kribbs et al., 1992; Engelman et al., 1992; Sforza and Lugaresi, 1994). This may indicate the relative insensitivity of these tests as behavioral measures of treatment outcome, as compared with the measures (i.e., number of gaps) derived from the FCRTT. Since the present study demonstrated an adequate level of compliance with CPAP therapy and correction of sleep fragmentation in the OSAS group following two weeks of treatment, the role of sub-optimal CPAP treatment in the OSAS group (Kribbs et al., 1993b) can be ruled-out as factors contributing to persistent daytime sleepiness. However, as suggested by Kribbs et al. (1993a), the possibility remains that continued daytime sleepiness in OSAS subjects can be attributed to inadequate sleep duration, and extending the sleep period in these individuals may alleviate the ongoing daytime hypersomnolence. Another possible explanation, as suggested by Montplaisir et al. (1993) and Kribbs et al. (1993a), is that the

OSAS subjects suffer from irreversible anoxic brain damage which leads to persistent decrements in cognitive functioning. The demonstration of continued neuropsychological deficits in other studies, particularly in executive function tasks (Engleman et al., 1993; Bedard et al., 1993), parallels the present finding of the persistent ability to rapidly to descend into sleep, which may also reflect permanent neurological changes in OSAS patients. Furthermore, it is possible that evaluation over a longer period of treatment with a greater number of subjects may yield more significant findings.

In addition, the observed MWT mean sleep latencies in the control group (33.3 ± 12.94 minutes) are consistent with recent normative data in healthy normal sleepers (Doghramji et al., 1996), which found an average sleep latency of 35.2 ± 7.9 minutes. This slight difference in values may be attributed, once again, to the different criteria which was adopted for the present study (one epoch of any stage of sleep). A more direct comparison can be made for the matched control group with data obtained by Doghramji et al. (1997, submitted for publication), who used a more lenient criteria for defining sleep onset, which used the first epoch of any stage of sleep. This yielded mean sleep latency values that were even closer to the control group in the present study (32.6 ± 9.9 minutes). Furthermore, there was evidence for discordance on sleep latency measures on the MSLT and the MWT for some of the matched control subjects, as well. Three out of 8 control subjects displayed mean sleep latencies on MSLT of less than 10 minutes; MWT mean latencies were all 40.0 minutes. Therefore, this adds evidence that these two objective tests of daytime functioning may be tapping different processes.

A surprising finding in the present study, was the fact that the CPAP/Melatonin group had consistently lower sleep latency values on all MWT sessions, compared to the CPAP/Placebo group. In particular, the last testing session of the day (15:00 hours), displayed a further reduction in sleep latency in the CPAP/Melatonin condition following treatment (from Baseline value of 6.0 down to 5.8 minutes). The CPAP/Placebo group went from 9.3 minutes at Baseline to 10.4 minutes following treatment. The diminished capacity to maintain wakefulness in the CPAP/Melatonin group could possibly be a result of some residual behavioral sedating properties associated with melatonin, or an interaction with the internal clock mechanism, whereby sleep tendency was shifted to an earlier time of the day. Therefore, the increased pressure to sleep which may have resulted from melatonin ingestion may have overridden the subjects' motivational drive to maintain wakefulness during the day. This finding was quite unexpected since there is no evidence of prolonged daytime carryover effects of nocturnal melatonin administration.

In summary, the lack of the effect of melatonin on body temperature and sleep parameters may have been due to the already high circulating levels of endogenous melatonin present at night in the study group. The level of melatonin production/synthesis was not assessed prior to treatment; clearly, this is important with regard to timing and dosage of melatonin administration. Given the fact that the OSAS subjects demonstrated a high propensity to sleep compared to matched controls, further reductions in the nocturnal sleep latency in the sub-group who received melatonin was not expected. The finding of a daytime carry-over effect of melatonin (as evidenced on the MWT) was quite surprising,

given the documented lack of such effects in other studies with nocturnal melatonin administration. This suggests that the CPAP/Melatonin group may have experienced the behavioral sedating properties associated with the substance, without evidencing any significant effects on their nocturnal sleep.

In terms of cognitive functioning, unlike the findings of Montplaisir et al. (1992) and Bedard et al. (1993), significant differences between the OSAS group at Baseline and the matched controls on measures of cognitive performance were not observed. Based upon studies which have shown specific deficits in OSAS patients' abilities to formulate goals, make plans, and carry those plans out (termed "executive functions") (Engleman et al., 1994; Bedard et al., 1991), the present study attempted to focus on the class of neuropsychological tests that tap into the domain of the "executive functions". The Baseline OSAS group only demonstrated significant differences from the matched control group on three out of 16 measures: general IQ of the WAIS-R, Trail Making - Part B (time), and delayed recall on the Rey-Osterreith Complex Figure Test. The finding on the Trail Making was not unexpected, since decrements on this task have been demonstrated in several studies of untreated apneics.

Significant improvement in the OSAS group following treatment was observed on three measures: Block Design of the WAIS-R; Digit-Symbol of the WAIS-R; and Trail Making - Part B of the Halstead-Reitan battery. All these measures impact heavily upon the constructive ability and psychomotor performance of the individual, which is also associated with the executive functions. While these findings are not surprising, it is interesting to note

that only one of these tests (Trail Making Test - Part B) indicated impaired performance in the OSAS group prior to treatment compared to the Baseline control group. This could indicate that even though OSAS subjects were not obviously deficient before treatment, there was still room for improvement. While the actual values on the Mazes test of the WISC-R did not differ significantly in the Baseline OSAS group compared to the controls, there was a non-significant increase in the number of impulsive errors observed in the OSAS group at Baseline. In their approach to complete the mazes, OSAS subjects demonstrated perseverative tendencies. Subjects tried the same unsuccessful approach repeatedly. In some instances, this resulted in a failure to complete the maze trial (4 OSAS subjects at Baseline; 3 OSAS subjects during Post-Treatment). None of the matched controls failed to complete any of the maze trials. This perseverative quality, or mental inflexibility, is characteristic of a sub-set of individuals in the OSAS patient group. This perseverative quality in responding was also seen in a group of apneics (Naegele et al., 1995), where they found a significantly decreased ability to initiate new mental processes and to inhibit automatic processes. This may be partial evidence in support of the findings of Bedard et al. (1993) and Engleman et al. (1993) that there may be some persistent deficits in tasks which require planning and foresight. Therefore, while overall neuropsychological test measures did not reveal significant loss of cognitive functioning in the untreated OSAS group, subjects were found to have subtle losses in tests of executive functioning compared to the control group, which became comparable to the control values after two weeks of continuous treatment. Furthermore, it is possible that we did not observe more significant changes following treatment due to the short treatment period (2 weeks). Perhaps, changes in cognitive

functioning would be evidenced over a longer period of time with continued CPAP usage. It is also possible that some of the observed Post-Treatment changes were a result of practice effects over the period of treatment.

The present study differed from studies by Montplaisir et al. (1992) with regard to their selection of subjects based upon nocturnal oxygenation criteria. Montplaisir et al. pre-selected their apneic population on the basis of the percentage of time spent during sleep at oxygen saturation levels of less than 80%. Therefore, in their study, in order to qualify as "severely apneic", OSAS patients must have spent more than 5% of the Total Sleep Time at saturation levels of less than 80%. In the present study, this distinction was not made in the OSAS group of subjects. Average Baseline values in the OSAS group for the percentage of time at oxygen saturation level below 80% in the present study were $5.7\% \pm 8.4\%$. Post-Treatment, this value fell to $0.02\% \pm 0.04$, which was similar to the control group level of $0.02\% \pm 0.05\%$. The percentage of time spent below 80% in other studies exploring deficits in cognitive function in patients with OSAS range from 0.1-22.5% (Bedard et al., 1991). Instead, in the present study, OSAS subjects were categorized as having "moderate-severe" apnea based upon the number of respiratory events per hour of sleep (AHI criteria > 25 events/hour). It is possible that the different criteria for disease severity accounted for the lack of significant findings in neuropsychological test measures in this study, and if so, adds further evidence to the argument that low levels of nocturnal oxygen saturation (hypoxemia) play an important role in the development of cognitive dysfunction in individuals with OSAS.

Subjective measures of mood and self-ratings of daytime sleepiness were also found to differ between the OSAS group and the matched control group. The Baseline values for the OSAS group on the Beck Depression Inventory are consistent with findings reported in a group of severe obstructive apneics (Bell, 1995). The observed values on the Beck Inventory of 8.1 ± 4.2 are comparable to Bell's findings (8.1 ± 7.3), indicating in both samples a mild degree of depressive symptomatology. While Beck scores are somewhat reduced in the OSAS group following treatment (6.0 ± 5.7), there is still a failure to reach levels reported by the matched controls. This may be a result of the short treatment duration, or indicative of a persistent depressive quality in this group of OSAS patients. The Stanford Sleepiness Scale was used to assess changes in the level of sleepiness over the course of a day. The Stanford Sleepiness Scale has been shown to be affected by OSAS (Regestein et al., 1987) and is sensitive to changes observed in OSAS following CPAP treatment (Rajagopal et al., 1986). This subjective measure of sleepiness is also affected by sleep deprivation and fragmentation of sleep (Bonnet, 1985). The Post-Treatment finding of a significant time effect upon subjective sleepiness ratings (increased sleepiness at 23:00 hours in all groups), is consistent with findings of Fröberg (1977), where peak sleepiness was reported close to the trough of body temperature in subjects kept in temporal isolation conditions. At 23:00 hours, most of our subjects began to experience a significant decline in their body temperature. Furthermore, our repeated measures analyses bore out the fact that, post-treatment, the CPAP/Melatonin and CPAP/Placebo groups were distinctively different in terms of their reported level of sleepiness at 23:00 versus 09:00, 13:00, and 14:00 hours.

This is also consistent with Monk et al's. (1983) finding that minimum subjective sleepiness occurs approximately 6 hours after waking (subjects in the present study awakened between 7:00-7:30 hours). Overall, the findings in the present study suggest that subjective "sleepiness" was consolidated at 23:00 for all subjects following the course of treatment.

In addition, the Epworth Sleepiness Scale has previously been shown to distinguish normal sleepers from subjects with OSAS and narcolepsy, and indicates improvement toward normal values in CPAP-treated OSAS patients (Smolley et al., 1993). While OSAS subjects in the present study were significantly different from the matched controls prior to treatment (14.0 ± 5.2 versus 4.1 ± 2.2), they failed to reach normative levels on the Epworth Sleepiness Scale following the course of treatment (12.0 ± 5.0). These levels are comparable to the findings of Johns (1992) (14.3 ± 3.6) in a group of untreated apneic patients. However, following a treatment period of 3 months, Epworth Sleepiness Scale scores in Johns' study decreased to 7.4 ± 4.1 . Initially, it was thought that the persistently high values on the Epworth Sleepiness Scale may be due to the sub-set of subjects receiving adjuvant melatonin at night; however, when the two treatment conditions were examined separately, they were virtually identical. This residual subjective sleepiness is not surprising, given the context that certain activities listed on the questionnaire are more likely to unmask the physiological sleepiness of the individual. Also, improvement on the Epworth Sleepiness Scale, indicating lower levels of daytime sleepiness, may be observed with a longer treatment duration.

In the present study, the values for the derived estimates of the temperature rhythm, respiratory events index (AHI), sleep fragmentation measures, hypoxemia indices and objective daytime performance measures were subjected to a series of correlations to assess the predictive value of these measures in terms of treatment outcome. Correlational analyses between Baseline physiological and Baseline daytime performance measures indicated that lower minima values on measures of nocturnal oxygen desaturation at Baseline assessment was associated with an increased number of gaps/lapses in responding on the Four Choice Reaction time task. Furthermore, the Baseline minimum desaturation level was highly predictive of estimating treatment response on the Four Choice Reaction Time task in the OSAS group (change scores following treatment) ($r = 0.72$). This significant positive relationship suggested that the more severely impaired apneic (as indicated by lower desaturation minima), had a lower degree of improvement on the performance task than an apneic with higher values of desaturation minima. This could be inferred to mean that the OSAS subjects who displayed greater levels of oxygen desaturation were experiencing more permanent changes in brain functioning, as evidenced by the lack of improvement on daytime testing following treatment. In contrast, OSAS subjects with higher minima values may have a better response to treatment, since the apneic disorder is possibly detected at a stage where changes in brain functioning are transitory in nature. These findings are consistent with those of Bedard et al. (1991) who demonstrated that the nocturnal oxygen minimum desaturation measure was highly correlated with measures on the Four Choice Reaction Time test (i.e., number of gaps and reaction time) in a group of moderate to severe apneics. Therefore, nocturnal hypoxemia indices (i.e., minimum oxygen desaturation) are

highly predictive of performance on a short-duration reaction time task.

Furthermore, in the present study, the most significant correlations for Baseline sleep latency measures on the MWT were the Baseline measures of sleep fragmentation (FACE and ACTIFACE) and the respiratory events index (AHI). This was also demonstrated by Poceta et al. (1990), where it was found that the measure of respiratory arousal index was the most important diagnostic correlate of daytime sleepiness, as measured by the MWT. These findings are consistent with the notion that the ability to maintain wakefulness during the day is highly dependent upon nocturnal sleep continuity (Stepanski et al., 1984; Bonnet, 1986). Therefore, measures of apnea-hypopnea frequency (AHI) could be considered to be more related to sleep fragmentation, per se, than they are to hypoxemia level (although there could be an interaction between the two respiratory parameters). In addition, the significant positive association between Baseline measures of desaturation events per hour of sleep (DESATIND) and change scores on the MWT ($r = 0.58$) also lends support to the idea that the more frequent the desaturation events, which may also be more related to the level of sleep fragmentation, the greater the response to treatment (as indicated by greater improvement in the ability to maintain wakefulness following treatment).

Correlations between change scores on the various classes of measures indicated that the most significant relationships existed between actigraphic-derived measures of sleep continuity (ACTIFACE) and mean sleep latency values on the MWT. The lack of a significant correlation with the NPSG-derived value of sleep continuity (FACE) and scores

on the MWT could partly be attributed to the fact that there was a fairly restricted range of improvement noted in NPSG-derived (FACE) values following treatment in the OSAS group (mean FACE values increased by approximately 7 minutes in the OSAS group). There was a more dynamic range of improvement noted in sleep continuity observed with the measures of ACTIFACE (increased by average of 19 minutes following treatment). Therefore, the higher degree of correlation reported with actigraphy-derived measures of sleep continuity and daytime performance may be due to the greater range of observed values using this measure versus NPSG-derived values. In addition, it is possible that NPSG-derived measures of sleep continuity, which are based upon shifts to a lighter stage of sleep or awakening, are not necessarily associated with the same degree of gross body movements, as would be detected via actigraphy. Therefore, the more subtle indications of arousal that are associated with FACE, may not contribute as much to the impairment in the overall ability to maintain wakefulness in OSAS subjects, as do the larger arousals detected with actigraphy.

V. CONCLUSION:

In conclusion, deviations from the matched control group were observed in the OSAS group at Baseline for every group of study parameters. Repeat studies were performed to see if short-term CPAP treatment had any impact upon the various physiological, cognitive performance or subjective measures under investigation. Furthermore, it was part of the study design to ascertain if any additional beneficial effects could be derived by adding

melatonin to the treatment regimen. While many of the neuropsychological test measures and subjective ratings of sleepiness (Epworth Sleepiness Scale) and mood (Beck Depression Inventory) were not significantly changed in the OSAS group following two weeks of continuous treatment, sleep latency values on the MSLT and MWT, sleep fragmentation measures, and temperature measures responded favorably to treatment. However, following treatment, the level of daytime sleepiness (as assessed by the MSLT and MWT) was still appreciably higher in the OSAS group as compared to the matched control group. This finding suggests lack of complete “normalization” in the OSAS group after treatment.

Therefore, the specific issues raised in the present study can be answered as follows:

1) Do OSAS patients demonstrate a disturbance in their nocturnal temperature rhythm compared to matched controls? The findings of the present study support the hypothesis that chronic sleep fragmentation and disrupted sleep architecture in untreated obstructive sleep apnea syndrome contributes to a disturbance in the nocturnal temperature rhythm, as evidenced by decreased amplitude of the derived temperature rhythm compared to a group of age- and sex-matched non-complaining sleepers. Other temperature measures (i.e., phase or mean temperature values) were not significantly different from the matched control group prior to treatment.

2) Does short-term CPAP treatment alone result in normalization of the nocturnal temperature rhythm in OSAS subjects? Significant improvement was noted in the

amplitude of the temperature rhythm following two weeks of continuous CPAP treatment in the OSAS group. However, contrary to expectation, melatonin did not enhance the effect of CPAP therapy with regard to the observed change in nocturnal temperature amplitude. However, a modest, though non-significant phase-advancing effect of melatonin was noted following treatment. This failed to reach significance due to the wide range of variability in the timing of the temperature nadir in OSAS subjects Post-Treatment in both treatment conditions.

3) Does adjuvant exogenous melatonin administration augment the effects of acute CPAP treatment in OSAS patients as evidenced by decreased levels of daytime sleepiness and cognitive impairment? This was ascertained by comparing daytime performance measures for CPAP/Melatonin and CPAP/Placebo conditions following two weeks of treatment. An unexpected finding in the present study, was the fact that the CPAP/Melatonin group had consistently lower sleep latency values on all MWT sessions, compared to the CPAP/Placebo group. In particular, the last testing session of the day (15:00 hours), displayed a further reduction in sleep latency in the CPAP/Melatonin condition following treatment (from Baseline value of 6.0 down to 5.8 minutes). Sleep latency values on the MSLT following treatment were not significantly different in the CPAP/Melatonin condition compared to the CPAP/Placebo condition. No significant improvement was noted following treatment in the CPAP/Melatonin condition compared to the CPAP/Placebo condition on measures of the Four-Choice Reaction Time Test (i.e., number of gaps) or on any specific tasks which involve “executive functions” (i.e., Trail Making - Part B; Picture

Arrangement of the WAIS-R). These findings were contrary to the expected outcome of further improvement in the CPAP/Melatonin condition compared to the CPAP/Placebo condition on objective measures of daytime performance, which would parallel the changes in the nocturnal temperature rhythm.

4) Can the severity of the apneic disorder as measured by the daytime vigilance deficits and subsequent response to treatment be predicted by the degree of the nocturnal temperature disturbance, respiratory events index, sleep fragmentation, or nocturnal hypoxemia indices? While several sleep fragmentation parameters (i.e., NPSG-derived measures of FACE and actigraph-derived measures of ACTIFACE) and respiratory indices (i.e., AHI, minimum nocturnal oxygen desaturation, number of oxygen desaturation events per hour of sleep) had prognostic value in our study subjects, temperature measures bore little to no relationship with these parameters. Significant correlations were not reached primarily due to the large individual differences observed in derived temperature measures of phase and mean. Perhaps evaluation over a longer period of treatment with a greater number of subjects may yield more significant findings. Furthermore, with particular regard to the changes observed in the timing of the temperature minima following treatment, it would be valuable to re-evaluate these OSAS patients after several months of continued CPAP use, to determine if these changes in phase were transitory or more permanent in nature.

Table 1. Sociodemographic characteristics of the three groups.

SOCIODEMOGRAPHIC MEASURES	CONTROLS (n = 8)	OSAS BASELINE (n = 16)	OSAS POST-TX. (n = 14)
Gender	7 M / 1 F	11 M / 5 F	10 M / 4 F
Age	39.50 (9.04)	41.00 (11.20)	41.14 (11.49)
Education(years)	15.75 (0.71)	14.50 (1.75)	14.57 (1.70)
Body-Mass Index*	25.76 (3.64)	35.20 (6.61)	35.52 (6.69)
CPAP Use (hrs/nt)	-	-	5.49 (0.85)

*Body-Mass Index = Weight (kg) / Height² (meter²)

Data are means and standard deviations (SD).

Table 2. Comparisons of sociodemographic and subjective data.
(MANOVA with post-hoc Scheffé tests and paired-sample t test results)

SOCIODEMOGRAPHIC AND SUBJECTIVE DATA	A CONTROLS (n = 8)	B OSAS BASELINE (n = 16)	C OSAS POST-TX. (n = 14)	F VALUES	P	A-B SCHEFFÉ TESTS	A-C	B-C PAIRED t TESTS (n = 14)
Education (EDUC)	15.75 (0.71)	14.50 (1.75)	14.57 (1.70)	1.89	-	-	-	-
Epworth (ESS)	4.13 (2.17)	14.00 (5.18)	12.00 (5.01)	12.30	****	****	**	-
Beck (BDI)	0.75 (1.16)	8.13 (4.16)	6.00 (5.70)	1.10	**	**	*	-
Memory (MEM)	1.75 (9.04)	2.06 (31.9)	14.71 (23.48)	7.37	-	-	-	*

Data are means and standard deviations (SD).

*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001

Table 3. Comparisons of sociodemographic and subjective data.
(Repeated measures ANOVA results - Condition X Time)

SOCIODEMOGRAPHIC AND SUBJECTIVE DATA	CPAP/MELATONIN BASELINE (<i>n</i> = 6)	CPAP/MELATONIN POST-TX. (<i>n</i> = 6)	CPAP/PLACEBO BASELINE (<i>n</i> = 8)	CPAP/PLACEBO POST-TX. (<i>n</i> = 8)
Education (EDUC)	14.33 (1.97)	-	14.75 (1.58)	-
Epworth (ESS)	15.00 (6.78)	11.83 (7.31)	13.00 (2.88)	12.13 (2.90)
Beck (BDI)	10.17 (5.56)	7.00 (6.23)	6.75 (2.71)	5.25 (5.57)
Memory (MEM) *	-0.67 (28.86)	18.17 (24.38)	-3.75 (29.16)	12.13 (24.11)

Data are means and standard deviations (SD).

**p* < 0.05, repeated measures ANOVA, Time effect.

Table 4. Traditional sleep and respiratory measures.
(Paired-sample t test results)

SLEEP AND RESPIRATORY MEASURES	A CONTROLS (n = 8)	B OSAS BASELINE (n = 16)	C OSAS POST-TX. (n = 14)	B-C PAIRED t TESTS (n = 14)
TST	376.75 (66.71)	326.28 (52.89)	371.93 (51.74)	*
SLAT	14.06 (20.35)	11.97 (8.30)	12.04 (8.89)	-
REMLAT	99.06 (36.45)	140.59 (77.96)	77.45 (27.20)	**
SE	91.51 (4.02)	80.46 (13.07)	91.34 (5.31)	**
WASO (minutes)	31.69 (11.18)	76.44 (59.44)	37.11 (24.32)	**
ST1%	7.00 (3.20)	24.92 (17.25)	9.34 (6.58)	**
ST2%	60.39 (7.18)	55.14 (14.57)	51.38 (9.81)	-
ST3%	11.84 (4.03)	6.97 (5.54)	12.31 (9.52)	*
ST4%	2.59 (4.02)	1.21 (2.22)	5.36 (8.01)	-
REM%	18.19 (5.34)	11.76 (6.85)	21.61 (6.28)	***
AHI	6.20 (2.77)	66.60 (35.05)	3.61 (3.61)	****
DESATIND	1.66 (0.95)	44.73 (35.80)	3.96 (3.97)	***
MINDESAT	88.0 (2.14)	69.94 (13.02)	87.07 (4.80)	****
PCT<80	0.02 (0.05)	5.73 (8.40)	0.02 (0.04)	*

Data are means and standard deviations (SD).

*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001

Table 5. Traditional sleep and respiratory measures.
(Repeated measures ANOVA results - Condition X Time)

SLEEP AND RESPIRATORY MEASURES	CPAP/MELATONIN BASELINE (n = 6)	CPAP/MELATONIN POST-TX. (n = 6)	CPAP/PLACEBO BASELINE (n = 8)	CPAP/PLACEBO POST-TX. (n = 8)
TST	324.08 (57.49)	365.50 (63.85)	322.00 (53.79)	376.75 (44.70)
SLAT	15.83 (10.93)	9.75 (8.76)	10.63 (5.95)	13.75 (9.17)
REMLAT**	128.50 (66.78)	68.00 (38.08)	170.50 (81.88)	85.06 (14.00)
SE**	80.18 (12.45)	91.42 (5.27)	80.66 (14.37)	91.29 (5.70)
WASO (minutes)	79.25 (48.79)	37.00 (23.52)	82.06 (73.37)	37.19 (26.52)
ST1‡	23.43 (12.89)	9.88 (7.99)	28.05 (21.99)	8.92 (5.86)
ST2‡	57.77 (7.32)	49.32 (10.21)	53.21 (19.68)	52.93 (9.89)
ST3‡	6.52 (5.96)	13.42 (10.80)	6.03 (5.62)	11.49 (9.11)
ST4‡	1.62 (3.44)	7.03 (8.20)	0.84 (1.28)	4.10 (8.19)
REM‡	10.67 (5.27)	20.37 (7.28)	11.88 (6.64)	22.55 (5.75)
AHI	72.83 (27.41)	4.75 (5.35)	68.68 (42.53)	2.76 (1.37)
DESATIND	42.17 (31.59)	4.68 (2.63)	55.14 (39.65)	3.41 (4.85)
MINDESAT	64.33 (14.15)	86.67 (4.41)	71.38 (12.39)	87.38 (5.34)
PCT<80	9.09 (12.22)	0.03 (0.06)	4.64 (4.90)	0.01 (0.03)

Data are means and standard deviations (SD).

**p < 0.01, repeated measures ANOVA, Time effect.

Table 6. Comparison of physiological measures.
(MANOVA with post-hoc Scheffé tests and paired-sample t test results)

TEMPERATURE AND SLEEP MEASURES	A CONTROLS (n = 8)	B OSAS BASELINE (n = 16)	C OSAS POST-TX. (n = 14)	F VALUES	P	A-B SCHEFFÉ TESTS	A-C SCHEFFÉ TESTS	B-C PAIRED t TESTS (n = 14)
Amplitude	1.40 (0.15)	1.11 (0.32)	1.36 (0.28)	3.92	*	-	-	*
Mean	37.08 (0.34)	37.21 (0.32)	37.18 (0.24)	0.54	-	-	-	-
Phase (minutes)	220.00 (88.03)	305.86 (157.62)	311.29 (136.61)	1.30	-	-	-	-
FACE	9.20 (3.72)	3.84 (2.31)	10.17 (4.42)	12.46	****	**	-	***
SE	91.51 (4.02)	80.46 (13.07)	91.34 (5.31)	4.78	*	*	-	**
SLAT	14.06 (20.35)	11.97 (8.30)	12.04 (8.89)	0.07	-	-	-	-
REMLAT	99.06 (36.45)	140.59 (77.96)	77.45 (27.20)	7.13	**	-	-	**
REMI	177.00 (77.74)	74.86 (38.55)	148.07 (63.15)	9.34	***	**	-	**
REMD	2.57 (0.68)	2.10 (0.80)	1.85 (0.71)	2.41	-	-	-	-
ACTIFACE	24.11 (16.60)	5.79 (4.98)	24.77 (13.15)	12.67	****	**	-	***

Data are means and standard deviations (SD).

*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001

Table 7. Comparison of physiological measures.
(Repeated measures ANOVA results - Condition X Time)

TEMPERATURE AND SLEEP MEASURES	CPAP/MELATONIN BASELINE (n = 6)	CPAP/MELATONIN POST-TX. (n = 6)	CPAP/PLACEBO BASELINE (n = 8)	CPAP/PLACEBO POST-TX. (n = 8)
Amplitude*	0.97 (0.29)	1.28 (0.25)	1.22 (0.31)	1.42 (0.31)
Mean	37.19 (0.42)	37.21 (0.24)	37.23 (0.24)	37.16 (0.25)
Phase (minutes)*	403.33 (157.31)	259.67 (98.22)	232.75 (119.20)	350.00 (154.18)
FACE**	3.35 (1.87)	9.96 (3.20)	4.03 (2.79)	10.32 (5.37)
SE**	80.18 (12.45)	91.42 (5.27)	80.66 (14.37)	91.29 (5.70)
SLAT	15.83 (10.93)	9.75 (8.76)	10.63 (5.95)	13.75 (9.17)
REMLAT**	128.50 (66.78)	68.00 (38.08)	170.50 (81.88)	85.06 (14.00)
REMI***	141.17 (91.69)	127.83 (54.16)	161.25 (144.64)	163.25 (68.52)
REMD	2.03 (0.81)	1.63 (0.38)	1.87 (0.85)	2.02 (0.87)
ACTIFACE***	4.03 (2.75)	18.42 (7.13)	5.14 (5.06)	29.54 (14.98)

Data are means and standard deviations (SD).

*p < 0.05, **p < 0.01, ***p < 0.001, repeated measures ANOVA, Time effect
for all significant measures, with exception for Phase (Interaction effect)

Table 8. Mean sleep latencies (minutes) for individual testing sessions of the MSLT and MWT.

MEAN SLEEP LATENCY SCORES	A CONTROLS (n = 8)	B OSAS BASELINE (n = 16)	C OSAS POST-TX. (n = 14)
MSLT 1	12.58 (6.88)	4.21 (2.23)	5.65 (3.29)
MSLT 2	10.59 (6.32)	5.14 (9.55)	8.26 (4.30)
MSLT 3	8.93 (5.89)	3.75 (2.63)	6.67 (5.55)
MSLT 4	8.42 (5.46)	5.41 (3.59)	6.78 (5.36)
MWT 1	33.83 (12.13)	10.25 (5.92)	15.85 (9.90)
MWT 2	33.83 (12.76)	8.28 (7.65)	13.77 (10.94)
MWT 3	32.75 (13.59)	7.53 (7.86)	14.67 (12.59)
MWT 4	32.83 (13.61)	7.57 (5.91)	8.24 (9.54)

Data are means and standard deviations (SD).

Table 9. Mean sleep latencies (minutes) for individual testing sessions of the MSLT and MWT. (across treatment conditions)

MEAN SLEEP LATENCY SCORES	CPAP/MELATONIN BASELINE (n = 6)	CPAP/MELATONIN POST-TX. (n = 6)	CPAP/PLACEBO BASELINE (n = 8)	CPAP/PLACEBO POST-TX. (n = 8)
MSLT 1	4.67 (1.99)	5.31 (4.25)	4.29 (2.62)	5.92 (2.63)
MSLT 2	4.08 (2.33)	8.03 (6.15)	6.58 (5.93)	8.44 (2.69)
MSLT 3	4.39 (1.75)	5.03 (1.25)	2.96 (2.56)	7.90 (7.22)
MSLT 4	4.42 (2.08)	6.47 (3.52)	5.88 (4.53)	6.58 (6.67)
MWT 1	9.78 (7.33)	10.39 (6.48)	9.98 (3.90)	19.96 (10.36)
MWT 2	5.86 (3.37)	9.64 (6.75)	9.35 (9.42)	16.88 (12.80)
MWT 3	5.97 (3.87)	8.70 (6.52)	8.90 (10.82)	19.15 (14.50)
MWT 4	6.03 (3.96)	5.83 (3.11)	9.31 (7.46)	10.04 (12.39)

Data are means and standard deviations (SD).

Table 2a. Change scores (Post-Tx. minus Baseline) for individual testing sessions of the MSLT and MWT. (Repeated measures ANOVA results - Condition X Time)

MEAN SLEEP LATENCY CHANGE SCORES	CPAP/MELATONIN (n = 6)	CPAP/PLACEBO (n = 8)
MSLT 1	0.64 (4.01)	1.62 (3.79)
MSLT 2	3.95 (4.40)	1.85 (5.22)
MSLT 3	0.64 (2.40)	4.94 (6.46)
MSLT 4	2.06 (3.22)	0.71 (5.83)
MWT 1	0.61 (3.69)	9.98 (11.34)
MWT 2	3.78 (6.49)	7.52 (18.59)
MWT 3	2.72 (8.96)	10.25 (14.55)
MWT 4	-0.20 (3.45)	0.73 (7.30)

Data are means and standard deviations (SD).
No significant Time or Condition effect on MSLT or MWT; trend for Condition effect on MWT.

Table 10. Individual trial data on Four-choice Reaction Time Test for the three groups.

FCRTT DATA	A CONTROLS (n = 8)	B OSAS BASELINE (n = 14)	C OSAS POST-TX. (n = 14)
# Gaps			
Trial 1	2.86 (6.28)	24.23 (32.82)	12.21 (24.37)
Trial 2	2.88 (4.55)	27.64 (34.82)	11.21 (18.34)
Trial 3	2.63 (3.42)	22.43 (27.65)	9.50 (14.10)
Trial 4	4.50 (7.69)	19.50 (23.32)	10.57 (18.64)
Trial 5	3.88 (7.32)	14.15 (16.86)	12.67 (18.97)
Mean RT (msec)			
Trial 1	398.3 (94.1)	609.6 (269.8)	461.6 (105.2)
Trial 2	380.9 (47.9)	644.8 (356.2)	446.4 (94.9)
Trial 3	374.8 (45.2)	585.3 (252.2)	427.9 (72.7)
Trial 4	382.9 (69.5)	496.5 (128.2)	428.3 (92.5)
Trial 5	371.1 (51.6)	469.4 (114.4)	419.0 (86.6)
Accuracy			
Trial 1	98.48 (1.94)	98.47 (2.56)	98.93 (0.99)
Trial 2	97.95 (2.31)	98.16 (2.79)	98.34 (2.00)
Trial 3	97.67 (3.91)	98.11 (1.49)	98.40 (2.18)
Trial 4	97.17 (4.57)	98.13 (1.53)	98.36 (0.97)
Trial 5	96.64 (6.16)	98.42 (0.84)	98.08 (1.29)
Throughput			
Trial 1	153.92 (27.80)	112.30 (39.66)	133.57 (24.09)
Trial 2	156.21 (17.38)	109.43 (40.59)	136.78 (24.02)
Trial 3	158.06 (16.26)	115.16 (38.75)	141.28 (21.38)
Trial 4	155.76 (22.33)	125.22 (28.53)	142.45 (23.54)
Trial 5	158.15 (16.95)	132.13 (28.62)	144.76 (23.13)

Data are means and standard deviations (SD).

Table 11. Neuropsychological test measures. (Blocked Design)
(Paired-sample t test results)

NAME OF TEST	A CONTROLS (n = 8)	B OSAS BASELINE (n = 8)	C OSAS POST-TX. (n = 8)	A-B B-C PAIRED t TESTS (n = 8)
IQ	110.00 (11.71)	98.25 (14.12)	104.63 (17.82)	* -
VERBIQ	110.63 (11.50)	99.38 (14.88)	97.63 (12.60)	- -
PERFIQ	106.25 (10.79)	102.63 (16.75)	105.13 (16.24)	- -
PICTARR	9.75 (1.91)	9.00 (2.73)	9.13 (3.36)	- -
BLKDES	11.50 (2.27)	9.63 (3.20)	10.38 (3.25)	- *
OBJASS	9.50 (2.83)	8.50 (2.78)	9.62 (2.83)	- -
DIGSYMB	10.63 (3.38)	9.13 (2.70)	10.50 (3.46)	- *
VERBFL	36.13 (11.19)	40.38 (5.40)	41.75 (11.61)	- -
TRAILSB	55.38 (23.93)	82.63 (31.30)	64.38 (28.22)	** *
STORYIMM	12.06 (3.27)	10.38 (3.54)	10.88 (4.96)	- -
STORYDEL	10.56 (3.53)	8.25 (4.23)	8.88 (4.67)	- -
PURDUE (dom.)	15.50 (2.00)	13.13 (1.73)	13.88 (2.80)	- -
MAZES (# errors)	3.25 (2.76)	5.38 (3.96)	4.88 (3.40)	- -
REYCOPY	33.50	31.88	30.69	- -
REYIMM	21.88	15.38	17.31	- -
REYDEL	21.88	15.13	15.81	* -

Data are means and standard deviations (SD).

*p < 0.05, **p < 0.01

Table 12. Comparison of neuropsychological and daytime performance measures.
(MANOVA with post-hoc Scheffé tests and paired-sample t test results)

NEUROPSYCHOLOGICAL AND PERFORMANCE MEASURES	A CONTROLS (n = 8)	B OSAS BASELINE (n = 16)	C OSAS POST-TX. (n = 14)	F VALUES	P	A-B SCHEFFÉ TESTS	A-C SCHEFFÉ TESTS	B-C PAIRED t TESTS (n = 14)
FCRTT (#gaps/sq.trans)	3.04 (2.34)	7.60 (4.90)	5.26 (4.23)	3.30	*	*	-	**
MWT	33.31 (12.94)	8.41 (3.98)	13.14 (7.50)	26.08	****	****	****	*
MSLT	10.13	4.63	6.78	6.55	**	**	-	**
TRAILS B (sec.)	55.38	88.50	78.21	2.86	-	-	-	-
PERF IQ	106.25	101.14	102.93	0.26	-	-	-	-
PICTARR	9.75	8.79	9.00	0.35	-	-	-	-

Data are means and standard deviations (SD).
*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001

Table 13. Comparison of neuropsychological and daytime performance measures.
(Repeated measures ANOVA results - Condition X Time)

NEUROPSYCHOLOGICAL AND PERFORMANCE MEASURES	CPAP/MELATONIN BASELINE (n = 6)	CPAP/MELATONIN POST-TX. (n = 6)	CPAP/PLACEBO BASELINE (n = 8)	CPAP/PLACEBO POST-TX. (n = 8)
FCRT** (#gaps/sq.trans)	8.70 (5.14)	6.10 (4.05)	6.77 (4.88)	4.63 (4.52)
MWT*	6.91 (2.73)	8.64 (3.53)	9.39 (4.63)	16.51 (8.07)
MSLT**	4.39 (1.53)	6.21 (3.03)	4.93 (2.54)	7.21 (4.23)
TRAILS(B (sec.)	83.17 (29.81)	70.33 (31.80)	92.50 (33.72)	84.13 (37.79)
PERFIQ	96.50 (19.52)	100.33 (17.84)	104.63 (15.32)	104.88 (17.38)
PICTARR	7.67 (2.34)	7.67 (2.42)	9.63 (2.97)	10.00 (2.88)

Data are means and standard deviations (SD).

*p < 0.05, repeated measures ANOVA, Condition and Time effect

**p < 0.01, repeated measures ANOVA, Time effect

Table 14. Correlation values for Baseline physiological measures with Baseline daytime performance measures.

	AHI	DESATIND	MINDESAT	FACE	ACTIFACE	AMPL	MEAN
MWT	-.71***	-.53	.62**	.71***	.74*****	.24	.08
FCRTT (# gaps)	.50	.41	-.58**	-.24	-.42	-.21	-.09

p < 0.01, *p < 0.001, ****p < 0.0001

Table 15. Correlation values for change scores of physiological measures and change scores of daytime performance measures.

	AHI	DESATIND	MINDESAT	FACE	ACTIFACE	AMPL	MEAN
MWT	-.18	-.60*	.36	.12	.74**	.34	-.24
FCRTT (# gaps)	.40	.42	-.56*	-.24	-.51	-.39	.17

*p < 0.05, **p < 0.01

Table 16. Correlation values for Baseline physiological measures with change scores of daytime performance measures.

	AHI	DESATIND	MINDESAT	FACE	ACTIFACE	AMPL	MEAN
MWT	.18	.58*	-.42	-.14	-.51	.01	.10
FCRTT (# gaps)	-.40	-.45	.72**	.40	.58*	-.002	.05

* $p < 0.05$, ** $p < 0.01$

Figure 1. Average Fitted Temperature Data for the Three Groups

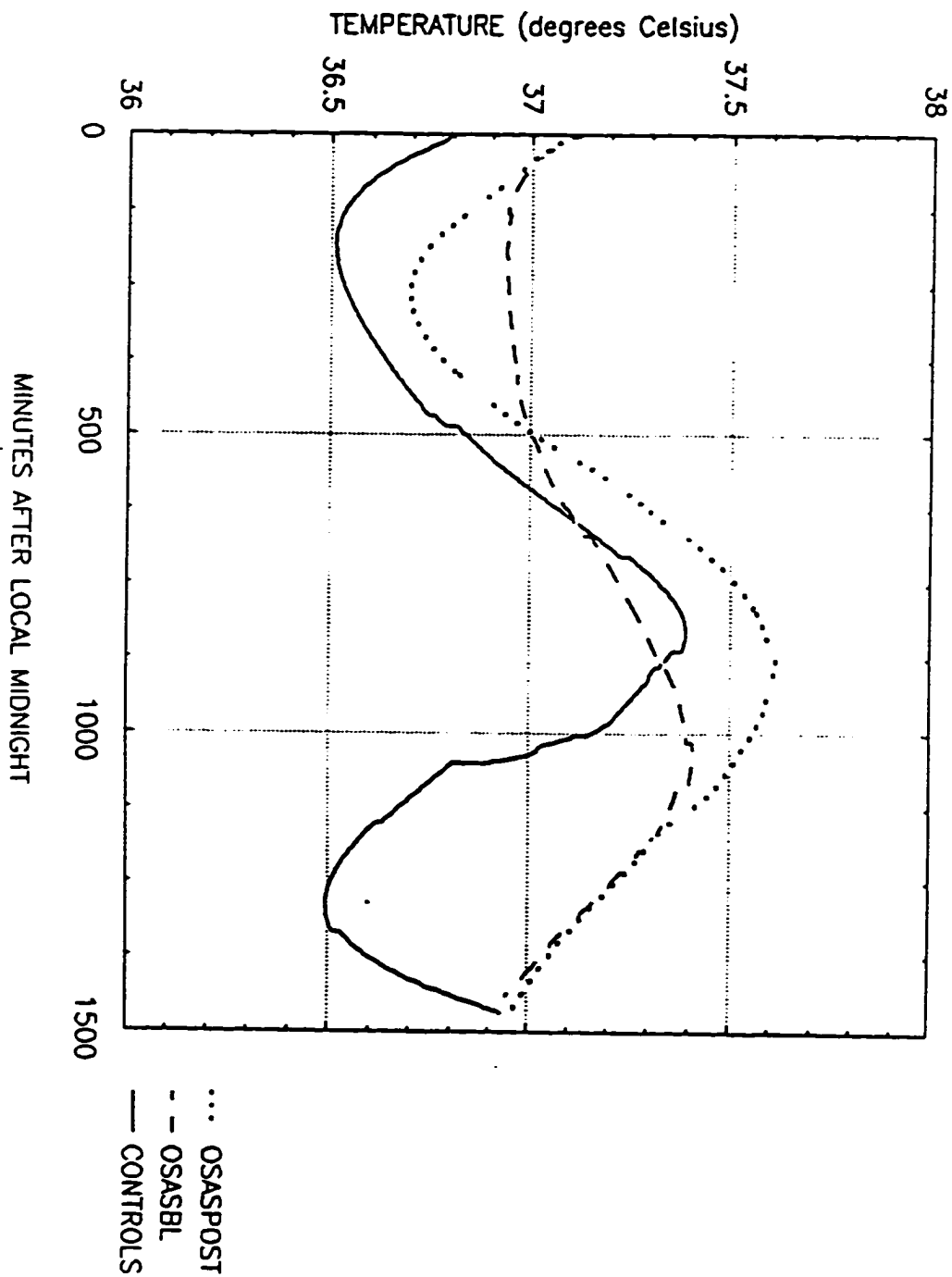


**Figure 2. Sleep Stage Percentages
(Controls v. OSAS Baseline and Post Tx.)**

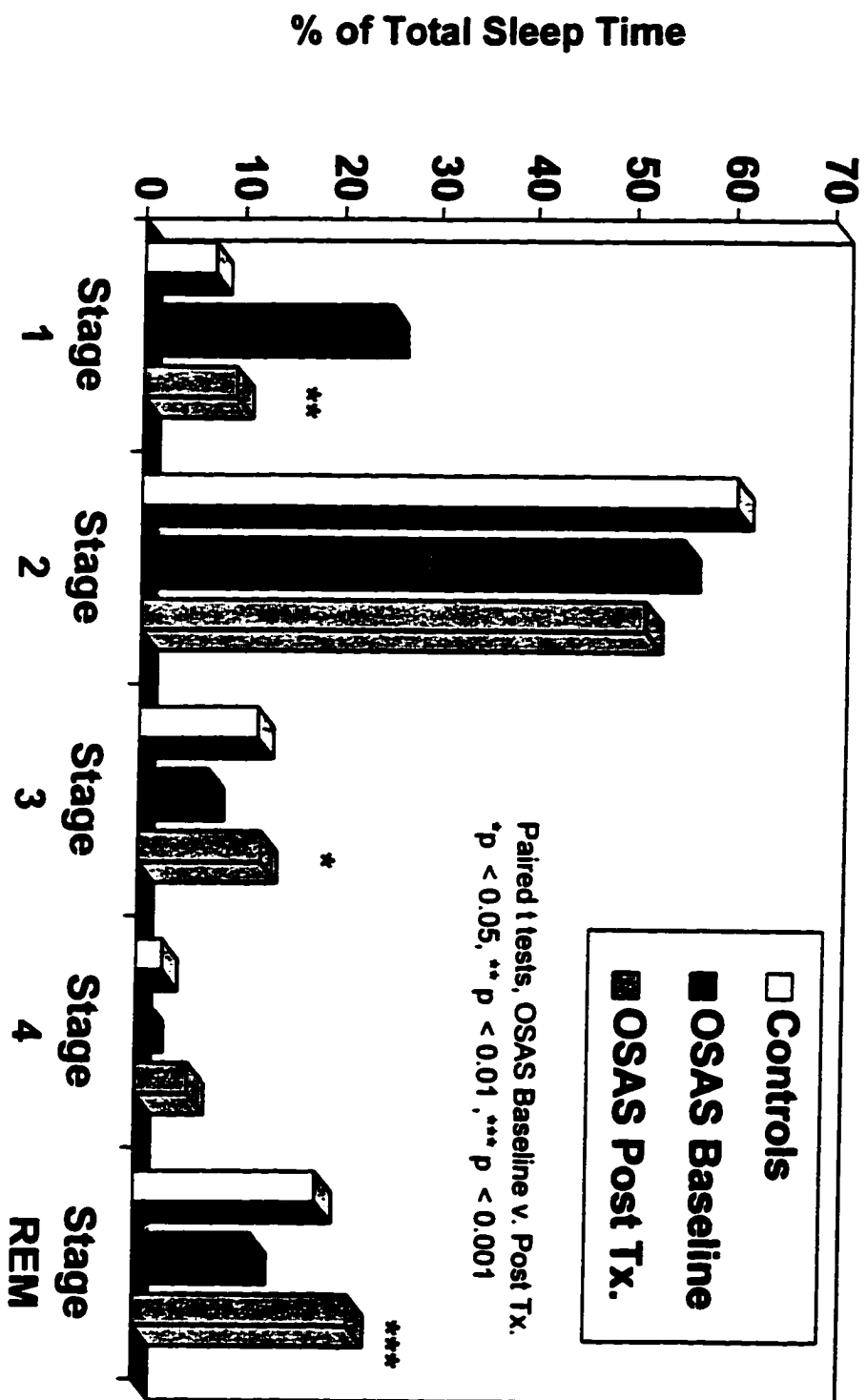


Figure 3. Sleep Stage Percentages - Change Scores in OSAS Group (across treatment conditions)

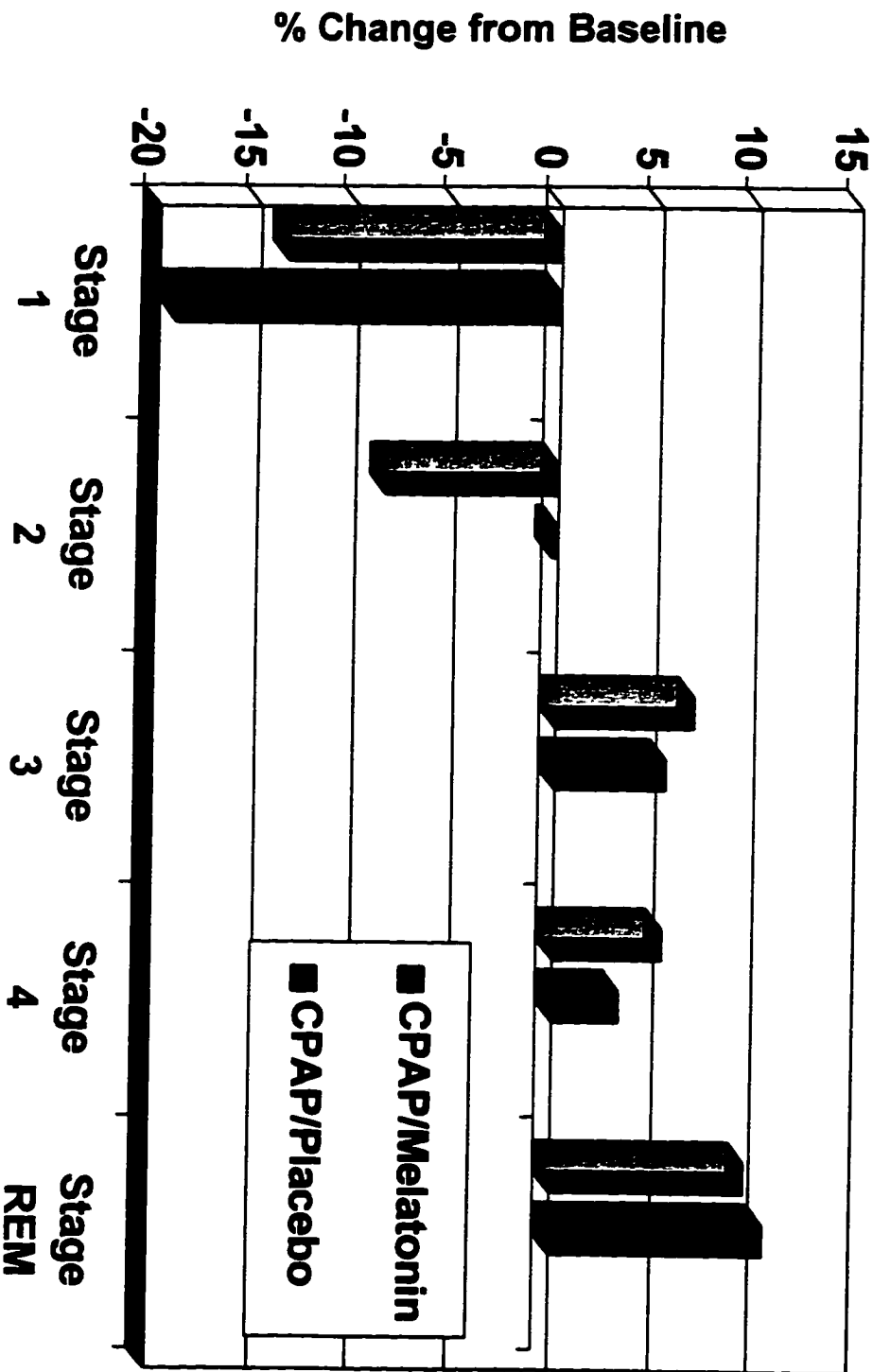


Figure 4. MSLT Data (across sessions)

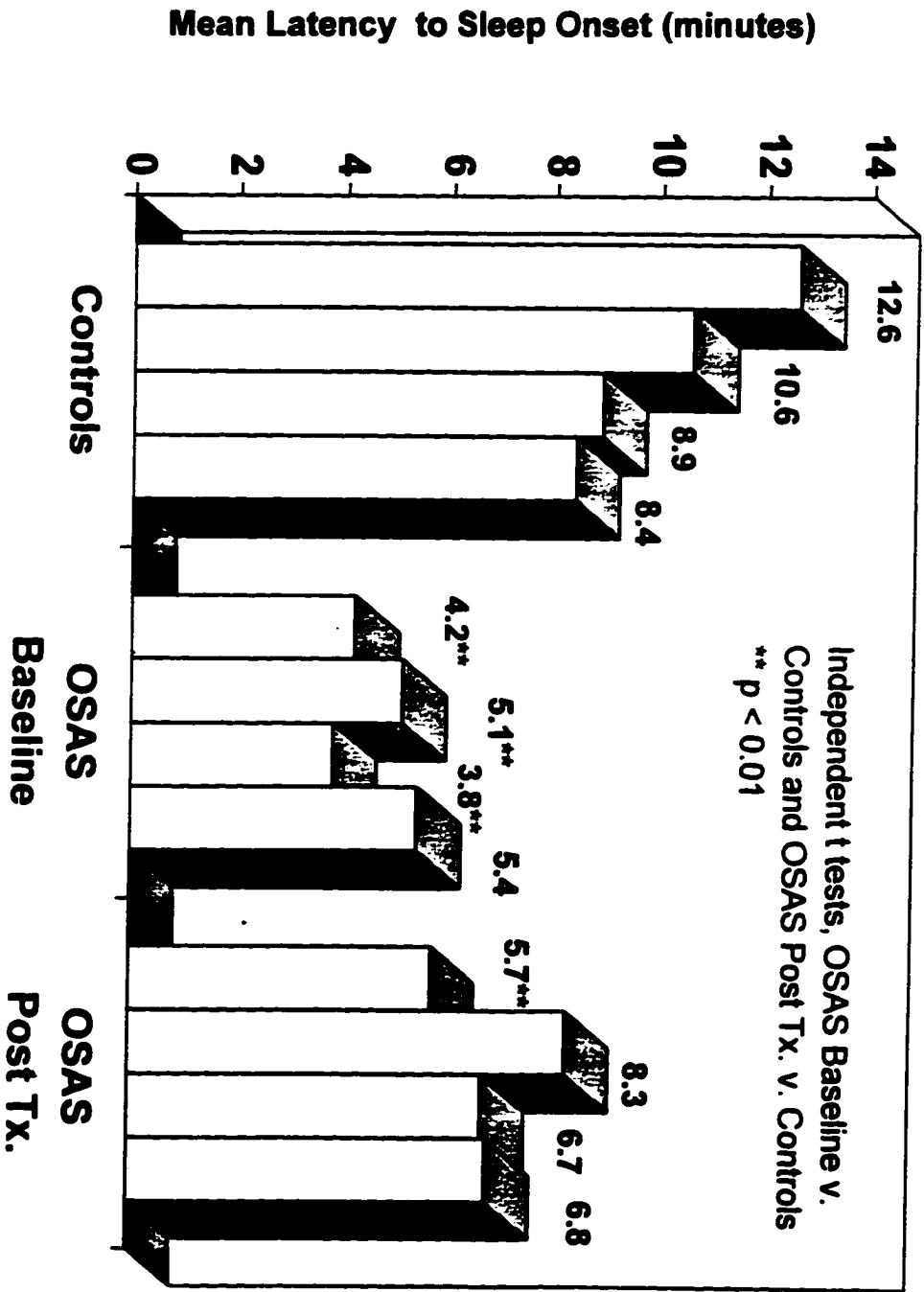


Figure 5. MWT Data (across sessions)

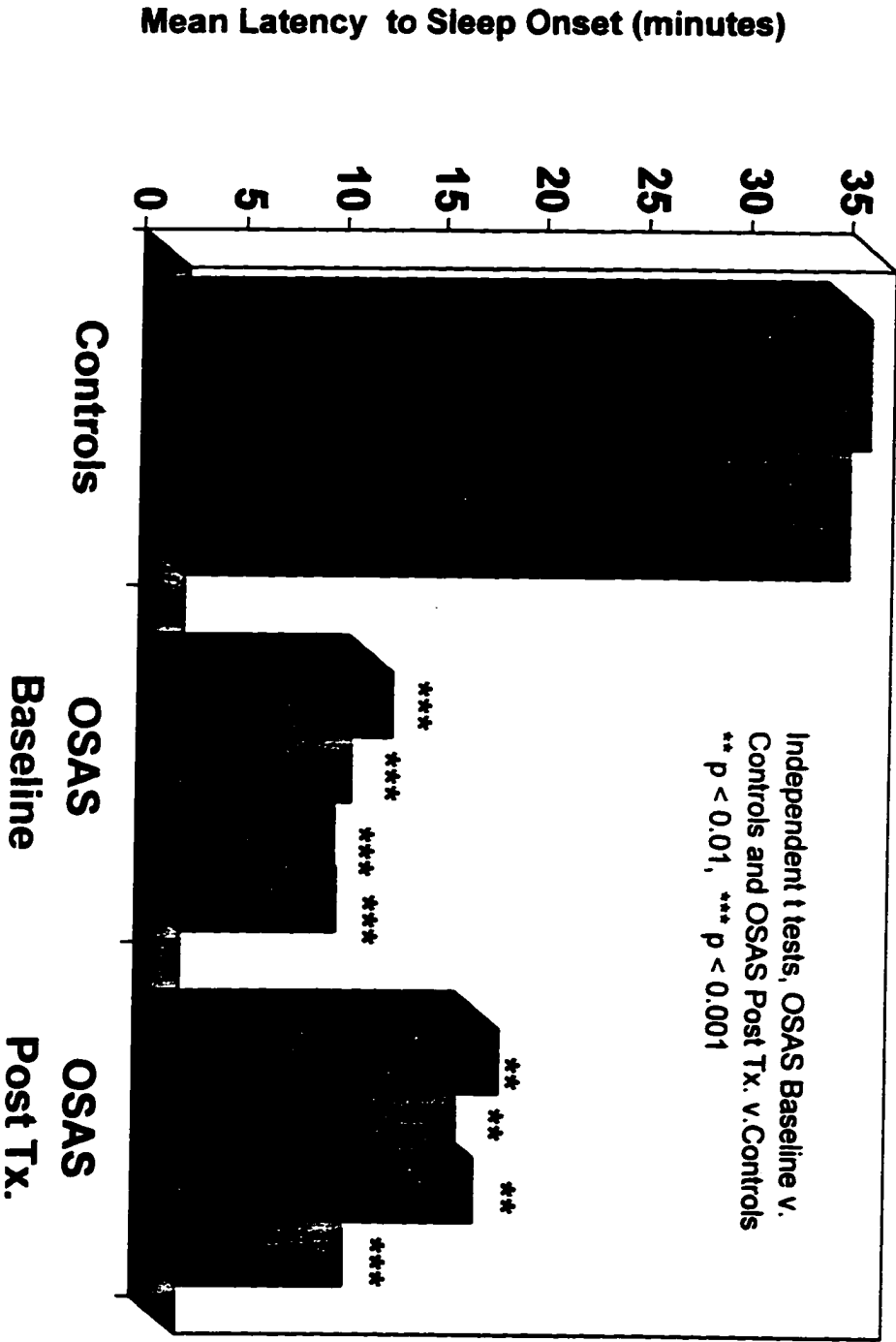
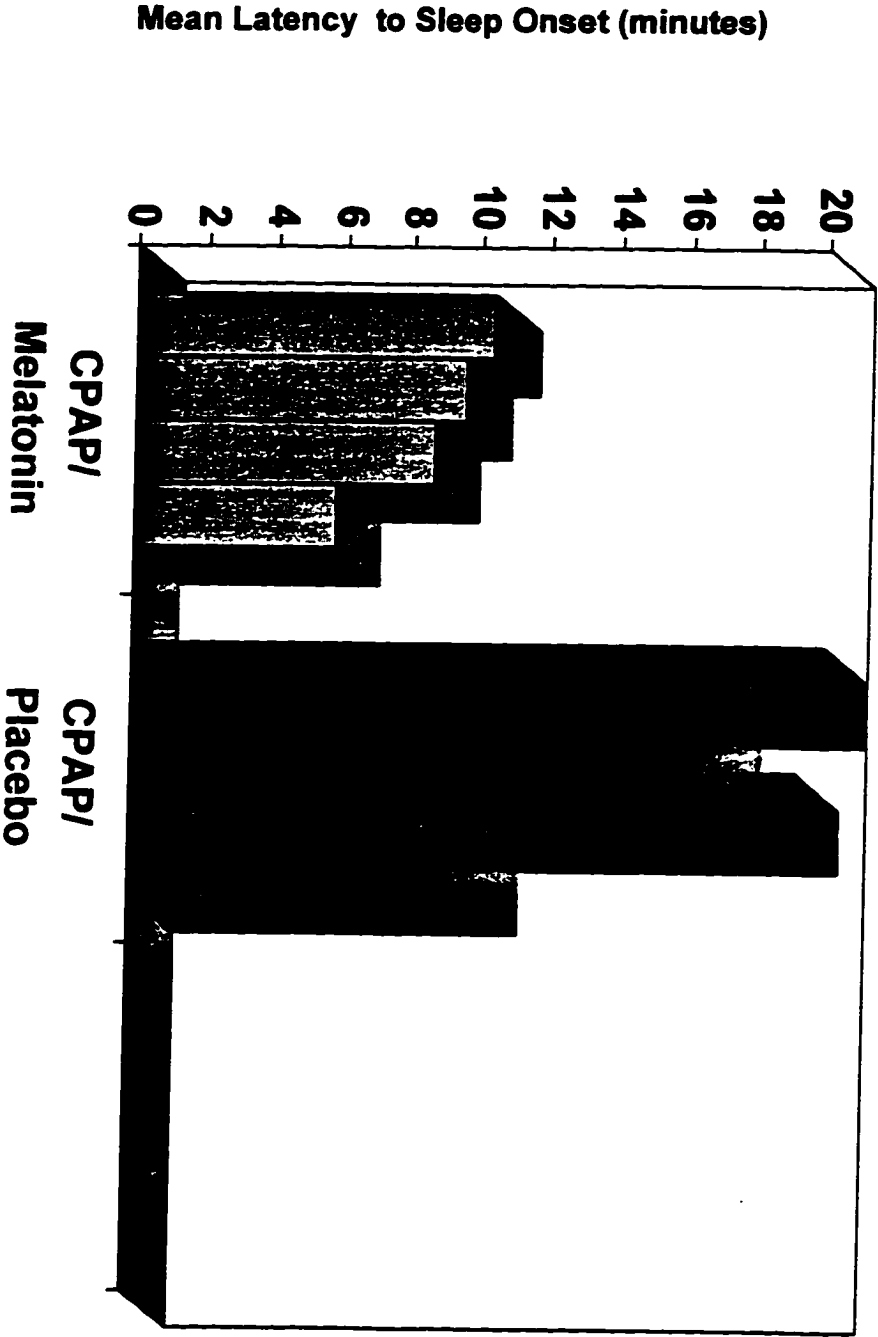


Figure 6. MWT Data - Post Tx. (condition x sessions)



APPENDIX I

PROTOCOL SCHEDULE

Baseline and Post-Treatment phases of the study will conform to the following schedule:

- 3:00-6:30 P.M - Subject arrival to the Sleep Disorders Center/GCRC
- Necessary forms to be filled-out for GCRC
 - Psych.interview and intake information (Beck Depression Inventory, Self-rating Scale of Memory Function, Epworth Sleepiness Scale, Handedness Inventory)
 - Administration of the WAIS-R
 - Neuropsychological testing battery:
 - A) Mazes of the WISC-R
 - B) Trail Making of the HRB (parts A & B)
 - C) Verbal Fluency Test (letters C,F,L)
 - D) Rey-Osterreith Complex Figure (Copy)
 - E) Rey-Osterreith Complex Figure (Immediate Recall)
 - F) Stories of the Wechsler Memory Scale (Immediate Recall)
 - G) Purdue Pegboard
 - H) Rey-Osterreith Complex Figure (Delayed Recall)
 - I) Stories of the Wechsler Memory Scale (Delayed Recall)
- 6:30 P.M. - DINNER
- 7:30-9:30 P.M.- Three practice sessions of the FCRTT (to be given over 2 hour span)
- 9:30 P.M. - Placement of electrodes and other monitors
- 11:00 P.M - Beginning of the sleep recording (NPSG)
-
- 7:00 A.M - Up-time
- 7:30 A.M. - BREAKFAST
- 8:40 A.M. - FCRTT #1
- 9:00 A.M. - MWT #1
- 10:00 A.M. - MSLT #1
- 10:40 A.M - FCRTT #2
- 11:00 A.M. - MWT #2
- 12:00 P.M. - MSLT #2
- 12:20 P.M. - LUNCH
- 12:50 P.M. - FCRTT #3
- 1:00 P.M. - MWT #3
- 2:00 P.M. - MSLT #3
- 2:40 P.M. - FCRTT #4
- 3:00 P.M. - MWT #4
- 4:00 P.M. - MSLT #4
- 4:40 P.M. - FCRTT #5

-Subjects will complete the SSS prior to each MSLT, MWT and FCRTT testing session, and upon going to bed and arising.

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