THE CIRCADIAN RHYTHMS OF CORE BODY TEMPERATURE, SERUM CORTISOL AND SERUM MELATONIN: PATTERNS OF DISRUPTION IN CHRONIC INSOMNIA OF CHRONOBIOLOGIC ORIGIN

by

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CIRCADIAN CORRELATES OF DISORDERED SLEEP

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Abstract

After specific causes of chronic insomnia have been ruled out, (e.g., nocturnal myoclonus, drug abuse) there remain a substantial number of patients who have been inexplicably sleepless and fatigued for many years. We wished to learn whether the chronicity of insomnia in these patients was associated with phase-shifts in circadian rhythm, a disorder of internal timekeeping. We identified three types in a pilot study: a) phase-delay, b) phase-advance, and c) an arrhythmic group.

Delayed sleep-phase insomnia (DSPI) has been well described. It is characterized by an intractably late sleep-onset and awakening time, a consistent sleep time-frame, and an essentially normal sleep EEG.

Advance sleep-phase insomnia (ASPI) is a much rarer phenomenon, with few reported cases. Sleepiness becomes overpowering in the late afternoon or early evening, followed by a very short sleep onset latency and an exceedingly early awakening time. The EEG is essentially normal.

Amplitude disorders and arrhythmias are even more rare under normal environmental conditions. They have been described but not carefully examined. We identified two patients in the pilot study who displayed a complete arrhythmia of their core temperature cycle. Their sleep was marked by variable onset latencies, and severe difficulties with sleep maintenance. Sleep-time averaged less than two hours per night, and was associated with a substantial reduction in slow-wave sleep.

Though these patterns sound easy to recognize, they are often obscured in patient reports. For example, social schedules requiring

early rising for work in spite of phase-delay or explanations that patients develop to better account for their predicament may disguise true patterns. We tried to detect the more intractable features in patient histories and reported patterns of sleep and to predict which type of phase disturbance each might have.

The physiological aspects of the circadian phase disturbance was examined using the sleep-wake cycle (14 x 24-hours), core body temperature (5 x 24-hours), serum cortisol (1 x 24-hours) and serum melatonin (1 x 24-hours) as circadian rhythm parameters. Monitoring was carried-out on three patient groups identified in the Sleep Disorders Clinic as being: 1) phase-delayed, 2) phase-advanced, or 3) arrhythmic.

The phase-delay group showed a significant delay in the acrophase of core temperature rhythm, but not serum cortisol. The phase advance group showed a significant advance in the acrophase of both rhythm parameters. The arrhythmic group displayed a highly variable core temperature acrophase but intact 24 hour waveforms. Serum cortisol had no discernable rhythmic component in this group.

The circadian rhythm of serum melatonin showed a significant acrophase delay for all patient groups. This was an unexpected result, as the phase control of melatonin secretion is thought to originate from oscillators providing phase information to the other parameters measured in this study.

Also important was the finding that there was no significant difference in the amplitude or period length for all three circadian rhythm parameters. Thus, "phase-disorders" seen in the present study possibly represent a defect in circadian rhythm timing but not in the

generation of the rhythms themselves. However, in patient Group 3 the substantial increased point to point random variation in core temperature and the arrhythmic nature of the serum cortisol secretion could imply involvement of rhythm timing and/or generation.

Since these phase disorders were associated with a consistent and unidirectional phase change in the circadian pattern of melatonin secretion, it is hypothesized that the pathology in these patients may lie at the level of the suprachiasmatic nucleus. Hyposensitivity to light could interfere with melatonin "offset" signals, while allowing other pacemakers to move toward their endogenous cycling frequencies. Thus, phase delayed, or advanced, or a more drastic rhythm anomaly might ensue.

This study also indicates that it may be possible to identify each of the three types of rhythm disturbance on the basis of clinical features that can be obtained by interview. The most important aspects appear to be: 1) chronicity of symptoms, 2) consistency of symptoms, 3) pattern of symptoms, 4) psychiatric and medical histories, and 5) recent drug histories. In addition, polysomnographic studies are required to exclude specific disorders such as nocturnal myoclonus.

Exogenous melatonin administration was carried out on this patient sample. A double-blind, placebo-controlled, 14-day trial showed a significant improvement of both total sleep time and day-time alertness with a 75 mg oral dose of melatonin as compared to placebo. This is the first study to demonstrate significant clinical soporiphic effects of exogenous melatonin in chronic insomniac patients.

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At the end of such a long and complex body of work, acknowledging all of those you gave me a 'leg-up' along the way would surely fill almost as many pages as the thesis itself. However, space allows only the mention of a few individuals who I feel have played a pivotal role in my academic life for the past several years.

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Cycles

by which I do not mean
what young men ride on,
but rather what we live by
and our expectations.

I mean the cosmic and the personal,
animal, vegetable, and mineral,
earth's circumambulation of the sun,
sun's certainty of east and west,
the wax and wane of moon,
the southward flight of birds,
May dogwood and Fall's bare trees,
the morning high of cortisol,
prolactin's nightly pulses,
et cetera, et cetera, et cetera,

by which I mean to say
that the unique and quintessential I
am envious.

(Samuel Sterns)

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INTRODUCTION

The term "chronophysiology" has been coined to describe those physiologic processes which display a cyclic pattern of activity. In humans, the frequency of these rhythmic activities varies considerably. If the periods are calculated as the reciprocal of the frequency, there is a range of nine orders of magnitude as one moves from EEG-activity (0.1 sec) through the cardiac (1.0 sec), respiratory (5.0 sec), sleep-wake (24 hours) and menstrual cycles (28 days), to hibernation (365 days) (Moore-Ede et al., 1982). The EEG, cardiac, and respiratory cycles are often referred to as ultradian rhythms since their frequency is shorter than 24 hours. The sleep-wake (24-hour) cycle is known as a circadian rhythm (circa:about, dian:day), while hiberation constitutes a circannual rhythm. Other terms such as circaseptan (about 7 days) and circatrigentan (about 30 days) (Halberg, 1982) further illustrate the magnitude of the study of "chronophysiology".

While it may appear simpler to have a physiologic process preprogrammed to operate at a given level at all times, this is not the case. Evolution has carved these levels into peaks and troughs thereby subserving a supply and demand operation. To improve efficiency, a preprogrammed message which becomes entrained with the changes in the external or internal milieu allows the organism to make a change of its own accord in anticipation of a regular stimulus such as light and darkness. The most important result is the efficient use of energy which is the prime concern of any organism in a competitive environment.

The astronomer Jean Jacques d'Ortous de Mairan provided the first clues regarding the endogenous origin of circadian rhythms in

1729 (Takahashi and Katz, 1982). He placed a light-sensitive heliotrope plant in a sealed room where no light could penetrate. He noted that the plant still opened its petals in the early morning in preparation for sunrise, and closed them at night. This suggested that the plant had been entrained by a particular zeitgeber (a German neologism meaning "time-giver") which he concluded to be sunlight, and that the rhythmic activity persisted in the absence of this zeitgeber.

For almost 150 years, experiments probing biologic rhythms were confined primarily to plants and insects. It was not until 1866 that the endogenous nature of these rhythms was studied in mammals. Moore-Ede et al. (1982) quote William Ogle as writing this description of body temperature in humans: "There is a rise in the early mornings while we are still asleep, and a fall in the evenings while we are still awake, which cannot be explained by reference to any of the hitherto mentioned influences. They are not due to variations in light; they are probably produced by periodic variations in the activity of the organic functions" (p. 14).

In humans, the sleep-wake cycle is the most obvious of all the circadian rhythms. In some lower organisms, the cyclic precision of this phenomenon is dramatically demonstrated where the onset of the "sleep-period" occurs within a two or three minute range during the standard 24-hour cycle (Takahashi and Zatz, 1982). With evolution there is an escape from this strict regime as the reflex arcs dwindle, and are gradually replaced by higher conscious processes. In humans, this culminates in a multitude of intertwined, cyclic phenomena giving rise

to a less specific time of sleep onset. Most of these rhythms are entrained with but not dependent on external environmental cues. The zeitgebers simply act to maintain synchrony between and within the existing endogenous oscillations.

This concept of many endogenous rhythms acting in concert provides the foundation on which the following study is based. Some of the questions which will be considered are: if one circadian rhythm is disrupted (e.g., the sleep-wake cycle), can other rhythms also be shown to be disrupted; are the disruption of these other rhythms a cause or an effect; is this disruption a reflection of some functional change; is the change reversible?

Insomnia is one of the most prevalent complaints in all of the industrialized nations. In Los Angeles, as many as 42.5% of the population surveyed complained of poor sleep (Hartmann, 1978). The number of hypnotic sleeping pills consumed every year is exceeded only by apsirin consumption. These sleeping agents do not cure the problem, however, they simply override it. The cure or effective management of this ubiqitous affliction may come from an understanding of the basic physiology of the sleep-wake cycle and how this cycle interacts with the endogenous and exogenous influences to produce synchrony in one person and dysynchrony in another.

The present study examined four rhythm parameters in an attempt to examine the nature of the circadian disruption that may accompany chronic insomnia. These measurements included core temperature (samples once-per-minute for five days), the sleep-wake cycle (by subjective

reports for 14 days, and by polysomnograph for three days), cortisol, and melatonin (both sampled once-per-hour for 24 hours).

The following account (Chapter 2) of all four variables is a detailed review of the basic physiology which can be considered the foundation on which the hypotheses for the present work is based. Some concepts are more central to the overall theme of this work than others, however, much of the material is alluded to in the discussion as the new data is interpreted in light of an existing theoretical framework. It is my intention to briefly summarize the basic properties of endogenous circadian oscillations, with particular regard to previous studies related to the timing of the various physiologic functions to be measured. These aspects will be reviewed with three principle questions in mind:

(1) Is the observed, disordered rhythm of the particular physiologic variable endogenous in origin?

(2) If so, is this disorder a direct consequence of another, separate periodic function or behaviour?

(3) How (hypothetically) can order be regained by manipulation of these disparate periodic entities?

CHAPTER 2

THE NATURE OF SLEEP AND SLEEP-ASSOCIATED VARIABLES

PART I

DEFINING THE VARIABLES

A. Sleep

A universal problem in sleep research centres around the difficulty in determining the function of sleep. It is possible that sleep is an instinctive behaviour that serves primarily as an adaptive response to external stimuli (Webb, 1974). Alternately, it may be part of some endogenous restorative process (Adam, 1979). It may even be a combination of both views (Daan et al., 1984). In any event, it is unlikely that sleep is a phylogenetically new behaviour unique only to mammals. Observations of sleep behaviour indicate that a sleep-like process is present in some sub-mammalian species (Tobler, 1984).

Most of the current theories of sleep (i.e., functional, neurochemical, neurophysiological, chronobiological, etc.) are based on animal studies. The alacrity with which these concepts have been applied to models of human sleep has been premature. In these comparative studies the criteria used to identify sleep often have been based on purely behavioural observations. For example, neither the relative degree of muscle tone nor organized motor responses are sufficient in defining the relative state of wakefulness. It follows that atonia, catatonia, and immobility do not necessarily reflect in any way a relative state of sleep. That is not to imply these observations are unimportant, but rather that they must be placed within the context of other important physiological variables. As with animal studies, the extent of phenomenological knowledge about human sleep encompasses a

wide variety of bodily changes, all loosely woven into the transition from the waking states. The question becomes, "which of these phenomena are essential manifestations of the sleep process, and which passively follow the sleep-wake cycle itself?"

The following is what this author considers specific features of sleep at the present time:

A: Operational definitions:

1. Sleep is a state of reduced orientation to environmental stimuli such that behavioural responses become poorly directed or limited.
2. Spontaneous internally generated nervous activity predominates within the brain (not normally expressed overtly), giving rise to a specific progression of electroencephalographic and hormonal patterns not seen in the waking state.

   In humans, and many other mammals, sleep is composed of successive stages, with sleep onset most faithfully characterized by the commencement of Stage-2 sleep (Rechtschaffen and Kales, 1968). The sleep episode may be broadly divided into two categories -- slow wave sleep (SWS) comprised of spindles, or high-voltage, slow, synchronized EEG patterns; and paradoxical sleep (PS) which shows a waking EEG pattern characterized by fast, low-voltage, desynchronized activity with the addition of rapid eye movements (REM) and an electromyogram (EMG) that

indicates total absence of skeletal muscle tone (postural atonia). The term paradoxical derives from the fact that although the EEG most resembles a waking pattern, the subject shows the highest waking threshold to external stimuli.

B: Probable functions:

3. Since sleep is normally followed by a sense of restoration, it logically follows that some, as yet undefined, neuronal restoration occurs as well.
4. The occurrence of these discharge patterns have both a homeostatic and circadian component that fits the organism neatly into an adaptive temporal niche.

Neural Regulation of Sleep

Waking and sleeping can be divided into two behavioural states, one of environmental vigilance and another where a suspension of vigilance predominates. Thus, both states may depend on a functional interplay between two antagonistic neuronal systems or networks. One can envisage an arousal or activating system and a hypnogenic or deactivating system. Both systems appear to be topographically concentrated in the brain stem with a rostral extension to the diencephalon (Hobson et al., 1986).

Superimposed on the generation of a sleep-wake cycle by these two networks is another cycle which gives rise to a rhythmic transition between PS and SWS. It is generally accepted that PS occurs as an endogenously generated rhythm in all terrestrial placental animals,

including humans (Tobler, 1984). What has been suggested by Sakai et al. (1981) is the presence of multiple neuronal circuits with particular emphasis on the monoaminergic-cholinergic interaction in the active generation and inhibition of PS. Based on the work of Sakai et al. (1981) and Sastre et al. (1981) it is proposed that separate PS-off and PS-on neuronal networks exist so that periods of PS could be projected into the existing SWS at regular intervals via these systems.

The neurophysiological data and the neurochemical data imply that sleep is an active process. Internally generated neuronal programs must be activated and maintained if a state of wakefulness is to remain supressed. Similarly, after the active induction of SWS, the program network of PS becomes periodically active within the sleep episode.

The exact function of sleep remains a mystery. Many hypotheses have been proposed, but none have been entirely validated. These include: the Restorative Theory (Oswald, 1970; Hartmann, 1973); the Protective Theory (Moruzzi, 1966); the Energy Conservation Theory (Zepelin and Rechtshaffen, 1974; Berger, 1975); the Ethological Theory (Meddis, 1975; Webb, 1974); and the Instinctive Theory (McGinty, 1971; Moruzzi, 1966). It is possible that the essential elements of all these proposals are valid. However, the complex interactions between the homeostatic and circadian components of sleep control indicate that its function is multifaceted.

Homeostatic vs Circadian Principles

The sleep-wake cycle in humans constitutes the most obvious circadian rhythm parameter. In most animals, the activity-rest cycle

often closely approximates the timing of sleep and wakefulness. However, more rigorous indices are required for accurate assessment. This is especially true of an insomniac population where sleep may be completely absent from an objective rest period, and in psychiatric populations where the relationship between the major states of sleep is radically altered. This raises the question of how sleep and its internal temporal organization may relate to circadian physiology.

Sleep deprivation (SD) has been the predominant "probe" for examining the homeostatic aspects of mammalian sleep. Humans deprived of sleep for a week or more, report impaired concentration, dizziness, irritability, hand tremors, and hallucinations (Dement, 1972). Chronically sleep-deprived rats became gradually weakened, with concomitant idiopathic epidermal damage, swollen paws, and decreased cortical activity. Death of most animals ensued within 30 days (Rechtschaffen et al., 1983). Such results imply that sleep subserves a vital physiologic role, but to date, no satisfactory theory as to the precise mechanisms involved has been promulgated. Intuitively, the primary function of sleep is repair and restoration. However, it does not logically follow that the sleep of animals engaged in a day of great physical and mental exertion is only marginally different than that of animals after a relatively uneventful day.

These observations lead to some interesting speculations. Perhaps sleep "resets" baseline neuronal functioning by discharging those neurons most quiescent during the preceding wake-period, while providing a quiescent period for those that have been most active. Except in cases of extreme sleep deprivation, the time required to reset CNS activity would be independent of prior waking-time or activity.

However, the propensity to sleep would be very dependent upon the length of prior wakefulness as the increasing synaptic or metabolic imbalance of neuronal systems would increase the "pressure" for a transition from a waking to a sleeping state. This cumulative threshold of sleep propensity during a typical waking period followed by its dissipation during sleep most likely reflects the physiological demands of the CNS.

Results of recent studies indicate that the circadian component and the homeostatic component of sleep regulation represent separate and distinct principles (Tobler et al., 1983). It was established by Ibuka et al. (1977) that complete bilateral lesions of the suprachiasmatic nuclei (SCN) permanently abolished the circadian sleep-wake rhythm and the rest activity rhythm in rats. Thus, the SCN could be the driving pacemaker of the circadian sleep-wake cycle. Mistelberger et al. (1983) showed that in SCN-lesioned rats where sleep homeostasis was disrupted by total sleep deprivation for 24 hours, REM and SWS had returned to normal baseline values by the end of the second recovery-day. The results indicate that homeostatic regulation of sleep still occurs in SCN-lesioned animals. The main difference lies in the time of the sleep episode. Although the total sleep-time and percent of each sleep-stage remains constant in SCN-lesioned animals, the episodes are no longer consolidated into a night (nocturnal) or day (diurnal) time-frame, but rather a random napping pattern predominates.

Daan et al. (1984) have proposed a two-process model, based on the assumption that two separate entities underlie sleep regulation. One process is a function of total sleep requirement, while the other is controlled by a circadian pacemaker. Sleep propensity can be assumed to be a function of the interaction between these two processes.

B. Cortisol

Cortisol (hydrocortisone) belongs to the glucocorticoid group of adrenocortical hormones. Cholesterol is the precursor from which the adrenal steroids, such as cortisol, are synthesized. The hormones of the adrenal cortex play a vital role in homeostasis. Exogenous and endogenous stress situations quickly induce their release and enable the organism to cope with noxious stimuli and environmental changes (Martin, 1985).

Control of Cortisol Secretion

The basal and stress-induced secretion of cortisol is under the control of adrenocorticotropic hormone (ACTH) which is released from the adenohypophysis (anterior pituitary). ACTH stimulates the hydrolysis of cholesterol esters which are stored in lipid granules of the adrenal cortex (Seybert et al., 1980). The hydrolysed cholesterol is quickly converted by a number of enzymatic steps to hydrocortisone, and released into the blood-stream. The adrenal gland apparently has no mechanism for hormone storage, thus increased synthesis is accompanied by increased secretion.

The secretion of ACTH from the adenohypophysis is regulated by a hypothalamic corticotropin releasing factor (CRF) and by the glucocorticoids themselves (Vale et al., 1981). CRF (a 41-amino-acid peptide) is released from peptidergic neurons of the median eminence into the hypothalamohypophyseal-portal vessels and transported to the adenohypophysis where it stimulates ACTH release.

CRF secretion shows a rhythmic 24-hour secretory pattern that most certainly is reflected by cortisol fluctuation in the plasma. Jones et al. (1977) were the first to examine neurotransmitter regulation of CRF release. To summarize, acetylcholine (ACh) is stimulatory, and ACh neurons probably synapse on CRF-secreting cells. GABA is a potent inhibitor of CRF, and may also act directly. Other neurotransmitters such as serotonin (5-HT) and noradrenalin (NA) have indirect effects through their action on ACh neurons.

Since pituitary-adreno-cortical secretions follow a circadian rhythm, it is important to know how the regulation of these rhythms influences secretory patterns. Swanson and Cowan (1975) were able to demonstrate efferent connections of the suprachiasmatic nucleus (SCN) to various other hypothalamic areas, including the median eminence. Kafka et al. (1983) showed that the SCN was responsible for the circadian variation of neurotransmitter activity, including those neurons controlling the release of CRF. Earlier work by Moore and Eichler (1972) showed a complete loss of cortisol rhythmicity following SCN lesions in the rat.

Circadian versus Homeostatic Principles

Hydrocortisone has a wide range of activities with respect to its effect on various tissue and organ functioning. It plays a major role in carbohydrate metabolism, largely through the enhancement of gluconeogenesis from protein while limiting peripheral-glucose utilization. It also acts to inhibit, or at least limit, inflammatory

reactions. These two principal functions of cortisol give this hormone a vital role in preparing an organism to cope with physiologic stressors and returning it to baseline homeostatic conditions as quickly as possible. The labile nature of cortisol release is hardly surprising, considered from a functional point of view.

Under quiet baseline conditions, the plasma glucocorticoids follow 24-hour secretory distribution. This circadian pattern can be quickly disrupted by exogenous influences, such as feeding-time and significant stressors (ether vapor, immobilization, or novelty stress). Thus, the homeostatic process is quick to respond to physiologic demand when an increased plasma cortisol level becomes an essential part of an appropriate physiological response.

Although SCN lesions have been shown to abolish the rhythm of cortisol secretion, Krieger et al. (1977) showed that feeding-time could be used to resynchronize the rhythm of cortisol and temperature. Krieger (1980) later showed that ventromedial-hypothalamic lesions could abolish food-shifted adrenal rhythmicity. These studies suggest that the two rhythms are under the control of separate circadian oscillators.

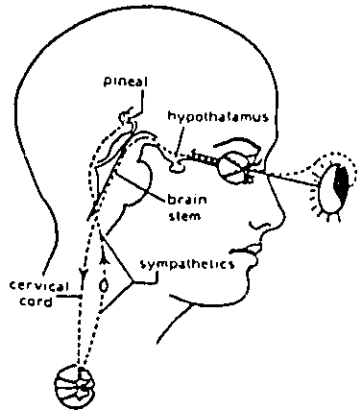
The major determinant of the circadian pattern of cortisol secretion appears to be dependent upon the inter-episode interval in the pulsatile secretory pattern (Krieger, 1979). Clustering of secretory episodes would constitute a peak. For cortisol this peak occurs between 0800 and 1100 hours. The lowest level is observed between 2000 and 0200 hours. The levels rise during the later half of the sleep episode, and reach peak levels usually around the time of awakening. Peak values for serum cortisol average between 150 and 200 ng/ml. The lowest values range from 3-5 ng/ml (Weitzman et al., 1971).

C. Melatonin

This hormone is secreted primarily from the pineal gland (epiphysis) which is a small, cone shaped endocrine organ attached to the roof of the third ventricle, lying rostral to the superior colliculus (Carpenter, 1985). The pineal gland synthesizes melatonin from a serotonin precursor via the action of N-acetyltransferase (NAT) and hydroxyindole-O-methyltransferase (HIOMT) (Klein and Berg, 1970). The activity of NAT is elevated at night and is acutely inhibited by exposure of the animal to light (Moore and Klein, 1974).

In most mammals, melatonin is paramount in the transduction of photic environmental time-cues. It plays a role in a variety of timekeeping functions including the synchronization of many yearly, monthly, and daily physiologic cycles (Tamarkin et al., 1985). The participation or influence of melatonin with regards to human timekeeping is still uncertain.

Figure 2.(i): Control of Melatonin Secretion



(Vaughan, 1984)

The activity of NAT affects the rate of synthesis and subsequent secretion of melatonin. The nocturnal rise in melatonin within the pineal is directly associated with an increase in the synthesis of noradrenaline (NA) in sympathetic nerve terminals within the gland itself (Craft et al., 1984). Specific beta-adrenoceptor (Craft et al., 1985) and to a lesser extent, alpha-adrenoceptor (Sugden et al., 1984), binding has been demonstrated. Both beta- (Moore et al., 1979) and alpha- (Lewy, 1986) adrenoceptor agonists and antagonists have been shown to greatly affect melatonin production.

It is apparent then, that the prevailing photoperiod must have its impact through the peripheral sympathetic nervous system. As for cortisol, the ultimate control of the rhythmic secretory pattern resides within the SCN. Based on indirect evidence, the pathway of innervation from the SCN to the pineal is as follows: relays pass to the tuberal region of the hypothalamus, the medial forebrain bundle, and spinal afferents to reach cells of the superior cervical ganglion. These pathways are based on studies where lesions to the superior cervical ganglion, the medial forebrain bundle, and the retrochiasmatic region of the hypothalamus block the stimulatory effects of the SCN upon pineal enzymes (Moore and Rapport, 1970; Moore and Klein, 1974). To a lesser extent, direct afferents from the SCN to the pineal via the paraventricular nuclei have been demonstrated in rats (Silverman et al., 1981).

The light-dark cycle synchronizes SCN activity via direct pathways from the retina (Moore and Lenn, 1972). When the eyes are

surgically removed or the optic nerves are cut, the rhythm of melatonin secretion free-runs (Reiter et al., 1971). If the SCN is ablated, then the rhythmic secretion of melatonin in higher mammals is arrested (Klein and Moore, 1979). Chik et al. (1987) have shown that, unlike core temperature and serum cortisol, rhythms of serum melatonin are resistant to entrainment by feeding schedules in rats.

Circadian Versus Homeostatic Principles

The pineal gland plays an integral role in the generation and maintenance of circadian rhythms among many non-mammalian vertebrates (Underwood, 1984). In mammals, however, pinealectomy (Px) has little or no effect on behavioural rhythmicity (Cheung and McCormack, 1982). It appears that the mammalian pineal acts primarily as a neuroendocrine transducer which synchronizes seasonal rhythmicity (e.g., breeding, pelage, etc.) with day-length (Tamarkin et al., 1985). There is evidence that suggests that the mammalian pineal is involved with the organization rather than the generation of circadian rhythms (Quay, 1972). Px rats and hamsters phase-shifted more rapidly than control animals to large shifts in photoperiods. Armstrong et al. (1986) have provided recent evidence that administration of melatonin to rats has a profound influence on the synchronization of various behavioural rhythms. Cassone et al. (1986) has shown this property of melatonin to be blocked by ablation of the SCN, which suggests that melatonin could mediate its effects, in part, via the SCN.

In humans, the function of melatonin remains undetermined. The results from animal work suggest that melatonin may act to synchronize and stabilize the phase relationship between the various circadian rhythms. There are many situations where desynchronization of circadian rhythms predominates -- jet-lag (Klein et al., 1972), shiftwork (Aschoff, 1978), and old-age (Wever, 1979). It is possible that exogenous melatonin administration-timed appropriately, could expedite resynchronization. A preliminary study by Arendt et al. (1986) showed that a 5 mg oral dose of melatonin, taken at 1800 hours (local-time) for 14 consecutive days improved jet-lag symptoms in eight out of eight subjects.

Without exception, the pineal gland metabolizes serotonin to melatonin during the dark-phase of the light-dark cycle (Reiter, 1984) regardless of the rest-activity cycle. The overall distribution of serum melatonin levels shows a sharp drop in concentration during the day. Peak levels (40-80 pg/ml) occur approximately 1.5-hours after average sleep-onset at approximately 0200 hours (Arendt et al., 1982).

D. Core Temperature

Core body temperature, at any moment, results from a balance between heat-gain and heat-loss. Moderate changes in metabolic rate produced by waking activities have little effect on temperature rhythms. Hildebrandt et al. (1974) demonstrated that circadian variation in heat-loss accounts for most of the circadian variation in temperature. He showed that the cutaneous circadian temperature rhythm was 180° out of phase with that of core temperature.

Central control of thermoregulation was first outlined by Benzinger et al. (1963). The two sites include the posterior hypothalamic "heat-maintenance-centre" which is a synaptic relay centre indifferent to thermal stimulation, and the anterior hypothalamic "heat-loss-centre" which is extremely sensitive to thermal stimulation. These centres act on simple feedback principles when core temperature falls outside of the thermoneutral zone. Both central and peripheral heat- and cold-sensitive neurons supply feedback information (in the form of firing rates) to the hypothalamic temperature regulatory centre.

Circadian Versus Homeostatic Principles

The question arises as to the physiological and evolutionary advantage of a core temperature circadian rhythm. The observation that nocturnal animals have peak temperatures at night supports the usefulness of higher waking temperatures and lower sleeping temperatures. However, this observation does not imply utility, only correlation. Without further evidence, it may be just an

epiphenomenon. However, it is likely that core temperature reflects overall metabolic rate within a biologic system. Most prominent is the rise in body temperature towards the end of sleep, before awakening; possibly a process of pre-adaptation for waking activity. Alternatively, changes in the thermoneutral set-point may be merely reflecting the multifaceted changes associated with changes of brain activity. These questions remain unanswered. However, the temperature rhythm currently provides enough data to indicate some basic properties of the endogenous circadian clock, and the way in which it is coupled to the external environment.

The light-dark (LD) cycle is the most powerful zeitgeber for the entrainment of core temperature cycles in all mammalian species (Daan and Pittendrigh, 1976), with the possible exception of humans (Aschoff, 1978). However, Czeisler et al. (1981a) showed that entrainment of human core temperature rhythm was possible under more rigorous lighting schedules. Sulzman et al. (1977) demonstrated that feeding-time could entrain core temperature rhythms in squirrel monkeys in the absence of lighting-cues. This could be an indirect effect by entrainment of a second hypothalamic pacemaker which is coupled to the SCN.

The pattern of the human core temperature cycle has been well documented (Gillberg and Akerstedt, 1982; Weitzman et al., 1981b; Winfree, 1982). The characteristic sinusoidal wave begins to fall after the peak (acrophase) which occurs in the late afternoon. The fall in temperature continues and becomes even more pronounced after sleep onset (Gillberg and Akerstedt, 1982). In the latter third of the sleep episode, the temperature wave reaches its nadir (trough) and begins to rise through awakening and on to the late afternoon where the acrophase again occurs.

PART II

CIRCADIAN RHYTHMS

A. The Circadian Timing System

The Circadian Oscillators

The ability of a particular time-cue to influence circadian rhythms is determined by the sensitivity of a pacemaker ensemble, such as the suprachiasmatic nuclei (SCN), to that time-cue (Enright, 1980). This is called "coupling-strength", and as the coupling-strength increases so does the influence of the time-cue on a given circadian cycle (Aschoff, 1979). Since the strongest time-cue is the light-dark cycle (LD), it suggests that a significant neural pathway might exist running from the retina, where light is detected, to the pacemaker neurons. Moore and Lenn (1972) injected tritiated amino-acids into the vitreous humour of the eyes of rats. The neuronal uptake and subsequent autoradiographic examination revealed the existence of a retino-hypothalamic tract which terminated specifically in the SCN of the hypothalamus. The SCN are a small pair of neuron clusters located in the anterior-ventral-hypothalamus (Moore, 1983). Their position just above and usually contiguous with the optic chiasm is what prompted the term "suprachiasmatic".

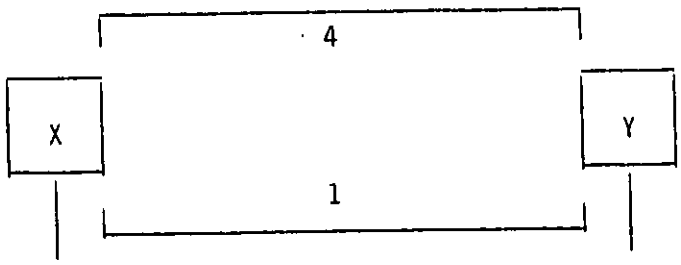
Initial theories envisioned all circadian rhythms to be under the control of one "master-clock", namely the SCN. Thus, state changes in the internal milieu occurred as the activity of this single circadian pacemaker oscillated rhythmically across a certain threshold. In an elaborate dissertation, Enright (1980) proposed a model whereby a single circadian oscillator could generate a remarkably precise circadian

output as a function of the summed stochastic output from the individual neuronal elements of a pacemaker ensemble.

Shortly after the single-oscillator proposal of the early 1960's, Aschoff (1967) demonstrated the phenomenon of internal desynchronization where core temperature and activity-rest cycles oscillated at different frequencies. Almost a decade later, Daan and Pittendrigh (1976) showed that "rhythm-splitting" occurred in animals housed in constant lighting conditions. The activity-rest cycle attained two separate and distinct rhythmic components. The evidence for rhythm splitting, and the fact that sleep-wake cycles exhibit ultradian, infradian, and circadian components distinct from the core temperature cycle led Wever (1979) to suggest that such data must indicate an interaction between at least two, coupled circadian oscillators. Further studies implied that while a proposed "Y-pacemaker" modulates the sleep-wake cycle, core temperature is modulated by a separate oscillator, an "X-pacemaker" (Czeisler et al., 1980b).

In an environment free of external time-cues, the rhythms driven by the two oscillators attained different intrinsic periods (Y 29.3 hours, and X 24.5 hours). This resulted in rhythm desynchronization, where rhythms generated by the Y-oscillator (sleep-wake, plasma growth hormone, urinary Ca++-excretion, etc.) seem to drift further from the entrained baseline rhythm period than those of the X-oscillator (core body temperature, plasma cortisol, urinary K+-excretion, etc.).

Also, Kronauer and colleagues (1982) have used mathematical models and computer simulation to demonstrate the possible interactions that may best explain the glut of human circadian data. They found that the data could be readily modeled if at least two Van den Pol oscillators were coupled so as to provide amplitude information to one another, with X having four times the coupling strength on Y than Y has on X.



REM sleep
Core body temperature
Plasma cortisol
Urinary K+-excretion
Plasma melatonin?

Slow-wave sleep
Skin temperature
Plasma growth hormone
Urinary Ca++-excretion
Plasma melatonin?

(Adapted from Moore-Ede et al., 1982)

It should be noted that the inclusion of plasma melatonin under the control of the Y-pacemaker is based on two lines of evidence. Direct neural connections have been demonstrated between the SCN and the pineal (Moore and Klein, 1979). Also, ablation of the SCN obliterates the rhythm of melatonin secretion in rats (Klein and Moore, 1975). However, data from Wever's lab (1983) show that during free-running conditions in humans, melatonin periodicity aligns itself with core temperature and cortisol. This would implicate the X-pacemaker in the

control of melatonin rhythmicity. It is these confounding results that necessitate the question mark beside melatonin in the preceding figure.

Kawato et al. (1982) extended on these concepts by proposing a three-oscillator model for the sleep-wake and temperature cycles. Although the addition of a third oscillator increased the "range" of the dual pacemaker theory, intuitively one would assume that the original "two-oscillator" proposal implied that there is more than one rather than specifically two. Increasing the degrees of freedom does not conceptually elaborate the original proposal of Wever's (1979).

The possible anatomical location of the X-pacemaker remains a mystery. Pickard and Turek (1983) have suggested that the second oscillator may exist within the SCN itself. This contrasts with the work of Fuller and colleagues (1981) who showed the circadian rhythms of body temperature persist after bilateral suprachiasmatic lesions in the squirrel monkey. This would indicate the presence of a major pacemaker ensemble outside the SCN in primates. The possible sites for the X-oscillator proposed by this group include the ventromedial and/or lateral hypothalamus (VMH, LH). Neural pathways between the SCN and VMH have been demonstrated (Moore, 1980). However, it should be cautioned that functional and anatomical estimations based on physiological or behavioural sequelae after destruction of the SCN are of limited value. Loss of function after lesioning or extirpation does not necessarily imply that the ablated structure is uniquely responsible for the generation of a particular function. Also, these studies have been carried-out on a limited number of species without rigorous quantitative histological verification regarding the extent of the lesions.

The Suprachiasmatic Nucleus

Van den Pol and Tsujimoto (1985) have analyzed the neurotransmitter of the SCN by immunocytochemical method with 33 antisera. Specifically, within the SCN the neuroransmitter-related antigens included bombesin, gastrin-releasing peptide, neurophysin, vasopressin, somatostatin, GABA, glutamate decarboxylase, vasoactive intestinal polypeptide (VIP), and serotonin. It is likely that vasopressin, somatostatin and VIP neurons comprise a significant population of SCN-interneurons (Moore, 1983). This interneuronal network is extensive as would be expected since the neurons or neuronal groups within the SCN represent mutually-coupled circadian components. These components could remain phase-locked via this interneuronal network. SCN afferents, such as 5-HT raphe, or retinal-hypothalamic tract neurons may act on the SCN to adjust its endogenous period length. Other substances within the SCN, such as GABA, could alter the impact of the efferents. The interneuronal network could coordinate the neuronal elements giving rise to the various SCN efferents. As stated by Van den Pol and Tsujimoto (1985), "the finding of many different putative neurotransmitters within the SCN serves to underscore the complex axonal organization of the nucleus" (p. 1049), which is still poorly understood.

Entrainment by Environmental Time Cues

Stable entrainment to an environmental cycle requires accurate perception of an exogenous time cue or zeitgeber (Aschoff, 1965). It is possible that some human circadian disorders occur by dysfunction at this level where environmental time-cues are somehow ineffective in the

entrainment of endogenous oscillators. Temperature, relative humidity, barometric pressure and an abundance of other physical variables can act as potential zeitgebers, as they vary diurnally. However, the most reliable index of environmental time has proven to be the light-dark cycle, followed by social-contacts and feeding-time (Wever, 1979).

It remains unclear as to what effect the light-dark cycle exerts on human circadian rhythms. Humans are manyfold less sensitive to dim-light than are the sub-human primates and nocturnal rodents examined to date (Moore-Ede et al., 1982). Czeisler and colleagues (1981a) found that bedrest/activity and core temperature rhythms were successfully entrained by imposed LD cycles with a maximum intensity of 500 lux. However, the action of light may not be strictly dependent on direct effects of light intensity. Rather, the secondary influences of light on sleep-wake cycle timing may be more a function of, 'if we can't see, we will tend to fall asleep.' In contrast to light within a common range of intensity found in normal indoor illumination, bright-light with an intensity above the threshold of melatonin suppression (>2500 lux) shows considerable effectiveness as a zeitgeber (Wever, 1983). An elegant study by Czeisler et al. (1986) showed that bright-light induced a 6-hour phase shift of core temperature and serum cortisol rhythms without affecting the timing of the sleep-wake cycle in humans.

This would follow animal studies where the threshold intensity for circadian entrainment of core temperature, cortisol, and melatonin is almost identical as that required for melatonin suppression (Brainard et al., 1982), assuming that the coupling mechanisms in animals and humans are similar. Rivest and Wurtman (1983) further support this with

their study of the effect of varying ambient light intensity as follows: rats exposed to dim lD or bright LD in 12:12-hour regimes showed identical suppression of melatonin with subsequent exposure to dim-light or bright-light. The continuous dim-light (0.1-0.3 $\mu W/cm^2$) was within the range of the minimum intensity required for entrainment of the pineal rhythm to the light-dark cycle.

In humans, the circadian system can be entrained to period lengths ranging from about 23 to 27 hours (Wever, 1979). This range of entrainment (ROE) is centred around the average free-running period length of 25 hours, and represents the maximum "resetting" capacity of the system in each direction (advance and delay). Whether the circadian period is advanced or delayed depends on the phase at which the zeitgeber is presented (DeCoursey, 1960). By measuring these differences a phase response curve (PRC) can be constructed where early evening light produces a phase-delay, while light late in the subjective night produces a phase-advance. See Figure 2(ii).

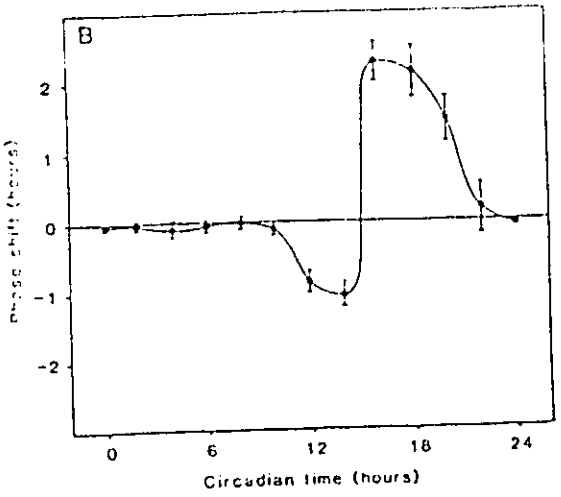


Figure 2(ii): A phase-response curve demonstrating the effect of 60-minute light pulses presented at various times relative to the hamster's rhythm of locomotor activity. (Takahashi and Katz, 1982)

A difference in the relative amplitude of individual PRC's may greatly affect the ROE. Czeisler et al., (1981b) proposed a model for phase-shift insomnia, based on an insufficient resetting capacity of patients such that their 24-hour environment may lie barely within their RUE. However, they may lack the additional phase-resetting capacity to shift the daily sleep episode to a new time. The sleep episode becomes "locked" at an inappropriate clock-time (phase-angle) as a consequence.

Of interest is the fact that the RUE can be increased with light of sufficient intensity (usually in the range required to suppress melatonin). Lewy et al. (1985a) has suggested that this could be used as a method of treatment for chronobiologic disorders including insomnia, where bright-light exposure at the appropriate portion of the PRC may allow patients to re-entrain. More recently, Czeisler et al. (1986, 1989) have established phase-dependent resetting capacity of human circadian rhythms in response to bright light, which has served to verify Lewy's proposal.

Properties of Circadian Rhythms

The generation of circadian rhythms in humans is endogenous in origin, as it is in all other animals. However, with the exclusion of environmental time-cues, the period length of the persistent circadian rhythmicity approaches and sometimes exceeds 25 hours (Aschoff, 1965). This would imply that the endogenous circadian period undergoes daily "corrections" to ensure the maintenance of a temporal relation between this oscillator and the 24-hour light-dark cycle imposed by the earth's rotation.

It has also been shown that the amplitude of a rhythm is an unambiguous measure of the oscillatory strength or the degree of persistence of this rhythm (Akerstedt, 1980; Reinberg et al., 1980). This means that "a rhythm is generally more persistent against disorder (e.g., internal desynchronization) the larger its amplitude" (Wever, 1980, p. 338). This theory arises from studies using core temperature and serum cortisol as circadian rhythm parameters. However, as persistent as these large amplitude rhythms are, they also demonstrate an increased inertia in subsequent phase shifting. Thus, the smaller the original amplitude of the rhythm the faster the entrainment period (Reinberg et al., 1980). It is possible, then, to predict the duration of re-entrainment after a time-cue shift (e.g., 180° sleep-wake inversion) as well as the subjective burdenings of the subject during this shift (Reinberg, 1978; Reinberg et al., 1980).

Circadian rhythm disorders most often are a concommitant of changes in these circadian rhythm properties. Alterations in the period length, amplitude, and "corrective" capacity of the circadian system could give rise to rhythm disruption, dissociation, or complete arrhythmicity. The pathology giving rise to these anomalies could be at the level of zeitgeber reception (i.e., impact of light on the SCN via the retinohypothalamic tract), the coupling between the two oscillators, or the output from the SCN itself. The precise nature of the pathognomonic changes that accompany circadian rhythm disorders remain undefined.

B. Sleep and Core Temperature

Many investigations involving core temperature rhythms have been reported. An invaluable study by Czeisler et al. (1980a) demonstrated the nature of the interaction between core temperature and the sleep-wake cycle. It appears that the duration and organization of human sleep depends on its circadian phase. That is, the length of a given sleep episode is highly correlated to the circadian phase of body temperature at which a free-running individual chooses to go to sleep. It was observed that 86.1% of all 151 awakening times of all subjects occurred on the rising phase of the temperature rhythm. This corresponds to the data from our preliminary study (MacFarlane, 1983). In both insomniac patients and controls, awakenings were consistently preceded by rises in core temperature.

The core temperature rhythms also alter the anatomy of the sleep pattern itself (Czeisler et al., 1980b). The amount of REM-sleep depends on the phase relationship between sleep and the temperature cycle, and REM-sleep propensity is closely coupled to the body temperature rhythm. It is capable of free-running with a period different from both 24 hours, and the average period of the sleep-wake cycle (Weitzman et al., 1980). The shift of REM-sleep to an earlier time within the daily sleep episode during the free-running state supports the concept that the timing of REM-sleep corresponds to the rising phase of the temperature rhythm. As the sleep-wake cycle shifts in the free-running state, the core temperature cycle remains closer to its original baseline. The 25-to 29-hour sleep-wake cycle now exhibits

a phase-delay in relation to core temperature, therefore the rising phase of the temperature rhythm is seen much earlier in the sleep episode. Concurrently, there is a clear shift in REM-sleep and a shift in waking-time to what was once the latter half of the individual's daily sleep episode before commencing on the free-running schedule. This is very similar to that change which is observed in patients who complain of "early morning arousal". There is a characteristic pattern of very short sleep-onset latency, and a very short REM-latency with a subsequent "too early" spontaneous awakening followed by either fragmented sleep or full arousal (Weitzman et al., 1980). It is also of interest that there is a corresponding cortisol phase-shift in this process. Again, our preliminary study (MacFarlane, 1983) demonstrated similar results. These data support the hypothesis that the sleep-wake cycle and core temperature cycle are under the phase control of separate oscillators.

Weitzman (1981) further showed that the quantity and quality of sleep in a given 60-minute time limit was dependent on the particular phase of the core temperature cycle at which this episode was allowed. Minimum and maximum sleep efficiency occurred at the acrophase and nadir of the wave respectively. Psychomotor task performance and vigilance also show maximum and minimum levels during a 24-hour cycle (Moses et al., 1978).

C. Circadian Control of Sleep and Core Temperature

The two-oscillator theory of Czeisler et al. (1981a) explained these findings as being a direct consequence of the interaction between the distinct free-running period of the X- and the Y-oscillators. The mathematical model posed by Kronauer et al. (1982) used these data to derive their equations. Two autonomous oscillators were inferred. One was a strong and less flexible oscillator indicated by the excursions of core temperature. The other was a somewhat "sloppy" more spontaneous oscillator reflected in the less predictable transitions between sleeping and waking. Each of these oscillators is thought to be responsible for the output of at least two smoothly varying quantities. Core temperature and sleep-wake cycles are the immediately obvious variables, while the others are not so apparent. However, sleep-wake may merely be a "passive" phenomenon brought about by the interaction of core temperature with some unobservable variant from the other oscillator. Wever's (1980) original proposal had emphasized the amplitude of the temperature rhythm, rather than specifically the phase, as being the most reliable dependent variable for the interpretation of the overall fraction of sleep time. He considered that temperature and sleep-wake fluctuations were a function of the summed output from both clocks. Thus, the recurrence of sleep onset is determined by the weighted sum of two independent circadian rhythms making regular threshold crossings. The two independent rhythms consist of the core temperature cycle with its consistent frequency and more variable amplitude, and the more conspicuous cycle (represented by sleep-wake) with it s consistent amplitude and more variable frequency.

This concept of a dependent interaction differed from Kronauer's model which presumed that sleep terminations where governed by the sleep-wake oscillator alone. However, the other, less flexible, 25-hour oscillator was capable of influencing the period length of the sleep-wake oscillator. When juxtaposed with slightly varying frequencies, a "beat phenomenon" has been proposed to explain the large, phasic variations in the sleep-wake oscillator frequency.

The two-oscillator model has been criticized on a number of grounds. Winfree (1982) examined sleep duration and wake duration by computer simulation using the two-oscillator model. He noted that this technique did not accurately account for sleep duration and wake duration with regard to their phase of onset in either of the two cycles. The model does not make a fundamental distinction between sleep and waking but rather assumes their presence to be entirely dependent upon successive threshold crossings. Although the phase of awakening relates in an orderly manner to the phase of prior sleep onset, sleep onset in no way relates to prior awakening when real data are plotted. This relationship is inadequately modelled using Kronauer's computer simulation.

Borbely (1982) also criticized the two-oscillator concept because of its inclusion of a circadian oscillator with a partly non-circadian and unusually broad frequency range. Although not stated, it follows intuitively that the proposed "beating" effect is a weak argument to account for this well documented property of the sleep-wake oscillator. In the physical world, the beating phenomenon occurs when the quality and frequency of two outputs is almost identical.

Obviously, the output of the proposed X- and Y-oscillators are qualitatively different.

Borbely (1982) re-introduced the concept of a single circadian oscillator model. His proposal, and the later work of Daan et al. (1984) and Winfree (1983) support a "two-process" model that is based on the assumption that two separate entities underlie sleep regulation. The logic of such a model is easily observed during periods of extended sleep deprivation where there is a waxing and waning of alertness and sleep propensity which seems to parallel the core temperature cycle. Thus, one process is a function of total sleep requirement, while the other is an oscillating threshold controlled by a circadian pacemaker. Sleep propensity can be assumed to be a function of the interaction between these two processes. They propose that a "pressure" for sleep (S) builds up during wakefulness (possibly as a result of an accumulation of a "sleep-promoting factor"), and subsequently declines during sleep. Sleep and wakefulness occur as S crosses an oscillating threshold (process C). At the nadir of C, the monotonic increase of S can more readily cross the threshold at which point sleep would be indicated. However, conscious input may overide this event, giving rise to a subsequent phase relationship where the zenith of the oscillating threshold once again exceeds S. Subjective sleep propensity would be observed to decline even in the absence of an intervening sleep episode.

Although elegant, this model was generally criticized for its inability to properly account for internal desynchronization, since a single oscillating threshold could only impose a single frequency on the

sleep-wake cycle. The quantum departure taken by the model was that it discarded the concept of the sleep-wake cycle itself being generated by a circadian pacemaker. Instead, a passive nature of S-oscillations was seen to more accurately reflect a cycle with such a large frequency range and an inherent sensitivity to exogenous factors. Daan and Beersma (1983) added a new element by including the assumption of a differential baseline influencing the level of C during wakefulness and during sleep. Thus, the "gain" of the system is in itself a "slave" oscillatory process dependent upon input from the same controlling pacemaker which can be affected (to a certain degree) by the relative state of consciousness. Such a variable would allow the range of frequencies which have been documented for the sleep-wake cycle.

Even with this important modification, the hypothesis has "fallen prey" to more recent data that is difficult to interpret in the context of this model. Lavie (1986) used ultra short sleep-wake schedules where subjects were on a 13/7 minute or a 7/13 minute sleep-wake schedule, before and after 24 hours of sleep deprivation for a period of 24 hours. For example, subjects on the 13/7 schedule were awake for 13 minutes, and then lying down trying to resist sleep for 7 minutes. The results showed the presence of an underlying ultradian rhythmicity giving rise to more than one "sleep-gate" during a 24-hour cycle. These data later confirmed by Strogatz et al. (1987), have unequivocally demonstrated a bimodal distribution of sleep propensity, with one peak at the temperature nadir, and another occuring 9 to 10 hours later. These studies contrast the view of Winfree (1982) that time of awakening is more predictable than time of sleep onset. Also,

the notion of the exponential increase or accumulation of "sleepiness" during waking with sleep onset triggered by the crossing of an upper threshold is hard to reconcile in light of these bimodal sleep gates. It is possible that some, as yet undefined ultradian element of the circadian component of the Borbely model is involved with this regulation. However, Strogatz et al. (1987) have hinted that such regulation can be adequately explained by the original two-oscillator theory.

Borbely et al. (1989) further modified his model where Process C is divided into an H-threshold which would define amplitude and phase, and an L-theshold which would define sleep termination. The difference in the present version is that H and L oscillate independently which adequately models the biomodal sleep gates. He argues, however, that these independent oscillations could still be generated by one circadian pacemaker.

The circadian control of sleep and temperature remains very much an unresolved issue. One must realize that although these original hypotheses are based on thorough and rigorous data collection, the conclusions are nothing more than speculation based on descriptive representation. With large inter-subject variations and a lack of understanding of what exactly is being measured, the theories must be approached with a cautious, yet open mind.

D. Sleep and Melatonin

The suggestions that melatonin secretion could participate in the sleep process are not new. Marczynski et al. (1964) were first to demonstrate melatonin's hypnotic effects when administered directly into the hypothalamus of cats. Several early studies reported sleep induction after melatonin administration (Anton-Tay et al., 1971; Barchas et al., 1967; Cramer et al., 1974). One investigator even went so far as to suggest that the pineal was a "tranquillizing organ" (Romijn, 1978). These effects of melatonin, however, were only observed at supraphysiologic levels after Quay (1968) had demonstrated that Px did not affect sleeping patterns in rats.

The renewed interest in the behavioural effects of melatonin do not seem to be related to any specific breakthrough. Vollrath et al. (1981) have shown the most significant effects to date. At a dose of 1.7 mg, intranasal administration of melatonin was shown to have a profound effect on sleep induction. Although no data were provided, even this small dose would likely give rise to plasma levels several-fold greater than normal physiologic peaks. Matthews et al. (1981) demonstrated plasma melatonin levels as high as 850 pg/ml after an oral dose of 2.5 mg. Lieberman (1986) demonstrated that the behavioural effects of exogenous melatonin lasted only three hours, even though plasma levels (up to 100 ng/ml) remained elevated for longer than this. It is possible that there is a threshold serum concentration which must be exceeded to exert pharmacologic effects. Below this, elevated concentration would lie within the physiologic range.

Melatonin's role in the sleep-wake cycle remains speculative.

Anton-Tay et al. (1969) demonstrated an increase in 5-HT levels after melatonin administration in rats. However, Holmes and Sugden (1982) found no gross changes in brain indolamine or catecholamine levels after administration of melatonin (20 ng/kg). Dubocovich (1985) subsequently demonstrated the melatonin administration resulted in a dose-related decrease in the Ca++-dependent release of striatal dopamine in rats. This could affect behavioural activation, as discussed in Part IA. Castroviejo et al. (1986) have noted changes in GABA binding to central cortex membranes after both Px and melatonin administration in rats. Coloma and Niles (1984) have also shown that melatonin increases [^{3}H]-muscimol binding at the GABA receptor in rat forebrain. Alteration of GABA binding could, in turn, affect 5-HT systems. Such a change would also have important implications as previously discussed.

It has also been proposed that endogenous melatonin could be involved in the regulation of the circadian timing of sleep (Wurtman and Lieberman, 1986). This is supported by data from Redman et al. (1983), where melatonin administered daily entrained the activity rhythms of rats kept in constant darkness. Wurtman and Lieberman (1985) fit their theory into the "two-process" model of sleep (Daan et al., 1984). The first process could reflect the accumulation of some sleep factor which is eliminated during sleep (Krueger, 1982b) while the second process ("circadian factor") could, in part, be mediated by the circadian secretion of melatonin. Arendt et al. (1984) reported that low doses (2 mg/day) administered at 5 pm could hasten evening sleepiness. The most important point, however, was that it took several days for the melatonin to have its effect.

Akerstedt et al. (1982) showed a strong circadian covariation between endogenous plasma melatonin levels and indices of fatigue and sleepiness during 64-hours of sleep deprivation. Birkland (1982) found that spontaneous nocturnal waking episodes (EEG desynchronization) to be significantly correlated to the occurrence of secretory episodes of melatonin. It was hypothesized that secretion in connection with spontaneous arousal could act to restore sleep in humans.

Other, less direct evidence implicates a relationship between pineal function and sleep. At puberty there is a substantial reduction in sleep quality (Dement and Carskadon, 1982), which is paralleled by a reduction in nocturnal melatonin secretion (Waldhauser et al., 1984). Brown et al. (1979) report a decline in nocturnal melatonin with advancing age, which is paralleled by a deterioration of normal sleep patterns (Zepelin, 1983).

Several recent studies have examined the capacity of the benzodiazepines to reset the mammalian circadian clock (Turek and Losee-Olson, 1987, 1986; Winfree, 1986). BDZs administered at a specific time of day can phase advance the circadian clock, while at another time it may act to delay the clock. When administration occurred in the mid-waking episode, there was no effect. However, this could result from activity bursts observed after BDZ administration, since immobilization can eliminate this effect in rats (Mrosovsky, 1988; VanReeth and Turek, 1989).

E. Circadian Control of Melatonin

Circadian studies of human pineal function have not always yielded reliable data. The reliability of the melatonin assay itself has always been questionable, and consistent between-laboratory results are rare. At present, there are three assay techniques used to assay acetylated and methoxylated indoles in plasma: radioimmunoassay (RIA), gas-chromotography-mass-spectometry (GCMS) and high-pressure liquid chromotography (HPLC). RIA has been the most widely employed method as developed by Rollag and Niswender (1976). The many modifications to the original technique (especially in the extraction phase) have led to a considerable variation in the levels of melatonin in human plasma and urine (Arendt, personal communication, 1988). It has become apparent that the efficiency of the RIA technique for melatonin is very dependent upon the specific modifications made by individual labs, as well as the competence and consistency of the technical staff running the assays.

For circadian studies, frequent sampling is required over a minimum period of 24-hours. Urinary melatonin is a much simpler measurement of circadian status, but is less reliable as it represents only a small percentage of the secretory product (Arendt et al., 1985). The more recent RIA techniques for measuring the major melatonin metabolite (6-hydroxymelatonin sulfate) (Arendt, 1985) and the continued development of an iodinated (rather than tritiated) tracer (Vakkuri et al., 1984) will most likely improve the discrepancies that a multitude of methodologies have introduced into the literature.

Even with the discrepancies, the abundance of animal melatonin data indicate that the SCN drives the endogenous rhythm of melatonin in

rodent and primates. An early study demonstrated that the N-acetyltransferase (NAT) rhythm is immediately abolished by denervation of the pineal gland (Moore and Klein, 1974). This implies that the rhythmic component of the gland is controlled by some unidentified external pacemaker. When the SCN is specifically ablated, the rhythmic production of melatonin is arrested in rodents (Klein and Moore, 1979). Therefore, it seems likely that the LD cycle synchronizes melatonin secretion by synchronizing neural activity in the SCN. This ensures that melatonin secretion occurs only during the dark phase for diurnal and nocturnal animals.

It is not clear whether the endogenous rhythm of melatonin in humans is under the discrete control of the SCN (Y-oscillator) and/or another pacemaker (possibly the X-oscillator). Initial studies on rats showed a direct neural connection between the SCN and the pineal via the superior cervical ganglion (Moore, 1980). This, coupled with ablation studies led to the speculation of SCN control. However, more recently, studies in humans have revealed that the rhythm of melatonin secretion can be desynchronized so as to dissociate from the sleep-wake cycle, aligning itself instead with the core-temperature and cortisol cycle. This technique of fractional desynchronization (Arendt et al., 1985; Wever, 1986) involves the gradual increases and decrease of zeitgeber period lengths (imposed day lengths). The implication is that circadian control of melatonin is under the auspices of the X-oscillator rather than the Y-oscillator. However, this would suggest that the anatomical substrate for human melatonin rhythm generation is in a different location or configuration than that of rodents and sub-human primates.

Perhaps the human pineal possesses more functional autonomy, and/or controlling input from an additional secondary pacemaker. This would explain why the melatonin rhythm desynchronized from both temperature and sleep-wake cycles in one of two subjects originally studied by Arendt et al. (1985). If this secondary input was from the so-called "strong" oscillator (X), during internal desynchronization the output from the X-pacemaker could dominate the picture. A simpler explanation may be that urinary excretion rates of melatonin metabolites are under the control of a separate oscillator than the pineal. Studies need to be done using plasma levels to yield more specific results. A recent study by Sharma et al. (1989) supports the view that melatonin and cortisol are under the control of separate pacemakers. This is based on the finding of an "internal phase drift" between cortisol and melatonin rhythms as a function of age. Furthermore, the ratio of the regression line slopes for melatonin and cortisol acrophases was 3.6:1, remarkably similar to proposed coupling strength of the X-and Y-pacemakers according to Kronauer et al. (1982). This supports Y-pacemaker control of the melatonin rhythm.

The early two-oscillator theory of Daan and Pittendrigh (1976) envisaged dual oscillators involved in the control of locomotor activity, with an evening oscillator controlling dusk activity, and a morning oscillator controlling dawn activity. The results of Illnerova and Vanecek (1982) indicate the rise in NAT activity at night and its decline in the morning may be similarly contrasted by morning (M) and evening (E) oscillators. This was based on the finding that one minute light pulses presented at various times in the subjective dark phase in

rats demonstrated the presence of two distinct phase response curves (PRCs). One curve was specific for the evening NAT rise and one specific for the morning decline of NAT activity. The PRC reflecting the phase-shift of the NAT decline one day after the initial light pulses had phase-delay as well as phase-advance components. However, the maximal values for phase-advance were consistently higher. This new component in the PRC profile for M, but not E, was assumed to indicate a mutual coupling between the two oscillators with the E having a greater impact on M than M on E.

The exact implications that the work of Illnerova and Vanecek (1982) has for entrainment of human melatonin production remains to be determined. Lewy et al. (1980) showed that light of sufficient intensity caused acute suppression of nightime melatonin production. Thus, there appears to be two separate and distinct effects of light on nocturnal melatonin production: entrainment and suppression. It is feasible that the existence of two PRCs for the NAT rhythm is a result of this "dual action" of light on a single oscillator, rather than separate oscillator control for each PRC (Lewy, 1985).

It is impossible, at the present time, to offer anything more than an educated speculation as to the circadian control of pineal melatonin production. It is highly unlikely that taking one step up the "evolutionary ladder" would competely "untie" the pineal from SCN control. However, one could assume that some additional controlling mechanism or the emergence of an existing one, has dramatically altered the "animal model" of pineal control and function in humans.

PART III

Chronic Insomnia

The etiology of insomnia is multidimensional. The major categories are: (1) psychophysiological (based on conditioned arousal responses); (2) accompanying psychiatric disorders; (3) use of drugs and alcohol; (4) sleep-induced respiratory impairment; (5) sleep-related (nocturnal) myoclonus and "restless legs"; (6) medical, toxic and environmental conditions; (7) childhood onset; and (8) other conditions including atypical sleep EEG (ASDC, 1979). These conditions are defined in an official classification. Thus, the array of clinical syndromes that fall under the heading of "insomnia" are often etiologically unrelated except for the presenting symptoms.

The present study examines the proposal that chronic insomnias are related to disorders in internal timekeeping, i.e., insomnia persists because the person is unable to coordinate the sleep-wake cycle to the mandatory impositions of everyday living. For normal people, this would include awakening at approximately 0700 hours, remaining awake and operating at a high level of performance throughout the day, and returning to sleep at approximately 2300 hours. Individuals afflicted with disturbances of their circadian system are incapable of this desirable schedule. They present with chronic problems with the initiation and/or maintenance of sleep with concomitant excessive daytime sleepiness or fatigue. Often they can identify a life event that marked the onset of the insomnia, but the symptoms have long outlasted the original precipitating factors.

In a pilot study (MacFarlane, 1983) three types of circadian disturbances were identified in these patients: (a) a phase delay, (b) a phase advance, and (c) an arrhythmic group.

Phase Delay

Delayed sleep-phase insomnia (DSPI) is a widely recognized clinical phenomenon. Since first described over 100 years ago, it has received little attention until Weitzman et al. (1981) described it as a chronobiologic disorder and proposed a drug-free treatment (Czeisler et al., 1981b). The disorder is characterized by an inappropriate phase relationship between the timing of the sleep-wake (SW) cycle and the circadian light-dark (LD) cycle. There are three distinguishing features: (1) sleep-onset and awakening time intractably later than desired; (2) actual sleep time lies within a similar time frame from night to night, and (3) essentially normal EEG tracing except for prolonged sleep onset latency (Czeisler et al., 1981b).

The exact course of delayed sleep-phase syndrome is unknown. It is apparent, however, that it is not a period disorder involving a lengthened period. Moore-Ede et al. (1982) point out that when sleep is allowed ad lib in these patients that the sleep-wake cycle is no longer delayed with respect to the self-selected schedule.

Phase Advance

This is a much more rare phase disorder characterized by a sleep-wake schedule that is phase advanced in relation to the LD

cycle. Our patients showed a shortened sleep-onset latency, as compared with normals, and report little difficulty in maintaining sleep. Sleepiness most often commences in the late afternoon or early evening, followed by a relatively normal sleep and an exceedingly early awakening time.

There is a marked paucity of literature on the phase advance syndrome. Most recently, Moldofksy et al. (1986) reported successful treatment of a patient with advanced sleep-phase syndrome (ASPS) by phase advance chronotherapy. Other isolated cases of severe ASPS have been reported (Kamei et al., 1979; Czeisler et al., 1986) but hardly enough to make it certain whether or not this syndrome represents a true clinical entity.

Wehr et al. (1979) demonstrated that ASPS was a common concomitant of major depressive disorder, and successfully employed phase advance chronotherapy as both a treatment for the insomnia and as an antidepressant. A shortening of the X-pacemaker's period has been implicated as a possible mechanism (Wever, 1979). Johnsson et al. (1979) provided evidence that lithium lengthens the free-running circadian period of core temperature in humans. However, it seems unlikely that primary ASPS is caused by a shortened pacemaker period as the phase relationship of sleep is stable but inappropriate.

Amplitude Disorders and Arrhythmias

These are characterized by either a marked reduction in circadian rhythm amplitude or the complete absence of a definable

circadian rhythm.

There is little documentation of these anomalies of circadian rhythmicity under normal environmental conditions. Lobban (1976) described rhythm amplitude reduction of urinary electrolytes in Eskimos living in an arrhythmic environment. Moore-Ede et al. (1982) suggest two explanations: firstly, the masking effect of light is removed in the circumpolar environment, and secondly, internal rhythm dissociation may reduce the amplitude of overt rhythms. Alternately, arrhythmicity may be secondary to some SCN pathology (Page et al., 1973) or failure of neural or endocrine transmission which would disrupt entrainment of secondary oscillators (Czeisler et al., 1977).

A pilot study by this author (MacFarlane, 1983) identified two patients, on the basis of tympanic temperature monitoring, who seemed to display a complete arrhythmia of their core temperature cycle. These patients showed by far the worst cases of insomnia that had ever presented in the clinic. Sleep was marked by variable sleep onset latency, and severe difficulty with sleep maintenance. These patients averaged less than two hours sleep per night, with large reductions in the percent of SWS and an associated increase in the percent of stage 1 sleep. Whether these patients represent a true clinical syndrome remains to be determined.

CHAPTER 3

RATIONALE AND HYPOTHESES

The control of the sleep-wake cycle is multifaceted. Thus, the timing of a multitude of intertwined cyclic phenomena becomes an essential feature of an appropriately phased cycle of sleeping and waking. As discussed in Chapter 2, the ultimate control of such rhythms falls under the auspices of the SNC, therefore, the control of a number of overt circadian variables are assumed to reflect the state of the circadian generating centres.

The presence of two (or more than one) generating centres is the working model for the present study. Moore-Ede et al. (1982) proposed two separate groups of circadian variables, assumed to be under the control of separate oscillators. In order to accurately assess the functioning of the circadian system, it is important to measure one or more variables from each group. That way, inter and intra-rhythm variability can be implied and tested.

In the present study, four circadian rhythm parameters were selected. Of those rhythms supposedly driven by the X-oscillator, core temperature and plasma cortisol were measured because they are the best defined and have the most robust rhythms. The best defined and most easily measured rhythm under the control of the Y-oscillator is the timing of the sleep-wake cycle itself. The rhythm of plasma melatonin was also measured in order to determine how it related to the other cycles.

Several hypotheses have been proposed for the present study based on the background literature and pilot study:

(1) Chronic late bedtimes and desire for or actual late arousals

will be associated with a phase delay of core temperature and plasma melatonin and cortisol rhythms.

(2) Chronic early awakening will be associated with a phase advance of all three rhythms.

(3) Patients who have variable sleep onset latencies, and exceedingly short sleep episodes (1-3 hours) may show a dissociation of the X-driven rhythms and the Y-driven rhythms.

According to the two oscillator model of circadian control, the pattern of disruption should be as follows: changes in the circadian pattern of serum cortisol will parallel changes in core temperature. Changes in the sleep-wake cycle should dissociate from both of these, and serum melatonin will parallel one or the other. Since the oscillators have been shown to be coupled to one another, then all four rhythm parametres should be displaced in parallel. The exception to this could be group (3) where a rhythm dissociation may exist. In this case, the rhythms could display a dissociation of two parallel rhythm pairs. This would be similar to a free-running condition where the masking effect of the sleep-wake cycle on inappropriately phased rhythm parameters could give rise to a reduction in rhythm amplitude, and a seeming arrhythmia in short-term monitoring, possibly present in our pilot study (MacFarlane, 1983).

The endogenous circadian disruption seen in chronic insomnia is an important venue in which to study the physiology of the circadian timing systems, and how it relates to this condition. In the data analysis, both rhythm amplitude and phase relationship (to clock time

and other circadian variables) should be considered.

Melatonin is thought to act at the BDZ-GABA receptor complex. Despite the abundance of data on the interaction of melatonin and sleep, there have been no attempts to test its possible therapeutic value in the treatment of the various insomnias. The drug is well tolerated, and its bioavailability appears unrestricted (Wetterberg, 1979). Supraphysiologic levels are rapidly obtained from 2.5 mg oral doses, and are sustained above normal values for several hours (Matthews et al., 1981). Thus, a controlled clinical trial of melatonin and placebo will becarried out after the subjects' phase disturbance had been characterized. That the effective dose range for patients might be different from that of controls will also be considered. Such a difference could imply that changes in BDZ receptors are a part of the overall sleep disturbance.

CHAPTER 4:
METHODS

PART I

Subject Selection

A total of 24 subjects took part in this study. This included 13 patients, nine control subjects, one patient excluded after non-restorative sleep syndrome was established, and one patient who was excluded after a drug dependency was established. All patients were selected from a group of chronic insomniacs who had been referred to Dr. J. M. Cleghorn, Co-Director of the Sleep Disorders Clinic at the McMaster Division, Chedoke-McMaster Hospitals, Hamilton, Ontario. A clinical history was taken at the time of referral and the patients were then screened in the Sleep Investigation Unit for one night where specific sleep disorders could be ruled out (i.e., nocturnal myoclonus, sleep apnea, etc.). Sleep diaries and daytime alertness scales were filled out by the patients for a minimum of one week prior to final assessment (Appendix B). At this time, patients were carefully selected based on the following inclusion criteria:

(1) Patients must be between the ages of 25 and 70 years of age.

(2) Patients must have a history of chronic insomnia (>4 years) that has long outlasted (>2 years) any of the original precipitating factors. The diagnosis of chronic insomnia included careful assessment of the following: i) sleep onset latency (SOL) > 90 minutes; ii) number of awakenings; iii) time of sleep termination; iv) total sleep time (TST); and v) impairment of subjective daytime alertness.

All patients were assessed to be in good general health. All neuroactive drugs which some patients may have been taking to manage their insomnia were withdrawn by Dr. Cleghorn two weeks prior to the study.

Patients were excluded from the study on the basis of their clinical assessment, as outlined by Cleghorn et al. (1983) which identified patients with major affective disorder, and depression or anxiety scores in the clinically significant range. Physiologic monitoring in the Sleep Disorders Laboratory effectively identified patients with insomnia of short duration, variations of normal, pseudoinsomnia, the non-restorative sleep syndrome, insomnia associated with phobias, insomnia secondary to medical and neurological disorders, and specific disorders of initiating or maintaining sleep, such as myoclonus.

When the screening data were complete, an attempt was made to allot each patient, based on clinical history, to one of the three groups as defined by clinical features and corresponding circadian temperature pattern in the preliminary study.

Predicted Group	Clinical History
Phase Delay	- long SOL - desire for late awakening time - normal sleep EEG
Phase Advance	- short SOL - early awakening time
Phase Dissociation	- variable SOL - variable awakening time - [illegible] total sleep time - [illegible] pattern

Each patient received a written explanation of the purpose and procedure of the project. A record of the communication of this information and the subject's consent to participate in the study was placed in the Medical Record (see Appendix C). The right of the subject to withdraw from the study or to refuse any procedure was made clear. All subjects were given $75.00 for their participation, although they were not informed of the fee payment until after completion of the monitoring.

Controls were selected from a group who had responded to an offer of $150.00 for their participation in the study. All potential candidates were interviewed, and a brief history was taken regarding their general physical and mental health, and sleep habits. All controls had been free of any medications for a minimum of eight weeks prior to the date of the study. None of the controls had a history of (or current) psychiatric illness or chronic medical illness, as defined by clinical histories and MMPI scales.

All female controls and patients were studied 17 days after the onset of menses in order to minimize monthly variations in melatonin secretion. Hariharasubramanian et al. (1985) have demonstrated a decrease in the mesor and a delay in the acrophase of melatonin at the mid-menstrual phase.

PART II

Study Design

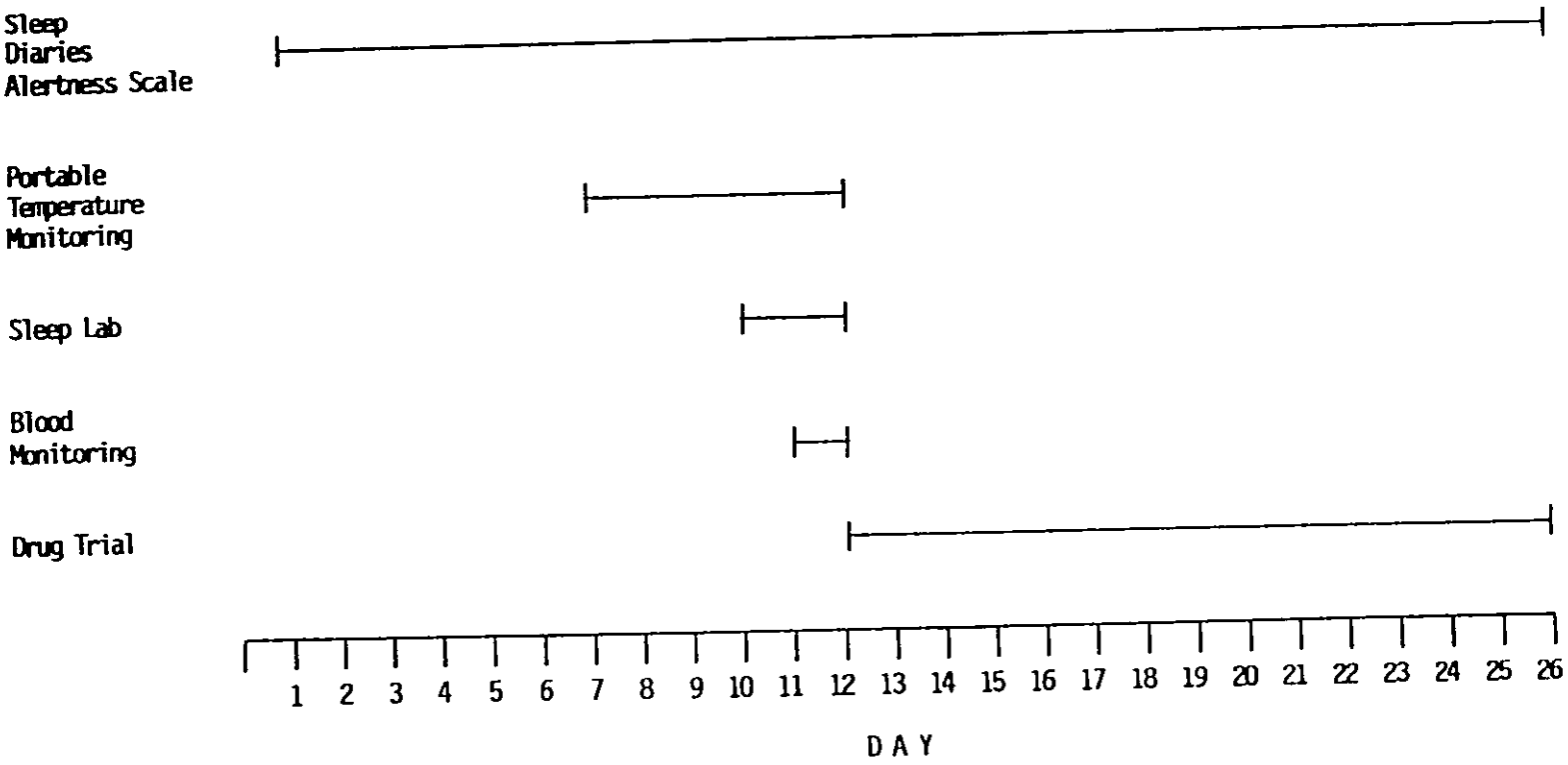
Patients and controls were required to spend 26 and 12 days respectively, within the experimental protocol. The breakdown of that time is shown in Figure 4(i).

Subjects were requested to maintain a strict schedule of lights out at or around 2300 hours and lights on at 0700 hours for the entire study. Subjects reported to the Sleep Lab at 0830 hours on Day 7, at which time they were instructed on the placement and maintenance of the rectal thermister and the Vitalog Recorder. After 15 minutes to stabilize, the temperature recording was started at precisely 0900 hours.

On the fourth day of portable monitoring, the subjects again reported to the Sleep Lab at 2100 hours. The following two nights and intervening day was spent in the Sleep Lab.

On Day 12 the drug trial with melatonin (see Appendix D) began for patients only. This was conducted as a double-blind, placebo-controlled trial with a single-crossover design. Patients were given two bottles, one marked "A" and the other marked "B". Neither the patients nor the investigators were aware which bottle contained melatonin, and which placebo. The patients were asked to take the "A" capsules from Day 12 to Day 18, and the "B" capsules from Day 19 to Day 25, each night at 2000 hours. Seven of the patients began with melatonin and six with placebo.

Figure 4(i): Outline of Study Design



PART III

Temperature Monitoring

Temperature measurements were originally to be obtained by a tympanic thermister, as with the 37-hour pilot study (MacFarlane 1983). After many attempts to develop this technique for a five day study (see Appendix E) it was decided that the rectal thermister was more accurate and practical for this particular study.

After insertion, the temperature probe (YSI^{tm} Series 400, Yellow Springs Instruments, Ohio) remained in place for 120 consecutive hours, and was removed only for bathing and defecating. The probe was connected to a Vitalog^{tm} PMS-8 (Vitalog Inc., Palo Alto, California) battery-operated physiologic monitor. This monitor, which is about the size of a pocket-calculator, could be worn easily in its own pouch, belted around the waist. During the recording period, temperature values were acquired and stored every minute. The PMS-8 receives its operating program from the Data Manager (Apple II^{tm} microcomputer) before each recording session. At the end of each recording session, the initialized temperature values were transferred onto a floppy-disc via the Data Manager.

This temperature monitoring system was tested for accuracy and stability as compared to a Beckman^{tm} Variance Mercury Thermometer which responds to temperature changes as small as 0.01°C. The tip of the rectal probe and the mercury thermometer were placed in proximity in a Grant^{tm} temperature controlled water bath for 168 consecutive hours (see Appendix F).

Simultaneous recordings of rectal and tympanic temperatures were carried out over an eight hour period on three subjects. Rectal temperature was acquired via the YSI rectal thermister and recorded by the Vitalog PMS-8. Tympanic temperature was acquired with a Mon-a-therm^{tm} tympanic thermocouple connected to a Labarge Mon-a-therm^{tm} Model 6000 quartz-readout resistance thermometer (IMED, Toronto). Temperature values were recorded every 10 minutes. These results verified previous reports of a significant correlation between tympanic and rectal temperature.

PART IV

Electroencephalographic Recordings (see Figure 4(ii))

The electroencephalographic tracings were recorded with a Grass[tm] Model-8, 16-channel polygraph. The tracings were organized as follows:

Channel	Parameter
1	EOG
2	EOG
3	EMG
4	EEG
5	EEG
6	EEG
7	EEG
8	ECG

The polygraphic tracings were scored on the basis of the criteria outlined by Rechtschaffen and Kales (1968). With the chart speed set at 15 mm/second, the scoring was done page by page, with each 30 cm page representing a 20 second "epoch". Each 20 second interval was scored by determining which sleep-stage constituted the largest percentage of that epoch. Sleep-stages were determined as follows:

Stage W (Wakefulness): The EEG contains alpha-activity and/or low-voltage mixed frequency.

Movement Time (MT): Scoring of the polygraph record is obscured by movements of the subject.

Stage 1: A relatively low-voltage, mixed frequency (2-14 H_z) with bursts of alpha-activity, and without rapid eye movements (REMs). Often difficult to distinguish from relaxed wakefulness.

Stage 2: Characterized by the presence of sleep-spindles and K-complexes on a background of relatively low-voltage mixed frequency EEG activity (2-7 H_z).

Stage 3: 20-50% high amplitude (>75 uV) low frequency (<2 H_z). Sleep-spindles may be seen.

Stage 4: Over 50% of the recording time is occupied by slow-wave activity (>75 uV, <2 H_z).

Stage REM: A relatively mixed low-voltage, mixed frequency EEG, similar to Stage 1, in conjunction with episodic eye movements, and a very low amplitude EMG.

Sleep records were scored and the following parameters were calculated: Sleep-onset latency **(SOL)**, REM-onset latency **(ROL)**, total sleep time **(TST)**, total waking time **(TWT)**, percent Stage 1 **(%S1)**, percent Stage 2 **(%S2)**, percent Stage 3 **(%S3)**, percent Stage 4 **(%S4)**, percent slow wave sleep - S3 and S4 **(%SWS)**, and percent REM sleep **(%REM)**.

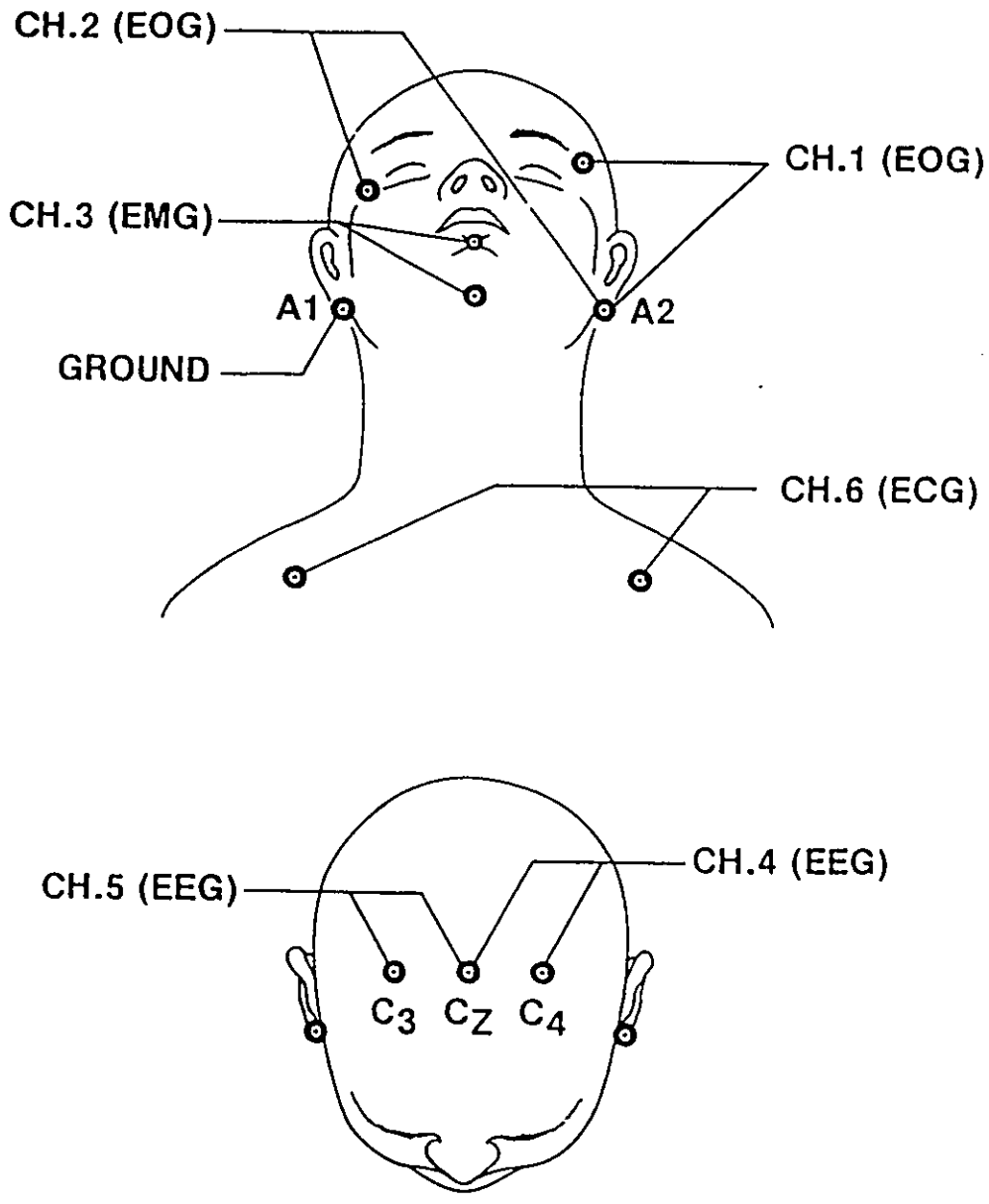


Figure 4(ii): Electrode Placement for Sleep EEG

PART V

Blood Sampling

An indwelling catheter (18 G x 1 1/4 inch Angiocath^{tm}) was inserted into an arm vein (usually the median cubital) one hour prior to the first blood sample to permit adaptation to the procedure. A normal saline intravenous drip was run at the rate of 30ml/hr. The blood samples (10ml each) were drawn every hour on the hour, refrigerated for three hours and centrifuged at 3000g in a Damon^{tm} refrigerated centrifuge. The serum was removed and stored at -20°C for 30 to 60 days before analysis.

It should be noted that a Hepalean Lok^{tm} (heparin-containing) indwelling catheter was not chosen for use in this study because of its interference with the radioimmunoassay for melatonin (Johansson et al., 1985). If melatonin were not measured, this would have been the most convenient method for serial sampling.

Melatonin Assay

Serum was removed from the freezer and allowed to warm up to room temperature. A portion of the serum was used for the determination of melatonin concentration. The method used for the assay (Pang et al., 1977) was modified by Brown (1983) as follows:

Extraction procedure: to 0.5 ml human serum in duplicate, 2.5 ml pesticide-grade dichloromethane (CH_2Cl_2) was added in 16 x 100 culture tubes (Maple Leaf Brand) and vortexed for 30 seconds. The mixture was centrifuged at 3000xg at 4°C in a Beckman Centrifuge Model J6, using a

Bucket Rotor. The aqueous phase was then aspirated-off in a fume hood, and tubes were placed in dry ice for 20 minutes to solidify any insoluable lipid. The solution was then decanted into 12x75 mm test tubes and the dichloromethane extract was evaporated to dryness in the Savant concentrator. Extracted samples were stored at -20°C in the dark until the following day.

0.5 ml Of the standards were transferred in duplicate to 12x75 mm test tubes. The following tubes were prepared:

tubes 1,2 - contained 0.5 ml de-ionized H_2O (total counts)

tubes 3,4 - contained 0.6 ml assay buffer (non-specific binding)

tubes 5,6 - contained 0.5 ml 0.1% gelatin buffer (zero-standard)

tubes 7,16 - 0.5 ml of each standard

tubes 17,end - 0.5 ml 0.1% gelatin buffer added to each dried down sample and vortexed

2 blank tubes with 2.5 ml CH_2Cl_2 evaporated and vortexed

50 uL Of [^{3}H] melatonin (Amersham, Oakville, Ontario, specific activity 79 Ci/mM) containing 2000 cpm added in 0.1% gelatin buffer to each tube, then 100 uL of antiserum (CIDtech, Hamilton, Ontario) was added to each tube (except tubes 1 - 4) and vortexed. After a two day incubation at 4°C, saturated ammonium sulphate (0.5 ml) was added to each tube with an Oxford pipettor (Model R) in an ice-bath and then vortexed gently, so as not to foam, and incubated at 4°C for one hour. The tubes were centrifuged for 20 minutes at 4000g at 4°C, then the supernatant was decanted and 0.55 ml de-ionized H_2O was added to each of the pellets and vortexed. 0.5 ml Of this solution was pipetted into 6

ml Wheaton plastic mini-vials and 5 ml of scintillation cocktail (Amersham, Arlington Heights, Ill.) was added. Vials were shaken well, and placed in a Beckman LS7000 liquid scintillation counter and the radioactivity was determined.

Cortisol Assay:

This was measured using the method of Brown (1982). Blood was centrifuged at 4000g for 20 minutes and the serum removed.

[^{3}H] Cortisol (Amersham, Oakville, Ontario) (86 Ci/mm) was diluted 1:20 with ethanol. The solution was then evaporated (approximately 125 uL ^{3}H cortisol) in a glass vial and 20 ml of 0.1 ml phosphate gelatin buffer was added. This solution contained approximately 2000 cpm in 50 uL.

Antiserum: Miles-Yeda (Rabbit-Anti-Cortisol -21-thyroglobulin, Naperville, Ill.) was reconstituted in vial with 5 ml phosphate gelatin buffer, and allowed to stand for 15 minutes. 5 ml of antibody reconstituted 1:10 with assay buffer was added to 32.5 ml of assay buffer giving 1:65 dilution, and added to tubes (100 uL).

A standard curve was constructed using cortisol (Sigma Chemical Co., St. Louis, Mo.) dissolved in methanol. This solution was further diluted with assay buffer (1:200). 0.1 ml of standards were transferred in duplicate to 12x75 mm tubes. Then 0.3 ml of gelatin phosphate buffer was added.

Then, 50 uL [^{3}H] cortisol (2000 cpm) was added to each tube and vortexed. 100 uL of antiserum (Miles-Yeda 1:65 dilution) was added to each tube (except tubes 1 - 4). All tubes were then incubated for 36 -

48 hours at 4°C. 0.55 ml $(NH_4)_2 SO_4$ Was added to all tubes (except tubes 1 and 2) which were then vortexed and incubated at room temperature overnight. The tubes were then centrifuged in a Beckman Model J6 centrifuge with a TYJR rotor, at 2500 x g for 30 minutes and decanted. The precipitate was then redissolved by adding 0.55 ml of de-ionized H_2O and vortexed. 0.5 ml Of this solution was transferred to 6 ml scintillation vials. After adding 5 ml scintillation cocktail (Ambersham, Ill.), vials were placed in a Beckman LS7000 liquid scintillation counter and radioactivity was determined.

Both the cortisol and the melatonin assays were tested for validity and precision (Rodbard, 1974). The parameters for these tests included: i) within-assay variability; ii) between-assay variability; iii) least detectable concentration; and iv) data on specificity (cross reactivity). The outcome of these measures is described below.

Validation of Assays:

Melatonin: The between-and within-assay coefficient of variation was calculated as follows (Rodbard, 1974):

$$\% \text{ variation} = \frac{\text{standard deviation}}{\text{mean}} \times 100$$

i) For intra-assay variation: 10% acceptable; 15% not acceptable; for inter-assay variation: 10% acceptable; 20% not acceptable (Walker, 1977). This is a general recommendation. The intra-assay coefficient variation for all assays used in this study was 8.1%. The inter-assay coefficient of variation for all assays used in this study was 14.8%.

ii) The limit of detection (sensitivity) was calculated as follows:

L/D = zero count (standard curve) - 2X standard deviation of all zero counts.

The limit of detection for all assays used in this study was approximately 10 pg/ml of melatonin.

iii) The results on cross-reactivity (specificity; Brown, 1985) are as follows:

Substance	% Cross-Reaction
Melatonin	100
N-acetylserotonin	0.01
Serotonin	<0.01
6-Hydroxymelatonin	0.95

This is a sample from a list of over 25 indole deriviates tested for cross-reactivity.

Cortisol: Intra- and inter-assay variability and specificity for cortisol was calculated as for melatonin.

i) Intra-assay co-efficient of variation was 11.75%.

ii) Inter-assay co-efficient of variation was 15.56%

iii) Limit of detection was approximately 10 ng/ml.

iv) The data on cross-reactivity (Miles-Yeda Ltd., Elkheart, Indiana) is are follows:

Substance	% Cross-Reaction
Cortisol	100
Corticosterone	11.7
Progesterone	6.9
Aldosterone	0.5

This is a sample from a list of 13 steroids tested for cross-reactivity.

PART VI

Data Analysis

The majority of rhythmic biologic data can be analyzed by modifying basic harmonic analysis to best suit the particular parameter. This is equivalent to best fitting sine waves computed by means of the least-squares technique (Nelson et al., 1979). The cosinor is an inferential statistical technique that provides a probability or p-value that indicates the significance of fit of the cosine curve. If p is .05 or less, fluctuation of the variable is presumed to be cyclic, and not random. Additional information from the analysis includes the three rhythmic parameters and their dispersions -- acrophase, mesor, and amplitude (Nelson et al., 1979).

The acrophase is the crest of the fitted cosine curve in relation to a predetermined reference point, such as local midnight (external acrophase), or the beginning of a sleep-phase (internal acrophase). Usually, the acrophase coincides with the time when the data values are highest (peak values). The mesor is the mean of the cosine curve fitted to the raw data. The amplitude is defined as one-half the total cosine excursion (i.e., vertical distance in °C) between mesor and peak.

The basic assumption for using this statistical method in the present study is that human circadian rhythms, such as core temperature, serum melatonin, and serum cortisol, exhibit repeated and consistent cycling; that is, a consistent period, amplitude and phase relationship with respect to clock-time (Wever, 1986). If the data do not show a

significant fit, the variable may still be rhythmic in nature; the waveform may simply have a different shape. However, it has been shown unequivocally that the vast majority of biologic rhythms can be analyzed successfully using the cosinor method (Nelson et al., 1979; Cornelissen et al, 1980; Halberg and Halberg, 1980). It is apparent that most cyclic data displays regularly repeating highs and lows (peaks and troughs) with transitional points joining the two -- a sinusoid. Cosinor analysis does not presuppose a perfect sine function. Rather, it models the data to the nearest sine function allowing the extraction of phase, amplitude, and period data whilst eliminating random variation.

Enright (1989) has recently alluded to inappropriate use of cosinor analysis. He warns that the cosinor statistic cannot prove or disprove the existence of a rhythm. Rather, it imposes a period, which for the present application is 24 hours, thereby providing estimates of period and phase. Like any other statistical method, there will be arbitrary mathematical "cut off" points (p-values) which will determine the level of significance. As long as one adheres to the basic assumption of this method, the interpretations are justified. Thus, where a rhythm exists with a pre-established tau, one can determine the "goodness of fit" of the raw data to the model cosine, and the level of significance for the phase and amplitude values of the fitted curve.

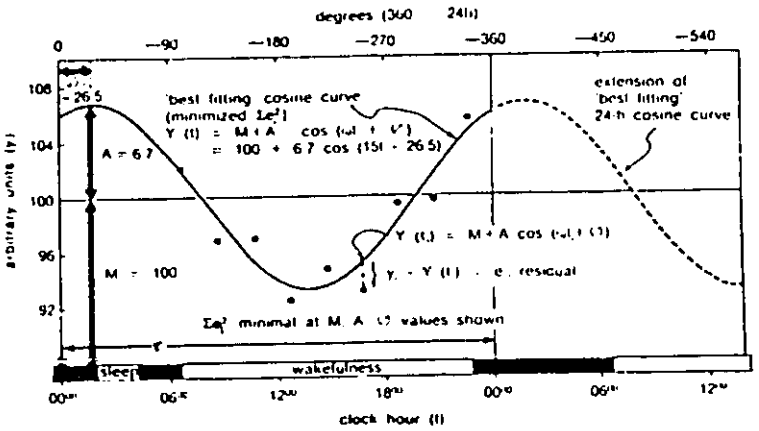
Cosinor Analysis:

All of the data analysis by this method uses a basic cosine function (Halberg et al., 1972).

$$f(t) = M + A \cos (wt + \theta)$$

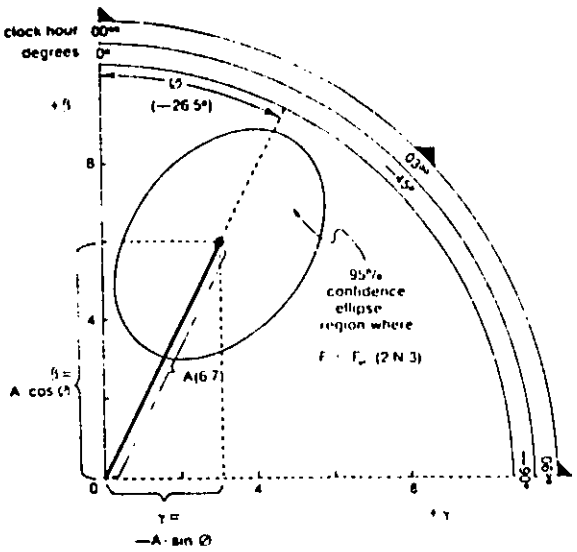
Where f(t) is a function of time as defined by M (mesor: the mean about which oscillation occurs); A (amplitude: distance between mesor and peak); w (angular frequency: degrees/unit time; 360° constituting a complete 24-hour cycle); and θ (acrophase: time of peak value, in degrees; Cornelissen et al., 1980) (see Figures below).

Figure 4(iii):



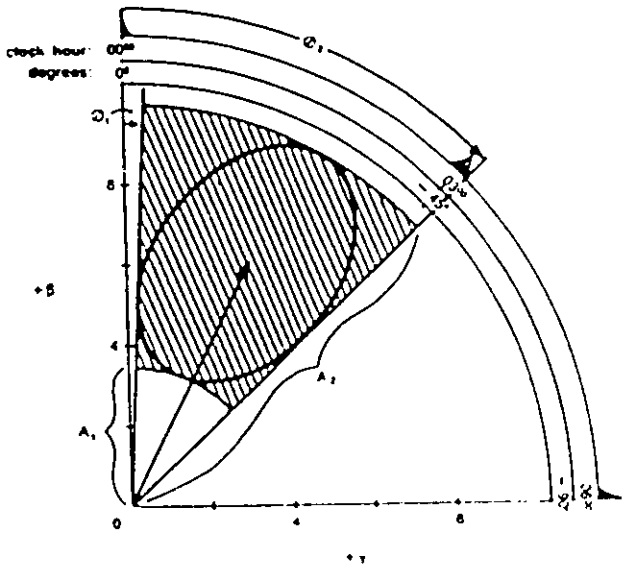
This information is then displayed as a cosinor plot. Amplitude and the computative acrophase are shown on a simple 24-hour cosinor clock, as shown below.

Figure 4(iv)



This allows the data to assume the form of a single vector which allows clear and simple comparison between a number of data sets. The 95% confidence ellipse gives a quick "eyeball test" of significance for the amplitude and the acrophase. Figure 4(v) demonstrates how this confidence ellipse should be interpreted.

Figure 4(v):



All cosinor analysis was done on an Apple II^{tm} microcomputer using a cosinor system program (Vokac, 1983). This consists of several subprograms -- 13 principal, 5 assembly language subroutines, and one initialization program. Since the maximum capacity for the system was 200 data points/cycle, an additional program had to be written which generated a storage file from the raw Vitalog data which was compatible with the cosinor program (MacFarlane and Wu, 1983). The 1,440 original time-points were reduced to 140 time-points, with each point being the average of 10 successive original time-points. These new values were stored as cosinor program "input" files.

The cosinor method was the principal statistical method used to determine if there is a significant difference in the circadian rhythm phase and/or amplitude between patients with chronic insomnia and controls. Thus, the cosinor was able to successfully determine the "goodness of fit" to a proposed sinusoidal model for circadian data. The progression from raw data to the final cosinor curve fitting is demonstrated in Appendix G.

The results of this analysis led to the necessity of employing other statistical methods, as it demonstrated some unforeseen results that were not part of the original hypothesis.

Hartley's F_{max} Test: This statistic tests for homogeneity of variance by comparing the equality of two variances (Winer, 1971). Some patients with variable acrophase angles show nonsignificant mean cosinor results despite significant single cosinor findings. The F-statistic was applied as:

$$F_{max} = \frac{\text{Mean Square (patient)}}{\text{Mean Square (control)}}$$

To ensure conservativism, the control with the largest variance of acrophase angle was used in all cases.

Best Fitting Tau: This analysis was applied to the same group of patients to test for significant changes in circadian period length (Tau). A range of frequencies (2 to 30 hours) at 30-minute increments were tested. The period length which best accounted for the data (e.g., highest %R and lowest p-value) was considered to be the significant period length for that particular single cosinor results. This was applied to each of the five single 24-hour recording periods of each patient. This was done in order to determine if the increase in acrophase angle variability could be accounted for by changes in the circadian rhythm period.

Two-Way Analysis of Variance (ANOVA): This was also applied to all of the circadian data for all subjects which allowed the effects of the experimental procedure on the various subject groups to be examined. In other words, did the chronic placement of a rectal thermister, 24-hour indwelling catheter, and other less dramatic elements of the protocol, have a differential effect on the phase, amplitude and mesor over the monitoring period in patient groups and controls?

Multivariate Analysis of the Variance (MANOVA): The final part of the experimental procedure involved a double-blind, controlled trial with a 75 mg dose of melatonin versus placebo. The two outcome (dependent) variables were sleep-time and daytime-alertness. Since there were two dependent variables, multivariate analysis of variance (MANOVA) was

applied. Both the MANOVA and ANOVA were carried out on a Digital Equipment Vax^{tm} in the CSU at the McMaster University Medical Centre. The statistical program package used in the analysis was BMDP^{tm}.

An additional statistic was applied to the melatonin data. Some view the episodic nature of melatonin secretion as displaying more of a "square-wave" pattern, thus eliminating the feasibility of cosinor-curve fitting. Melatonin onset times were determined by calculating the mean 24-hour plasma levels of melatonin in each individual. Onset was defined as that point at which plasma melatonin levels exceeded mean values by at least 20%. This point had to be one of at least two consecutive incremental increases (i.e. point #1 > x + 20%, point #2 > point #1). The time at point #1 would be considered melatonin onset. An unpaired t-test was used to compare the mean melatonin onset time of patients and controls.

Sample Size

In a pilot study (MacFarlane, 1983) there were 3 patient groups identified based on the circadian core temperature data -- a phase-delay group, a phase-advance group and an arrhythmic group. In order to establish each of these groups as a significant clinical entity, the present study set out to reexamine the groups with an adequate number of patients in each to allow for statistical analysis. The number of patient/group was calculated using the formula:

$$N/\text{group} = 2\,[(Z_{\alpha} + Z_{\beta})\ \sigma/\Delta]^2$$

Where α is the standard normal deviate corresponding to the probability of making a type-1 error; β is the standard normal deviate corresponding to the probability of making a type-2 error; is the standard deviation of the outcome variable in a normal population, in this case estimated from a pilot study (MacFarlane, 1983); and is the smallest difference between the control and patient means that is being sought.

$$N/\text{group} = 2\,[1.96 + 1.28]\ 42.6\ \text{min}/90\ \text{min}]^2$$

$$= 4.64$$

Therefore, 5 patients/group were required.

The determination of the control group size was calculated using the formula:

$$N = \frac{\text{number of patients}}{\sqrt{\text{number of groups}}}$$

$$= \frac{15}{\sqrt{3}} = 8.7$$

Therefore, 9 control subjects were required.

CHAPTER 5:
RESULTS

PART I

CONTROL SUBJECT DATA

Table 5(i): The raw temperature data for all controls were analyzed using the cosinor method previously described (Chapter 4). The results of that analysis are listed on Table 5(i). The individual subjects are listed as letters A through I, with the corresponding data for the five recording days provided.

The %R is an assigned value which indicates the percentage of the data which is accounted for by the cosine model. The **Mesor** is the mean value around which the temperature fluctuates. The **Amplitude** is defined as half the total temperature excursion, that is, the distance in °C from the mesor to the acrophase. The acrophase is the highest value on the fitted cosine curve. The point along the time axis at which the acrophase occurs is defined as **Phi** which is given in both degrees (360° = 24 hours) and clock hours and minutes.

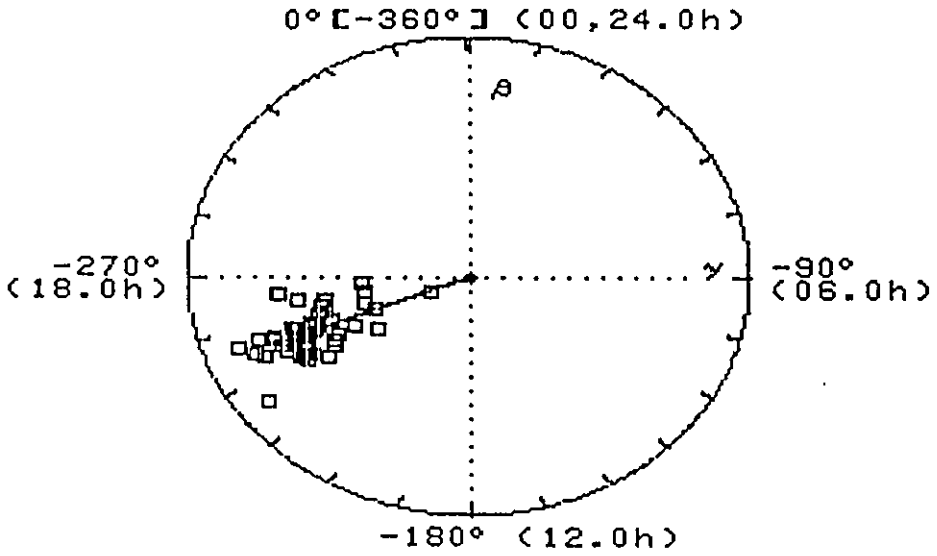
Table 5(i): Temperature Data - All Controls

Controls	Day	% R	Amp	Mesor	Phi (Degrees)	Phi (Hrs/Mins)
	1	65.5	0.42	37.07	-231.7	15.27
	2	54.8	0.36	36.91	-240.0	15.48
A	3	68.6	0.50	36.96	-269.0	17.56
	4	54.4	0.31	36.99	-245.5	16.22
	5	64.3	0.40	36.92	-254.4	16.58
	1	59.9	0.48	37.31	-241.3	16.05
	2	64.8	0.51	37.56	-241.0	16.04
B	3	74.7	0.41	37.46	-241.6	16.06
	4	69.0	0.42	37.43	-253.0	16.52
	5	78.8	0.40	37.51	-266.6	17.46
	1	29.0	0.29	37.19	-235.1	15.40
	2	48.6	0.56	37.08	-238.1	15.52
C	3	69.4	0.34	37.23	-243.2	16.13
	4	75.6	0.49	37.24	-264.1	17.36
	5	66.8	0.29	37.06	-265.2	17.41
	1	49.0	0.29	37.23	-252.8	16.51
	2	67.3	0.39	37.27	-259.0	17.16
D	3	87.0	0.48	37.27	-236.1	15.44
	4	64.3	0.28	37.28	-265.1	17.44
	5	67.3	0.39	37.27	-259.0	17.16
	1	90.4	0.60	36.97	-245.4	16.22
	2	83.9	0.65	36.95	-250.5	16.42
E	3	71.2	0.51	37.00	-245.1	16.20
	4	87.5	0.54	36.84	-246.4	16.25
	5	91.7	0.51	36.90	-245.0	16.19
	1	71.0	0.51	37.00	-250.5	16.42
	2	84.4	0.51	37.01	-260.4	17.22
F	3	86.9	0.46	36.92	-260.8	17.23
	4	80.7	0.47	36.88	-257.0	17.08
	5	70.7	0.45	36.90	-255.5	17.02
	1	72.7	0.45	36.99	-236.2	15.45
	2	57.4	0.39	37.01	-242.2	16.09
G	3	60.4	0.27	36.92	-248.7	16.35
	4	77.2	0.44	36.80	-250.5	16.42
	5	75.2	0.40	36.85	-252.8	16.51
	1	76.6	0.49	37.27	-242.9	16.11
	2	80.8	0.66	37.18	-233.7	15.35
H	3	86.3	0.59	37.07	-250.5	16.42
	4	82.4	0.50	36.82	-238.1	15.53
	5	88.8	0.52	36.70	-244.2	16.17
	1	78.3	0.45	36.90	-260.2	17.21
	2	83.3	0.48	36.98	-255.0	17.00
I	3	88.4	0.51	36.94	-250.5	16.42
	4	83.7	0.50	37.00	-248.7	16.35
	5	90.0	0.52	37.01	-250.0	16.40
Mean	1	65.8	0.44	37.11	-244.0	16.16
	2	69.5	0.50	37.13	-246.7	16.27
	3	76.9	0.45	37.08	-249.5	16.38
	4	74.9	0.44	37.03	-252.0	16.48
	5	77.0	0.43	37.01	-254.7	16.59

Figure 5(i) shows mean cosinor plot for all of the control temperature data, which totals 45 recording days (5 days x 9 controls; Table 5(i)). Each box represents the results from one recording day. A vector from the centre of the polar plot through the box to the polar axis defines the acrophase for that particular day. The distance of the box from the centre defines the relative amplitude. The confidence interval which forms an ellipse around the mean acrophase is buried in the data points and is therefore not visible. Phi 1 and Phi 2 are the two tangents to the confidence interval ellipse which define the 95% confidence interval on the cosinor clock.

This mean and confidence interval will be shown again in Part III when comparing patient data to control data.

Figure 5(i): Polar Plot of Temperature Data - All Controls



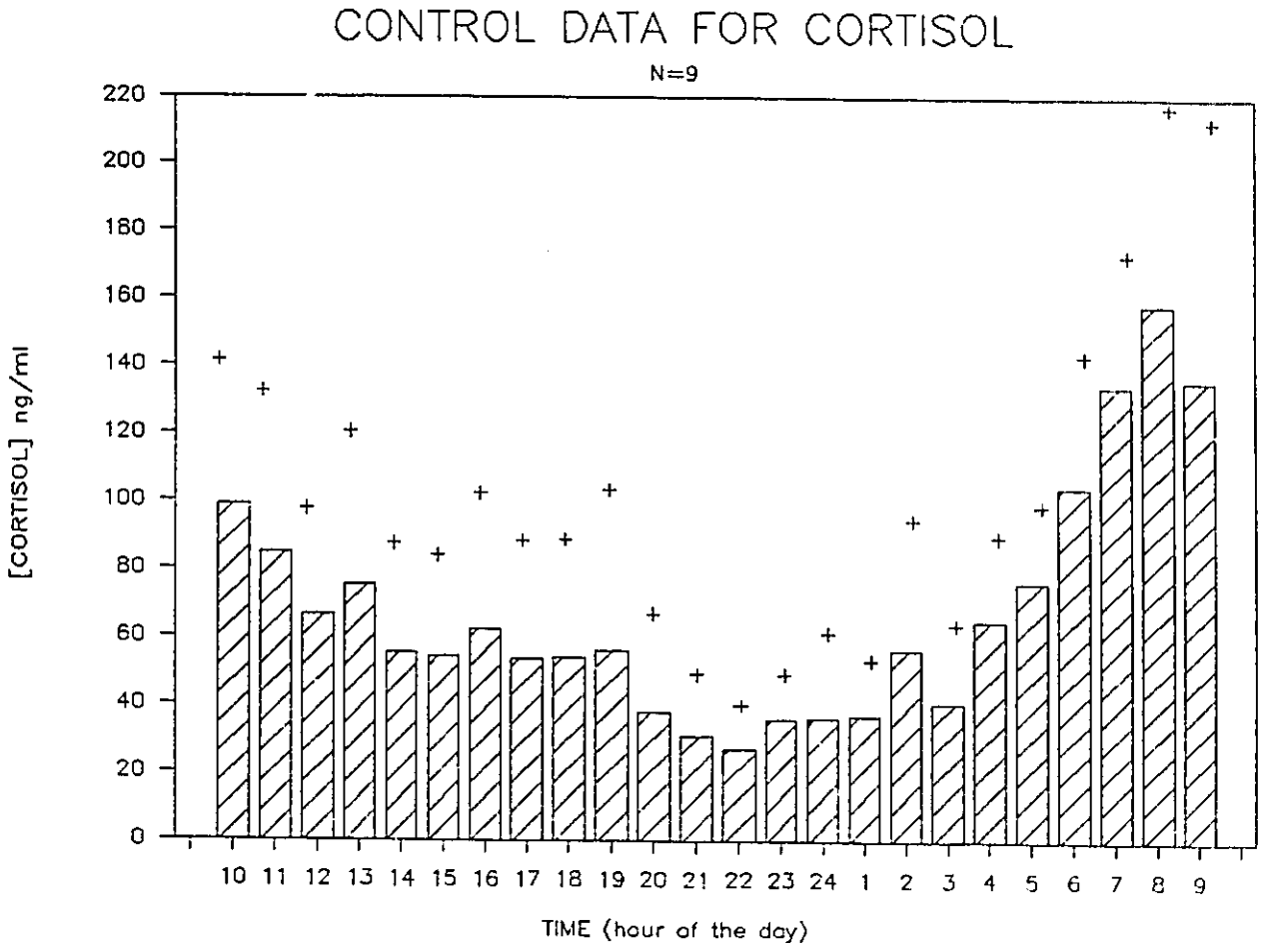
Mean Cosinor

% R	= 71.3	P-Level	= <0.001
Mesor	= 37.2	Amp	= 0.45
Phi (Deg.)	= -247.3	Hrs/Mins	= 16.29

Tangents from Pole to Ellipse:

Phi 1	= -244.7	Hrs/Mins	= 16.19
Phi 2	= -250.1	Hrs/Mins	= 16.40

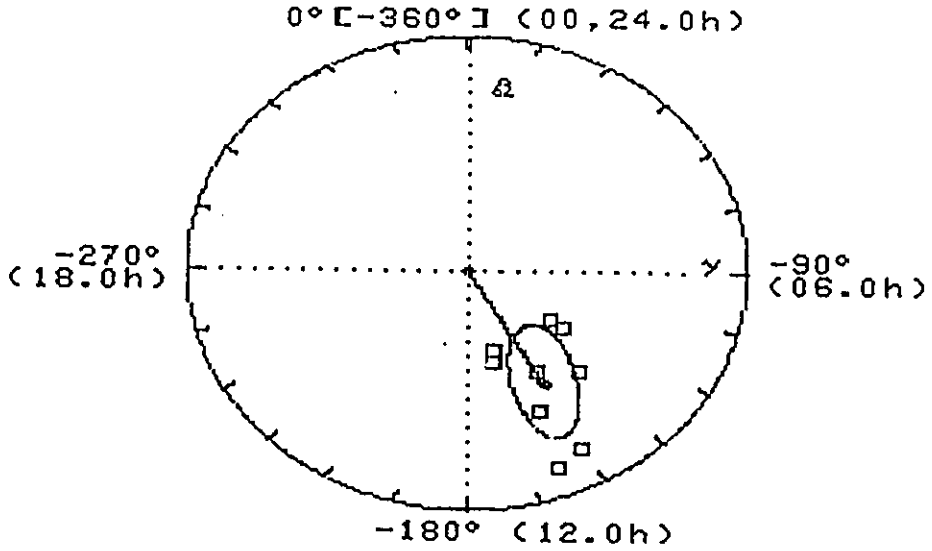
Figure 5(i)b: Cortisol Control Data



+ = Standard Error

Figure 5(ii) shows the mean cosinor plot for all control cortisol data. The coordinates of the individual acrophases are listed below (subjects A through I). The data for the mean cosinor of the individual control data are shown below that giving the coordinates of the ellipse.

Figure 5(ii): Polar Plot of Cortisol Data - All Controls

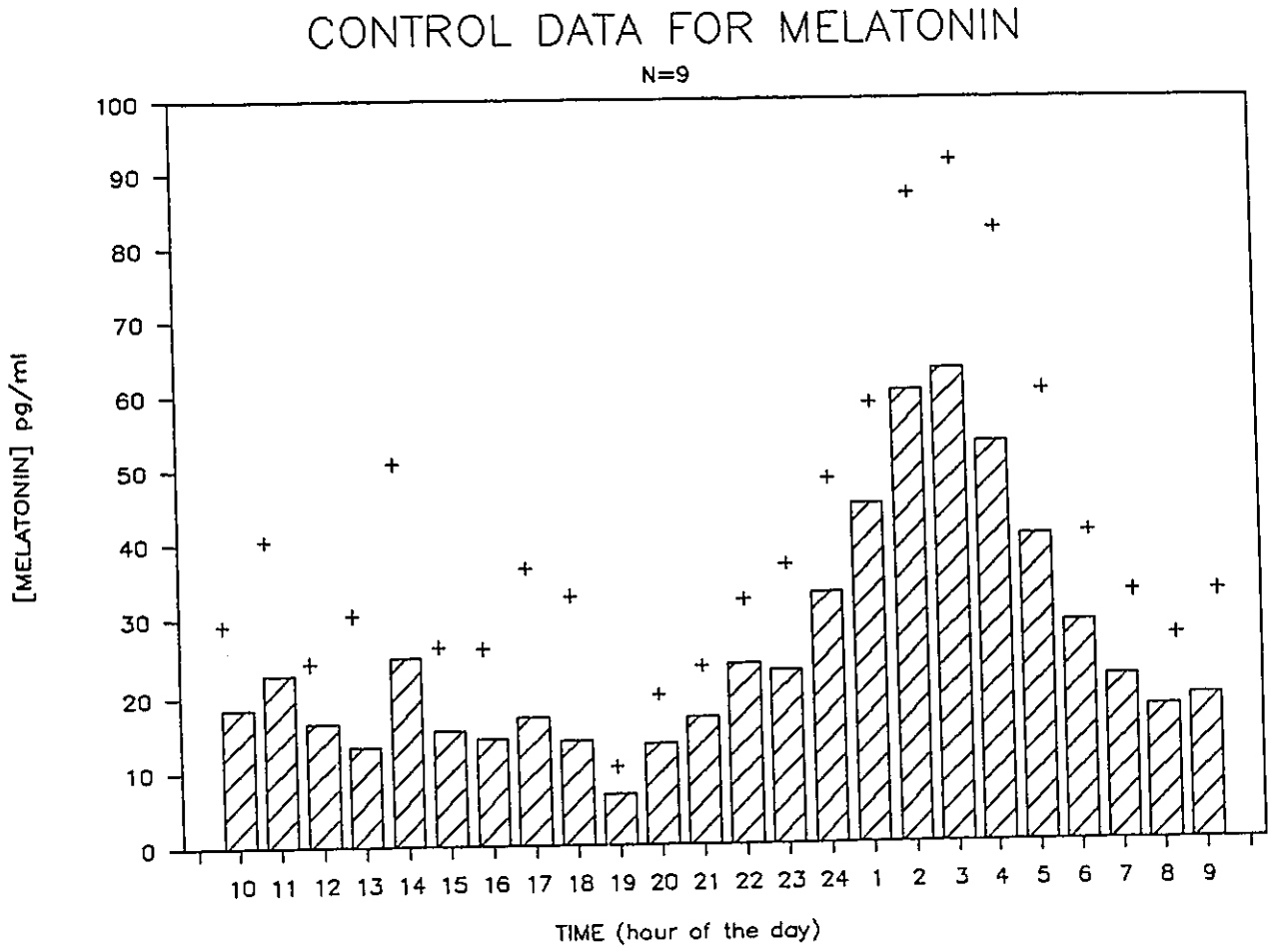


Subject	% R	AMP	MESOR	PHI (Degrees)	PHI (Hrs.Min)
A	71.0	44.73	69.50	-136.3	9.05
B	59.8	67.48	104.00	-157.9	10.32
C	42.1	27.45	33.23	-124.6	8.19
D	39.0	32.71	58.69	-124.8	8.20
E	39.5	29.80	87.51	-165.7	11.03
F	78.2	64.99	73.35	-150.4	10.02
G	70.9	26.76	64.28	-163.5	10.54
H	47.5	37.28	48.52	-148.4	9.54
I	77.5	49.70	51.75	-156.3	10.25
Mean	57.1	41.17	67.7	-148.6	9.45

Tangents from Pole to Ellipse:

Phi 1	= -130.7	Hrs/Min	= 8.43
Phi 2	= -161.2	Hrs/Min	= 10.45

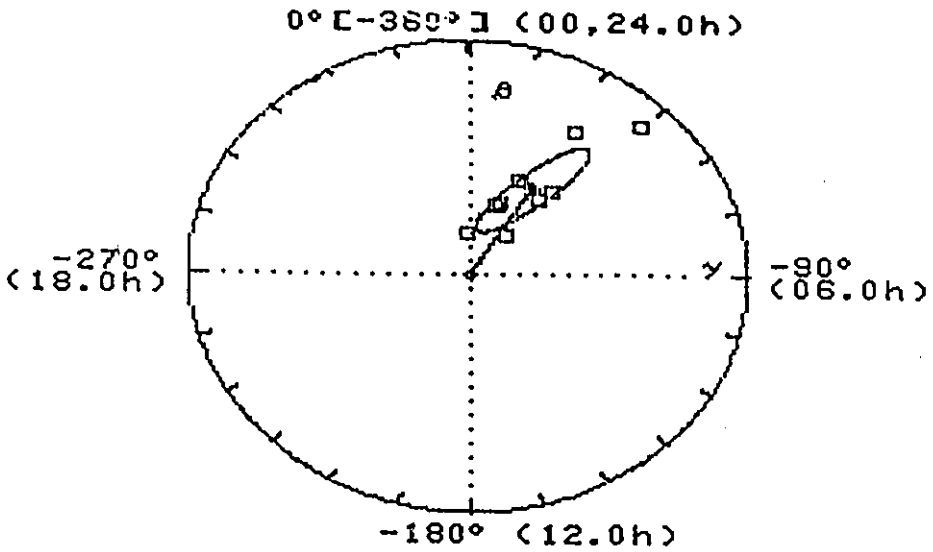
Figure 5(ii)b: Melatonin Control Data



+ = Standard Error

Figure 5(iii) shows the mean cosinor plot for all control melatonin data. The coordinates of the individual acrophases are listed below (subjects A through I). The data for the mean cosinor of the individual control data are shown below that giving the coordinates of the ellipse.

Figure 5(iii): Polar Plot of Melatonin Data - All Controls



Subject	% R	AMP	MESOR	PHI (Degrees)	PHI (Hrs.Min)
A	33.8	15.52	23.01	- 38.5	2.34
B	47.0	16.76	30.71	- 25.3	1.41
C	39.0	27.54	27.33	- 32.1	2.08
D	83.5	33.94	27.91	- 42.5	2.50
E	30.3	17.60	39.87	- 40.1	2.40
F	32.9	12.19	27.52	- 21.6	1.27
G	30.7	6.78	15.21	-357.6	23.50
H	72.5	8.05	12.29	- 40.0	2.40
I	40.7	15.21	11.50	- 18.7	1.15
Mean	45.6	18.35	21.93	- 32.9	2.12

Tangents from Pole to Ellipse:

Phi 1	= - 7.9	Hrs/Min	=	0.32
Phi 2	= - 42.5	Hrs/Min	=	2.50

PART II

INDIVIDUAL PATIENT DATA

In this section, the data from each individual patient are presented in their entirety. Each presentation will begin with a summary of the patient's history which was taken in the Sleep Clinic. At the end of the history, the patients are assigned to one of the three predicted groups (MacFarlane et al, 1983). The group assignment was done before the patient entered the study.

This is followed by a graphic representation of the subjective sleep diaries (Appendix B) maintained by the patients for a 14 day period. The black bars indicate those times at which the patients believed that they were asleep. (Note, rectal temperature monitoring begins on day 9.) Under this graph is a table showing the mean of the values graphed above, the mean of the corresponding values of a matched control subject, and the mean and standard error of all control subjects.

The third part of the case presentation is the sleep histograms for nights 13 and 14 where a full sleep EEG was recorded. This graph of clock time versus sleep stage provides a visual report on the sleep episode for a given night. This is followed by a table where total sleep time **(TST)**, sleep onset latency **(SOL)**, REM onset latency **(ROL)**, percent stages 1 through 4 **(%S1-S4)**, and **%REM** are given. Again, values for a matched control subject and the mean plus and minus standard error for all control subjects are provided.

Next, a mean cosinor polar plot of the temperature data for the

particular patient is shown. This represents the five consecutive recording days where rectal temperature was continuously monitored. Given below this is a table showing the parameters of the individual recording days.

The last part of the presentation shows the graphs and the corresponding polar plots of the hormone data from the 24-hour sampling period on the 14th day and night.

CASE PRESENTATION A

PATIENT A

Clinical History

Patient A is a 38 year old, caucasian female, married with two teenage children. She presented with a sleep problem which primarily involved a difficulty with sleep onset, although a lack of sleep maintenance had occasionally been a concomitant. Her history of chronic insomnia dated back at least eight and one-half years to her hospitalization for a depressive state.

Her past medical history includes a duodenal ulcer diagnosed in April, 1984, for which she takes Ranitidine, a histamine (H_2) receptor antagonist, Sucralfate, an anti-pepsin agent and a Belladonna Alkaloid (Donnatol) which is an anticholinergic. In addition, she had headaches, some of a tension sort, and she had been using transcutaneous muscle stimulation with some relief. However, she has had severe migraines with one lasting for three and one-half weeks in 1984, and evidently takes Amitriptyline 75 mg h.s. for this. Also, she takes Tetracycline for her acne for two weeks each month.

It appears that her difficulties are aggravated premenstrually and she has attended a PMS Clinic, and takes Progesterone injections intramuscularly 15 days out of the month. She and her husband feel that the swelling of the knees and breasts, and bad headaches associated with the PMS have marginally improved with this treatment. They believe that most of her medical problems occur in the premenstrual period.

This patient was subsequently studied in the Sleep Investigations Unit for an all night recording with a full electrode

compliment as were all the other patients in this study. This followed a period during which all psychoactive drugs were gradually reduced and eliminated. The recording showed no evidence of sleep apnea or myoclonus. Also, the patient was able to accurately ascertain sleep onset latency (SOL), number of awakenings and total sleep time (TST) compared to the EEG recording of normal controls.

Patient A had the most complex medical history of any patient within this study. However, the relentless eight year history of sleep onset insomnia made her an interesting candidate. She had a well defined pattern of napping or sleeplessness in the first half of the night, with more solid and restorative sleep episodes towards the early morning; this patient was assigned to the phase delay group.

From a psychiatric standpoint, the patient did not meet DSM III diagnostic criteria for major depressive disorder at the time of the study. However, she did meet the criteria for Borderline Personality Disorder. The MMPI profile (Appendix H) was one commonly seen in individuals with that disorder. The profile suggests that she avoids close interpersonal relationships, is anxious, feels alienated from others, is distrustful, and is unduly sensitive to criticism. She has a number of physical health complaints and bodily preoccupations. She feels incapable of coping with life's demands.

Sleep and Circadian Data

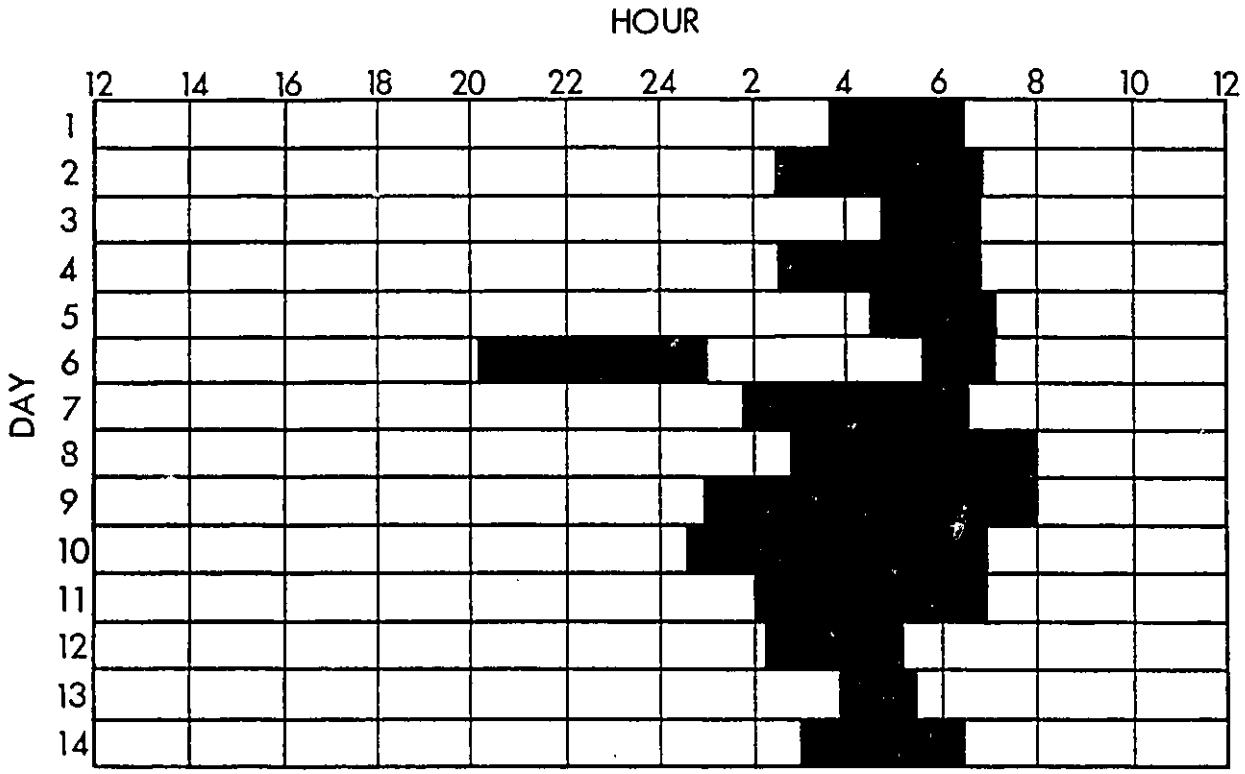
Sleep onset latency was greatly lengthened both on nights in which sleep was recorded and on her self report of 14 nights. Total

sleep time was markedly less than that for a matched control. The proportion of time spent in various sleep stages was not markedly deviant with the exception of Stage 3, in which she spent proportionately twice as much time as the matched control. Stage 2 was less than that of the control.

There was a pronounced phase delay in core temperature. The acrophase for cortisol was not deviant from that of the control. There was a slight delay in the acrophase for melatonin. The sleep disorder presented here is not particularly typical of patients with major depressive disorder.

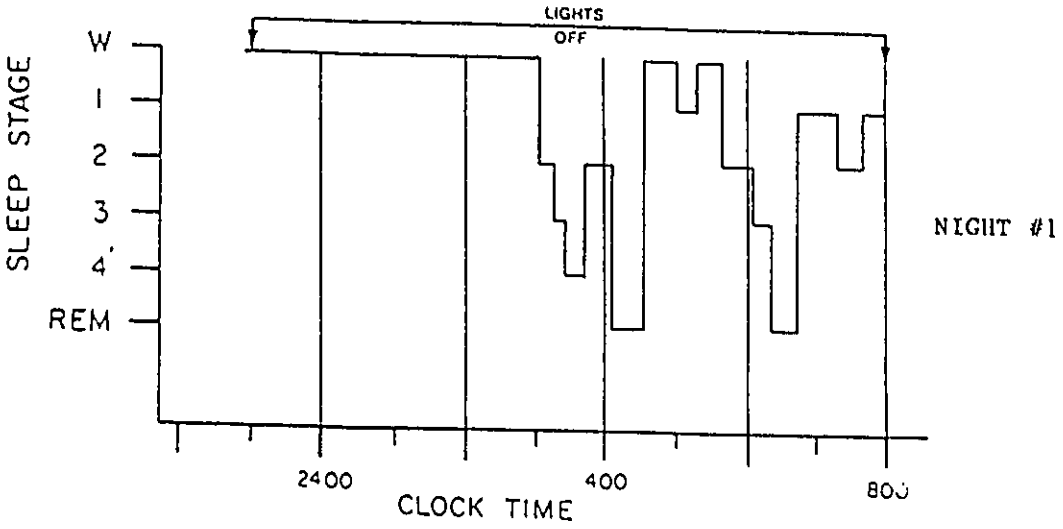
A clinical interpretation of the disorder would suggest that this patient's extreme emotional arousal with anger and sense of loneliness have been incompatible with sleep onset and very likely contributed to the onset of the phase delay insomnia. However, the phase delay persists during periods in which the patient is considerably improved and relatively untroubled, which is often the case during the first two weeks of her menstrual cycle.

Figure 5A(i): Subjective Sleep Diaries and Comparative Table -Patient A



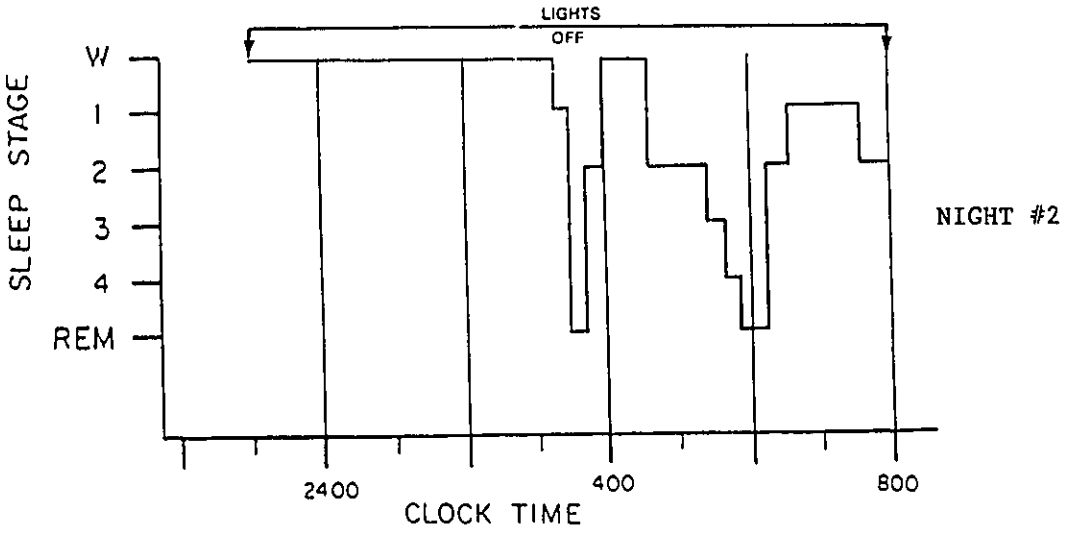
	MEAN OF SLEEP PARAMETERS		
	PATIENT A	MATCHED CONTROL	ALL CONTROLS ± SE
SOL (MIN.)	223.8	16.7	15.2 (± 2.7)
AWAKENINGS	2.1	.60	.33 (± .04)
TST (MIN.)	210.0	454.4	469.80 (±14.4)

Figure 5A(ii) Sleep Histograms and Comparative Table - Patient A



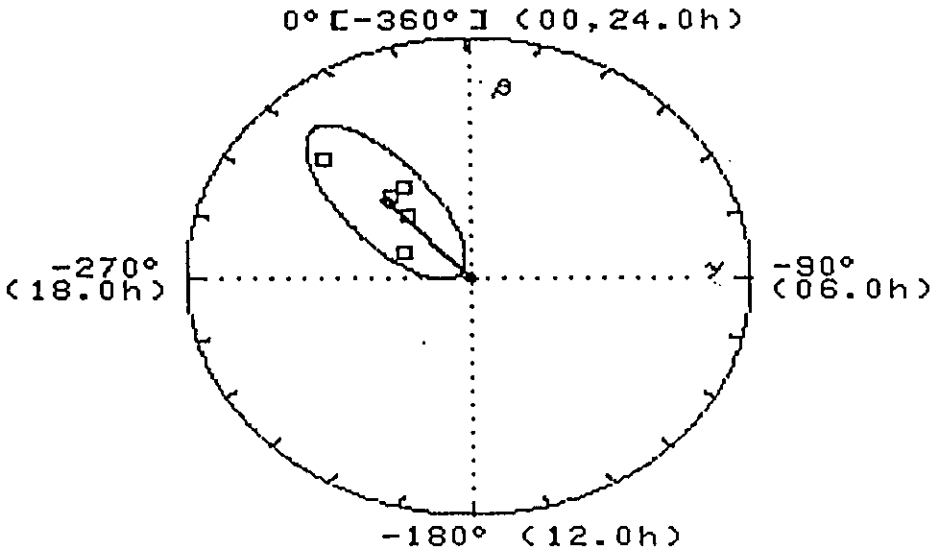
	PATIENT A	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #1	NIGHT #1	NIGHT #1
TST(Minutes)	130	439.5	454.2 (±15.2)
SOL(Minutes)	244	10.4	12.4 (±3.7)
ROL(Minutes)	30	75.0	77.4 (±21.1)
% S1	16.8	5.2	10.2 (±3.3)
% S2	36.2	47.7	49.2 (±6.4)
% S3	3.8	8.4	4.9 (±0.9)
% S4	14.6	14.1	13.0 (±4.1)
% REM	28.6	24.6	22.7 (±4.8)
# AWAKENINGS	4	1	2

Figure 5A(iii): Sleep Histograms and Comparative Table - Patient A



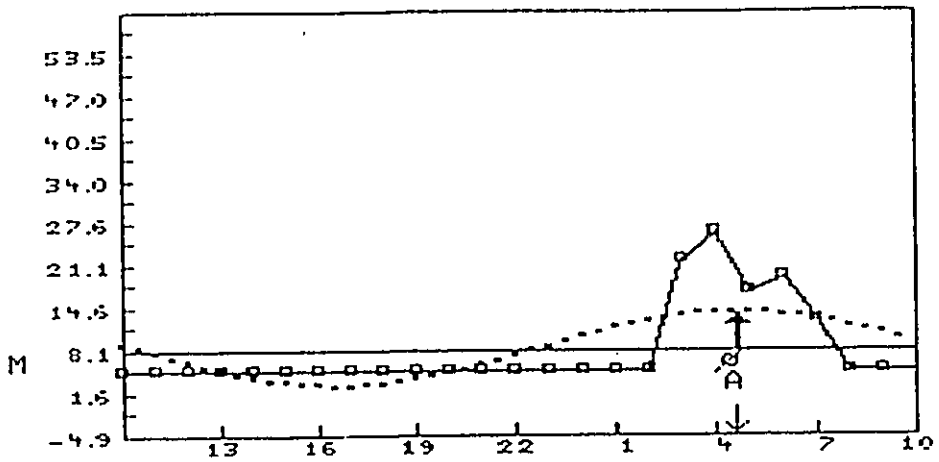
	PATIENT A	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #2	NIGHT #2	NIGHT #2
TST(Minutes)	119.6	424.4	422.0 (± 26)
SOL(Minutes)	238.5	15.2	18.5 (± 4.1)
ROL(Minutes)	10	81.3	93.7 (± 19.8)
% S1	24.3	4.9	6.1 (± 1.3)
% S2	39.0	49.8	46.2 (± 7.0)
% S3	9.4	13.3	11.3 (± 4.8)
% S4	10.6	13.7	12.8 (± 3.7)
% REM	16.6	18.3	23.6 (± 3.3)
# AWAKENINGS	3	5	4

Figure 5A(iv): Polar Plot of Temperature Data and Comparative Table - Patient A



	Patient A	Matched Control
% R	55.0	68.6
P-Level	0.029	0.004
Mesor	37.3	36.9
Amp	0.38	0.39
Phi (Deg)	-317.5	-245.8
Phi (Hrs/Min)	21.10	16.23

Figure 5A(v): Single Cosinor Plot for Melatonin and Comparative Table - Patient A (X-axis = Clock hours; Y-axis = pg/ml)



		Patient A	Matched Control
% R	=	41.50	30.70
P-Level	=	0.004	0.021
Amp	=	5.80	6.78
Mesor	=	7.96	15.21
Phi(Deg)	=	69.50	-357.6
Phi(H/Min)	=	4.38	23.50

Figure 5A(vi): Polar Plot of Melatonin - Patient A

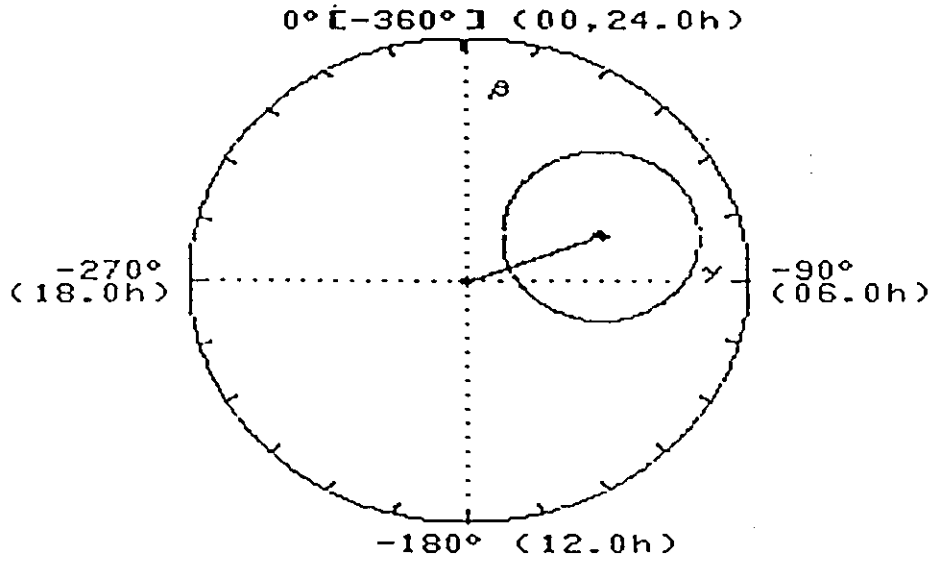
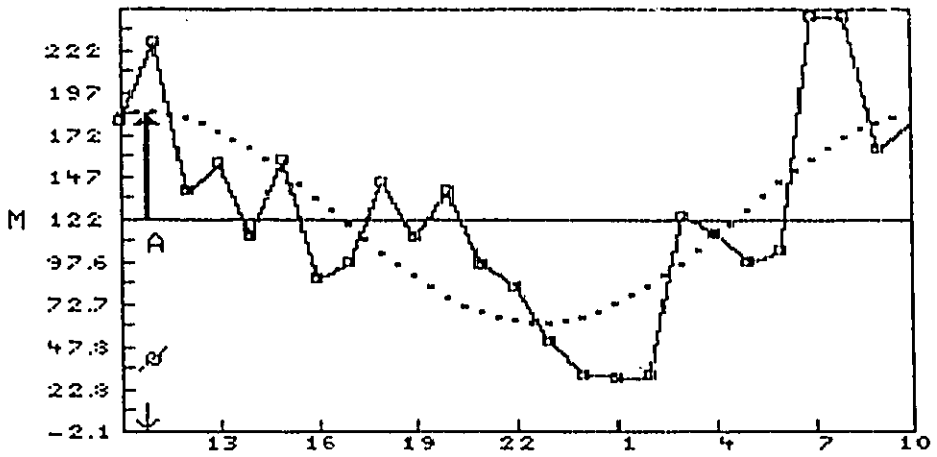


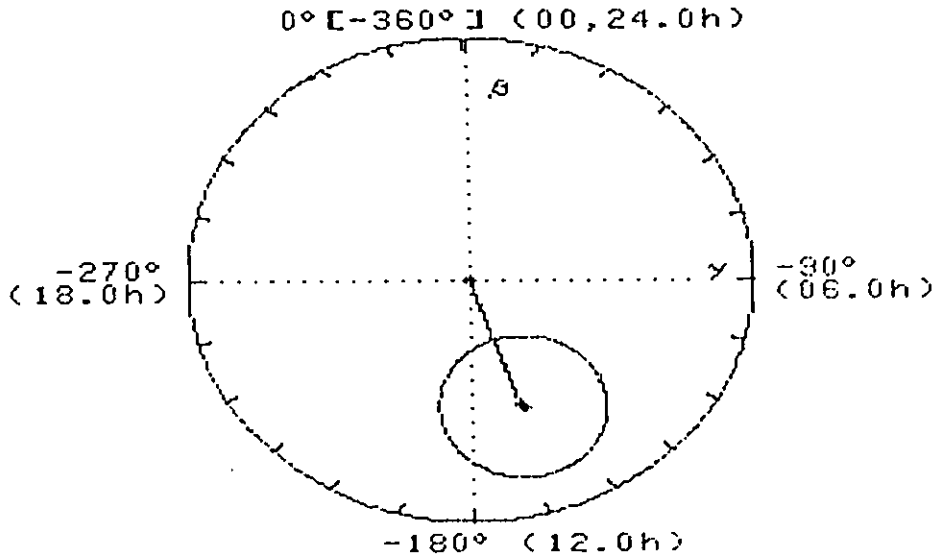
Figure 5A(vii): Single Cosinor Plot for Cortisol and Comparative Table - Patient A (X-axis = Clock hours; Y-axis = ng/ml)



· = Calculated points
▫ = Input data

		Patient A	Matched Control
% R	=	54.70	70.90
P-Level	=	<0.001	<0.001
Amp	=	61.71	26.76
Mesor	=	122.54	64.28
Phi(Deg)	=	-160.7	-163.5
Phi(H/Min)	=	10.43	10.54

Figure 5A(viii): Polar Plot of Cortisol - Patient A



CASE PRESENTATION B

PATIENT B

Clinical History

Patient B is a 25 year old, caucasian male, single, currently residing with his parents. His presenting complaint was a chronic difficulty with sleep onset. Left on a free-running schedule, he prefers to get up at about 1400 hours. By about 2000 hours he tends to feel more energetic. During the night while awaiting sleep onset, he can become quite depressed, very concerned about the effect that this problem has on his life.

This man has experienced this pattern of insomnia since age 15 (>10 years). The beginning of his problem was related to staying up late and "partying" with friends. He noticed a gradual emergence of morning fatigue and prolonged sleep onset latency while noting that his friends on the same schedule showed no such pattern,.

The medical and psychiatric history of this man was unremarkable. His mood overall was one of dysphoria, which he repeatedly related to his concern about the effect of the insomnia upon his life.

This patient claimed that the only thing that helped him sleep was a night of drinking, which occurred once every several weeks. He did not drink much coffee. He had taken a lot of pop containing caffeine, but had at times discontinued this with little effect. He had taken amitriptyline (Elavil) on one occasion for a period of time and Doxepin (Sinequan) on another. These medications helped induce sleep, but not maintain it. This patient was assigned to the phase delay group.

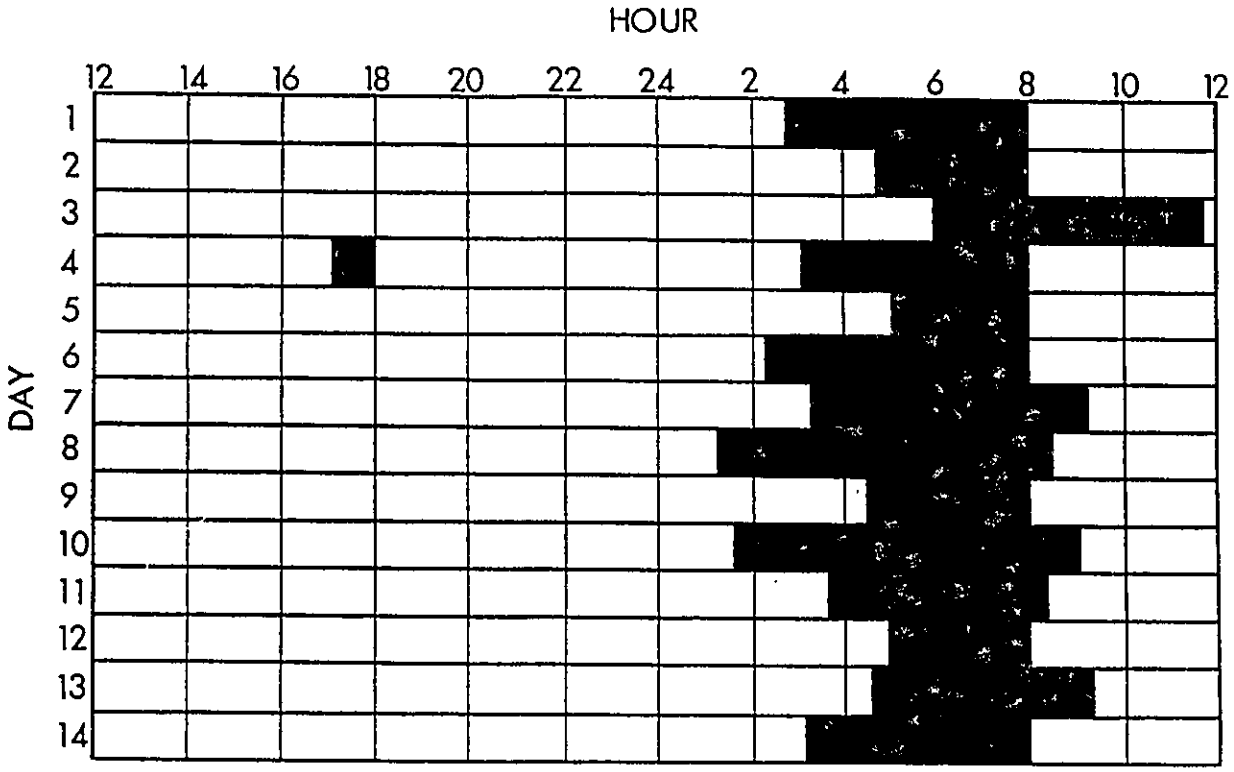
The MMPI reflected moderately severe degrees of depression, anxiety, attention and fearfulness and extreme social discomfort. He felt pessimistic and self deprecatory and mistreated, and alienated from his social environment. This patient would meet DSM III criteria for Dysphoric Disorder.

Sleep and Circadian Data

Self report indicated consistent phase delay and reduced total sleep time. EEG sleep recording confirmed this when compared with his matched control. Stage 1 time was much greater than in the control, and Stage 2, less. On the other hand, Stage 3 was much greater than in the control on night #1 but not on night #2. On night #1 he had no REM sleep and on night #2, 30.2%.

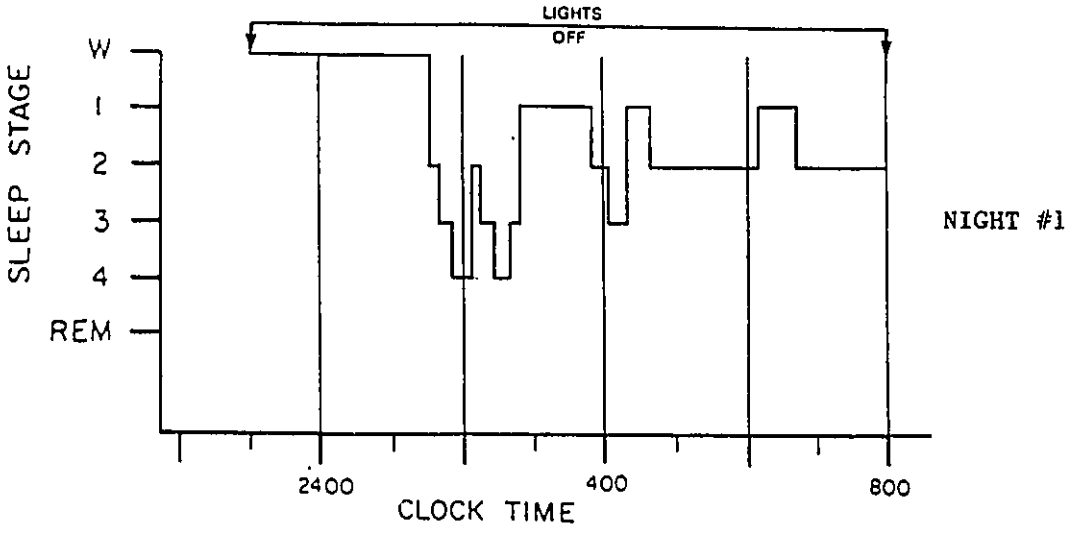
The acrophase of his temperature was at approximately 0600 hours as compared with 1103 hours for the matched control. The acrophase for melatonin was 0454 hours and cortisol at approximately 1230 hours as compared with 0240 hours for his matched control. Thus, sleep phase, temperature, and cortisol were all delayed.

Figure 5B(i): Subjective Sleep Diaries and Comparative Table -Patient B



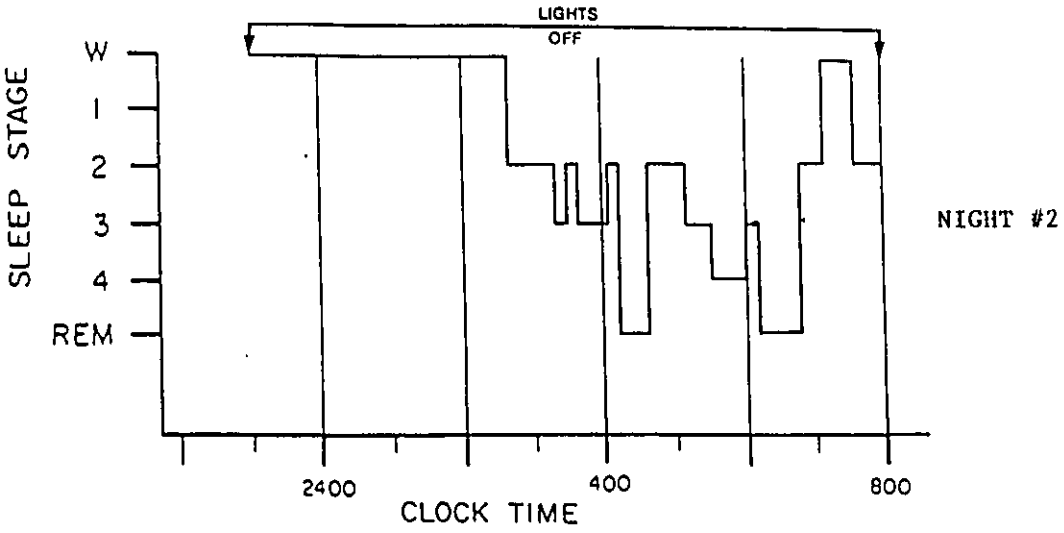
	MEAN OF SLEEP PARAMETERS		
	PATIENT B	MATCHED CONTROL	ALL CONTROLS ± SE
SOL (MIN.)	271.0	10.7	15.2 (± 2.7)
AWAKENINGS	.2	.4	.33 (± .04)
TST (MIN.)	288.9	414.8	469.80 (±14.4)

Figure 5B(ii): Sleep Histograms and Comparative Table - Patient B



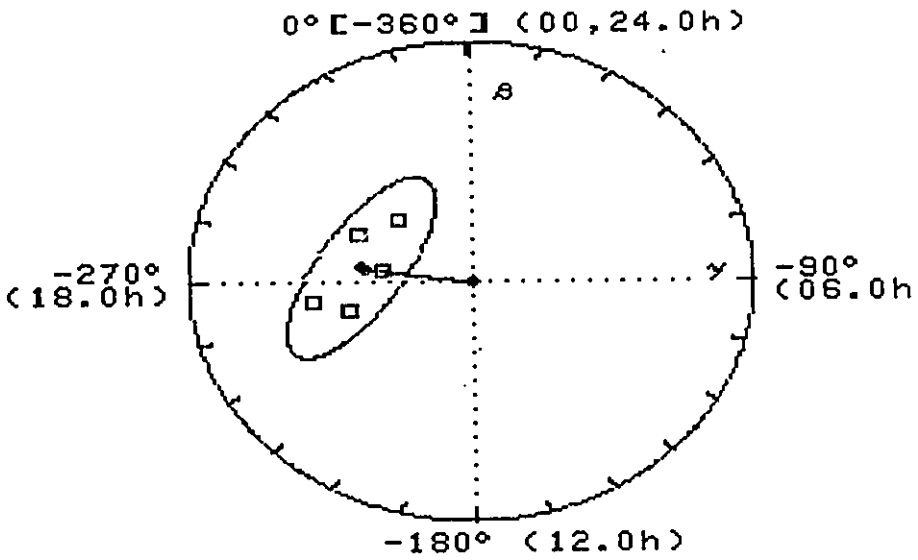
	PATIENT B	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #1	NIGHT #1	NIGHT #1
TST(Minutes)	418.0	450.4	454.2 (±15.2)
SOL(Minutes)	150.0	16.2	12.4 (±3.7)
ROL(Minutes)	---	88.5	77.4 (±21.1)
% S1	32.1	3.0	10.2 (±3.3)
% S2	34.4	50.2	49.2 (±6.4)
% S3	24.8	12.7	4.9 (±0.9)
% S4	8.7	14.1	13.0 (±4.1)
% REM	0.0	23.0	22.7 (±4.8)
# AWAKENINGS	0	2	2

Figure 5B(iii): Sleep Histograms and Comparative Table - Patient B



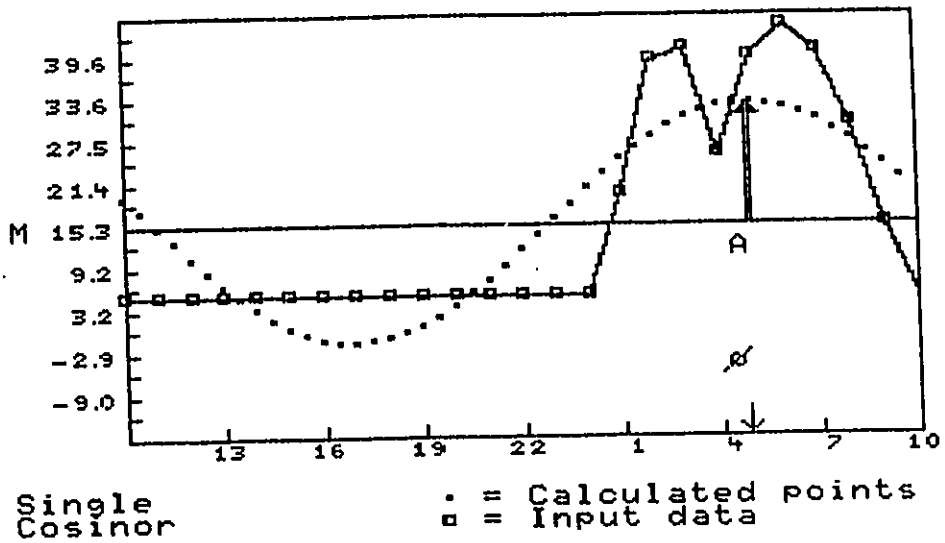
	PATIENT B	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #2	NIGHT #2	NIGHT #2
TST(Minutes)	247.9	445.5	422.0 (± 26)
SOL(Minutes)	220.0	10.5	18.5 (± 4.1)
ROL(Minutes)	85.2	85.7	93.7 (± 19.8)
% S1	6.7	4.2	6.1 (± 1.3)
% S2	42.5	49.8	46.2 (± 7.0)
% S3	15.8	14.7	11.3 (± 4.8)
% S4	14.8	10.3	12.8 (± 3.7)
% REM	20.2	25.2	23.6 (± 3.3)
# AWAKENINGS	4	5	4

Figure 5B(iv): Polar Plot of Temperature Data and Comparative Table - Patient B



		Patient B	Matched Control
% R	=	55.10	84.90
P-Level	=	0.009	<0.001
Amp	=	36.50	36.90
Mesor	=	0.59	0.56
Phi(Deg)	=	-251.1	-246.6
Phi(H/Min)	=	18.44	16.27

Figure 5B(v): Single Cosinor Plot for Melatonin and Comparative Table - Patient B (X-axis = Clock hours; Y-axis = pg/ml)



		Patient B	Matched Control
% R	=	69.30	30.30
P-Level	=	<0.001	0.023
Amp	=	17.24	17.76
Mesor	=	15.33	39.87
Phi(Deg)	=	-73.40	-40.10
Phi(H/Min)	=	4.54	2.40

Figure 5B(vi): Polar Plot for Melatonin - Patient B

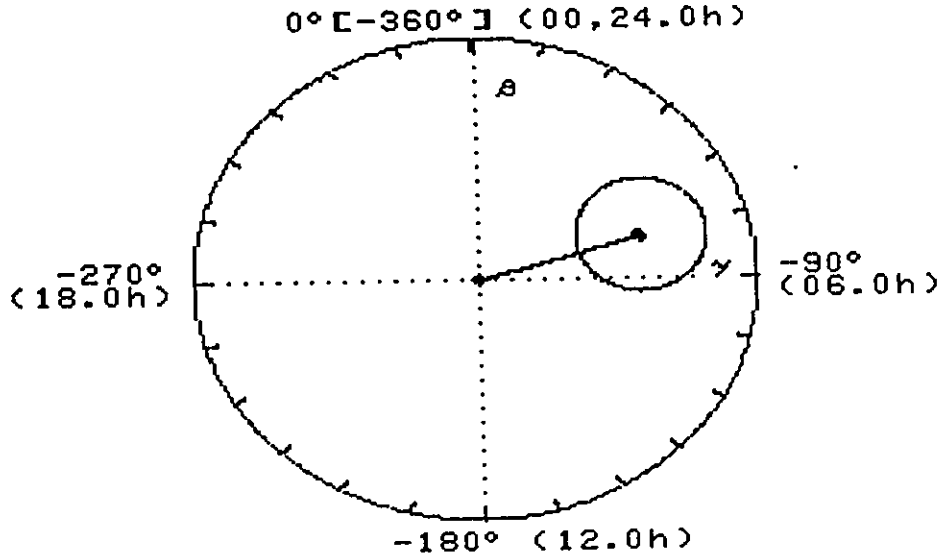
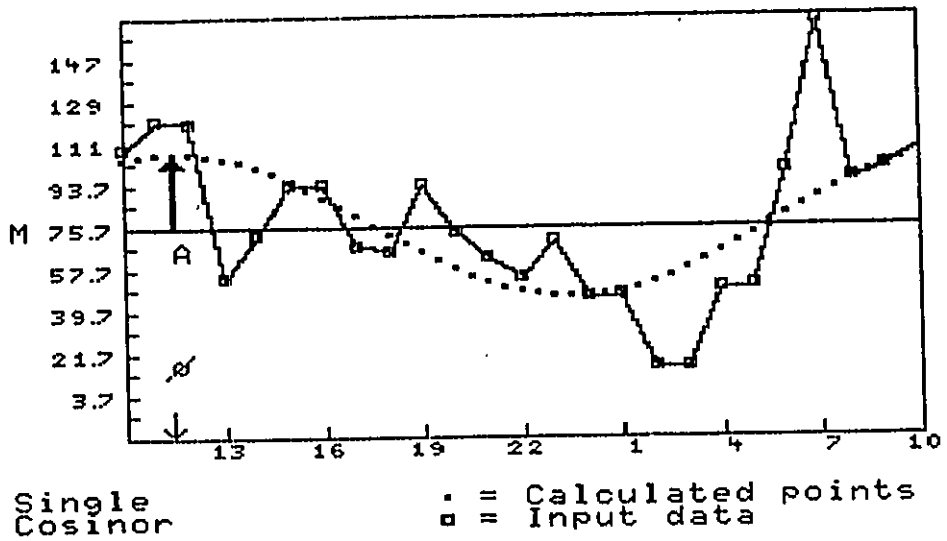
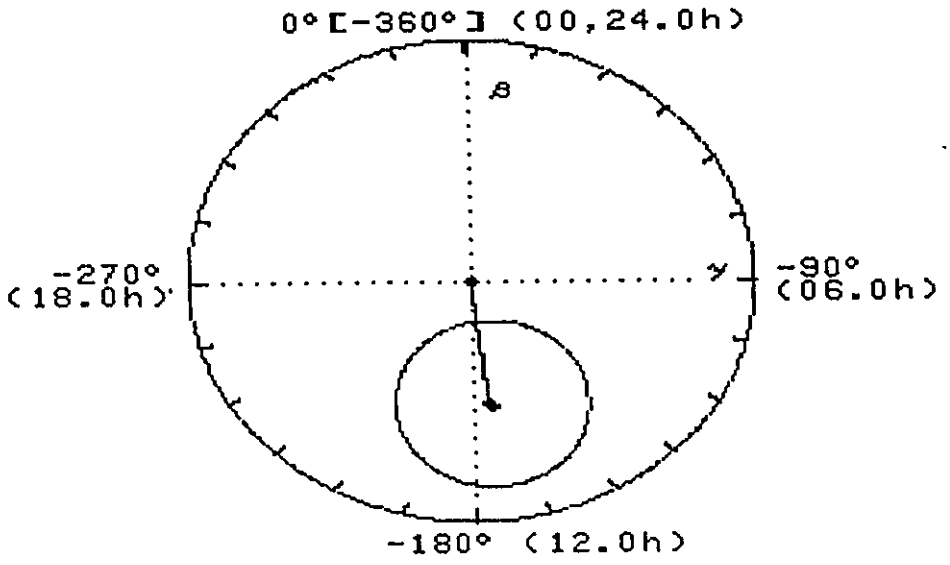


Figure 5B(vii): Single Cosinor Plot for Cortisol and Comparative Table - Patient B (X-axis = Clock hours; Y-axis = ng/ml)



		Patient B	Matched Control
% R	=	42.20	39.50
P-Level	=	0.003	0.005
Amp	=	30.09	29.80
Mesor	=	75.73	87.51
Phi(Deg)	=	-172.40	-165.7
Phi(H/Min)	=	11.29	11.03

Figure 5B(viii): Polar Plot for Cortisol - Patient B



CASE PRESENTATION C

PATIENT C

Clinical History

Patient C is a 25 year old, caucasian male, single, currently residing with his parents. His presenting complaint was a very delayed sleep onset. Once sleep onset had occurred, he could generally remain asleep. He reported sleep satiation on weekends and holidays when he was removed from the confines of a work schedule and allowed to sleep well into the day.

He was unable to recollect any particular events that may have precipitated an initial episode of insomnia, and claimed that this pattern of sleep disturbance had been life-long. Various relaxation exercises had helped in the past but the effects were transient.

This patient had a completely unremarkable medical and psychiatric history. He had tried nitrazepam (Mogadon) in the past, but stopped after several days because of an apparent hangover effect. His intake of coffee and alcohol were regular but modest.

The patient's insomnia was investigated further in the Sleep Investigation Unit, at which time specific sleep disorders were ruled out.

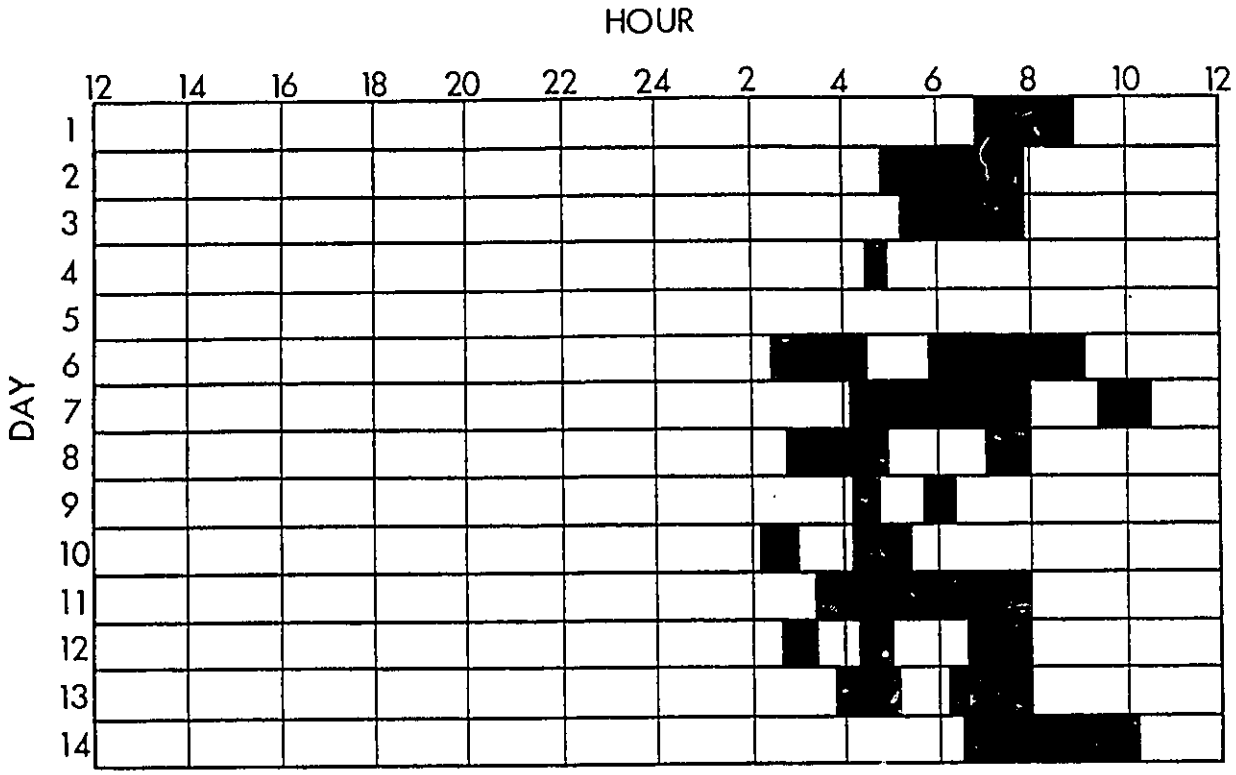
Since this patient showed a completely normal pattern of sleep, save for the delay in sleep onset latency, he was assigned to the phase delay group.

The MMPI revealed a person who feels alienated and rebellious and distant from others.

Sleep and Circadian Data

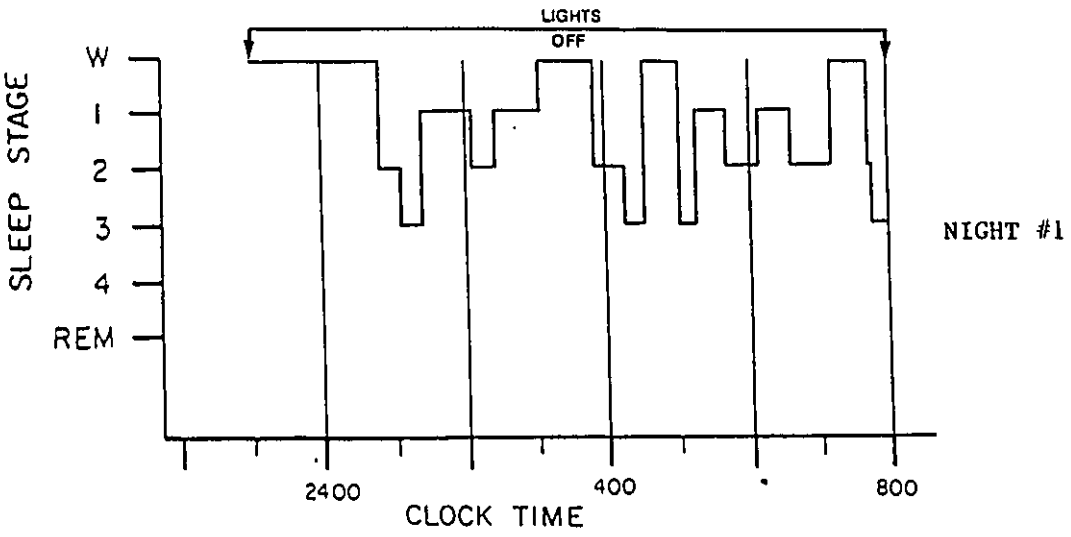
Self reported sleep patterns revealed markedly delayed sleep onset and very short total sleep times and this was confirmed by the sleep recordings. In this patient, as contrasted with patients A and B, there was no compensatory increase in Stage 3, which was actually reduced, and Stage 4 was absent. On night #1 there was no REM sleep, and on night #2, 22.8%. Temperature acrophase was markedly delayed to approximately 1900 hours and cortisol until approximately 0130 hours. Melatonin acrophase occurred at 0723 hours in marked contrast to the 0240 hour acrophase of the matched control. Thus, this patient demonstrated a delay in sleep phase, cortisol and temperature.

Figure 5C(i): Subjective Sleep Diaries and Comparative Table -Patient C



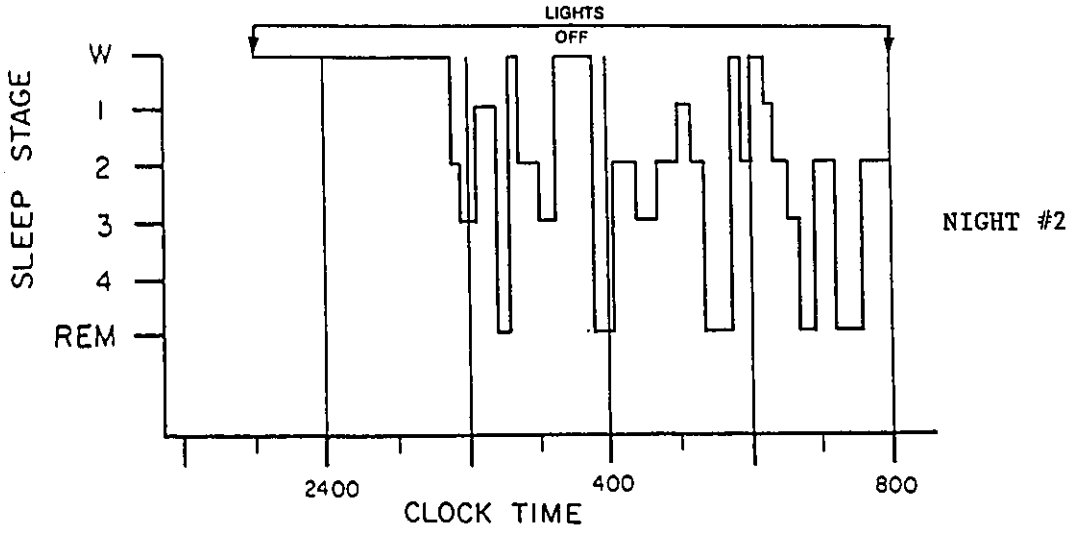
	MEAN OF SLEEP PARAMETERS		
	PATIENT C	MATCHED CONTROL	ALL CONTROLS ± SE
SOL (MIN.)	332.3	10.7	15.2 (± 2.7)
AWAKENINGS	3.0	0.4	.33 (± .04)
TST (MIN.)	162.8	474.8	469.80 (±14.4)

Figure 5C(ii): Sleep Histograms and Comparative Table - Patient C



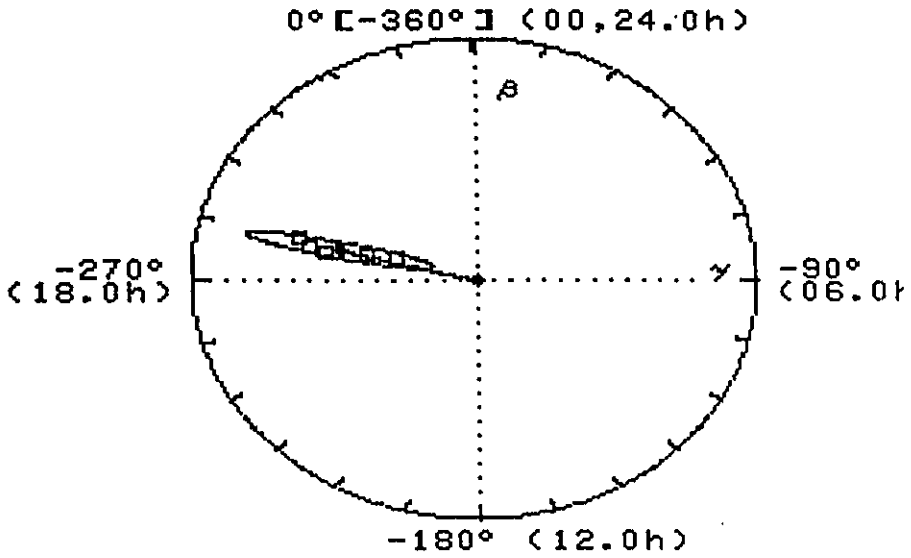
	PATIENT C	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #1	NIGHT #1	NIGHT #1
TST(Minutes)	282.0	439.5	454.2 (±15.2)
SOL(Minutes)	165.0	16.2	12.4 (±3.7)
ROL(Minutes)	---	88.5	77.4 (±21.1)
% S1	53.6	3.0	4.9 (± 0.9)
% S2	39.0	57.2	49.2 (±6.4)
% S3	7.4	12.7	10.2 (±3.3)
% S4	---	14.1	13.0 (±4.1)
% REM	---	23.0	22.7 (±4.8)
# AWAKENINGS	4	2	2

Figure 5C(iii): Sleep Histograms and Comparative Table - Patient C



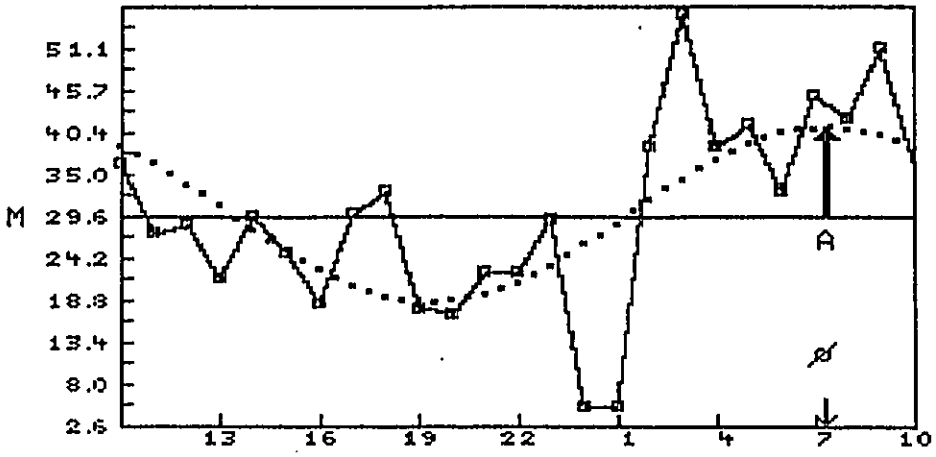
	PATIENT C	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #2	NIGHT #2	NIGHT #2
TST(Minutes)	250.0	445.5	422.0 (± 26)
SOL(Minutes)	166.0	10.5	18.5 (± 4.1)
ROL(Minutes)	14.0	85.7	93.7 (± 19.8)
% S1	15.2	4.2	6.1 (± 1.3)
% S2	48.7	45.6	46.2 (± 7.0)
% S3	13.3	14.7	11.3 (± 4.8)
% S4	---	10.3	12.8 (± 3.7)
% REM	22.8	25.2	23.6 (± 3.3)
# AWAKENINGS	4	5	4

Figure 5C(iv): Polar Plot of Temperature Data and Comparative Table - Patient C



		Patient C	Matched Control
% R	=	77.20	84.90
P-Level	=	0.014	<0.001
Amp	=	36.90	36.90
Mesor	=	0.58	0.56
Phi(Deg)	=	-284.2	-246.6
Phi(H/Min)	=	18.57	16.27

Figure 5C(v): Single Cosinor Plot for Melatonin and Comparative Table - Patient C (X-axis = Clock hours; Y-axis = pg/ml)



Single Cosinor

• = Calculated points
▫ = Input data

		Patient C	Matched Control
% R	=	40.30	30.30
P-Level	=	0.004	0.023
Amp	=	11.18	17.76
Mesor	=	29.58	39.87
Phi(Deg)	=	-110.6	- 40.1
Phi(H/Min)	=	7.23	2.40

Figure 5C(vi): Polar Plot for Melatonin - Patient C

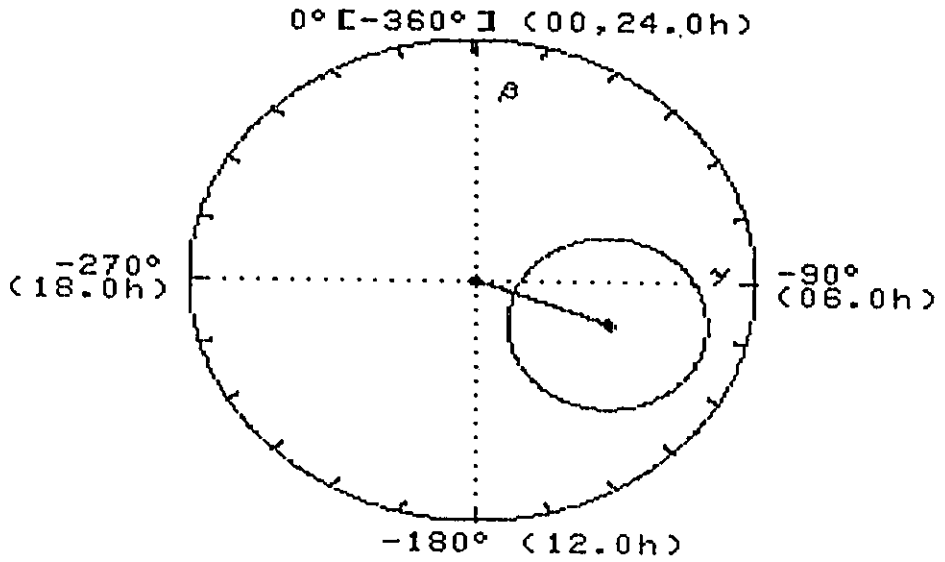
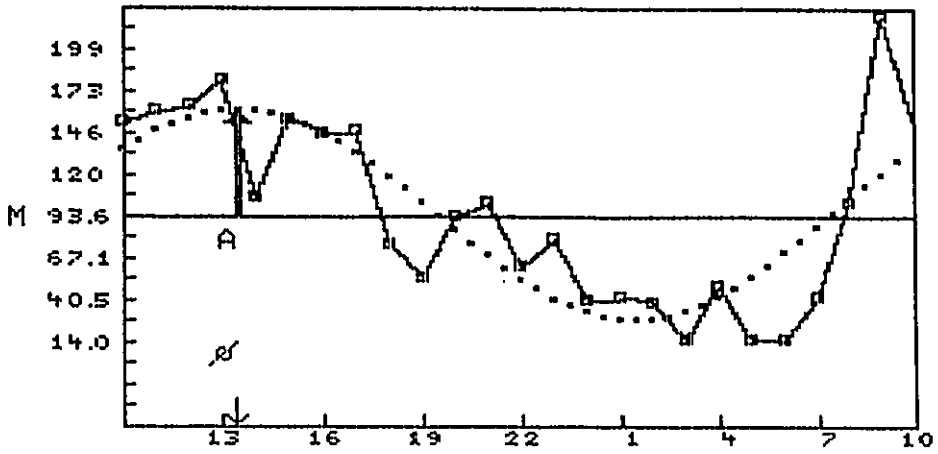


Figure 5C(vii): Single Cosinor Plot for Cortisol and Comparative Table - Patient C (X-axis = Clock hours; Y-axis = ng/ml)

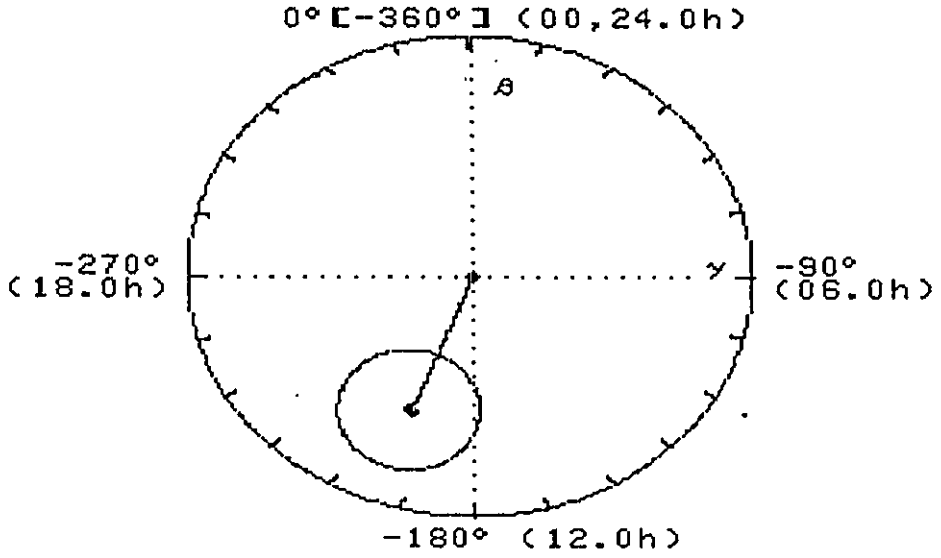


Single Cosinor

• = Calculated points
o = Input data

		Patient C	Matched Control
% R	=	65.20	39.50
P-Level	=	<0.001	0.005
Amp	=	66.30	29.86
Mesor	=	93.59	87.51
Phi(Deg)	=	-202.1	-165.7
Phi(H/Min)	=	13.27	11.03

Figure 5C(viii): Polar Plot for Cortisol - Patient C



CASE PRESENTATION D

PATIENT D

Clinical History

Patient D is a 29 year old, caucasian female, married, with no children. Her presenting complaint was a difficulty with sleep onset which would last several nights, followed by two or three nights of sleeping well. This pattern had been relentless over a four year period. Lights-out was generally around midnight, followed by a delayed sleep onset latency of one to five hours. Occasionally, she would experience a totally sleepless night.

She could not relate the onset to any specific events in her life, although she reported being married, leaving home, and moving from Montreal to Southern Ontario in 1980.

This patient had an unremarkable medical and psychiatric history. However, since the time of her marriage her activity level had dropped, and her food intake increased enough to result in a 120 lb. weight increase in just four years.

She had tried a variety of benzodiazepines in the past, but reported that they had a paradoxical effect and left her more alert. Her coffee and alcohol intake had been very modest.

She was subsequently studied in the Sleep Investigations Unit, at which time specific sleep disturbances were ruled out. A sleep diary was maintained by the patient at that time, which showed a phase delay of sleep onset of three to four hours on four nights and no sleep at all on two nights.

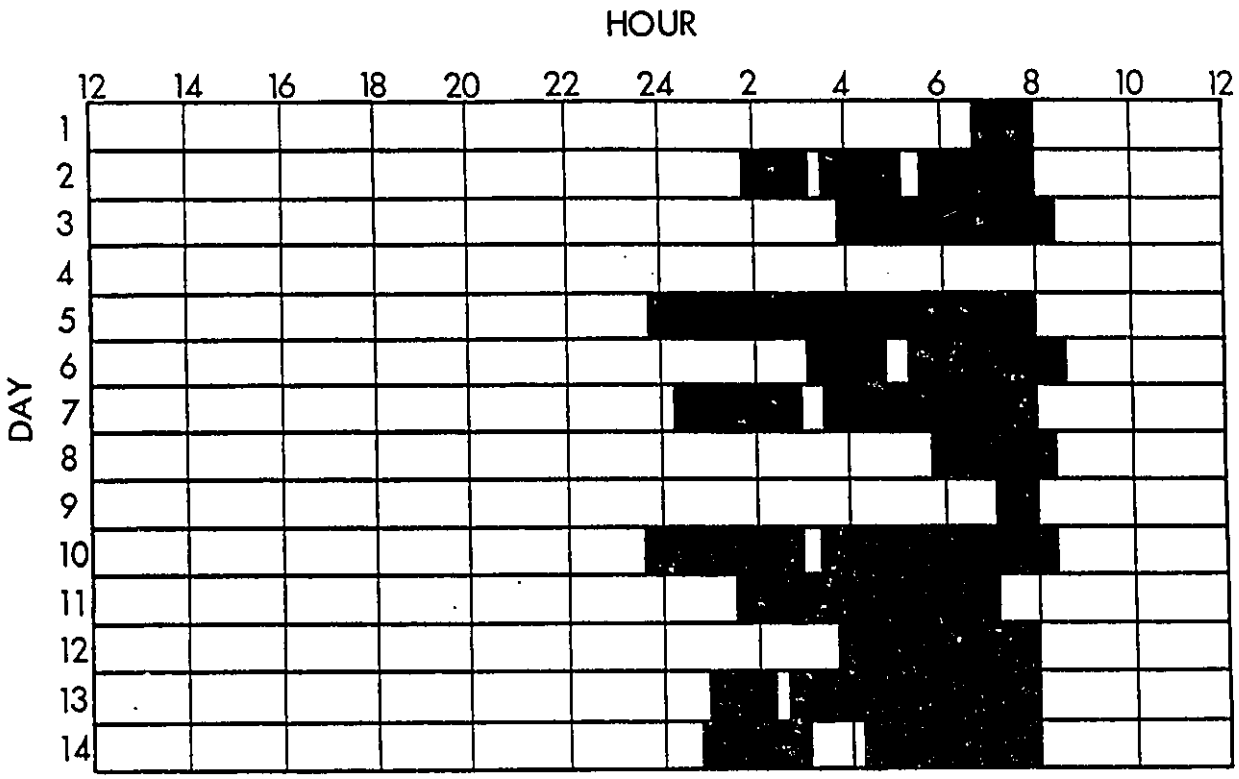
Since sleep episodes occurred most often in the early morning hours, she was assigned to the phase delay group.

MMPI revealed a profile commonly obtained by individuals with character disorders associated with impulsivity, arousal seeking, self indulgence, and resentment of limits.

Sleep and Circadian Data

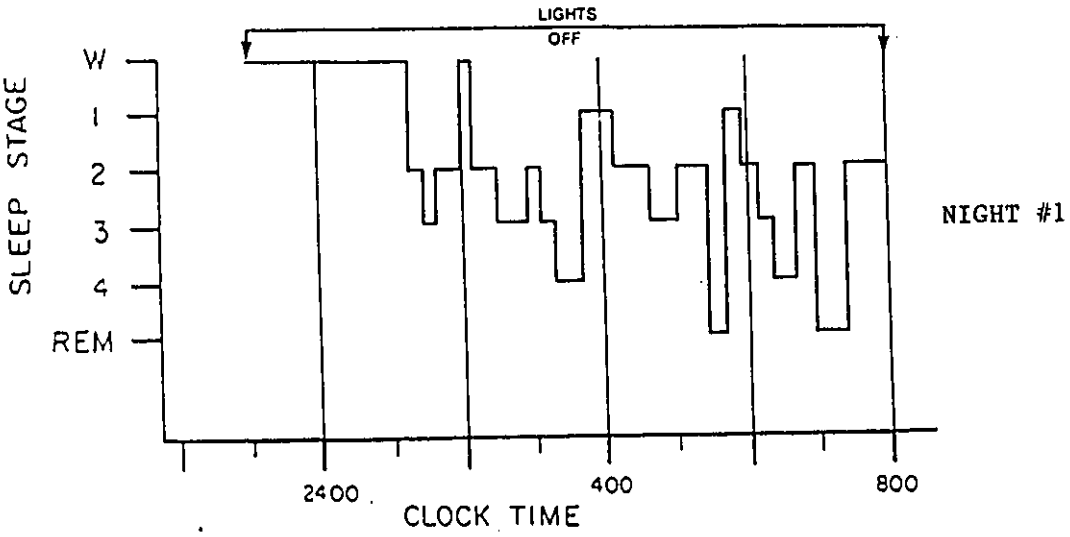
Self report revealed an irregular pattern of sleep onset, with some moderate to marked delay on 7 of 14 nights and a variable total sleep time. The EEG revealed a markedly delayed sleep onset but total sleep time was only moderately reduced as compared with a matched control. REM sleep was less than half that of the matched control, but otherwise the proportion of time spent in sleep stages was not remarkable. Temperature acrophase was moderately delayed to 1745 hours. The acrophase for cortisol was at 0905 hours and for melatonin 0208 hours. Phase delay was less pronounced in this patient than the others in the phase delay group.

Figure 5D(i): Subjective Sleep Diaries and Comparative Table -Patient D



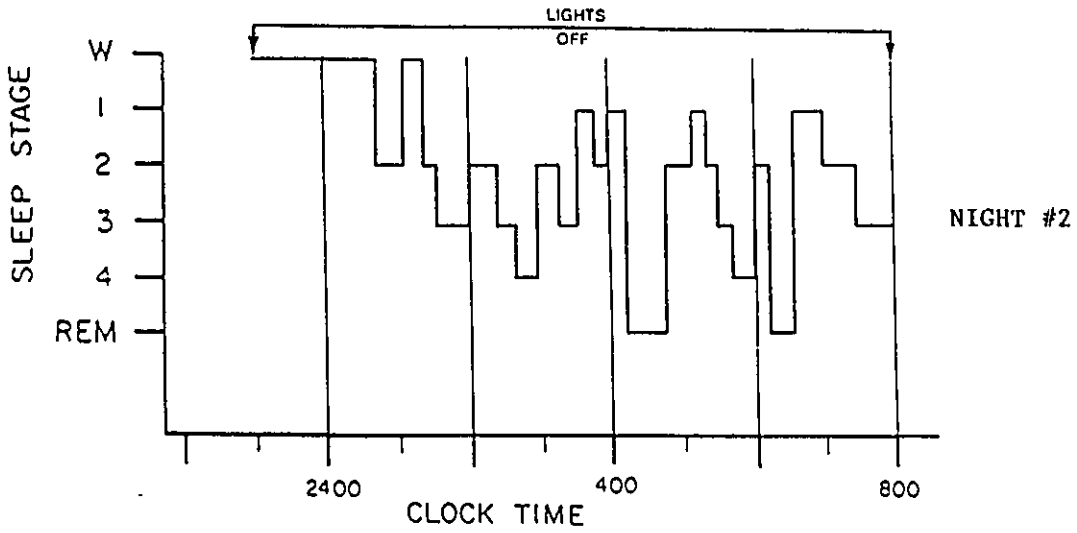
	MEAN OF SLEEP PARAMETERS		
	PATIENT D	MATCHED CONTROL	ALL CONTROLS ± SE
SOL (MIN.)	223.8	13.4	15.2 (± 2.7)
AWAKENINGS	2.7	.66	.33 (± .04)
TST (MIN.)	288.0	458.7	469.80 (±14.4)

Figure 5D(ii): Sleep Histograms and Comparative Table - Patient D



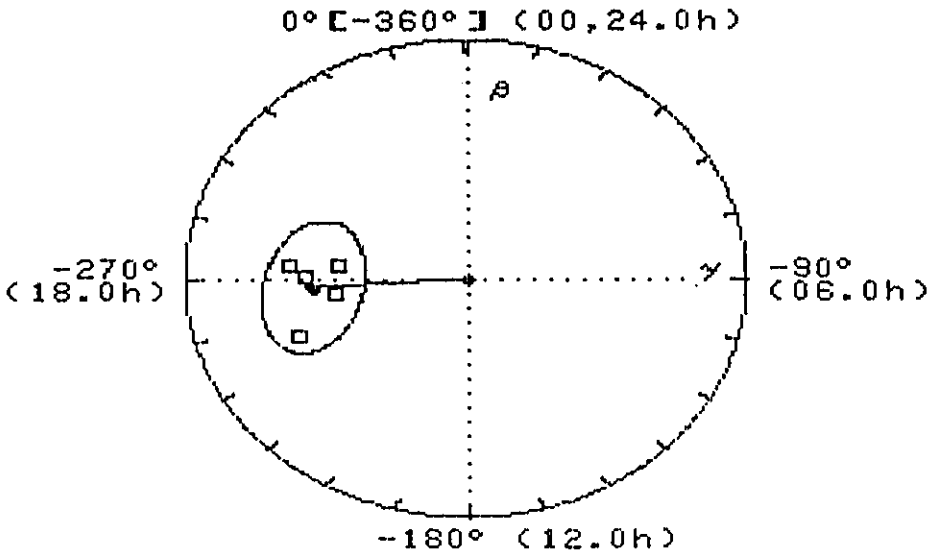
	PATIENT D	MATCHED CONTROL	CONTROL X ± SE)
	NIGHT #1	NIGHT #1	NIGHT #1
TST(Minutes)	415.0	460.0	454.2 (±15.2)
SOL(Minutes)	180.8	10.4	12.4 (±3.7)
ROL(Minutes)	264.0	88.1	77.4 (±21.1)
% S1	13.3	4.3	4.9 (± 0.9)
% S2	37.6	48.7	49.2 (±6.4)
% S3	26.0	11.4	10.2 (±3.3)
% S4	11.3	11.5	13.0 (±4.1)
% REM	11.9	24.1	22.7 (±4.8)
# AWAKENINGS	1	1	2

Figure 5D(iii): Sleep Histograms and Comparative Table - Patient D



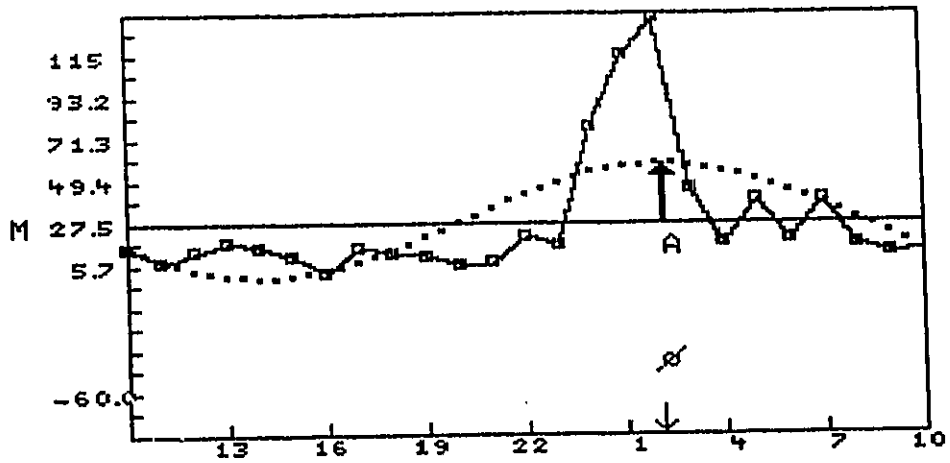
	PATIENT D	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #2	NIGHT #2	NIGHT #2
TST(Minutes)	431.0	438.4	422.0 (± 26)
SOL(Minutes)	135.0	15.3	18.5 (± 4.1)
ROL(Minutes)	185.4	83.4	93.7 (± 19.8)
% S1	26.0	6.7	6.1 (± 1.3)
% S2	38.3	48.3	46.2 (± 7.0)
% S3	15.6	12.2	11.3 (± 4.8)
% S4	10.1	10.4	12.8 (± 3.7)
% REM	10.0	22.4	23.6 (± 3.3)
# AWAKENINGS	1	4	4

Figure 5D(iv): Polar Plot of Temperature Data and Comparative Table - Patient D



		Patient D	Matched Control
% R	=	57.30	69.40
P-Level	=	0.002	<0.001
Amp	=	37.20	37.30
Mesor	=	0.40	0.44
Phi(Deg)	=	-266.1	-248.0
Phi(H/Min)	=	17.45	16.32

Figure 5D(v): Single Cosinor Plot for Melatonin and Comparative Table - Patient D (X-axis = Clock hours; Y-axis = pg/ml)



Single Cosinor

• = Calculated points
▫ = Input data

		Patient D	Matched Control
% R	=	39.0	47.0
P-Level	=	0.006	0.001
Amp	=	29.38	16.76
Mesor	=	27.54	30.71
Phi(Deg)	=	- 32.10	- 25.3
Phi(H/Min)	=	2.08	1.41

Figure 5D(vi): Polar Plot for Melatonin - Patient D

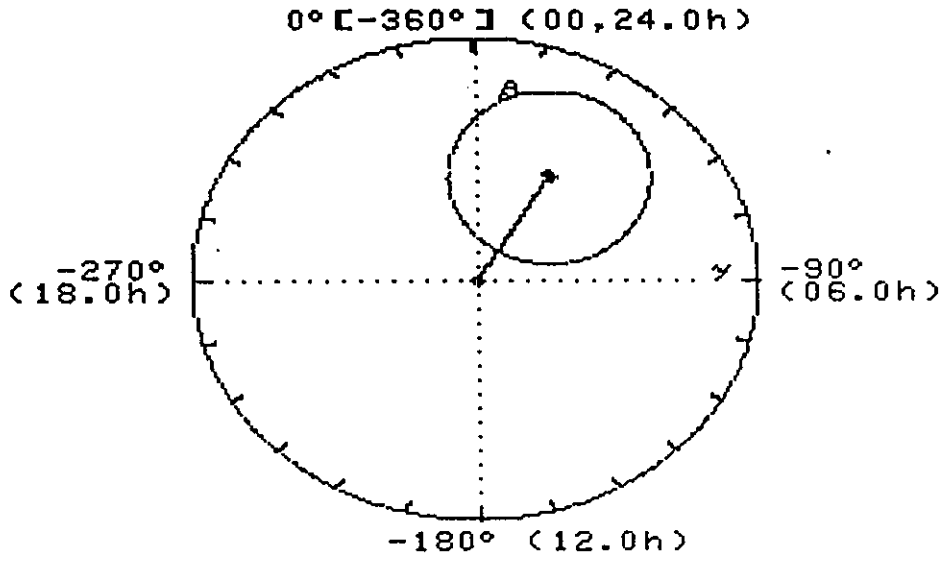
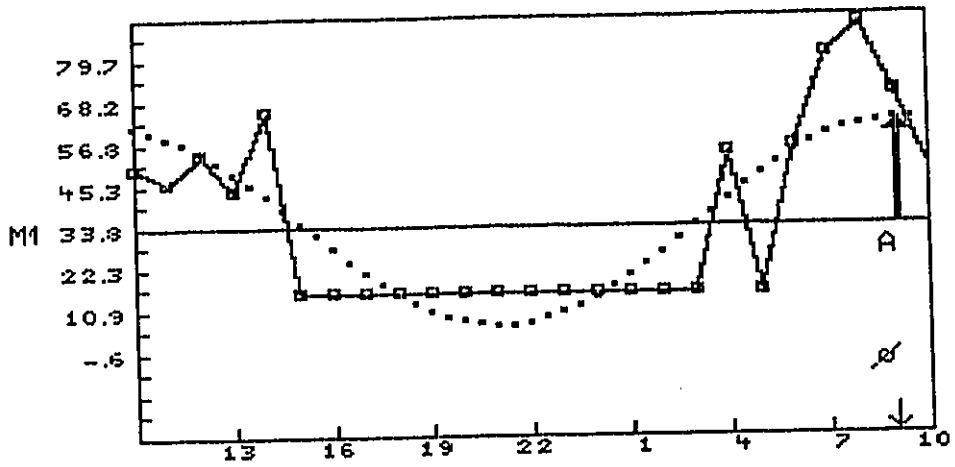


Figure 5D(vii): Single Cosinor Plot for Cortisol and Comparative Table - Patient D (X-axis = Clock hours; Y-axis = pg/ml)

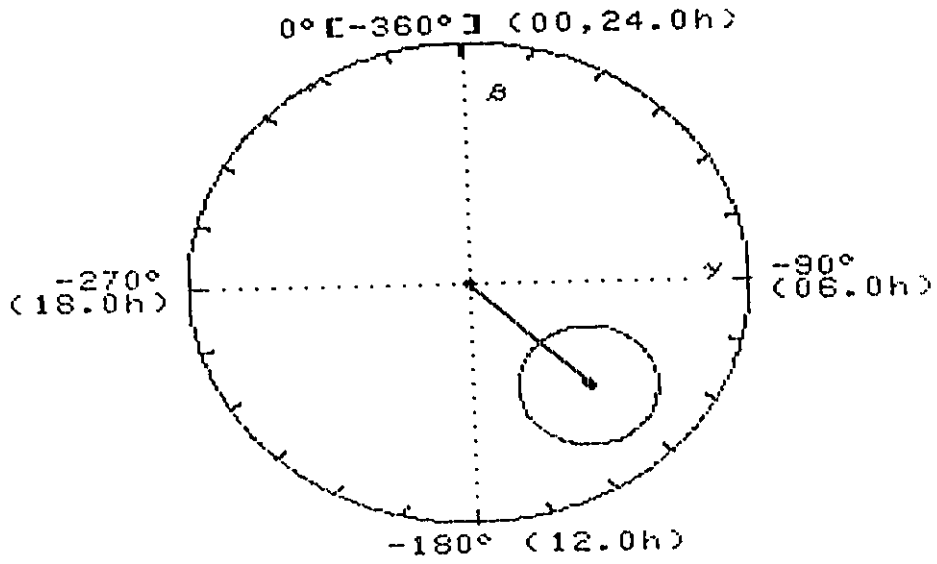


Single Cosinor

. = Calculated points
▫ = Input data

		Patient D	Matched Control
% R	=	66.30	59.80
P-Level	=	<0.001	<0.001
Amp	=	22.74	67.48
Mesor	=	33.81	104.0
Phi(Deg)	=	-136.3	-157.9
Phi(H/Min)	=	09.05	10.32

Figure 5D(viii): Polar Plot for Cortisol - Patient D



CASE PRESENTATION E

PATIENT E

Clinical History

Patient E is a 30 year old, caucasian female, single, currently living alone. Her presenting complaint was a dramatic delay in the time of sleep onset. She felt she slept best in the early morning, but always awoke with a feeling of muscle tension, especially in her back and shoulders.

She remembered the onset of this pattern occurring about seven years previously, during a period when she was living in her native England. She found that her sleep onset time gradually became delayed until 0500 hours. She would sleep until 0700 hours, go to work, function at a rather "low level" in the morning, but by the afternoon, pick up a little more initiative.

This patient had a completely unremarkable medical and psychiatric history. She had been involved in a deep muscle relaxation exercise class prior to the study, but this was of little benefit. She has been wary of any sort of medication, and was proud to report that she had never taken any sleeping pills. Also, she never consumed coffee, and had only an occasional alcoholic beverage.

This patient was observed in the Sleep Investigations Unit, at which time she displayed no specific sleep disorders. With a clear history of prolonged sleep onset latency she was assigned to the phase delay group.

MMPI revealed a profile of a moderate degree of distress, characteristic of individuals with chronic health problems, as well as people who have sleep maintenance disorders. She feels depressed,

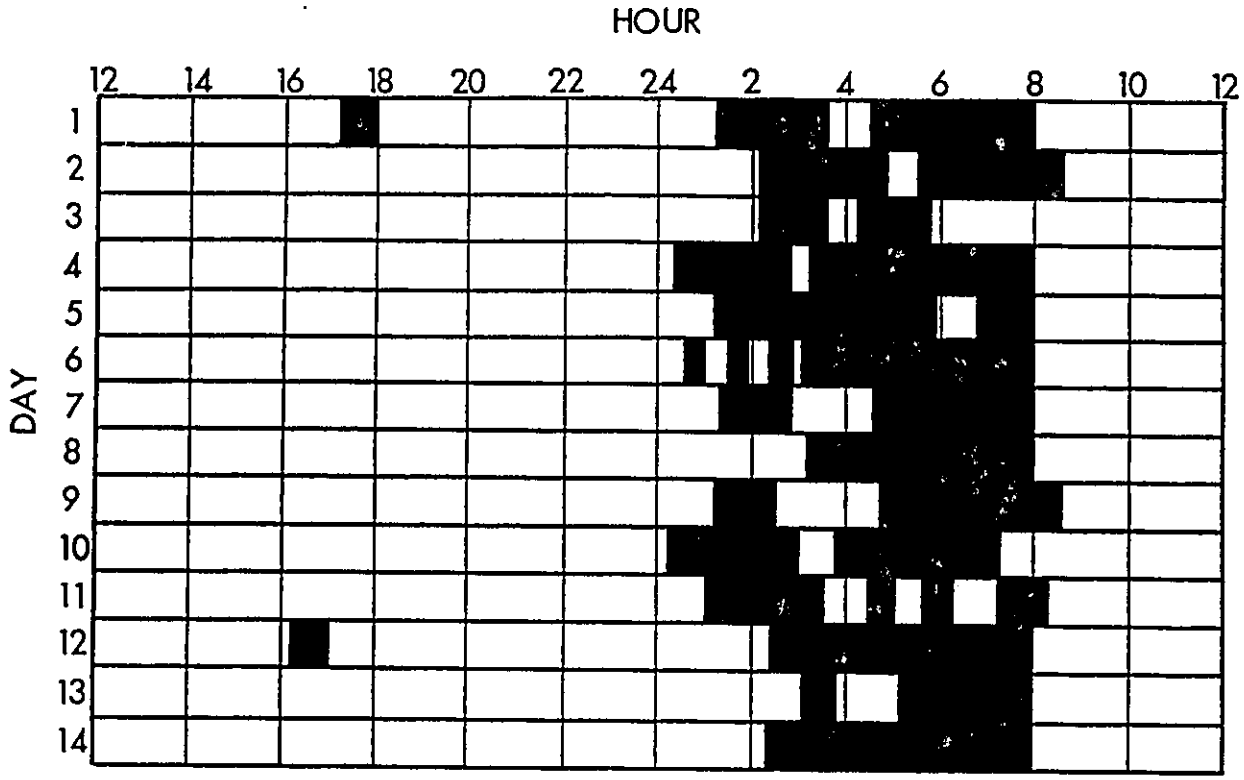
mentally inefficient, uncomfortable although she has a fair amount of energy. She is irritable, tense, weak and fatigued, but also demonstrates a fair degree of optimism and denial of psychological problems.

Sleep and Circadian Data

Sleep onset occurred at approximately 0200 hours on 11 of the 14 nights, and between 0000 hours and 0100 hours on the other three. EEG recording revealed a markedly delayed sleep onset time and a significant reduced total sleep time compared with the normal control. She spent proportionately more time in Stage 1 and 4 than the control and less time in REM sleep on night #2.

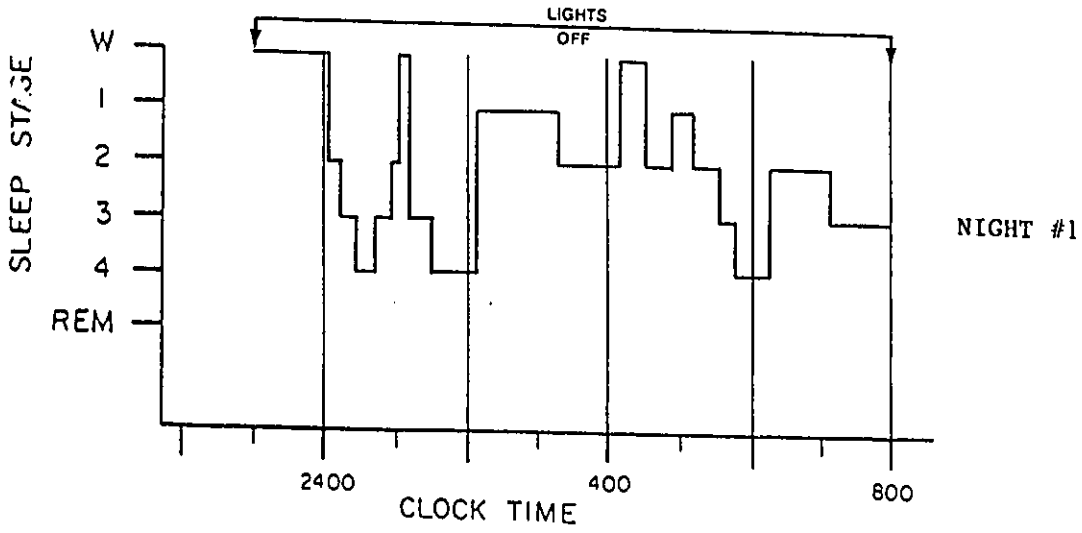
Temperature acrophase occurred at approximately 1800 hours. The cortisol and melatonin acrophases occurred at 0100 hours and 0500 hours, respectively.

Figure 5E(i): Subjective Sleep Diaries and Comparative Table -Patient E



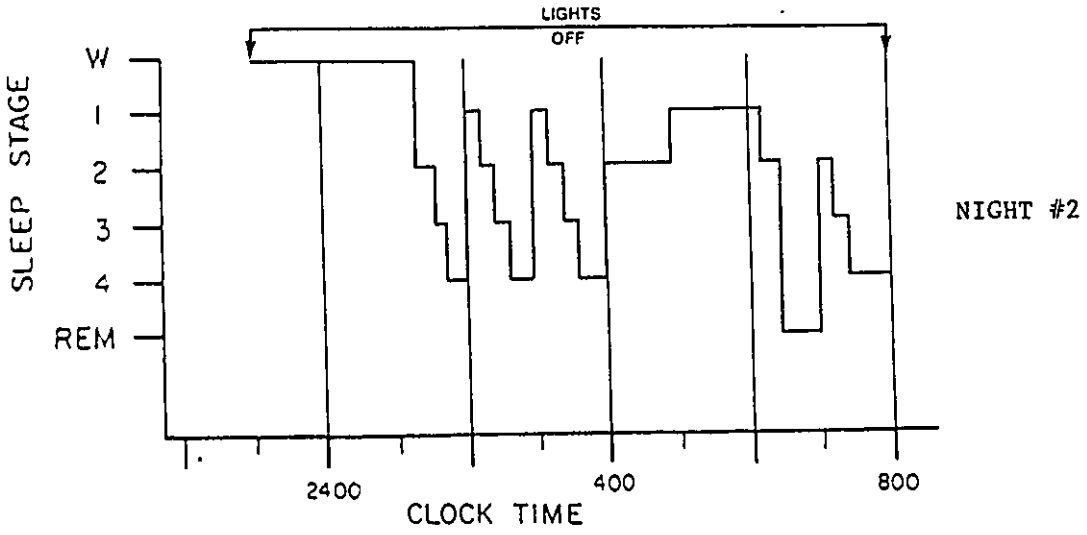
	MEAN OF SLEEP PARAMETERS		
	PATIENT E	MATCHED CONTROL	ALL CONTROLS ± SE
SOL (MIN.)	146.0	13.4	15.2 (± 2.7)
AWAKENINGS	2.4	.66	.33 (± .04)
TST (MIN.)	318.0	458.7	469.80 (±14.4)

Figure 5E(ii): Sleep Histograms and Comparative Table - Patient E



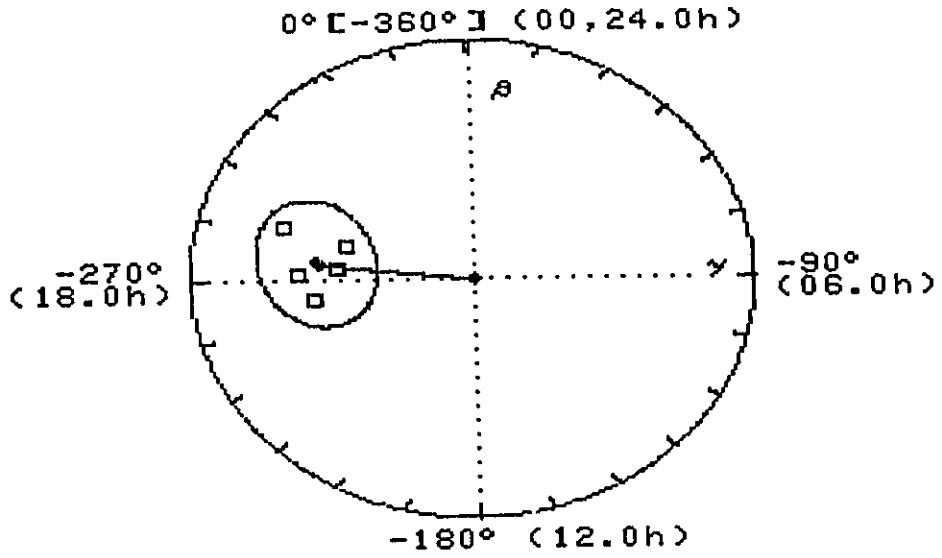
	PATIENT E	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #1	NIGHT #1	NIGHT #1
TST(Minutes)	426.0	460.0	454.2 (±15.2)
SOL(Minutes)	65.0	10.4	12.4 (±3.7)
ROL(Minutes)	---	88.1	77.4 (±21.1)
% S1	24.5	4.3	4.9 (± 0.9)
% S2	38.8	48.7	49.2 (±6.4)
% S3	7.5	11.4	10.2 (±3.3)
% S4	29.2	11.5	13.0 (±4.1)
% REM	---	1.0	22.7 (±4.8)
# AWAKENINGS	2	2	2

Figure 5E(iii): Sleep Histograms and Comparative Table - Patient E



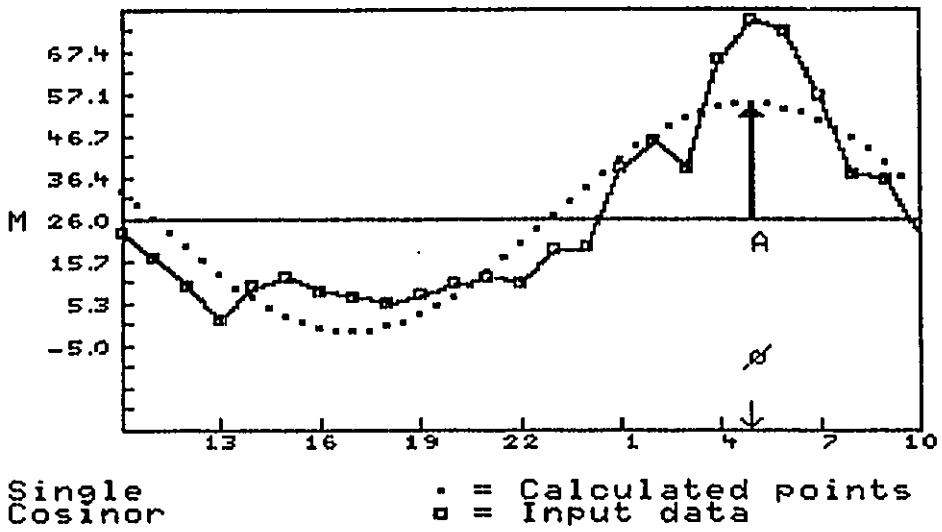
	PATIENT E	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #2	NIGHT #2	NIGHT #2
TST(Minutes)	392.0	438.4	422.0 (± 26)
SOL(Minutes)	145.0	15.3	18.5 (± 4.1)
ROL(Minutes)	318.0	83.4	93.7 (± 19.8)
% S1	27.5	6.7	6.1 (± 1.3)
% S2	22.4	48.3	46.2 (± 7.0)
% S3	12.4	12.2	11.3 (± 4.8)
% S4	26.5	10.4	12.8 (± 3.7)
% REM	11.2	22.4	23.6 (± 3.3)
# AWAKENINGS	0	4	4

Figure 5E(iv): Polar Plot of Temperature Data and Comparative Table - Patient E



		Patient E	Matched Control
% R	=	67.90	69.40
P-Level	=	0.004	<0.001
Amp	=	37.30	37.30
Mesor	=	0.37	0.44
Phi(Deg)	=	-276.4	-248.0
Phi(H/Min)	=	18.26	16.32

Figure 5E(v): Single Cosinor Plot for Melatonin and Comparative Table - Patient E (X-axis = Clock hours; Y-axis = pg/ml)



		Patient E	Matched Control
% R	=	80.1	47.0
P-Level	=	<0.001	0.001
Amp	=	27.91	16.76
Mesor	=	26.02	30.71
Phi(Deg)	=	- 74.20	- 25.3
Phi(H/Min)	=	4.57	1.41

Figure 5E(vi): Polar Plot for Melatonin - Patient E

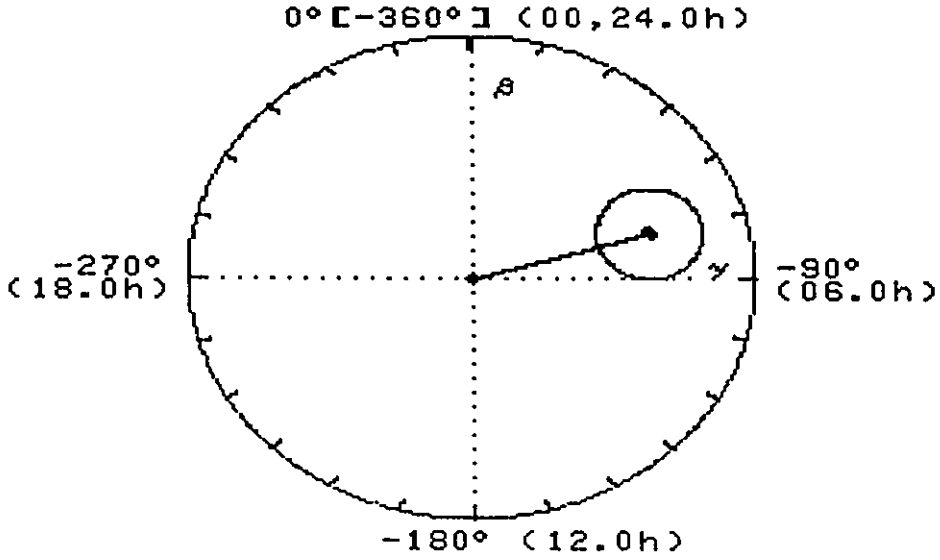
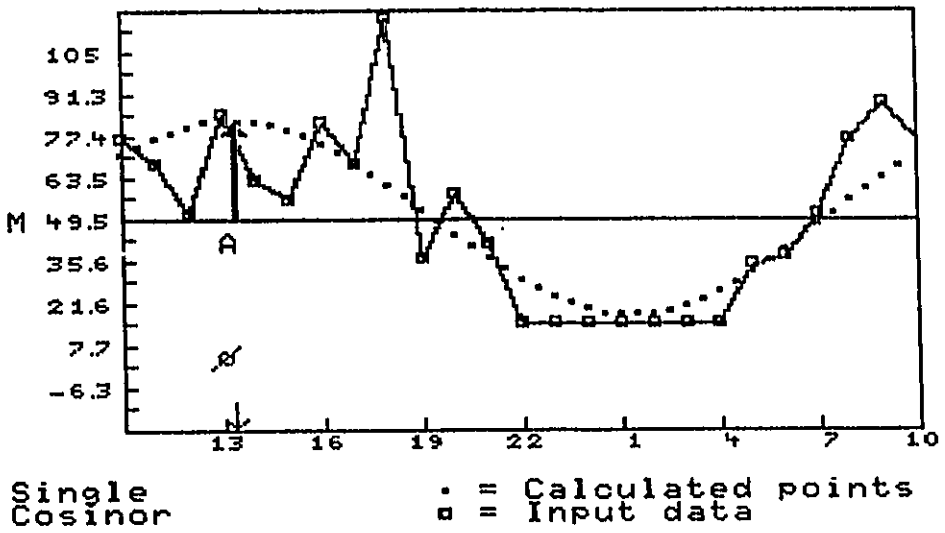
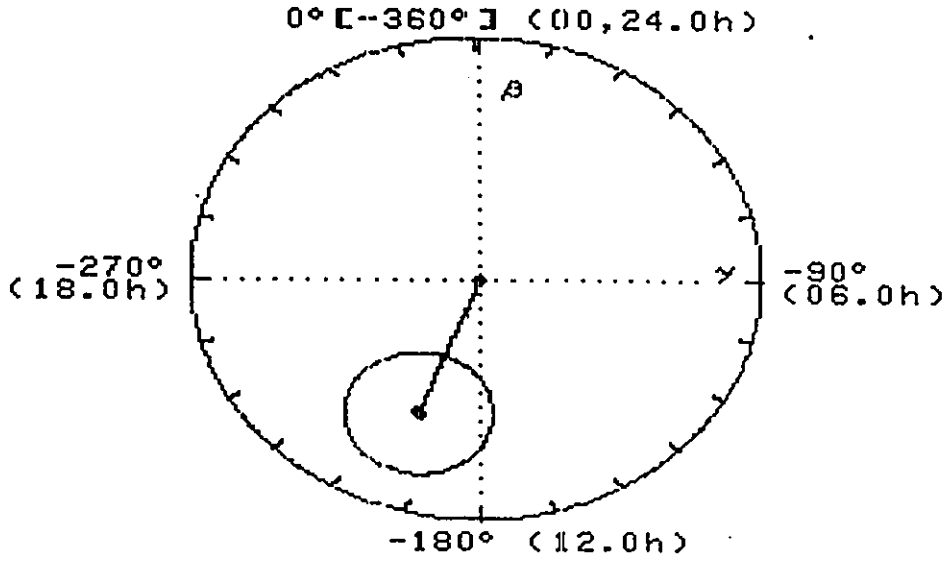


Figure 5E(vii): Single Cosinor Plot for Cortisol and Comparative Table - Patient E (X-axis = Clock hours; Y-axis = ng/ml)



		Patient E	Matched Control
% R	=	64.50	59.80
P-Level	=	<0.001	<0.001
Amp	=	32.38	67.48
Mesor	=	49.52	104.0
Phi(Deg)	=	-199.8	-157.9
Phi(H/Min)	=	13.19	10.32

Figure 5E(viii): Polar Plot for Cortisol - Patient E



CASE PRESENTATION F

PATIENT F

Clinical History

Patient F is a 26 year old, caucasian male, married with no children. His presenting complaint was a difficulty with sleep maintenance. He reported sleep onset latency ranging from 15 minutes to three hours, depending on his level of apprehension regarding lack of sleep and daytime functioning. After four or sometimes five hours, he awoke in the early morning, and was unable to return to sleep. This pattern was constant, even on weekends and holidays.

This pattern of insomnia began about six years previously when this patient entered university. He could recall no particular events associated with the initial development of the symptoms, but rather an insidious onset possibly brought about by excessive worry about his performance in school.

The medical and psychiatric histories of this man were unremarkable. He consumes very little alcohol, and no other drugs except for hypnotics. He has tried lorazepam (Ativan) 0.5 mgs, and oxazepam (Serax) 30 mgs, but found no benefit. Flurazepam (Dalmane) 30 mgs was effective for one week only.

This patient was difficult to assign to a group since he reported periodic difficulties with sleep onset. However, this complaint was not nearly as frequent or consistent as his early morning awakening, and for this reason he was assigned to the phase advance group.

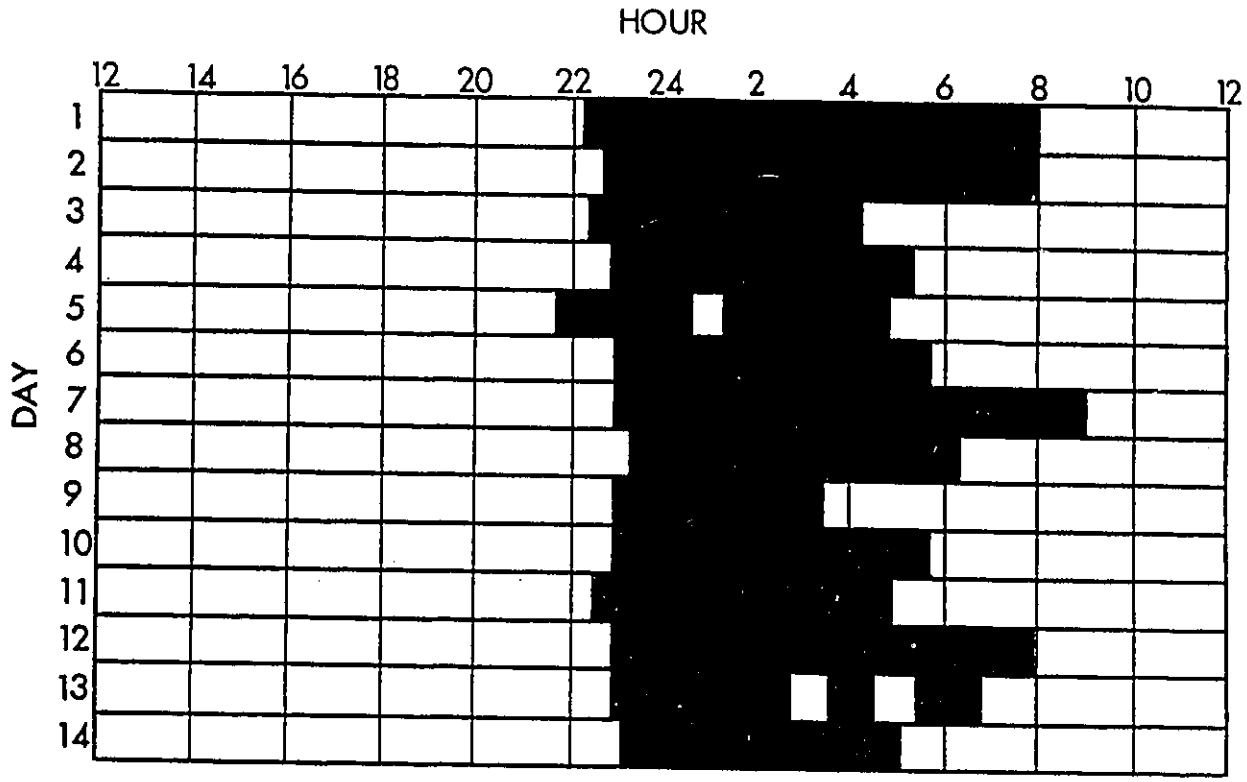
This patient revealed an MMPI profile characteristic by

evasiveness, denial and depression, often felt weak and fatigued and tense, worried about his school work. He did not demonstrate a markedly pathological profile.

Sleep and Circadian Data

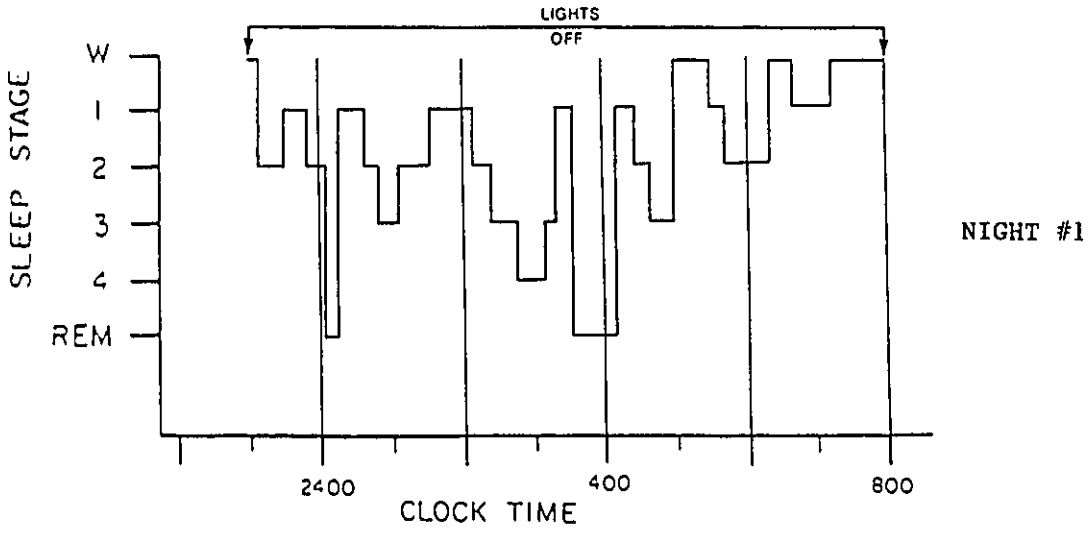
His sleep diary revealed rapid sleep onset at between 2200 and 2300 hours. He slept five to six hours on eight of 14 nights but 10 hours or more on the other nights. In the Laboratory his sleep onset was very prompt and total sleep time was somewhat less than the matched control but not markedly so, and averaged just less than six hours. He spent significantly more time in Stage 1 and less time in Stage 4 than the matched control, and less time in REM. Temperature acrophase occurred at 0300 hours; cortisol at approximately 0730 hours; and melatonin at 0530 hours. The temperature data are consistent with a prediction of phase advance; however, the nearly normal total sleep time is only mitigated by the relatively short time in slow wave and REM sleep.

Figure 5F(i): Subjective Sleep Diaries and Comparative Table -Patient F



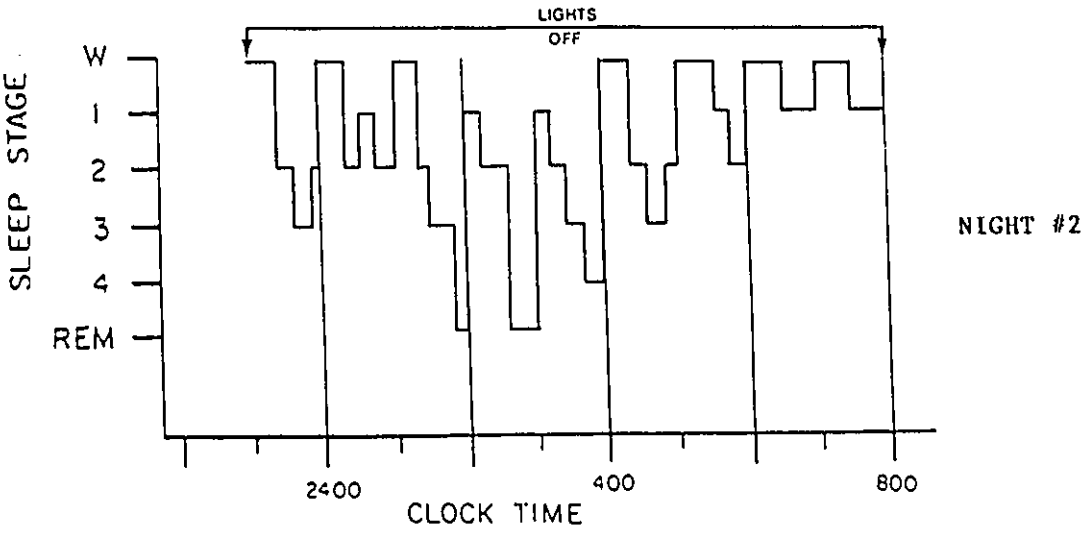
	MEAN OF SLEEP PARAMETERS		
	PATIENT F	MATCHED CONTROL	ALL CONTROLS ± SE
SOL (MIN.)	< 10.0	16.7	15.2 (± 2.7)
AWAKENINGS	.8	.24	.33 (± .04)
TST (MIN.)	403.7	474.4	469.80 (±14.4)

Figure 5F(ii): Sleep Histograms and Comparative Table - Patient F



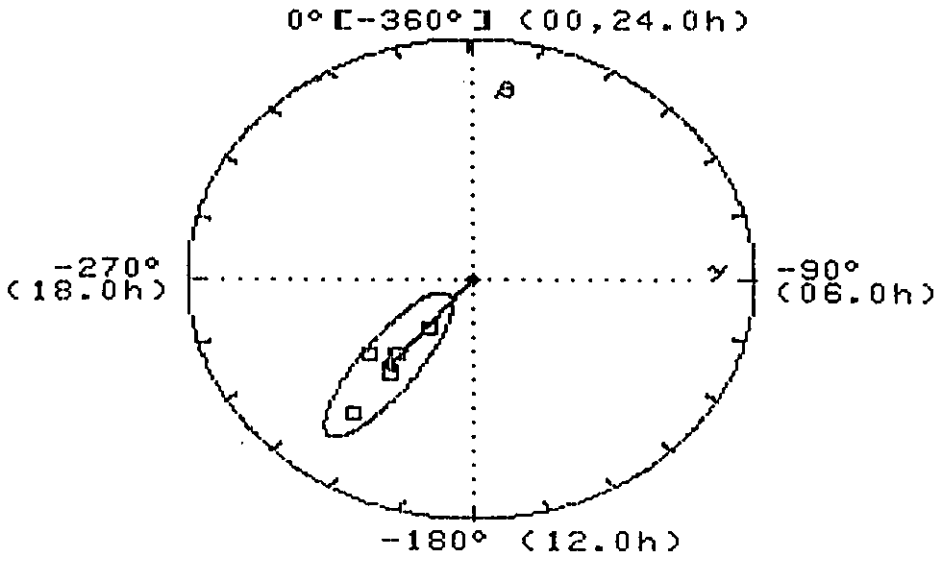
	PATIENT F	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #1	NIGHT #1	NIGHT #1
TST(Minutes)	415.0	437.7	454.2 (±15.2)
SOL(Minutes)	10.0	17.5	12.4 (±3.7)
ROL(Minutes)	55.0	96.0	77.4 (±21.1)
% S1	34.0	4.8	4.9 (± 0.9)
% S2	33.0	44.8	49.2 (±6.4)
% S3	16.9	12.7	10.2 (±3.3)
% S4	4.8	14.0	13.0 (±4.1)
% REM	11.3	23.7	22.7 (±4.8)
# AWAKENINGS	4	0	2

Figure 5F(iii): Sleep Histograms and Comparative Table - Patient F



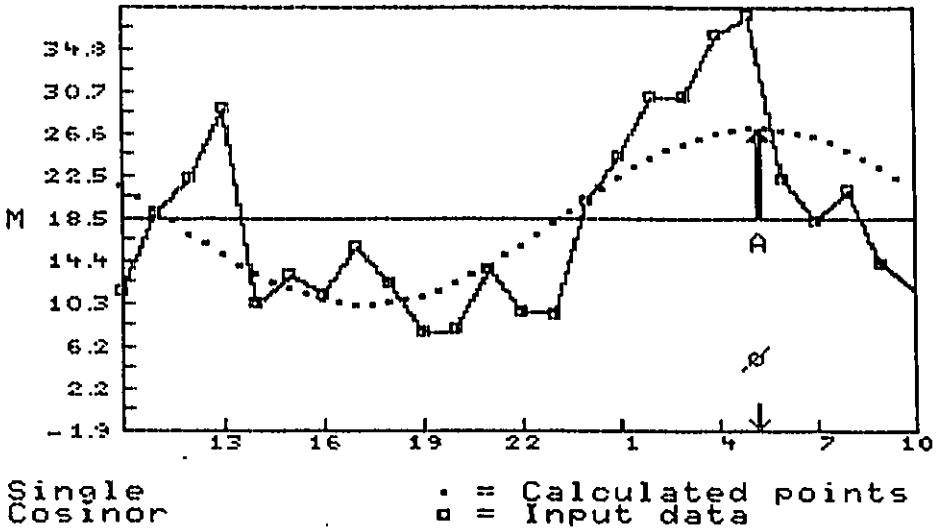
	PATIENT F	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #2	NIGHT #2	NIGHT #2
TST(Minutes)	385.0	450.5	422.0 (± 26)
SOL(Minutes)	20.0	27.3	18.5 (± 4.1)
ROL(Minutes)	115.0	88.7	93.7 (± 19.8)
% S1	35.1	7.4	6.1 (± 1.3)
% S2	36.4	44.5	46.2 (± 7.0)
% S3	16.8	11.3	11.3 (± 4.8)
% S4	5.2	9.8	12.8 (± 3.7)
% REM	6.5	27.0	23.6 (± 3.3)
# AWAKENINGS	6	2	4

Figure 5F(iv): **Polar Plot of Temperature Data and Comparative Table - Patient F**



		Patient F	Matched Control
% R	=	57.40	84.90
P-Level	=	0.036	<0.001
Amp	=	37.30	36.90
Mesor	=	0.42	0.56
Phi(Deg)	=	-225.5	-246.6
Phi(H/Min)	=	15.02	16.27

Figure 5F(v): Single Cosinor Plot for Melatonin and Comparative Table - Patient F (X-axis = Clock hours; Y-axis = pg/ml)



		Patient F	Matched Control
% R	=	46.80	30.30
P-Level	=	0.001	0.023
Amp	=	8.49	17.76
Mesor	=	18.46	39.87
Phi(Deg)	=	- 78.90	- 40.10
Phi(H/Min)	=	5.16	2.40

Figure 5F(vi): Polar Plot for Melatonin - Patient F

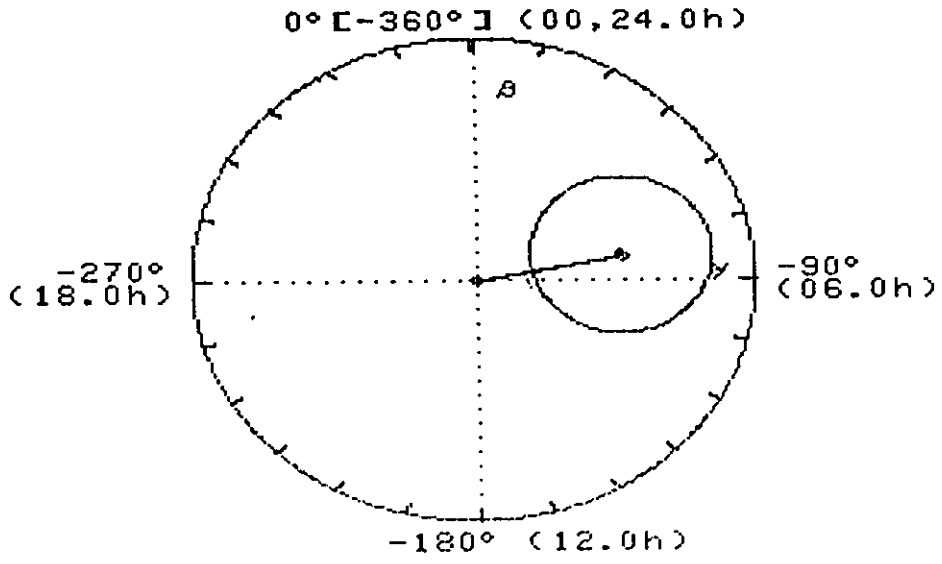
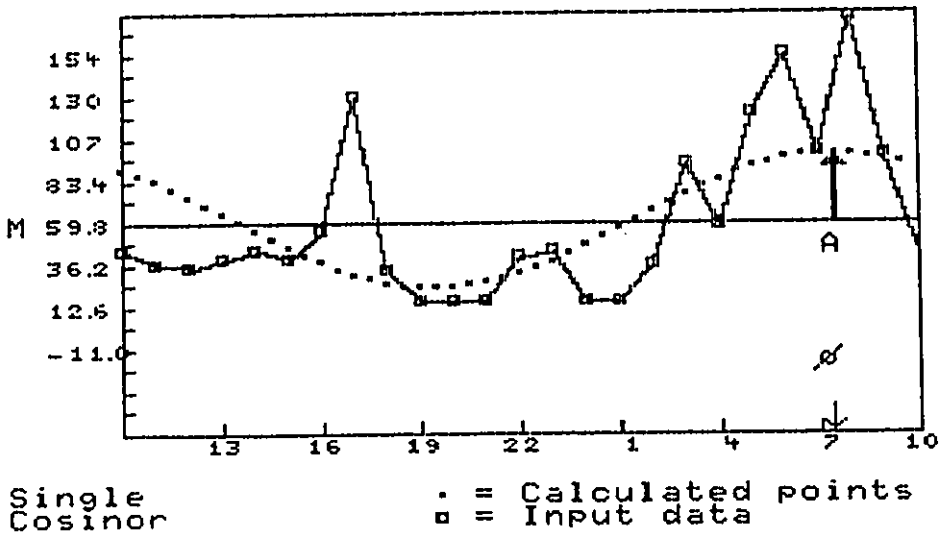


Figure 5F(vii): Single Cosinor Plot for Cortisol and Comparative Table - Patient F (X-axis = Clock hours; Y-axis = ng/ml)

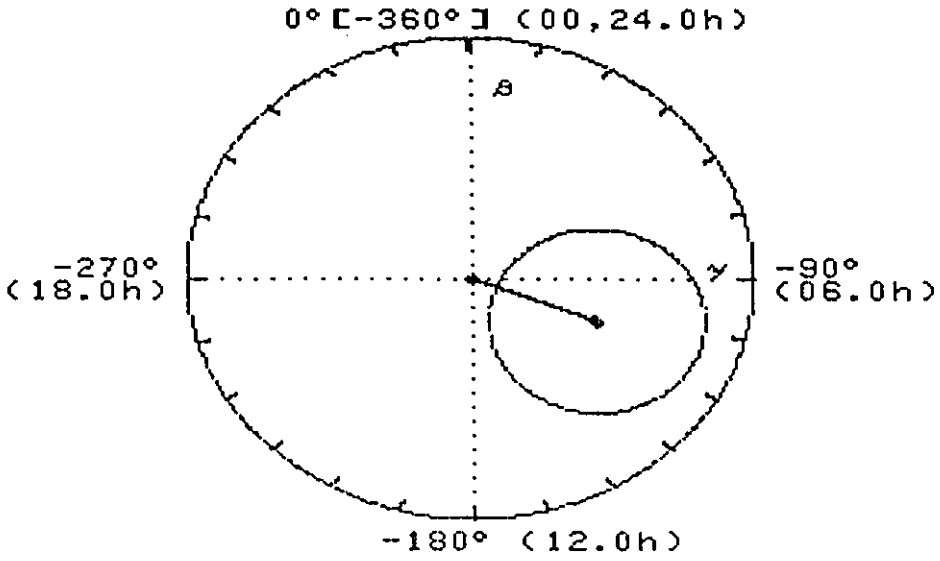


Single Cosinor

. = Calculated points
▫ = Input data

		Patient F	Matched Control
% R	=	34.60	39.50
P-Level	=	0.012	0.005
Amp	=	57.30	29.80
Mesor	=	59.79	87.51
Phi(Deg)	=	-111.8	-165.7
Phi(H/Min)	=	7.27	11.03

Figure 5F(viii): Polar Plot for Cortisol - Patient F



CASE PRESENTATION G

PATIENT G

Clinical History

Patient G is a 31 year old, Asian Indian female, married, with two children. Her presenting complaint was a chronic problem with sleep maintenance interspersed occasionally with total insomnia. On those nights that she did sleep, sleep onset latency was within normal limits. This was followed by one or two hours of sleep, and then she would awaken, occasionally able to nap for several short intervals before awakening with her family at 0730 hours. She reported being incapable of napping during the day even when lying in a darkened room.

This disturbed pattern had been most severe during the past year, but present in a less dramatic form during the last six years. She reported working shifts 10 years earlier with no apparent difficulty, but gradually becoming unable to adjust to her changing work schedule.

This woman showed an unremarkable medical history. Although the psychiatric history appeared normal, she demonstrated a strikingly flat affect at all times during her involvement with the Clinic and with this study. It was also apparent that difficulties such as sleeping problems were not seen in her culture in psychological or physiological terms, but in religious, supernatural terms. Treatment was often expedited by a priest involving the use of rituals to correct whatever violation of religious beliefs that had been commited giving rise to the present state. It is impossible to say what role these ideations may have played in this woman's insomnia. It is also important to note that her

subjective assessment of total sleep time was occasionally much less than that recorded by EEG.

She had tried a variety of benzodiazepines with little or no effect. Subsequently, chlorpromazine (50 mgs) and later Gammahydroxy-butyrate were tried but the side effects of daytime sedation and morning nausea were intolerable.

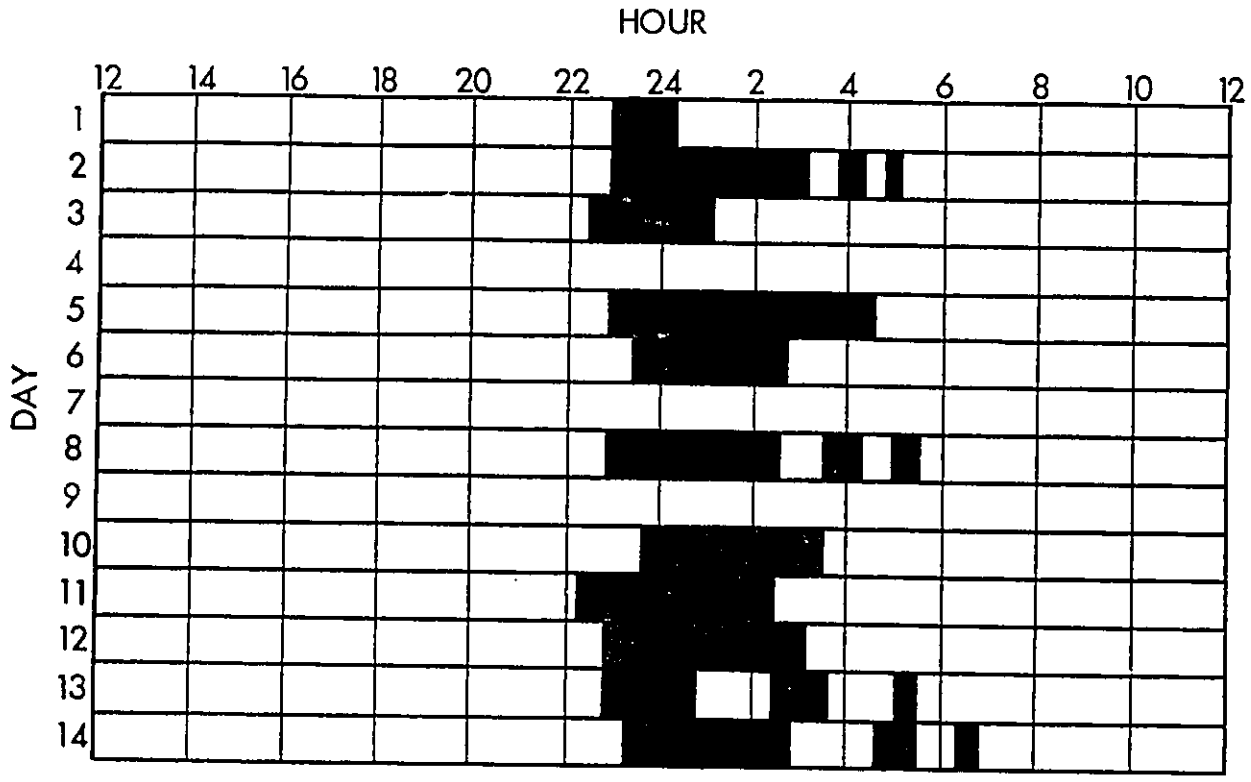
Except for the occasional sleepless night, this patient's sleep pattern resembled that predicted for the phase advance group.

This patient did not complete an MMPI. On examination, however, her preoccupation with her insomnia and belief that it reflected a serious illness, despite reassurance and various behavioural and pharmacological interventions, meant that she met DSM III criteria for Hypochrondiasis, in spite of the reality of her insomnia.

Sleep and Circadian Data

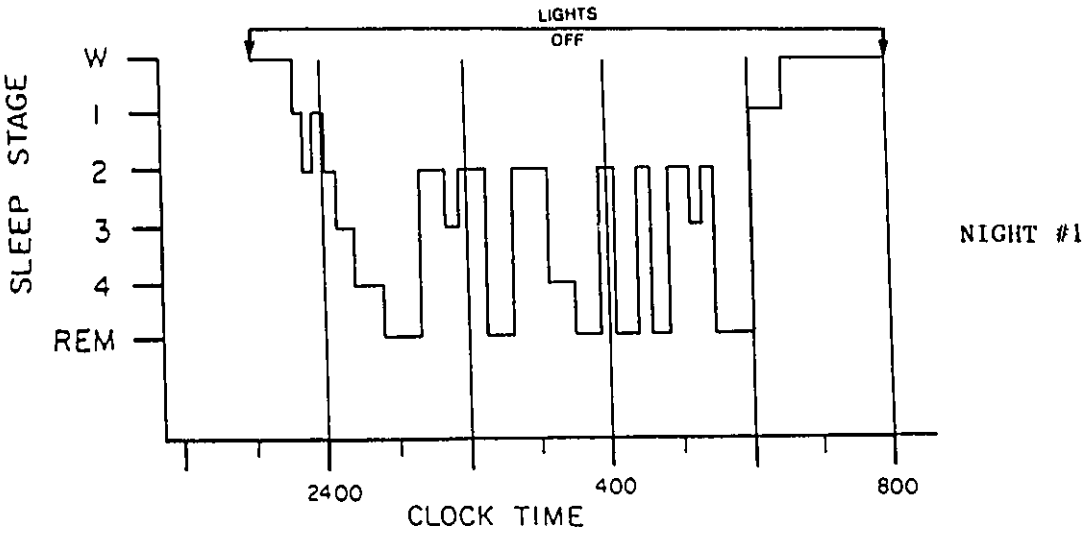
Data revealed that sleep onset was quite prompt but the total sleep time reported in her sleep diary was very markedly reduced to approximately four hours or less on most nights. In the Laboratory, however, she slept slightly more or less than six hours on two successive nights. She spent less time in Stages 3 and 4 on one of those nights than the matched control, and much more time in REM sleep. Temperature acrophase was at 0400 hours, cortisol at 0800 hours, and melatonin did not represent a cyclic pattern. The objective indices revealed a mild disturbance of sleep and the subjective reports revealed markedly greater distress. She thus conformed with the category of subjective complaint of insomnia without objective findings, according to the diagnostic classification of sleep and arousal disorders.

Figure 5G(i): Subjective Sleep Diaries and Comparative Table -Patient G



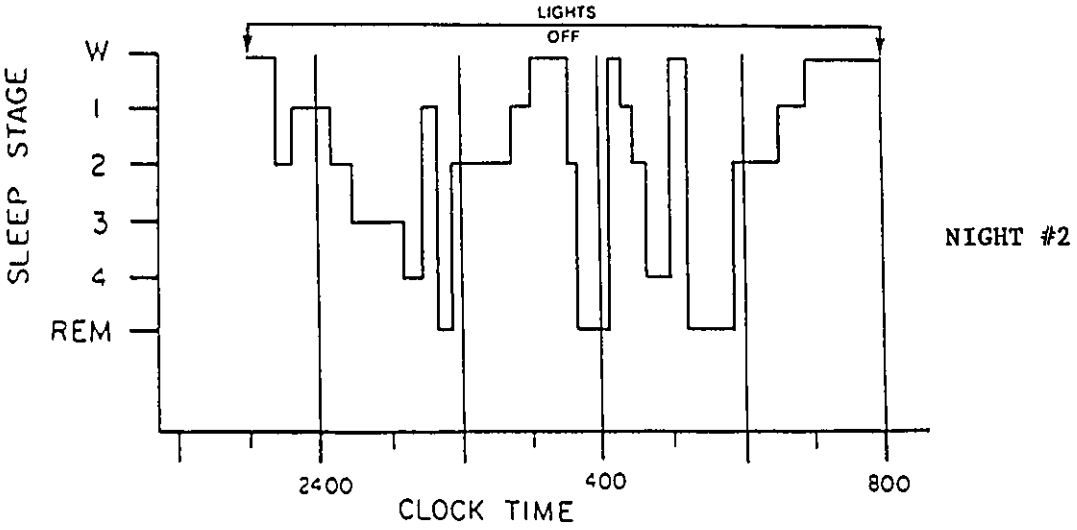
	MEAN OF SLEEP PARAMETERS		
	PATIENT G	MATCHED CONTROL	ALL CONTROLS ± SE
SOL (MIN.)	12.5	13.4	15.2 (± 2.7)
AWAKENINGS	1.8	.66	.33 (± .04)
TST (MIN.)	168.9	458.7	469.80 (±14.4)

Figure 5G(ii): Sleep Histograms and Comparative Table - Patient G



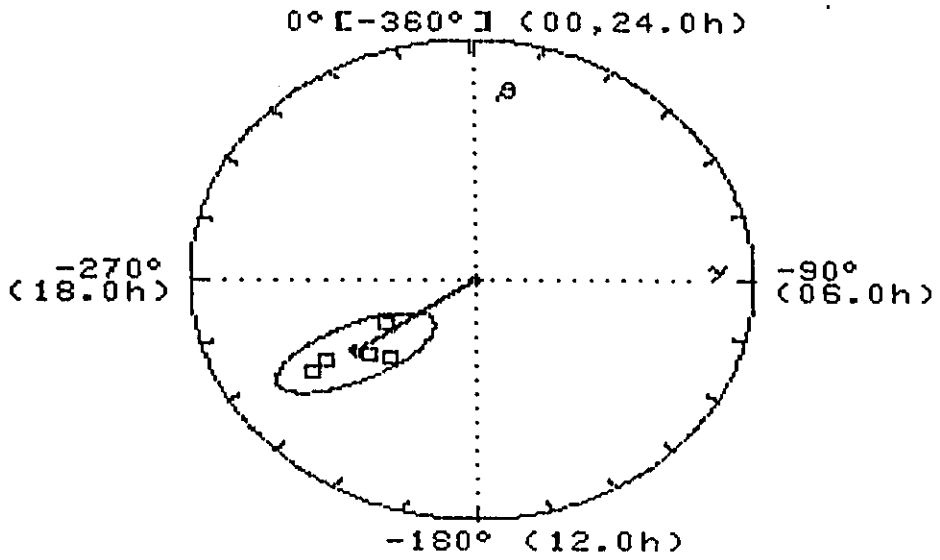
	PATIENT G	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #1	NIGHT #1	NIGHT #1
TST(Minutes)	384.0	460.0	454.2 (±15.2)
SOL(Minutes)	35.0	10.4	12.4 (±3.7)
ROL(Minutes)	88.7	88.1	77.4 (±21.1)
% S1	4.6	4.3	4.9 (± 0.9)
% S2	38.2	48.7	49.2 (±6.4)
% S3	2.2	11.4	10.2 (±3.3)
% S4	7.5	11.5	13.0 (±4.1)
% REM	47.5	24.1	22.7 (±4.8)
# AWAKENINGS	0	1	2

Figure 5G(iii): Sleep Histograms and Comparative Table - Patient G



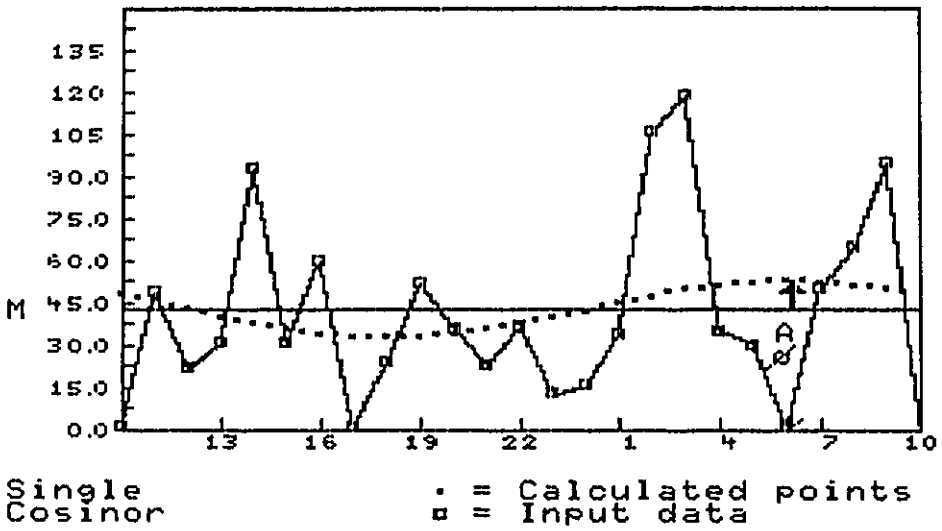
	PATIENT G	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #2	NIGHT #2	NIGHT #2
TST(Minutes)	345.3	438.4	422.0 (± 26)
SOL(Minutes)	23.0	15.3	18.5 (± 4.1)
ROL(Minutes)	147.0	83.4	93.7 (± 19.8)
% S1	6.4	6.7	6.1 (± 1.3)
% S2	50.4	48.3	46.2 (± 7.0)
% S3	12.1	12.2	11.3 (± 4.8)
% S4	8.3	10.4	12.8 (± 3.7)
% REM	22.8	22.4	23.6 (± 3.3)
# AWAKENINGS	5	4	4

Figure 5G(iv): Polar Plot of Temperature Data and Comparative Table - Patient G



		Patient G	Matched Control
% R	=	70.40	69.40
P-Level	=	0.003	<0.001
Amp	=	37.20	37.30
Mesor	=	0.38	0.44
Phi(Deg)	=	-233.0	-248.0
Phi(H/Min)	=	15.32	16.32

Figure 5G(v): Single Cosinor Plot for Melatonin and Comparative Table - Patient G (X-axis = Clock hours; Y-axis = pg/ml)



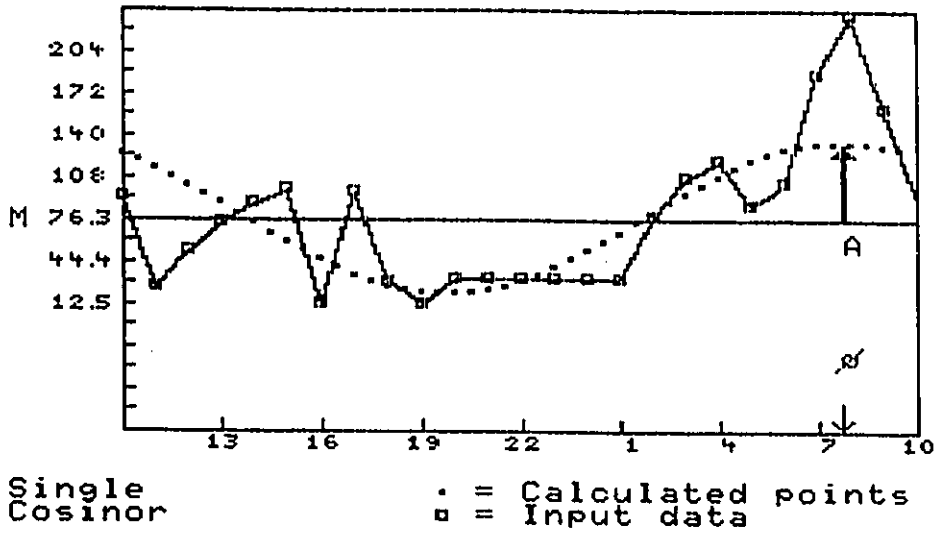
		Patient G	Matched Control
% R	=	4.80	47.0
P-Level	=	0.598 (N.S.)	0.001
Amp	=	9.90	16.76
Mesor	=	42.26	30.71
Phi(Deg)	=	- 91.60	- 25.30
Phi(H/Min)	=	6.06	1.14

Figure 5G(vi): Polar Plot for Melatonin - Patient G

AS P>0.05, THE DATA ARE ASSUMED TO BE VARIABLE AND NOT CYCLIC,

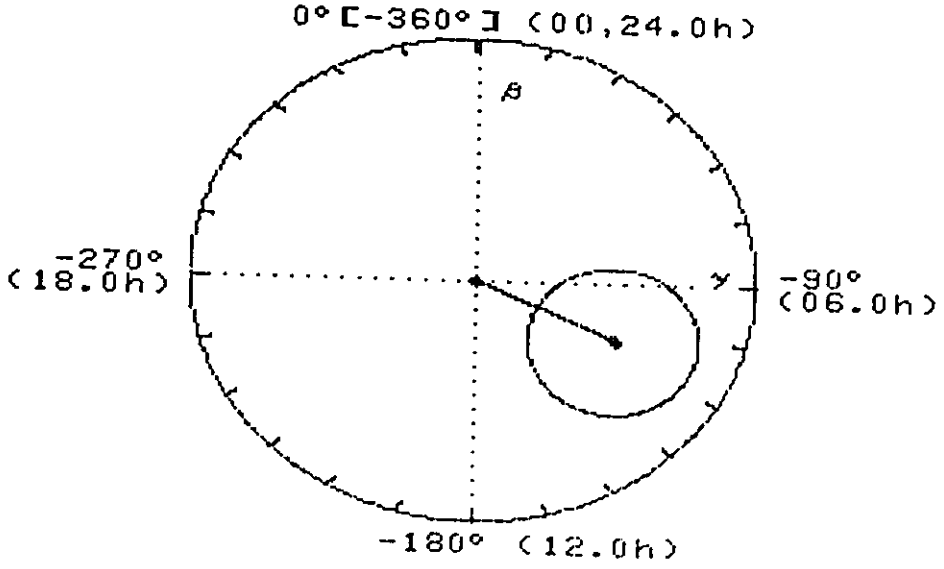
THEREFORE, NO POLAR PLOT IS SHOWN

Figure 5G(vii): Single Cosinor Plot for Cortisol and Comparative Table - Patient G (X-axis = Clock hours; Y-axis = pg/ml)



		Patient G	Matched Control
% R	=	52.30	59.80
P-Level	=	<0.001	<0.001
Amp	=	57.90	67.48
Mesor	=	76.30	104.0
Phi(Deg)	=	-113.3	-157.9
Phi(H/Min)	=	7.33	10.32

Figure 5G(viii): Polar Plot for Cortisol - Patient G



CASE PRESENTATION H

PATIENT H

Clinical History

Patient H is a 54 year old, caucasian male, divorced, currently living with a girlfriend. His presenting complaint was early morning awakening. His pattern was described as early to bed, short sleep onset latency, and then awakening around 0230 hours with no further sleep. He reported his average total sleep to range from two to three hours. He recalled a time several years previous when he would stay awake watching television until 0200 to 0300 hours then fall asleep and awake at 0600 hours.

This man had experienced a problem with sleep maintenance for as long as he could remember. He could recall no particular life-events which might have contributed to the onset of these disturbed sleeping patterns.

When he was subsequently studied in the Sleep Investigations Unit, specific disorders were ruled out. However, the proportion of time spent in slow-wave sleep was 7%, markedly below the normal 25% for this stage.

He showed an unremarkable medical and psychiatric history. He takes no alcohol, coffee, or medications of any description.

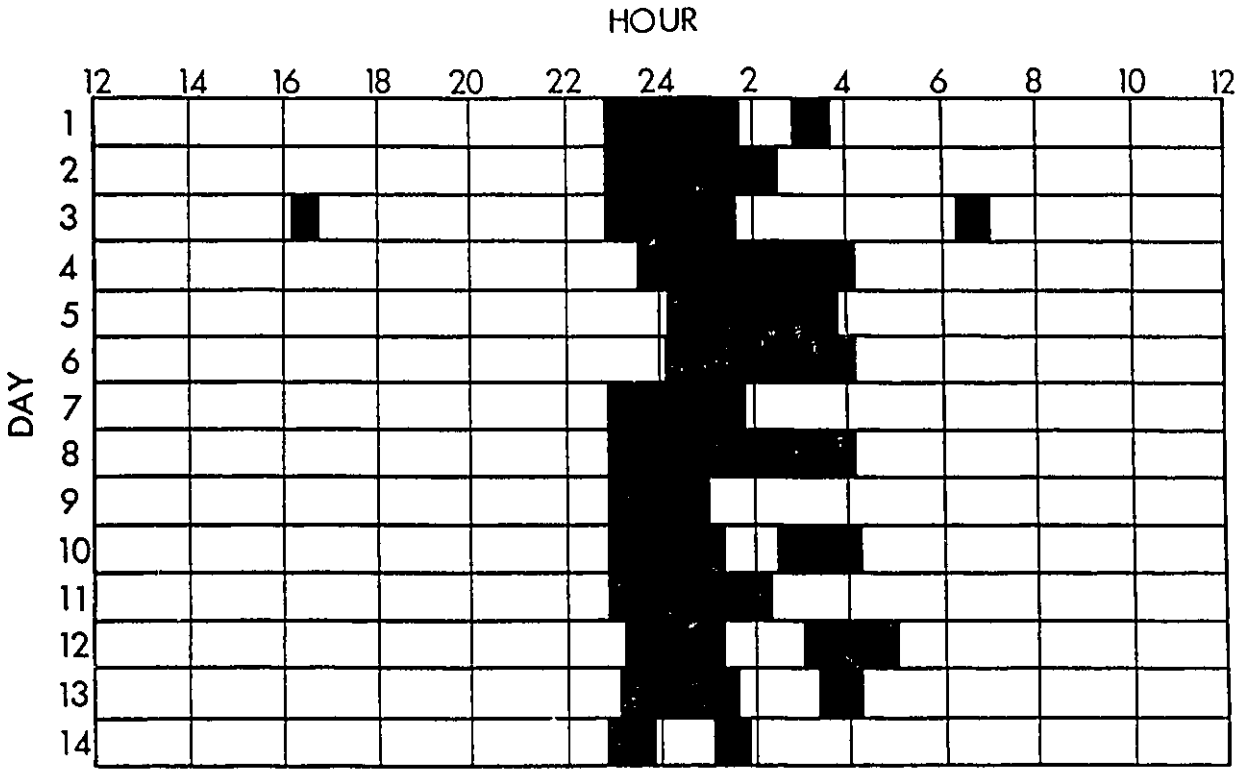
With a history of short sleep onset latency, and a tendency to awaken early, this patient was assigned to the phase advance group.

The MMPI profile was essentially within normal limits. None of the scales were elevated.

Sleep and Circadian Data

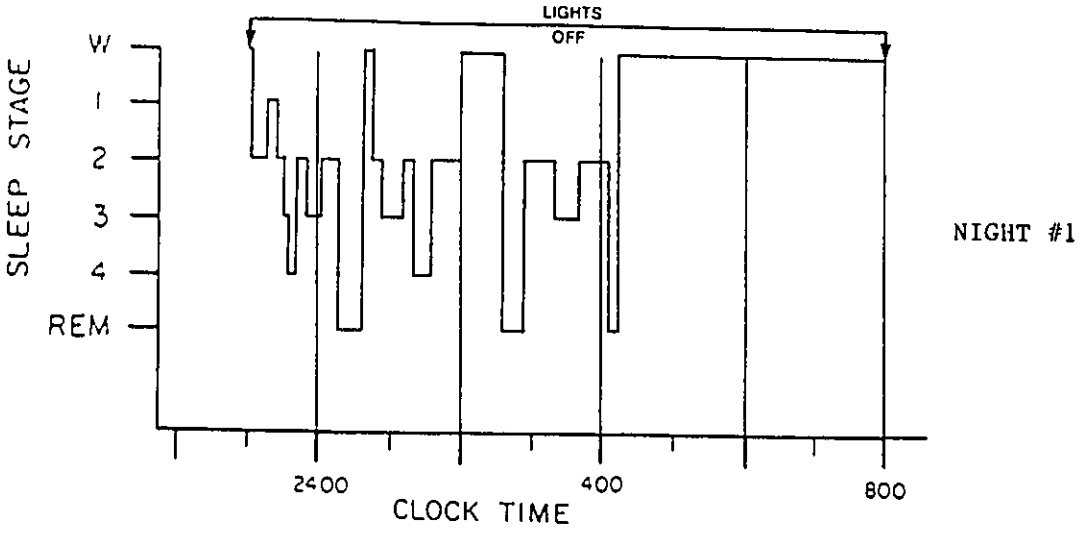
Sleep diary revealed reasonably rapid sleep onset and a total sleep time of less than 3 1/2 hours on the average, with awakenings at 0400 hours or earlier. In the Laboratory, sleep onset was also prompt. Stage 3 was markedly greater than the matched control, but REM% markedly less. Temperature acrophase occurred at 0300 hours which was consistent with phase advance. Cortisol, however, peaked at 0800 hours and melatonin at 0500 hours.

Figure 5H(i): Subjective Sleep Diaries and Comparative Table -Patient H



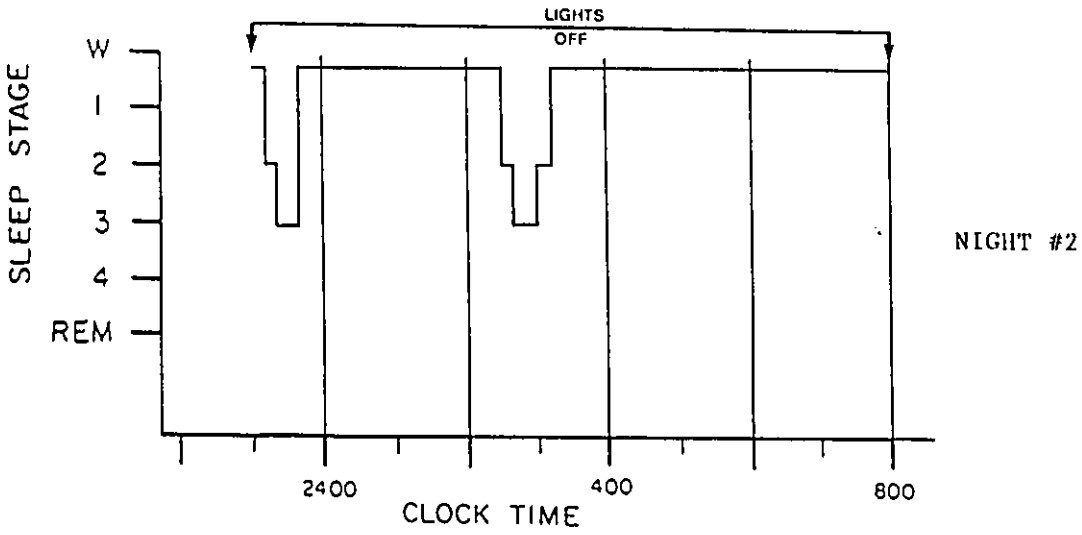
	MEAN OF SLEEP PARAMETERS		
	PATIENT H	MATCHED CONTROL	ALL CONTROLS ± SE
SOL (MIN.)	17.0	17.1	15.2 (± 2.7)
AWAKENINGS	2.6	1.8	.33 (± .04)
TST (MIN.)	203.5	427.2	469.80 (±14.4)

Figure 5H(ii): Sleep Histograms and Comparative Table - Patient H



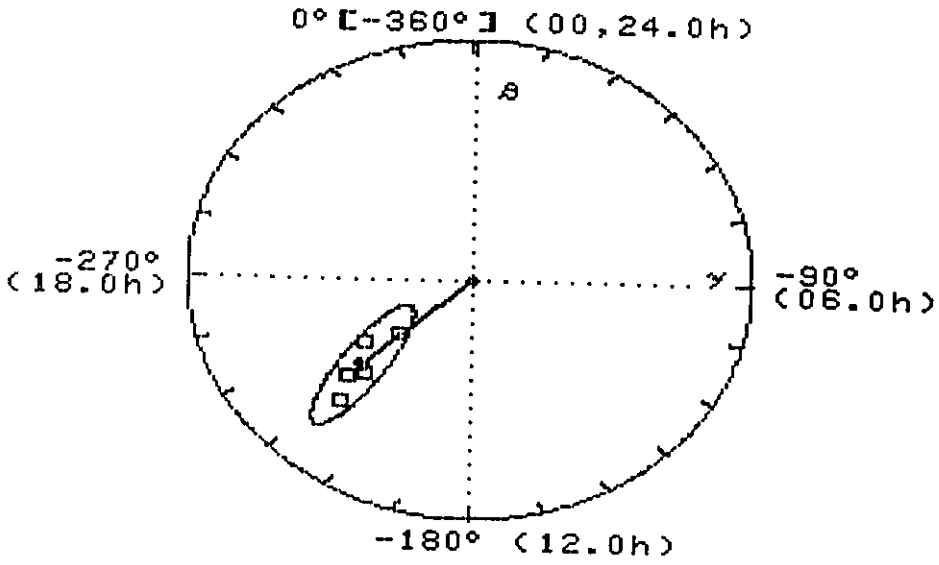
	PATIENT H	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #1	NIGHT #1	NIGHT #1
TST(Minutes)	282.3	437.1	454.2 (±15.2)
SOL(Minutes)	< 10.0	17.4	12.4 (±3.7)
ROL(Minutes)	76.1	88.4	77.4 (±21.1)
% S1	2.1	5.5	4.9 (± 0.9)
% S2	59.8	48.7	49.2 (±6.4)
% S3	19.0	9.1	10.2 (±3.3)
% S4	4.5	8.8	13.0 (±4.1)
% REM	14.6	27.9	22.7 (±4.8)
# AWAKENINGS	4	2	2

Figure 5H(iii): Sleep Histograms and Comparative Table - Patient H



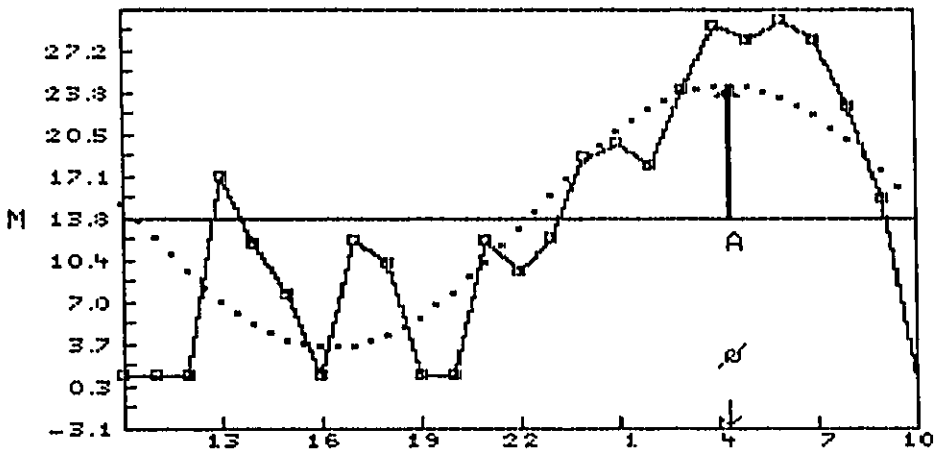
	PATIENT H	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #2	NIGHT #2	NIGHT #2
TST(Minutes)	79.0	409.4	422.0 (± 26)
SOL(Minutes)	12.2	23.8	18.5 (± 4.1)
ROL(Minutes)	---	81.4	93.7 (± 19.8)
% S1	---	8.7	6.1 (± 1.3)
% S2	46.3	49.3	46.2 (± 7.0)
% S3	53.7	7.4	11.3 (± 4.8)
% S4	---	8.5	12.8 (± 3.7)
% REM	---	26.1	23.6 (± 3.3)
# AWAKENINGS	2	2	4

Figure 5H(iv): Polar Plot of Temperature Data and Comparative Table - Patient H



		Patient H	Matched Control
% R	=	67.90	83.0
P-Level	=	0.005	0.001
Amp	=	36.90	37.0
Mesor	=	0.47	0.55
Phi(Deg)	=	-226.1	-241.7
Phi(H/Min)	=	15.04	16.07

Figure 5H(v): Single Cosinor Plot for Melatonin and Comparative Table - Patient H (X-axis = Clock hours; Y-axis = ng/ml)



Single Cosinor

· = Calculated points
▫ = Input data

		Patient G	Matched Control
% R	=	87.50	72.50
P-Level	=	<0.001	<0.001
Amp	=	22.40	37.30
Mesor	=	21.23	0.44
Phi(Deg)	=	- 69.50	- 40.0
Phi(H/Min)	=	4.38	2.40

Figure 5H(vi): Polar Plot for Melatonin - Patient H

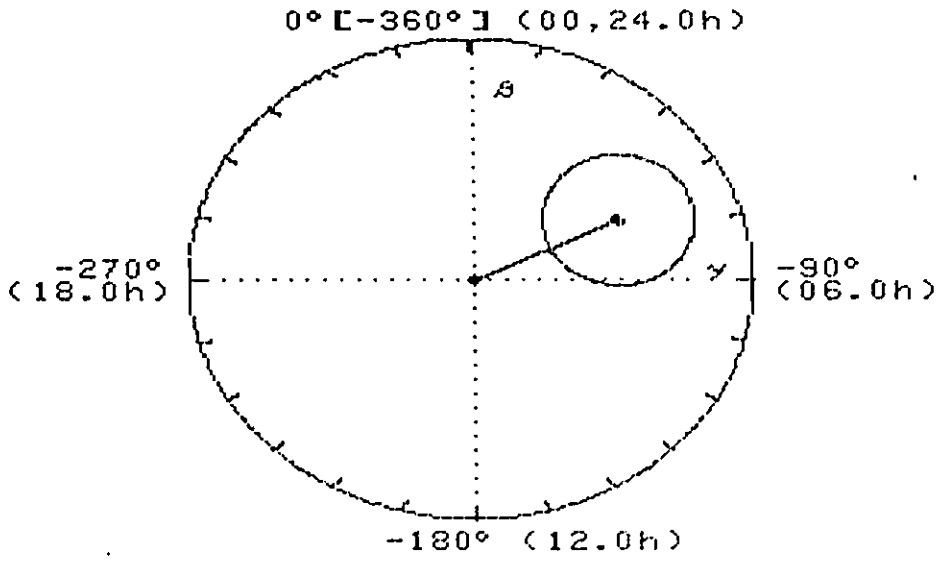
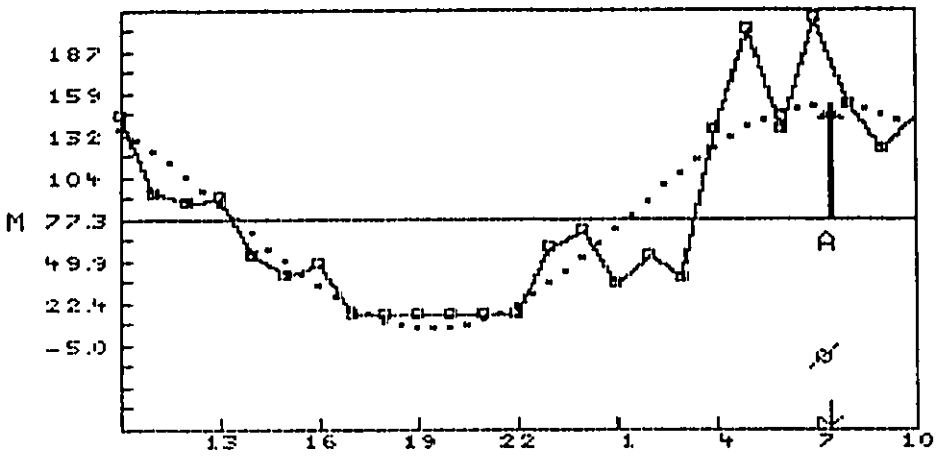
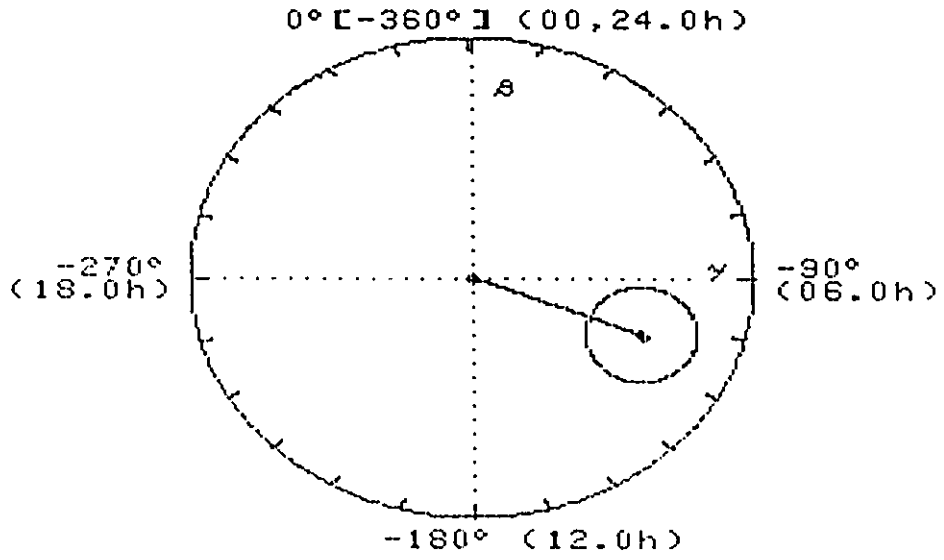


Figure 5H(vii): Single Cosinor Plot for Cortisol and Comparative Table - Patient H (X-axis = Clock hours; Y-axis = ng/ml)



		Patient H	Matched Control
% R	=	55.90	47.50
P-Level	=	<0.001	0.001
Amp	=	45.01	37.28
Mesor	=	49.68	48.52
Phi(Deg)	=	-114.6	-148.4
Phi(H/Min)	=	7.39	9.54

Figure 5H(viii): Polar Plot for Cortisol - Patient H



CASE PRESENTATION I

PATIENT I

Clinical History

Patient I is a 29 year old, caucasian female, married with two children. Her presenting complaint was a difficulty with sleep maintenance. Her usual pattern involved a very short sleep onset latency followed by an awakening two to three hours later. Often this was when she would arise and try to occupy herself until her family awakened at 0700 hours. Occasionally, she would fall into a pattern for several days where she would fall back to sleep in the early morning hours due to what she described as "sheer exhaustion".

This pattern of sleep disturbance was life-long. She could recall the similarities to her childhood sleep patterns in great detail. Her mother even noted that her sleep was disrupted during her infancy.

This patient has an unremarkable medical history. Her psychiatric history is marked by a single period of depressive symptoms which were treated by her family doctor in 1981, but never followed-up. She felt that her symptoms were most likely a reaction to her heavy school schedule coupled with her insomnia.

This patient had taken doxepin (Sinequan) 150 mgs, which improved her sleep for the first time ever. However, she had not had this medication since 1981. She takes Propranolol (40 mgs x 2) when suffering from an occasional migraine. She consumes very little alcohol or coffee.

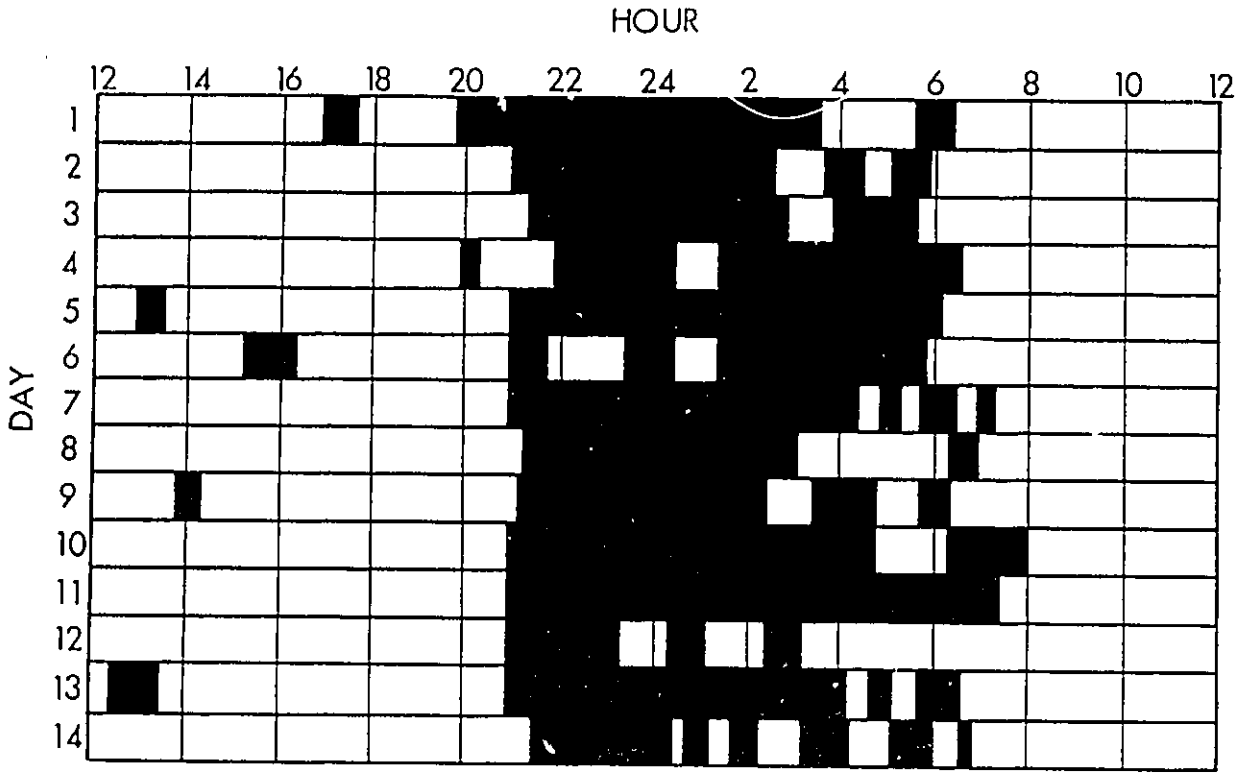
Since this patient's sleep disturbance involves only early morning awakening, she was assigned to the phase advance group.

The MMPI revealed only one elevated scale. This suggested that the patient was suspicious and brooding and may feel that she is not getting what is coming to her. At the same time, she appeared socially competent. There was no evidence of major affective or anxiety symptoms.

Sleep and Circadian Data

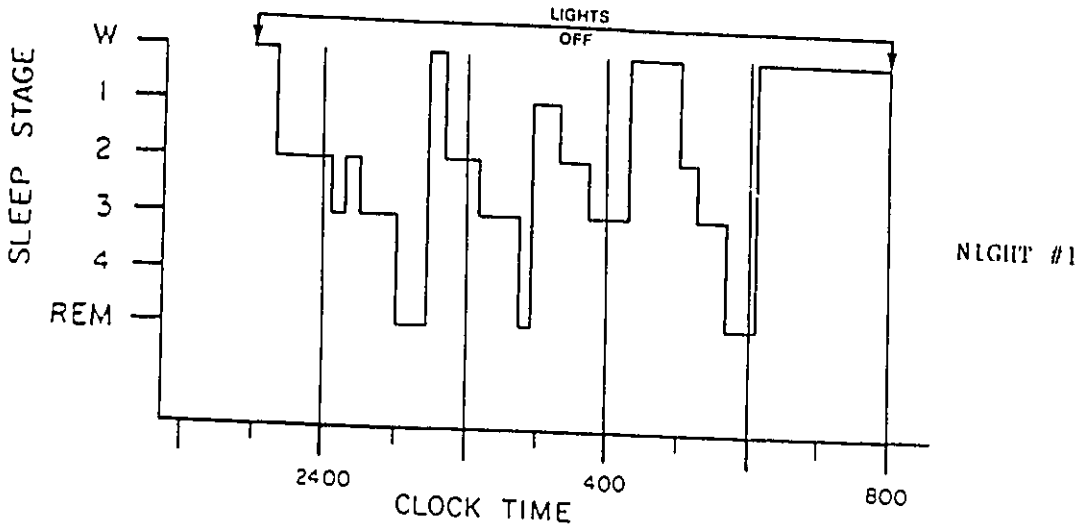
Subjective reports indicated a rapid sleep onset with total sleep time approximately an hour and a quarter less than the matched control. This was more pronounced in the Laboratory. On night #1 the proportion of time spent in Stage 3 was much greater than in the control, but Stage 4 was less than that of the control on both nights. On night #2, she spent much more time in Stage 1 and less time in REM sleep than the control. Circadian temperature acrophase occurred at approximately 0230 hours, cortisol at 0800 hours, and melatonin at 0400 hours. The temperature data are thus consistent with a prediction of phase advance.

Figure 5I(i): Subjective Sleep Diaries and Comparative Table -Patient I



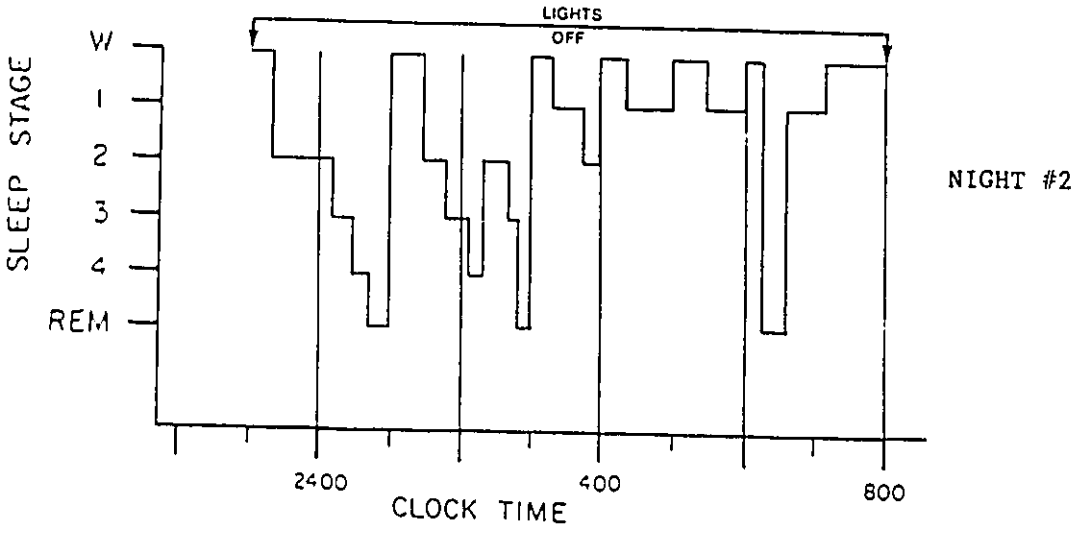
	MEAN OF SLEEP PARAMETERS		
	PATIENT I	MATCHED CONTROL	ALL CONTROLS ± SE
SOL (MIN.)	8.7	16.7	15.2 (± 2.7)
AWAKENINGS	3.2	.60	.33 (± .04)
TST (MIN.)	378.0	454.4	469.80 (±14.4)

Figure 5I(ii): Sleep Histograms and Comparative Table - Patient I



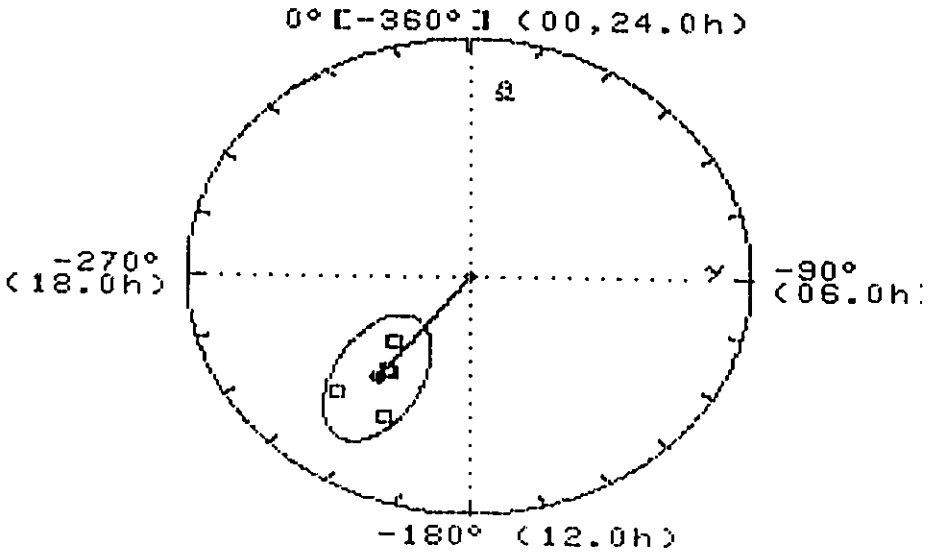
	PATIENT I	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #1	NIGHT #1	NIGHT #1
TST(Minutes)	345.5	439.5	454.2 (±15.2)
SOL(Minutes)	18.4	10.4	12.4 (±3.7)
ROL(Minutes)	97.0	75.0	77.4 (±21.1)
% S1	7.2	5.2	4.9 (± 0.9)
% S2	39.9	47.7	49.2 (±6.4)
% S3	32.9	8.4	10.2 (±3.3)
% S4	---	14.1	13.0 (±4.1)
% REM	20.0	24.6	22.7 (±4.8)
# AWAKENINGS	4	1	2

Figure 5I(iii): Sleep Histograms and Comparative Table - Patient I



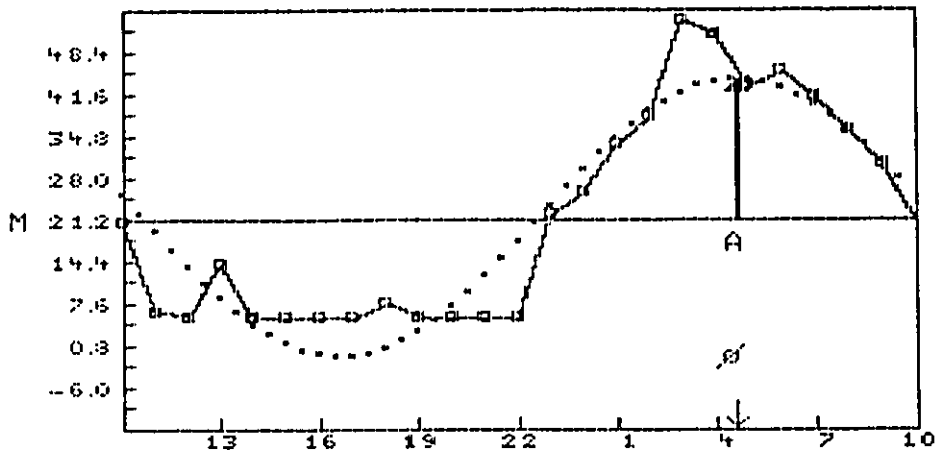
	PATIENT I	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #2	NIGHT #2	NIGHT #2
TST(Minutes)	294.0	424.4	422.0 (± 26)
SOL(Minutes)	22.5	15.2	18.5 (± 4.1)
ROL(Minutes)	74.2	81.3	93.7 (± 19.8)
% S1	40.1	4.9	6.1 (± 1.3)
% S2	32.4	49.8	46.2 (± 7.0)
% S3	13.9	13.3	11.3 (± 4.8)
% S4	5.1	13.7	12.8 (± 3.7)
% REM	8.5	18.3	23.6 (± 3.3)
# AWAKENINGS	8	5	4

Figure 5I(iv): Polar Plot of Temperature Data and Comparative Table - Patient I



		Patient I	Matched Control
% R	=	58.60	69.40
P-Level	=	0.007	<0.001
Amp	=	0.39	0.44
Mesor	=	37.30	37.30
Phi(Deg)	=	-219.0	-248.6
Phi(H/Min)	=	14.36	16.32

Figure 5I(v): Single Cosinor Plot for Melatonin and Comparative Table - Patient I (X-axis = Clock hours; Y-axis = ng/ml)



		Patient I	Matched Control
% R	=	60.0	47.0
P-Level	=	<0.001	0.001
Amp	=	10.54	16.76
Mesor	=	13.75	30.71
Phi(Deg)	=	- 65.2	- 25.3
Phi(H/Min)	=	4.21	1.41

Figure 5I(vi): Polar Plot for Melatonin - Patient I

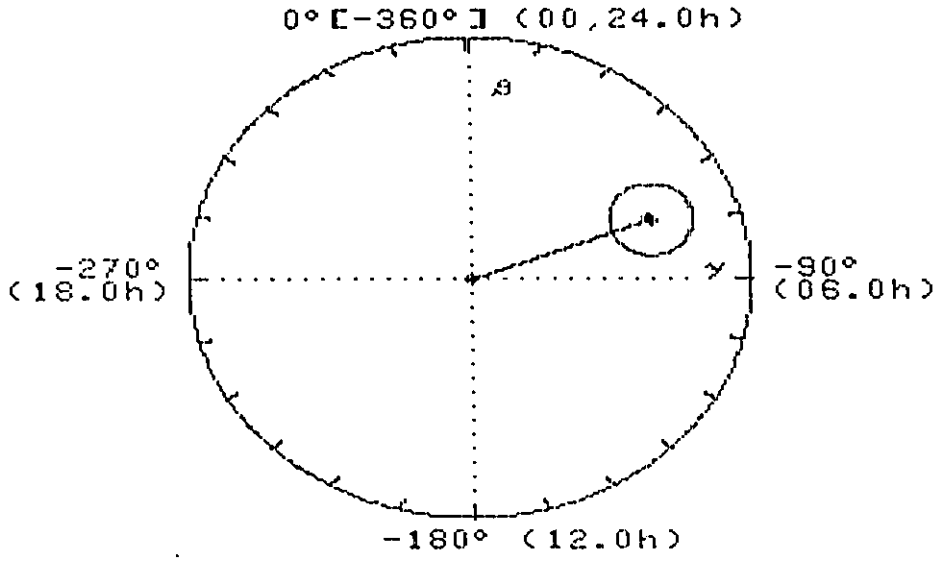
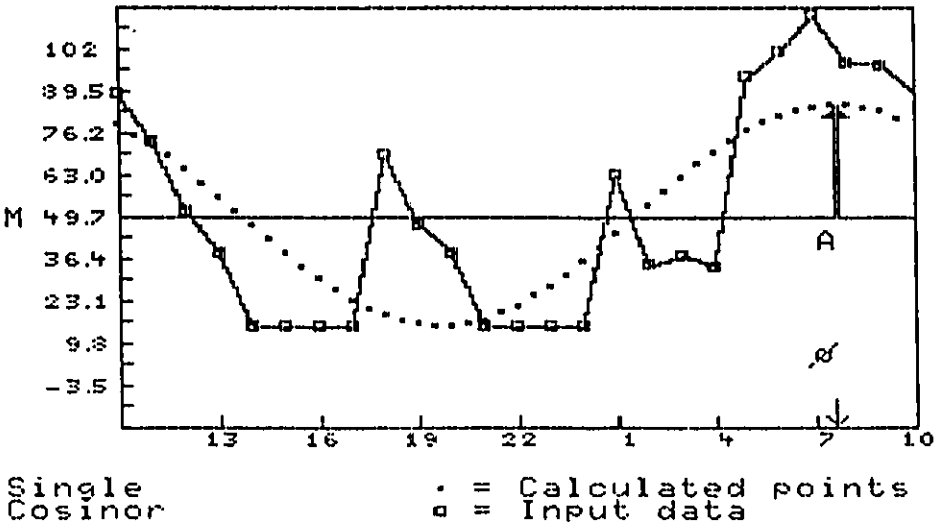
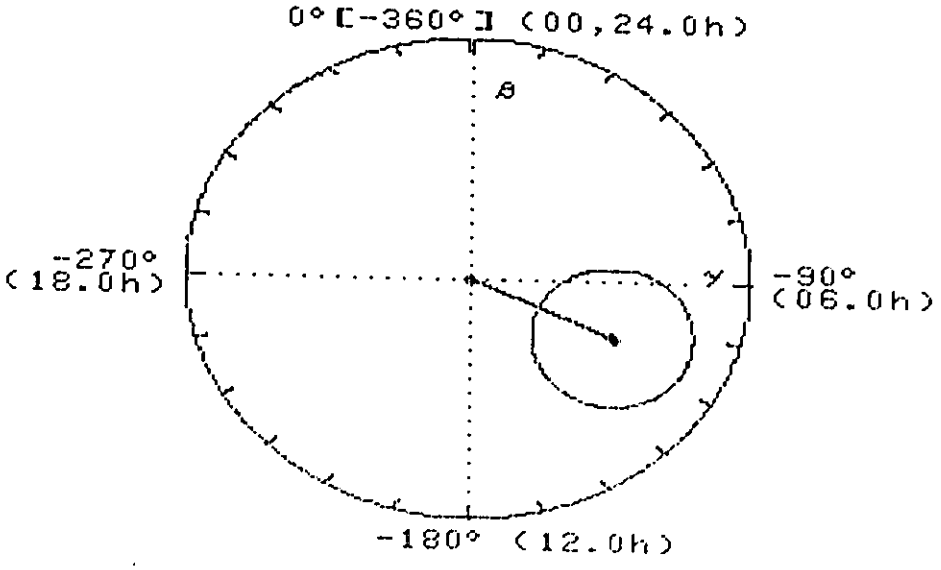


Figure 5I(vii): Single Cosinor Plot for Cortisol and Comparative Table - Patient I (X-axis = Clock hours; Y-axis = ng/ml)



		Patient I	Matched Control
% R	=	77.90	59.80
P-Level	=	<0.001	<0.001
Amp	=	68.05	67.48
Mesor	=	77.32	104.0
Phi(Deg)	=	-111.5	-157.9
Phi(H/Min)	=	7.26	10.32

Figure 5I(viii): Polar Plot for Cortisol - Patient I



CASE PRESENTATION J

PATIENT J

Clinical History

Patient J is a 65 year old, caucasian male, married with three adult children. His presenting complaint was a great difficulty with sleep onset and maintenance. Occasionally, he would have a relatively short sleep onset latency followed by a napping pattern throughout the night. This pattern was interspersed with total sleeplessness, often followed by a short sleep episode between 0800 and 1000 hours.

This pattern of sleep disturbance had been relentless since the age of 15 years. The only period of great relief was in 1977 when he began to sleep day and night. Later, he was discovered to be suffering from pernicious anemia. As treatment for the anemia progressed, the original pattern of sleep resumed.

This patient has an unremarkable psychiatric history. His medical history was also clear, except for anemia in 1977, and periodic asthma attacks which have been stabilized for at least 10 years. He appeared certain that the asthma attacks were not responsible for maintaining wakefulness.

This man had tried a dozen or more hypnotics at various times but these had simply left him groggy and unable to sleep. He regularly uses a narcotic cough suppressant, Hydrocodone Bitartrate (Tussionex) which has occasionally improved his sleep. He also regularly used a sympathomimetic inhalent, Isoproterenol-Phenylephrine HCl (Isuprel-Neo) for treatment of his asthma. He consumes no alcohol or coffee.

The mixed phenomenology of this patient's sleep disturbance

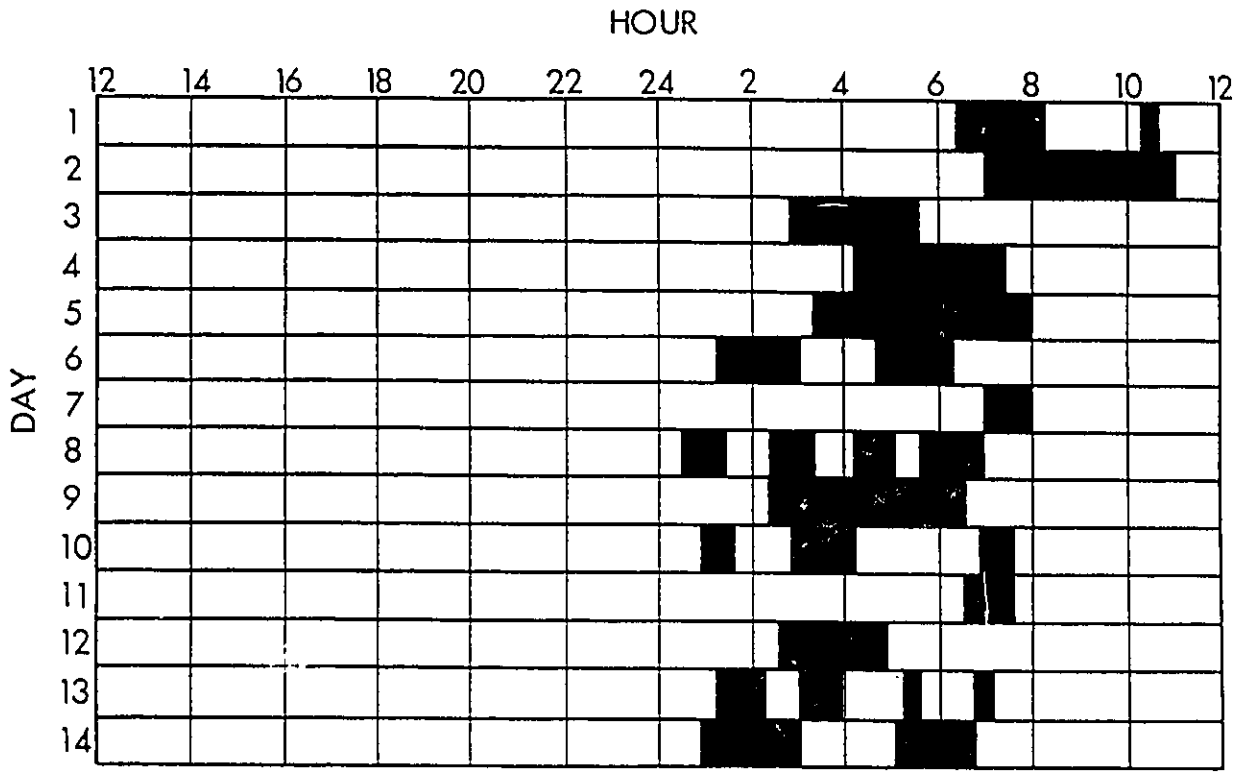
indicated that arrhythmicity of circadian pattern could exist. He was assigned to the arrhythmic group.

The MMPI revealed only one elevated scale, which usually signifies depression, which was denied.

Sleep and Circadian Data

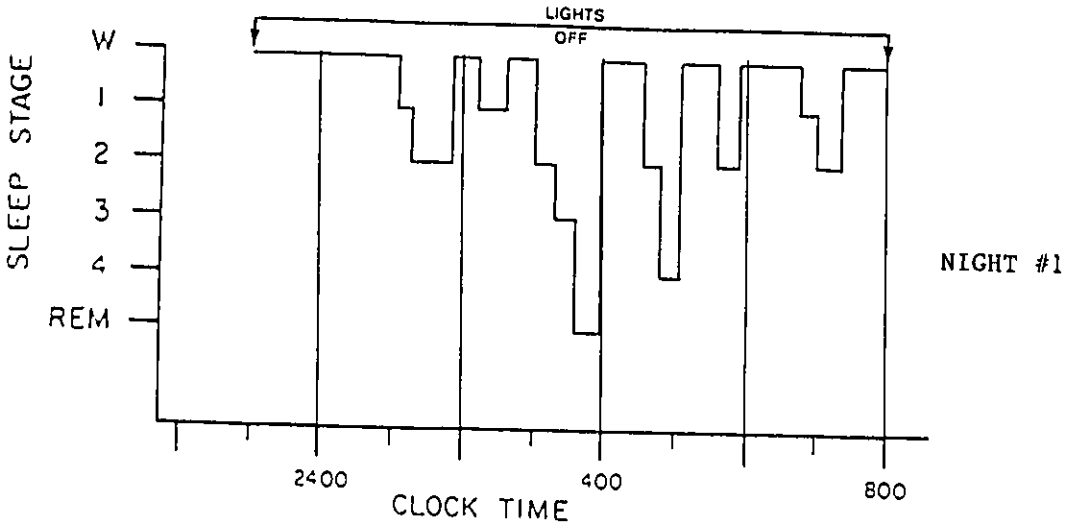
The EEG revealed a pattern that was confirmed on the patient's self report. That is, an intermittent napping pattern and a total sleep time of slightly more than three hours for an average of 14 nights, and a markedly delayed sleep onset time. Laboratory findings were consistent with subjective sleep reports. The patient spent much more time in Stage 1 on both nights than the control, and proportionately more time in Stage 3 on night #2. He spent relatively less time in REM sleep on both nights. Temperature acrophase occurred at a variety of different times and was of variable amplitude. Cortisol data were not cyclic. Melatonin acrophase occurred at just before 0600 hours but the curve explained only 37% of the variance. These data are consistent with the prediction that the patient has a variable noncircadian rhythm.

Figure 5J(i): Subjective Sleep Diaries and Comparative Table -Patient J



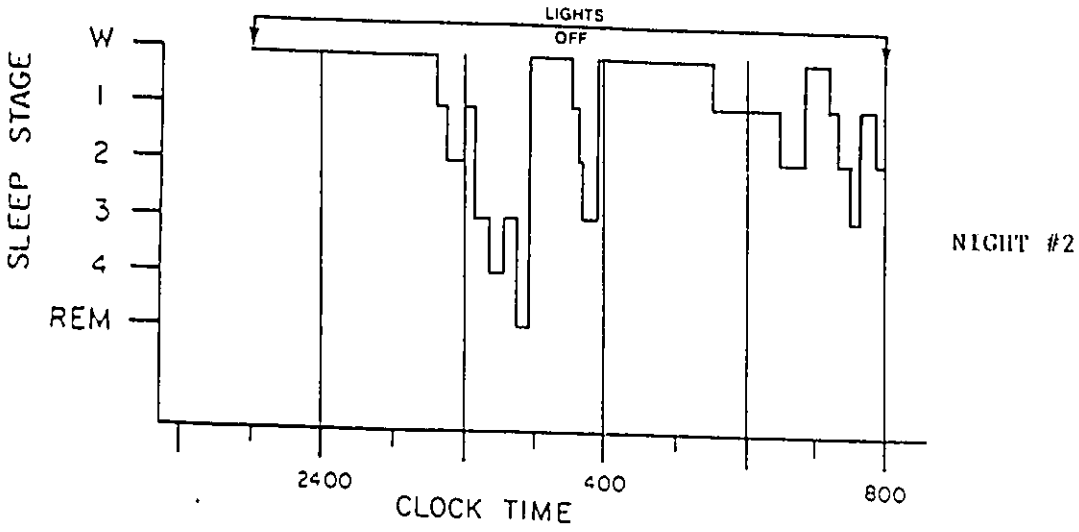
	MEAN OF SLEEP PARAMETERS		
	PATIENT J	MATCHED CONTROL	ALL CONTROLS ± SE
SOL (MIN.)	257.8	17.1	15.2 (± 2.7)
AWAKENINGS	4.2	1.8	.33 (± .04)
TST (MIN.)	174.5	427.2	469.80 (±14.4)

Figure 5J(ii): Sleep Histograms and Comparative Table - Patient J



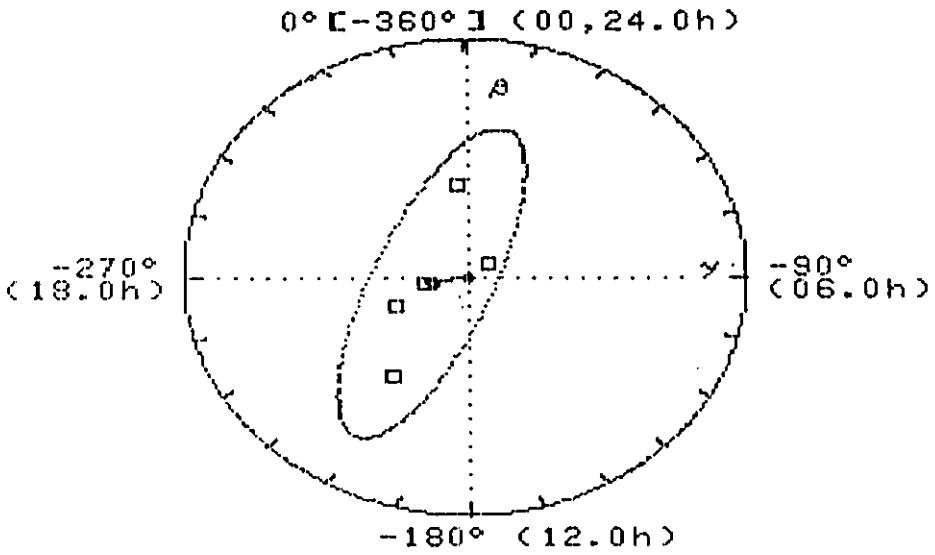
	PATIENT J	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #1	NIGHT #1	NIGHT #1
TST(Minutes)	211.4	437.1	454.2 (±15.2)
SOL(Minutes)	122.4	17.4	12.4 (±3.7)
ROL(Minutes)	156.0	88.4	77.4 (±21.1)
% S1	36.0	5.5	4.9 (± 0.9)
% S2	45.9	48.7	49.2 (±6.4)
% S3	5.2	9.1	10.2 (±3.3)
% S4	7.1	8.8	13.0 (±4.1)
% REM	5.8	27.9	22.7 (±4.8)
# AWAKENINGS	7	2	2

Figure 5J(iii): Sleep Histograms and Comparative Table - Patient J



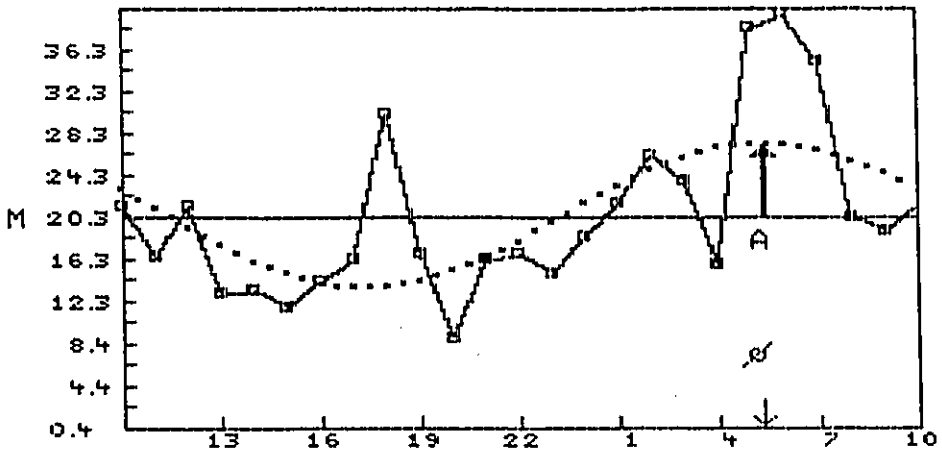
	PATIENT J	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #2	NIGHT #2	NIGHT #2
TST (Minutes)	204.0	409.4	422.0 (± 26)
SOL (Minutes)	160.8	23.8	18.5 (± 4.1)
ROL (Minutes)	85.2	81.4	93.7 (± 19.8)
% S1	41.6	8.7	6.1 (± 1.3)
% S2	25.9	49.3	46.2 (± 7.0)
% S3	22.5	7.4	11.3 (± 4.8)
% S4	3.4	8.5	12.8 (± 3.7)
% REM	6.6	26.1	23.6 (± 3.3)
# AWAKENINGS	4	3	4

Figure 5J(iv): **Polar Plot of Temperature Data and Comparative Table - Patient J**



		Patient J	Matched Control
% R	=	79.30	83.0
P-Level	=	0.335 (N.S.)	0.001
Amp	=	37.20	37.0
Mesor	=	0.32	0.55
Phi(Deg)	=	-237.8	-241.7
Phi(H/Min)	=	15.51	16.07

Figure 5J(v): Single Cosinor Plot for Melatonin and Comparative Table - Patient J (X-axis = Clock hours; Y-axis = pg/ml)



		Patient J	Matched Control
% R	=	37.00	72.5
P-Level	=	0.008	<0.001
Amp	=	6.86	8.05
Mesor	=	20.23	12.29
Phi(Deg)	=	- 80.6	- 40.0
Phi(H/Min)	=	5.22	2.40

Figure 5J(vi): Polar Plot for Melatonin - Patient J

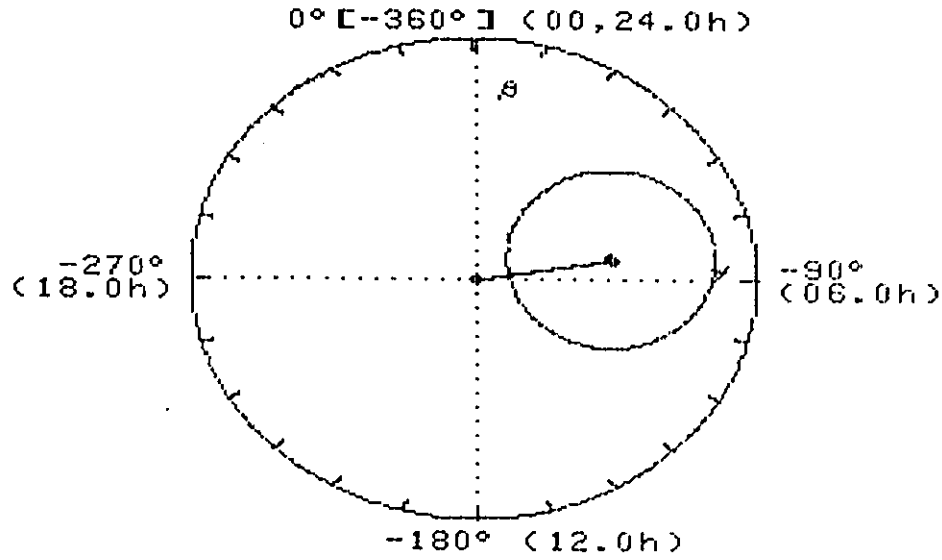
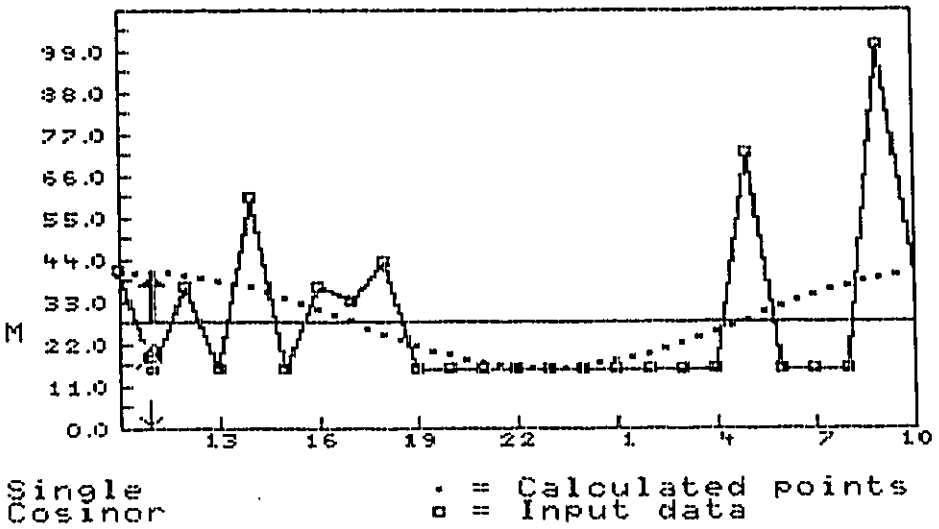


Figure 5J(vii): Single Cosinor Plot for Cortisol and Comparative Table - Patient J (X-axis = Clock hours; Y-axis = ng/ml)



		Patient J	Matched Control
% R	=	16.30	47.50
P-Level	=	0.155 (N.S.)	0.001
Amp	=	12.39	37.28
Mesor	=	27.50	48.52
Phi(Deg)	=	-163.8	-148.4
Phi(H/Min)	=	10.55	9.54

Figure 5J(viii): Polar Plot for Cortisol - Patient J

AS P>0.05, THE DATA ARE ASSUMED TO BE VARIABLE AND NOT CYCLIC,

THEREFORE, NO POLAR PLOT IS SHOWN

CASE PRESENTATION K

PATIENT K

Clinical History

Patient K is a 67 year old, caucasian male, married with two grown children. His presenting complaint involved a long history of difficulty with sleep maintenance, and an exceedingly short total sleep time. Most often, there was a rapid sleep onset, followed by 90 to 120 minutes of sleep. He then awakened for 60 to 120 minutes, and was usually able to acquire one more short sleep episode before arising. His day typically began between 0330 and 0600 hours. This pattern was occasionally interspersed with totally sleepless nights.

This patient had a 40 year history of serious and debilitating sleep disturbance. It started at a time when he was put under increasing pressure at his place of business. Although he is now retired and content with his lifestyle, his insomnia persists.

This patient had a long medical and psychiatric history. He has had recurrent depressions which respond to tricyclic antidepressants but the insomnia has persisted in the free intervals. The past several years have been free of these periodic depressive episodes but the insomnia has not improved. He has a history of alcoholism which he attributed partly to job stress, and also an attempt to "self medicate" with regards to his chronic insomnia. However, this has not been a problem for several years.

This man had tried many different benzodiazepines, but none had significantly enhanced his sleep. The only successful treatment began in 1975 when he was part of a double blind, controlled study of Gamma-

hydroxybutyrate (GAMMA-OH) (30 mg/kg). He has taken this drug intermittently since that time with continued success.

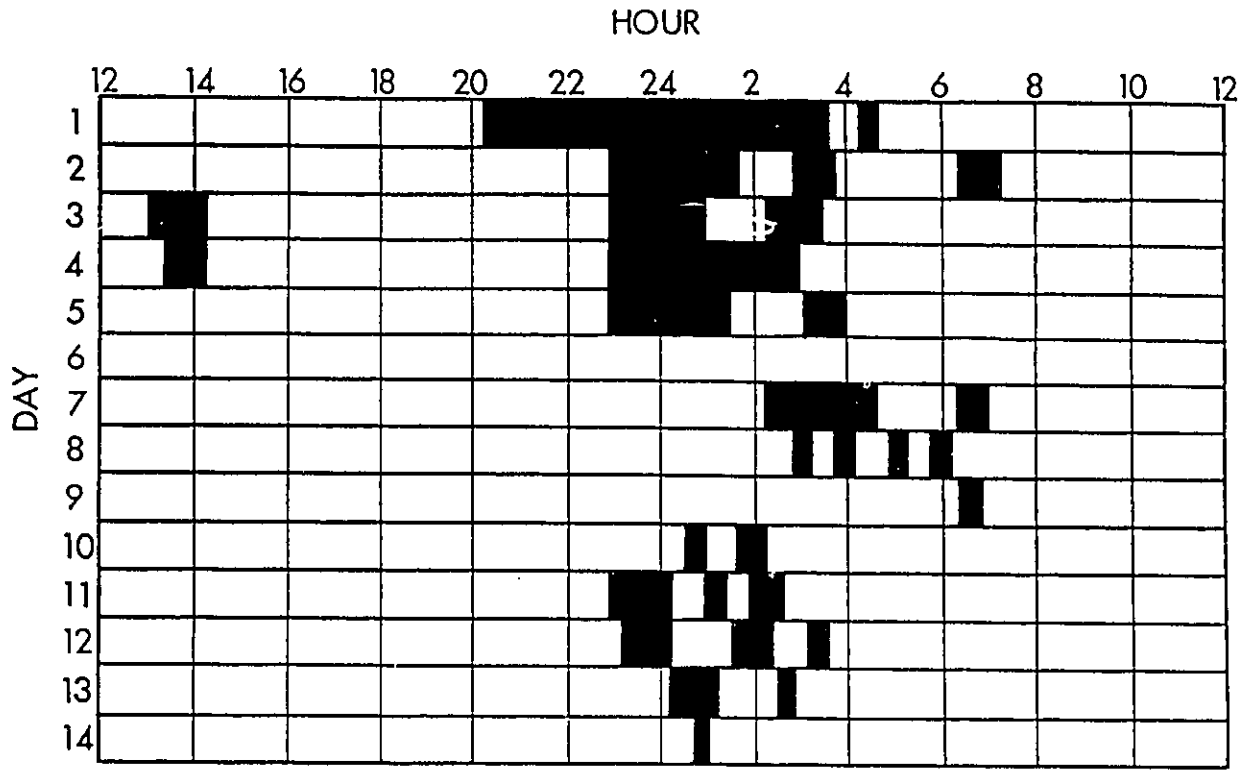
There was some irregularity in the reported pattern of sleep disturbance with this patient, but most often he reported short sleep onset latency, with early awakening. With some deliberation, he was assigned to phase advance group.

The MMPI revealed an elevated depression scale, worry, indecision, and feelings of inadequacy. At the time of the study the patient met the criteria for Dysthymic Disorder, but not major depression.

Sleep and Circadian Data

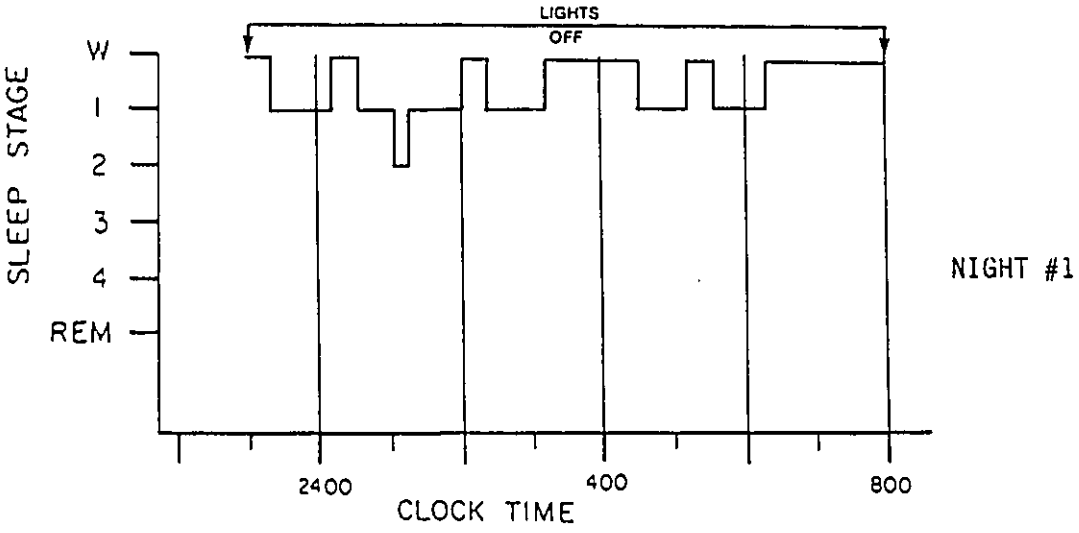
Subjective report indicated a markedly variable sleep onset and total sleep time. The means were greatly deviant from the matched control. In the Laboratory, the sleep onset latency was 15 and 20 minutes, markedly less than that reported. Total sleep time, however, was only slightly longer than that reported and much less than that of a normal control, 3 and 3.5 hours on nights #1 and #2, respectively. Much more time was spent in Stage 1, as compared to the control on both nights. On night #1, however, the patient spent 29.5% of his total sleep time in Stage 3, but on night #2, 0%. There was no REM sleep either night. The plot of the temperature data showed a variable distribution of acrophases on the various nights, ranging from 2200 hours to 0600 hours, all of which were of low amplitude and the curve explained only 25% of the variance. The cortisol data and the melatonin data were not cyclic. These observations were not consistent with the prediction that this patient had a phase advance circadian pattern.

Figure 5K(i): Subjective Sleep Diaries and Comparative Table -Patient K



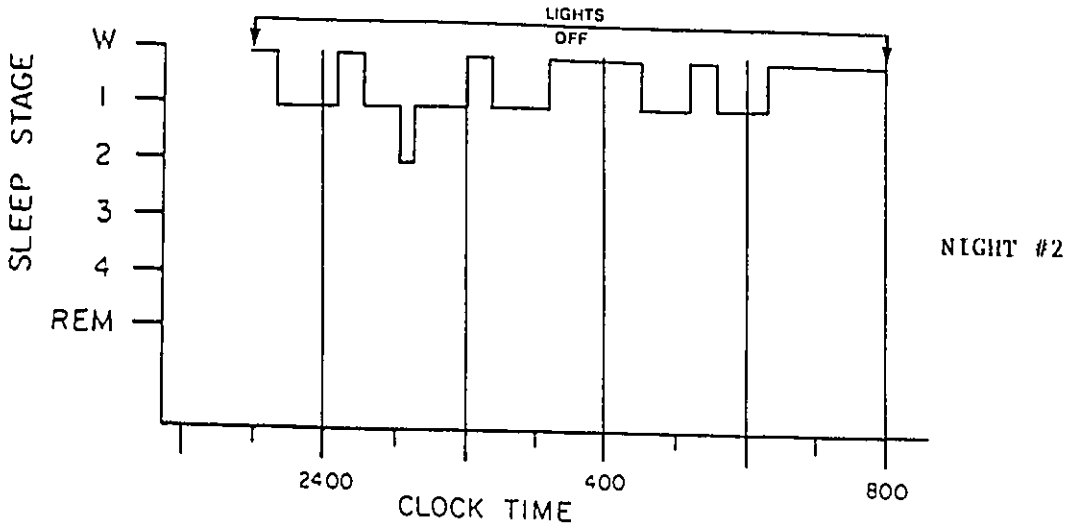
	MEAN OF SLEEP PARAMETERS		
	PATIENT K	MATCHED CONTROL	ALL CONTROLS ± SE
SOL (MIN.)	54.6	17.1	15.2 (± 2.7)
AWAKENINGS	4.7	1.8	.33 (± .04)
TST (MIN.)	149.6	427.2	469.80 (±14.4)

Figure 5K(ii): Sleep Histograms and Comparative Table - Patient K



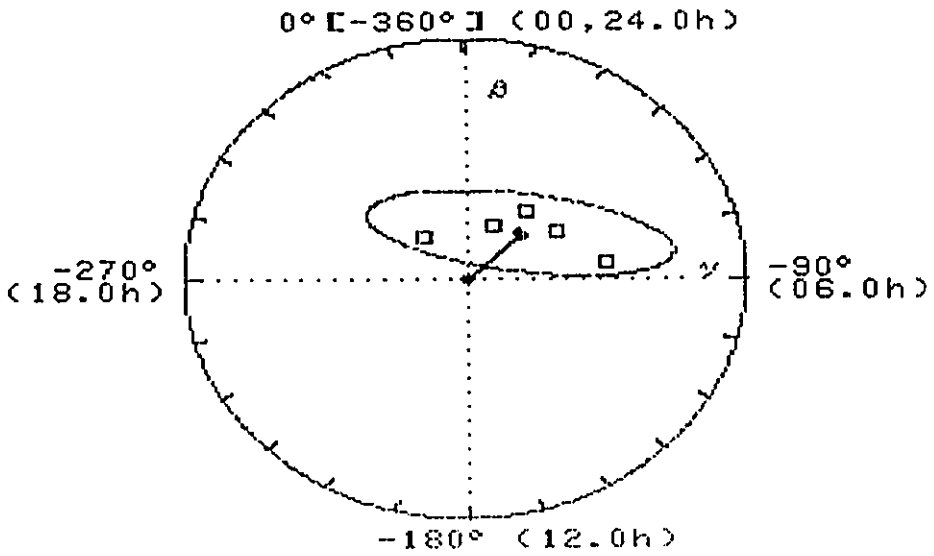
	PATIENT K	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #1	NIGHT #1	NIGHT #1
TST(Minutes)	168.9	437.1	454.2 (±15.2)
SOL(Minutes)	20.0	17.4	12.4 (±3.7)
ROL(Minutes)	---	88.4	77.4 (±21.1)
% S1	23.0	5.5	4.9 (± 0.9)
% S2	47.5	48.7	49.2 (±6.4)
% S3	29.5	9.1	10.2 (±3.3)
% S4	0	8.8	13.0 (±4.1)
% REM	0	27.9	22.7 (±4.8)
# AWAKENINGS	2	2	2

Figure 5K(iii): Sleep Histograms and Comparative Table - Patient K



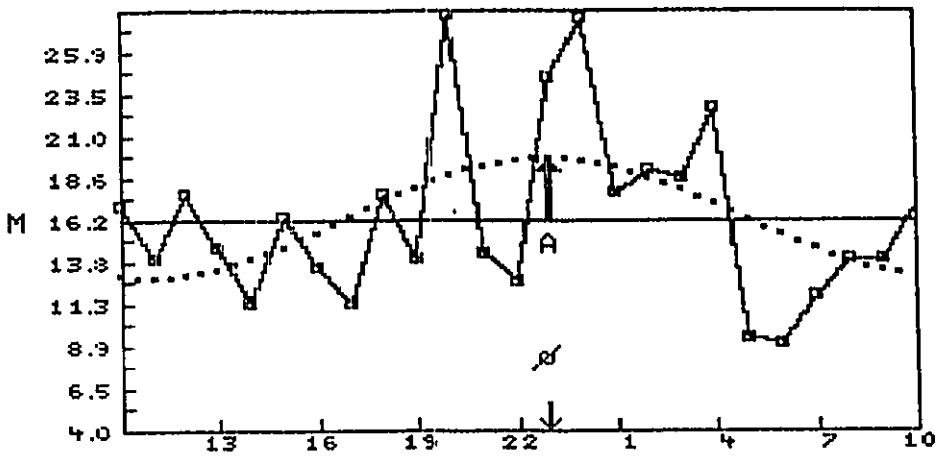
	PATIENT K	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #2	NIGHT #2	NIGHT #2
TST(Minutes)	210.4	409.4	422.0 (± 26)
SOL(Minutes)	15.3	23.8	18.5 (± 4.1)
ROL(Minutes)	---	81.4	93.7 (± 19.8)
% S1	95.5	8.7	6.1 (± 1.3)
% S2	4.5	49.3	46.2 (± 7.0)
% S3	0	7.4	11.3 (± 4.8)
% S4	0	8.5	12.8 (± 3.7)
% REM	0	26.1	23.6 (± 3.3)
# AWAKENINGS	7	3	4

Figure 5K(iv): Polar Plot of Temperature Data and Comparative Table - Patient K



		Patient K	Matched Control
% R	=	63.40	83.0
P-Level	=	0.048 (Marginal)	0.001
Amp	=	37.10	37.0
Mesor	=	0.36	0.55
Phi(Deg)	=	- 48.9	-241.7
Phi(H/Min)	=	3.16	16.07

Figure 5K(v): Single Cosinor Plot for Melatonin and Comparative Table - Patient K (X-axis = Clock hours; Y-axis = pg/ml)

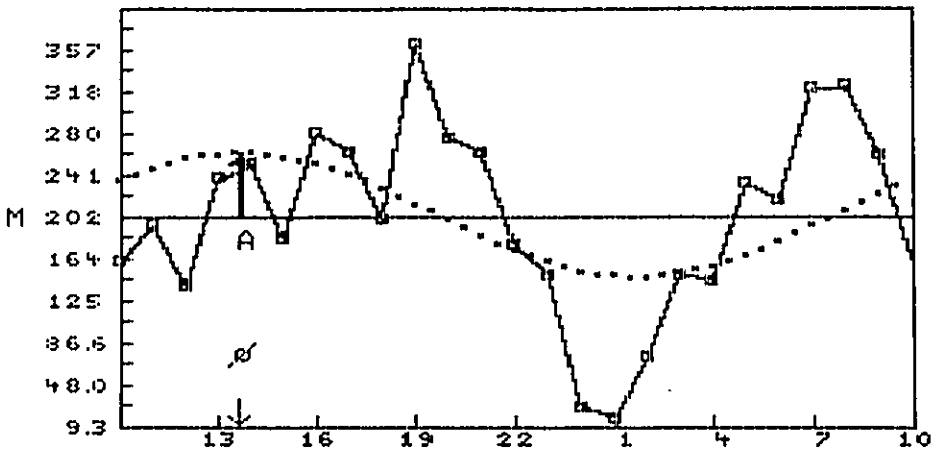


		Patient K	Matched Control
% R	=	27.80	72.5
P-Level	=	0.084	<0.001
Amp	=	3.47	8.05
Mesor	=	16.25	12.29
Phi(Deg)	=	-344.4	- 40.0
Phi(H/Min)	=	22.58	2.40

Figure 5K(vi): Polar Plot for Melatonin - Patient K

AS P>0.05, THE DATA ARE ASSUMED TO BE VARIABLE AND NOT CYCLIC, THEREFORE, NO POLAR PLOT IS SHOWN

Figure 5K(vii): Single Cosinor Plot for Cortisol and Comparative Table - Patient K (X-axis = Clock hours; Y-axis = ng/ml)



		Patient K	Matched Control
% R	=	22.00	47.5
P-Level	=	0.074 (N.S.)	0.001
Amp	=	57.29	37.28
Mesor	=	202.70	48.52
Phi(Deg)	=	-205.3	-148.4
Phi(H/Min)	=	13.41	9.54

Figure 5K(viii): Polar Plot for Cortisol - Patient K

AS P>0.05, THE DATA ARE ASSUMED TO BE VARIABLE AND NOT CYCLIC,

THEREFORE, NO POLAR PLOT IS SHOWN

CASE PRESENTATION L

PATIENT L

Clinical History

Patient L is a 48 year old, caucasian male, married with no children. His presenting complaint was a difficulty with sleep maintenance. There was also occasional difficulty with sleep onset, although he attributed this to anxious rumination of the day's activities, and apprehension of another insufficient night's sleep. He also reported a completely sleepless night once or twice per week.

This patient had a 30 year history of insomnia. It began during his early teenage years, coinciding with his first episode of endogenous depression.

This man has a long medical and psychiatric history. He was receiving treatment for hypertension and asthma which are kept under control by current medications. He also has a history of periodic depression which has responded favourably to tricyclic antidepressants, although the insomnia persists in the free intervals. At the time of his visit to the Clinic, it had been almost two years since the last depressive episode.

He currently takes a wide array of medications, including Propranolol (180 mgs) a beta-adrenoceptor antagonist for his hypertension, Bronkaid (epinephrine) inhalation for his asthma, Lorazipam (Ativan) 1 mg, and/or Triazolam (Halcion) 1 mg for his insomnia, and Doxepin (Sinequan) 25 mgs as required for periodic depression.

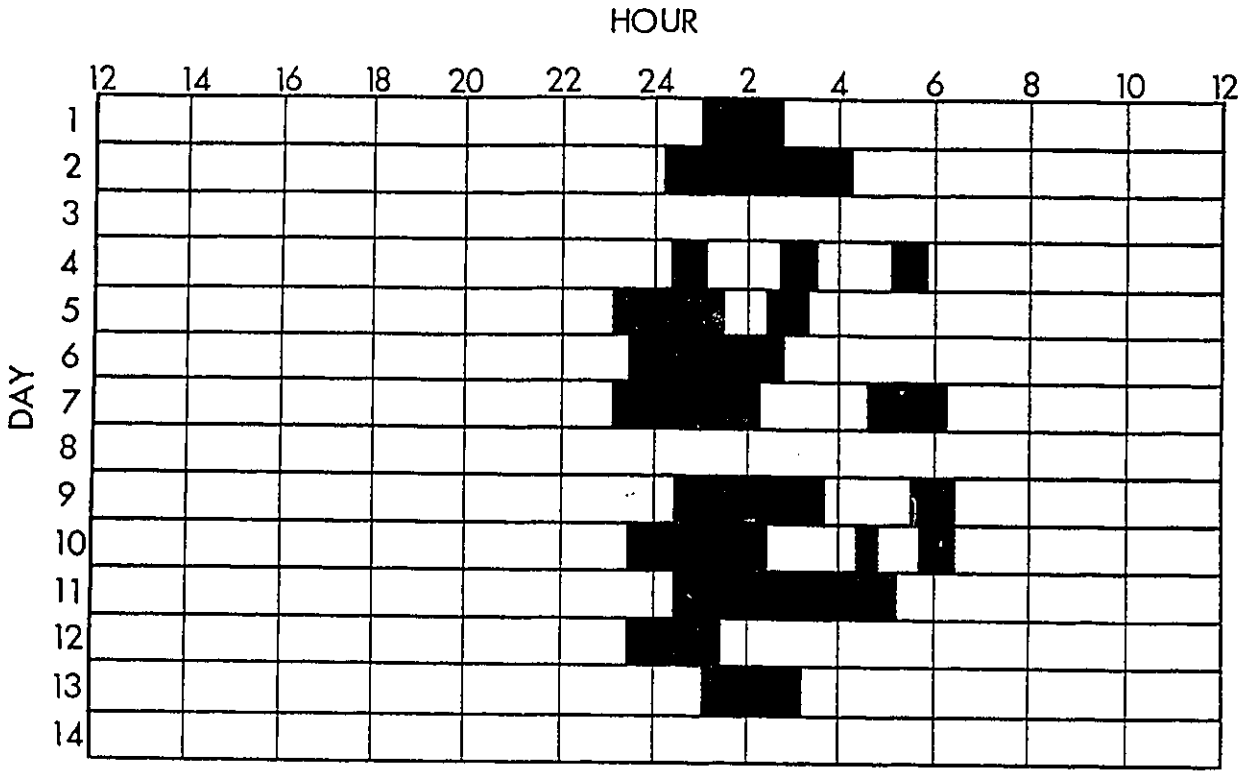
With a long history of early morning awakening interspersed only occasionally with increased sleep onset latency, this man was assigned to the phase advance group.

The MMPI major characteristics were apathetic depression, over-control, self doubt and helplessness, desire for greater recognition. Though not in a major depressive episode at the present time, the chronic symptoms and preoccupation with the insomnia and its debilitating effects would permit the DSM III diagnosis of Dysthymic Disorder.

Sleep and Circadian Data

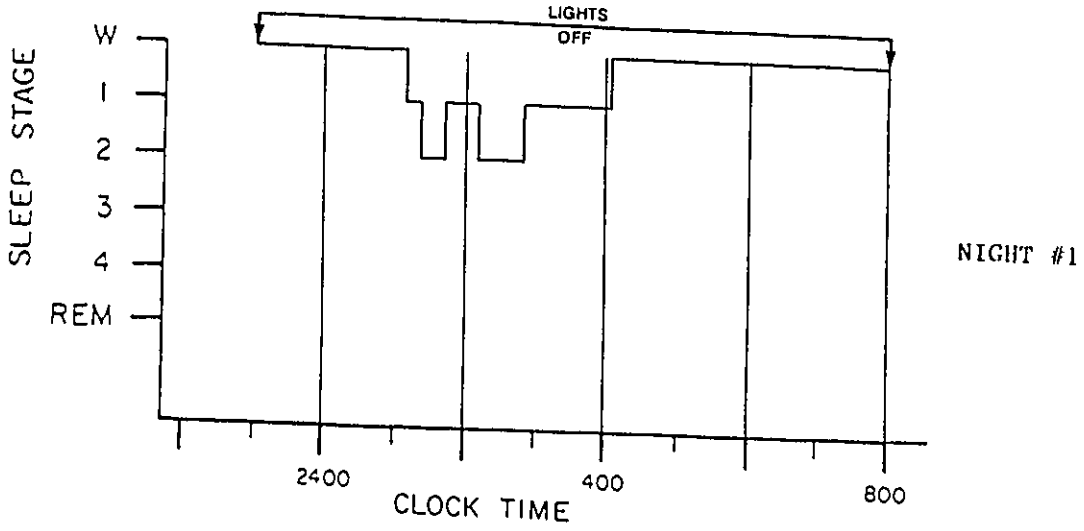
Subjective reports indicated a sleep onset time of approximately 48 minutes and a total sleep time of 140 minutes. In the Laboratory sleep onset time was much longer, 90 - 120 minutes. Total sleep time was slightly longer on night #1, and considerably shorter on night #2, than that reported subjectively in the sleep diaries. The patient had slept only in Stages 1 and 2 and spent no time in Stage 3, 4 or REM. Temperature acrophase occurred at a variety of times, from 1700 hours to 0900 hours and was of low amplitude on four of five nights. Cortisol showed no cyclical pattern. Melatonin peaked at 0400 hours and showed a robust cyclical pattern. These data were not consistent with the clinical prediction of a phase advance insomnia.

Figure 5L(i): Subjective Sleep Diaries and Comparative Table -Patient L



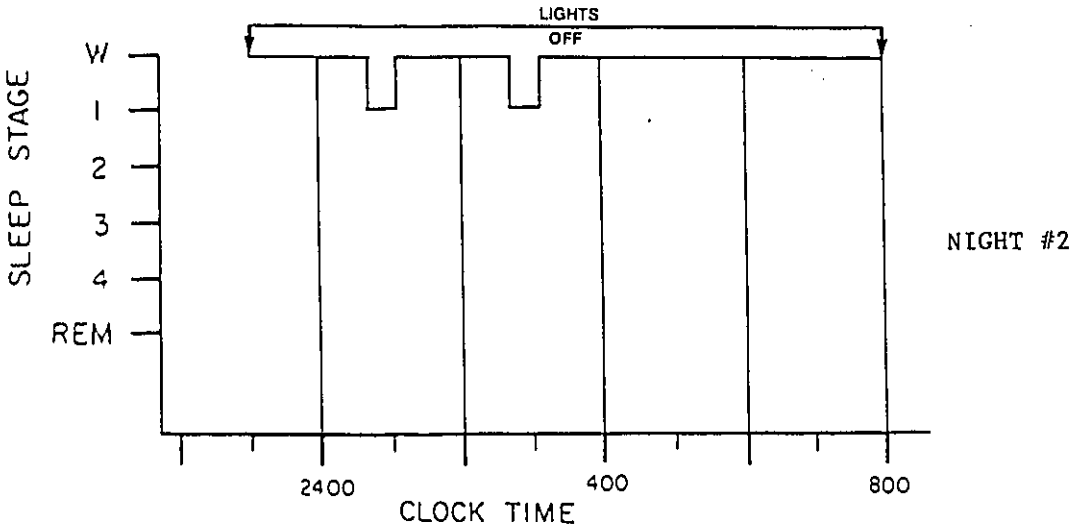
	MEAN OF SLEEP PARAMETERS		
	PATIENT L	MATCHED CONTROL	ALL CONTROLS ± SE
SOL (MIN.)	48.3	17.71	15.2 (± 2.7)
AWAKENINGS	2.8	1.8	.33 (± .04)
TST (MIN.)	141.6	427.2	469.80 (±14.4)

Figure 5L(ii): Sleep Histograms and Comparative Table - Patient L



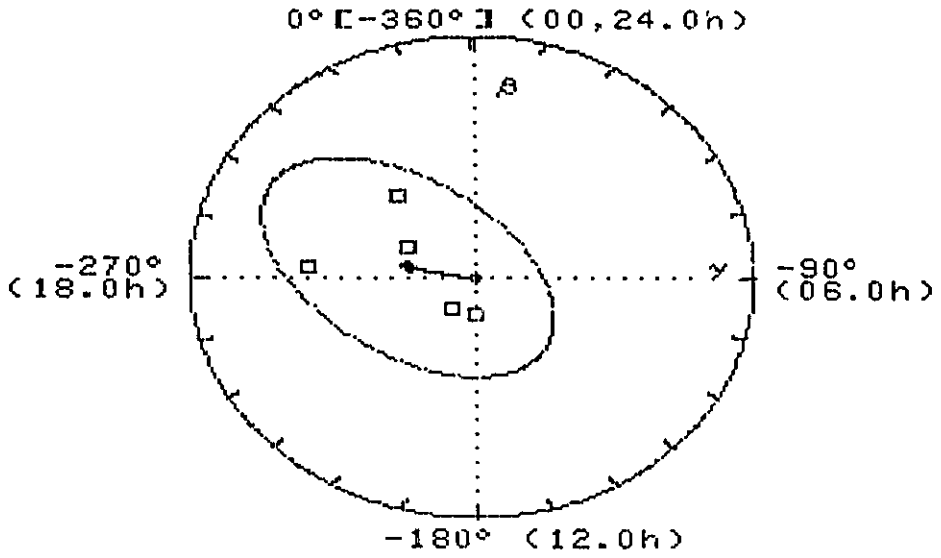
	PATIENT L	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #1	NIGHT #1	NIGHT #1
TST(Minutes)	189.0	437.1	454.2 (±15.2)
SOL(Minutes)	121.0	17.4	12.4 (±3.7)
ROL(Minutes)	---	88.4	77.4 (±21.1)
% S1	57.4	5.5	4.9 (± 0.9)
% S2	42.6	48.7	49.2 (±6.4)
% S3	---	9.1	10.2 (±3.3)
% S4	---	8.8	13.0 (±4.1)
% REM	---	27.9	22.7 (±4.8)
# AWAKENINGS	0	2	2

Figure 5L(iii): Sleep Histograms and Comparative Table - Patient L



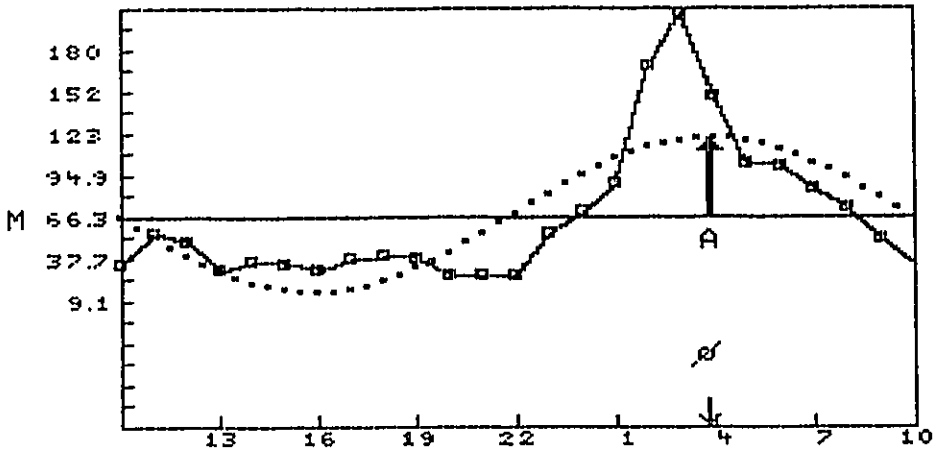
	PATIENT L	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #2	NIGHT #2	NIGHT #2
TST(Minutes)	54.3	409.4	422.0 (± 26)
SOL(Minutes)	93.0	23.8	18.5 (± 4.1)
ROL(Minutes)	---	81.4	93.7 (± 19.8)
% S1	100.0	8.7	6.1 (± 1.3)
% S2	---	49.3	46.2 (± 7.0)
% S3	---	7.4	11.3 (± 4.8)
% S4	---	8.5	12.8 (± 3.7)
% REM	---	26.1	23.6 (± 3.3)
# AWAKENINGS	2	3	4

Figure 5L(iv): Polar Plot of Temperature Data and Comparative Table - Patient L



		Patient L	Matched Control
% R	=	47.90	83.0
P-Level	=	0.47 (N.S.)	0.001
Amp	=	36.50	37.0
Mesor	=	0.49	0.55
Phi(Deg)	=	-279.5	-241.7
Phi(H/Min)	=	18.38	16.07

Figure 5L(v): Single Cosinor Plot for Melatonin and Comparative Table - Patient L (X-axis = Clock hours; Y-axis = pg/ml)



Single Cosinor

· = Calculated points
▫ = Input data

		Patient L	Matched Control
% R	=	61.70	72.5
P-Level	=	<0.001 (N.S.)	<0.001
Amp	=	52.52	8.05
Mesor	=	66.32	12.29
Phi(Deg)	=	- 58.3	- 40.0
Phi(H/Min)	=	3.53	2.40

Figure 5L(vi): Polar Plot for Melatonin - Patient L

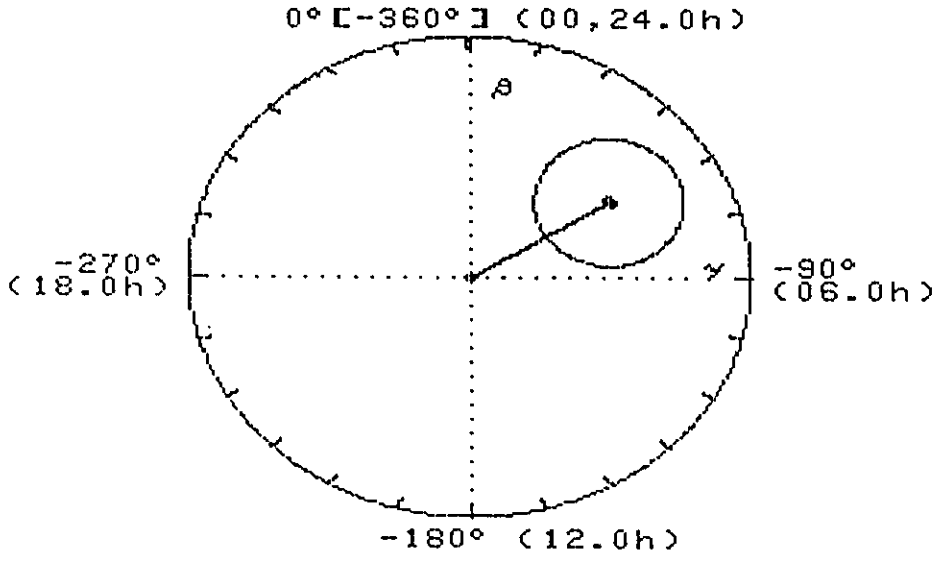
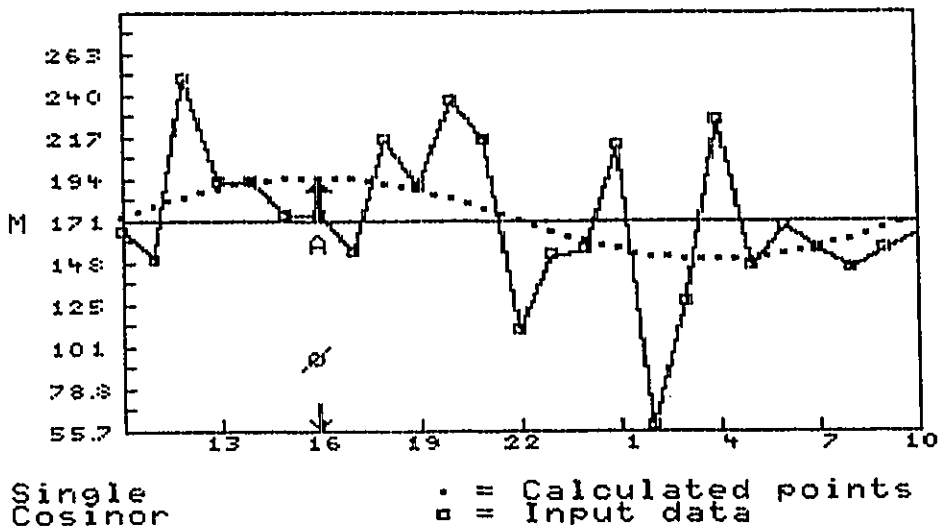


Figure 5L(vii): Single Cosinor Plot for Cortisol and Comparative Table - Patient L (X-axis = Clock hours; Y-axis = ng/ml)



		Patient L	Matched Control
% R	=	15.0	47.50
P-Level	=	0.181	0.001
Amp	=	22.87	37.28
Mesor	=	171.12	48.52
Phi(Deg)	=	-238.5	-148.4
Phi(H/Min)	=	15.54	9.54

Figure 5L(viii): Polar Plot for Cortisol - Patient L

AS P>0.05, THE DATA ARE ASSUMED TO BE VARIABLE AND NOT CYCLIC,

THEREFORE, NO POLAR PLOT IS SHOWN

CASE PRESENTATION M

PATIENT M

Clinical History

Patient M is a 41 year old, caucasian male, married, with one infant child. His presenting complaint was a chronic difficulty with sleep maintenance. Often there was also difficulty with sleep onset (1 - 6 hours) and almost as often his sleep episode was fractured by multiple awakenings of considerable duration.

This history of sleep disturbance was life long, although it had become considerably worse during the last 10 years. As a child, and occasionally at present, he has had nightmares that wake him up screaming, and also reports episodes of sleep walking. This suggests a history of slow wave sleep arousals resulting in night terrors and somnambulism.

This patient's psychiatric history was unremarkable. His medical history was clear except for a recent problem with idiopathic lumbar pain for which he had regular sessions in physiotherapy. It should be noted that at this time he reported a high degree of marital conflict for which he was also receiving therapy in a "couples clinic".

This patient takes no medications. Although benzodiazepines had been offered to him in the past, he had refused them. He consumes very little alcohol or coffee.

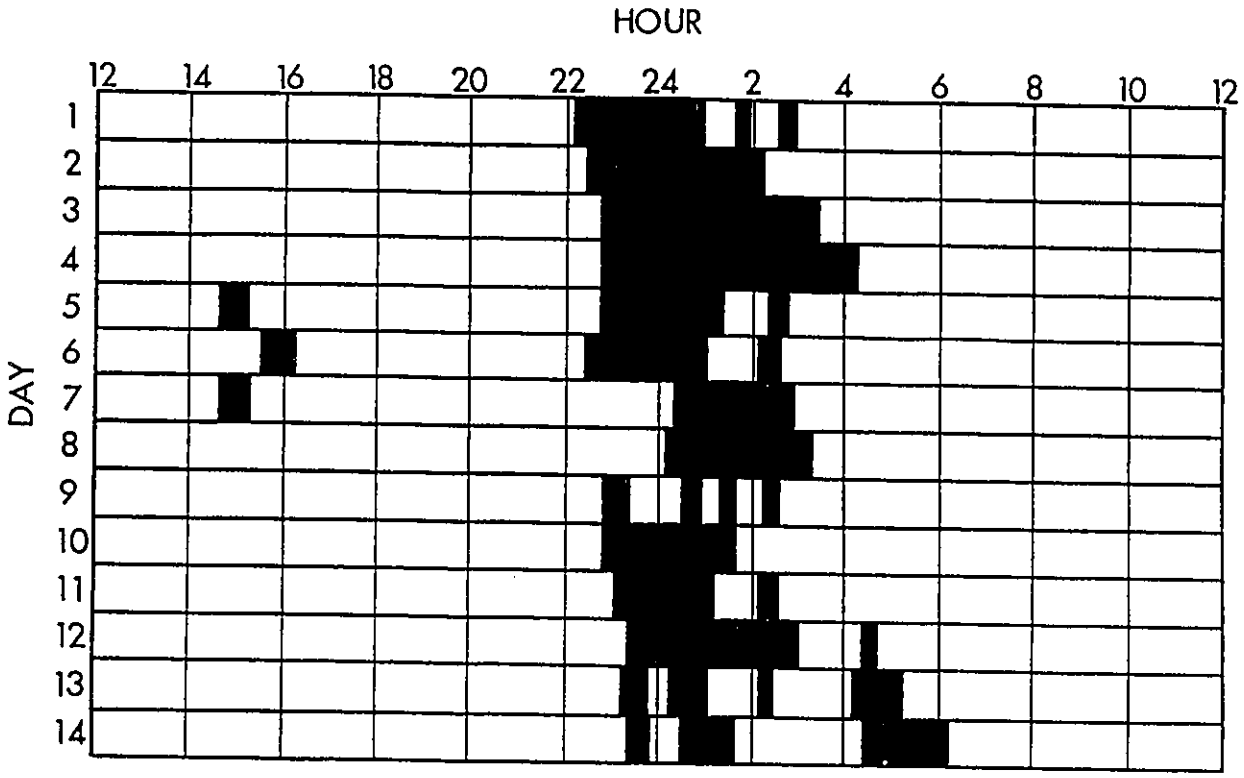
It was difficult to assign this patient to one of the predicted groups. The early morning awakening would imply a phase advance, but often there is a concomitant difficulty with long sleep onset latency and multiple awakenings. With reservations, this patient was assigned to the arrhythmic group.

The MMPI reflected significant affective symptoms and a great deal of tension and anxiety, and moderate to severe dysphoria, concern with his mental function, and brooding worry. He would meet criteria for Dysphoric Disorder.

Sleep and Circadian Data

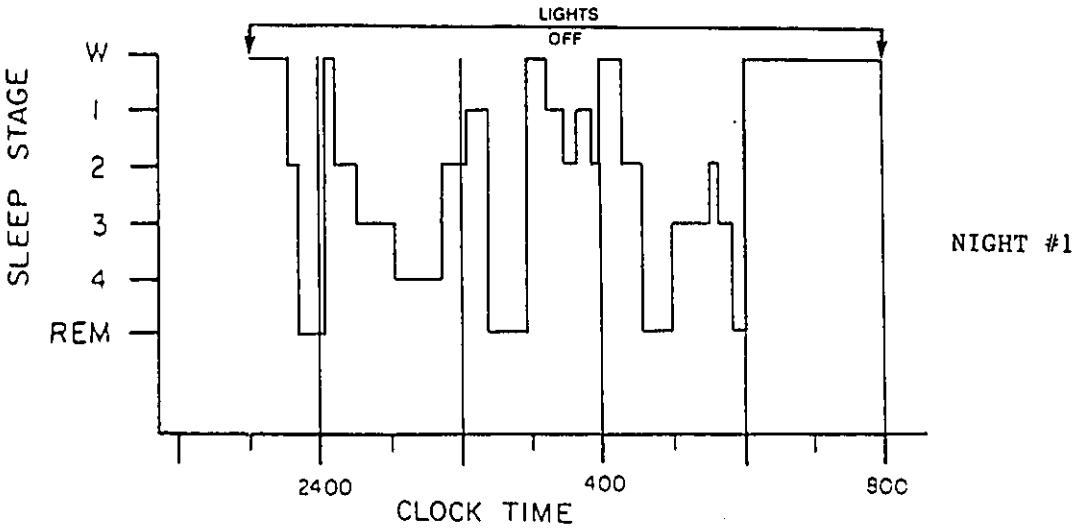
His subjective report revealed fairly prompt sleep onset but a very attenuated total sleep time averaging just over three hours. In the Laboratory his sleep onset time was slightly longer and his total sleep time was over four hours on both nights. He spent more time in Stage 1 than the control on both nights, and on night #1, much more time in Stage 3. Circadian temperature showed an acrophase occurring at a range of times from 0800 hours to 2000 hours and of small amplitude. Cortisol data were not cyclic. Melatonin peaked at approximately 0330 hours. These data are consistent with the variable noncircadian pattern.

Figure 5M(i): Subjective Sleep Diaries and Comparative Table -Patient M



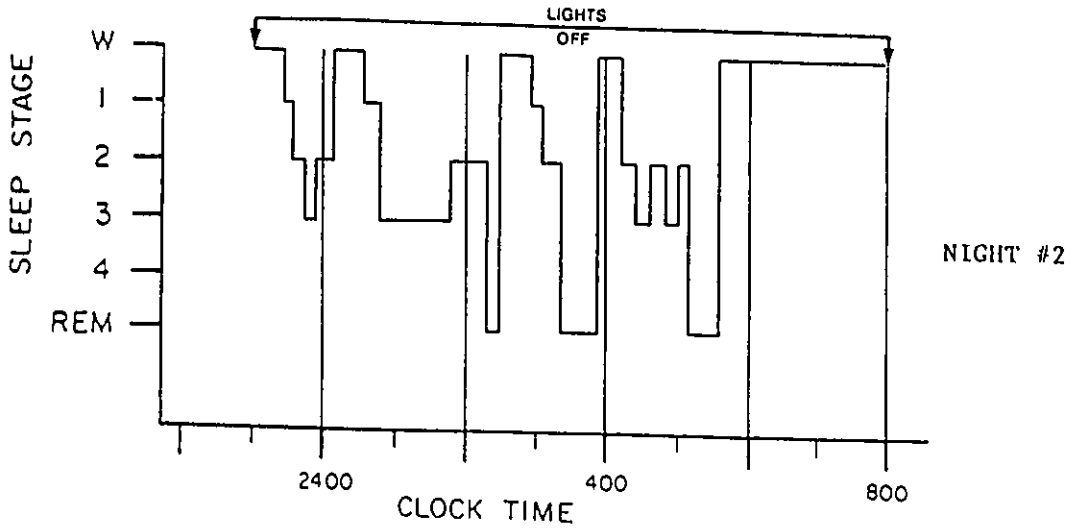
	MEAN OF SLEEP PARAMETERS		
	PATIENT M	MATCHED CONTROL	ALL CONTROLS ± SE
SOL (MIN.)	20.3	11.7	15.2 (± 2.7)
AWAKENINGS	4.3	.62	3 (± .04)
TST (MIN.)	198.2	441.3	469.80 (±14.4)

Figure 5M(ii): Sleep Histograms and Comparative Table - Patient M



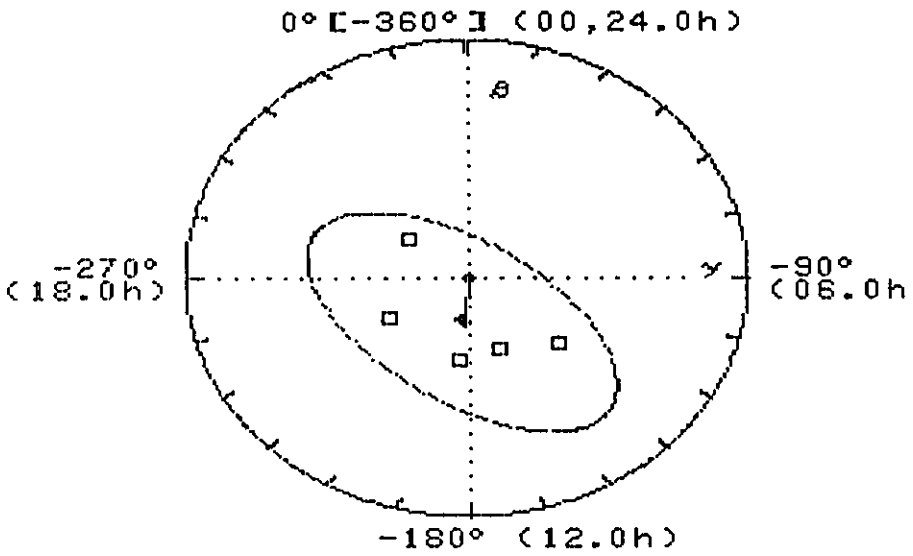
	PATIENT M	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #1	NIGHT #1	NIGHT #1
TST(Minutes)	259.6	437.4	454.2 (±15.2)
SOL(Minutes)	29.1	8.3	12.4 (±3.7)
ROL(Minutes)	168.0	88.7	77.4 (±21.1)
% S1	16.0	4.6	4.9 (± 0.9)
% S2	37.8	51.7	49.2 (±6.4)
% S3	29.1	9.1	10.2 (±3.3)
% S4	---	12.8	13.0 (±4.1)
% REM	17.1	21.8	22.7 (±4.8)
# AWAKENINGS	5	0	2

Figure 5M(iii): Sleep Histograms and Comparative Table - Patient M



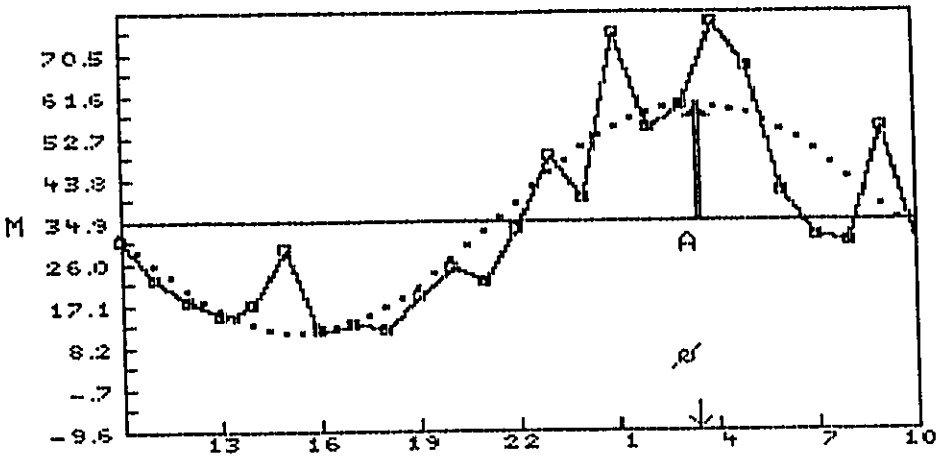
	PATIENT M	MATCHED CONTROL	CONTROL X ±(SE)
	NIGHT #2	NIGHT #2	NIGHT #2
TST(Minutes)	263.4	417.6	422.0 (± 26)
SOL(Minutes)	40.3	17.1	18.5 (± 4.1)
ROL(Minutes)	5.3	81.7	93.7 (± 19.8)
% S1	23.2	7.2	6.1 (± 1.3)
% S2	35.5	50.4	46.2 (± 7.0)
% S3	9.2	8.8	11.3 (± 4.8)
% S4	6.6	11.9	12.8 (± 3.7)
% REM	25.5	21.7	23.6 (± 3.3)
# AWAKENINGS	5	2	4

Figure 5M(iv): Polar Plot of Temperature Data and Comparative Table - Patient M



		Patient M	Matched Control
% R	=	52.90	78.7
P-Level	=	0.25 (N.S.)	<0.001
Amp	=	36.70	36.90
Mesor	=	0.21	0.48
Phi(Deg)	=	-189.1	-256.8
Phi(H/Min)	=	12.36	17.07

Figure 5M(vi): Single Cosinor Plot for Melatonin and Comparative Table - Patient M (X-axis = Clock hours; Y-axis = pg/ml)



Single Cosinor

· = Calculated points
□ = Input data

		Patient M	Matched Control
% R	=	73.60	32.90
P-Level	=	<0.001	0.015
Amp	=	23.98	12.19
Mesor	=	34.91	27.52
Phi(Deg)	=	- 51.6	- 21.6
Phi(H/Min)	=	3.56	1.27

Figure 5M(vi): Polar Plot for Melatonin - Patient M

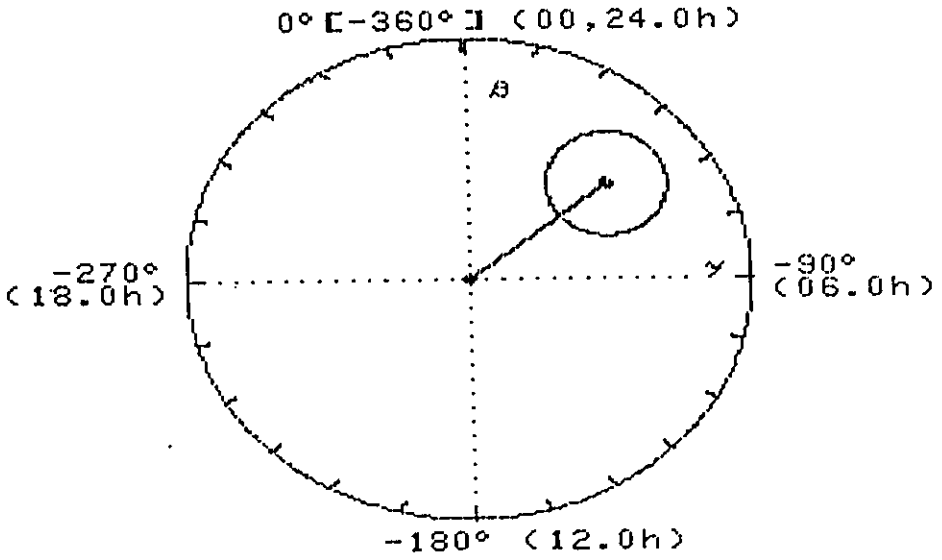
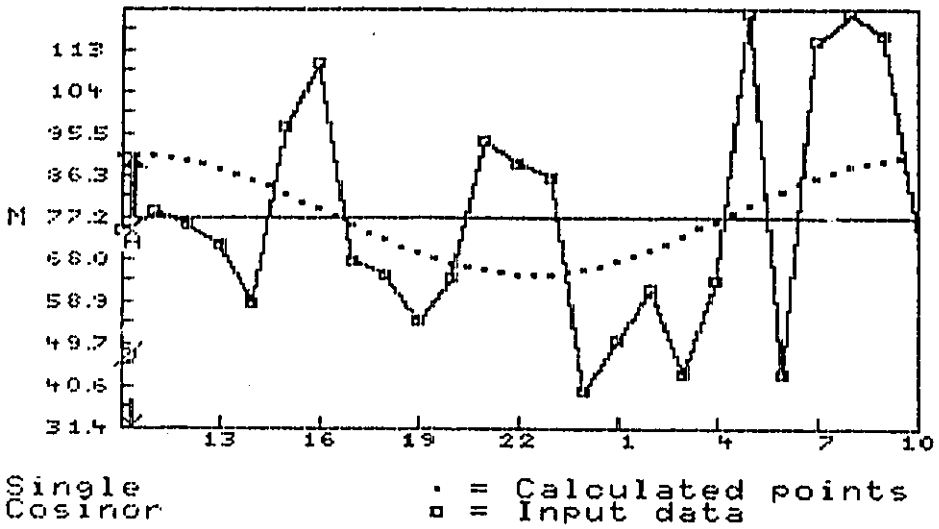


Figure 5M(vii): Single Cosinor Plot for Cortisol and Comparative Table - Patient M (X-axis = Clock hours; Y-axis = ng/ml)



		Patient M	Matched Control
% R	=	13.40	78.20
P-Level	=	0.222 (N.S.)	<0.001
Amp	=	13.01	64.99
Mesor	=	77.16	73.35
Phi(Deg)	=	-155.6	-150.4
Phi(H/Min)	=	10.22	10.02

Figure 5M(viii): Polar Plot for Cortisol - Patient M

AS P>0.05, THE DATA ARE ASSUMED TO BE VARIABLE AND NOT CYCLIC,

THEREFORE, NO POLAR PLOT IS SHOWN

PART III

SIGNIFICANT GROUP DATA

GROUP TEMPERATURE DATA

Patients A,B,C,D,E (Group 1) **Phase Delay**

Phase delay was predicted by these clinical features: chronic late bedtimes, long sleep and latency (>90 minutes) and excessive morning fatigue.

When the data from the appropriate subjects were analyzed by the cosinor method, the phase delay group, based on clinical evaluation, formed a congruent temperature phase delay group as shown in Figure 5(iv). Student's t-test comparing this group to the control group showed that there was a significant difference in the phase ($t = 4.91$, $p<0.01$) but not amplitude ($t = 0.166$, $p>0.05$).

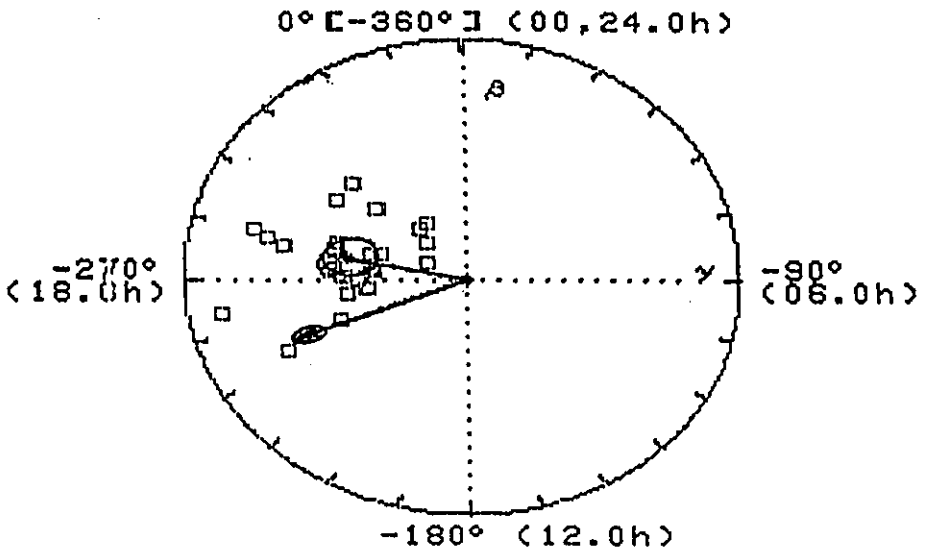
The phase delay cell was filled quickly and easily. In fact, within the first four months of the study there were already an adequate number of phase delayed subjects based on evaluation in the Sleep Disorders Clinic.

Table 5(ii): Temperature Data - Patient Group 1

Patients	Day	% R	Amp	Mesor	Phi (Degrees)	Phi (Hrs/Mins)
A	1	33.8	0.15	37.18	-294.3	19.37
	2	77.0	0.42	37.43	-318.6	21.15
	3	47.7	0.46	37.29	-228.8	21.55
	4	53.5	0.35	37.23	-317.5	21.11
	5	63.0	0.54	37.40	-313.4	20.54
B	1	42.0	0.48	36.47	-278.3	18.30
	2	60.0	0.55	36.64	-315.8	21.03
	3	48.5	0.69	36.38	-244.0	16.17
	4	54.5	0.87	36.51	-260.6	17.22
	5	70.4	0.77	36.55	-315.0	21.00
C	1	49.7	0.34	37.07	-287.1	19.08
	2	87.2	0.71	37.08	-283.3	18.53
	3	92.2	0.76	37.08	-285.4	19.02
	4	69.7	0.64	37.76	-281.7	18.47
	5	87.3	0.47	37.74	-284.7	18.59
D	1	82.0	0.34	37.20	-262.4	17.29
	2	82.1	0.47	37.28	-248.4	18.57
	3	24.5	0.46	37.34	-275.1	18.20
	4	50.5	0.33	37.18	-277.2	18.29
	5	47.5	0.42	37.00	-270.3	18.12
E	1	45.0	0.47	37.18	-288.2	19.13
	2	73.5	0.38	37.27	-268.0	17.52
	3	65.0	0.32	37.39	-275.5	18.22
	4	76.8	0.42	37.36	-261.7	17.27
	5	79.2	0.31	37.46	-289.9	19.12
Mean	1	50.5	0.36	37.02	-282.1	18.48
	2	76.0	0.51	37.14	-286.8	19.07
	3	55.6	0.48	37.30	-261.8	17.27
	4	61.0	0.52	37.41	-279.7	18.39
	5	69.5	0.50	37.23	-294.7	19.38

Figure 5(iv): Grand Mean of Delay Group Temperature Data (N=5)

N.B. control mean and confidence interval also shown (darkened ellipse)



% R	= 68.3	Mesor	= 37.0
P-Level	= <0.001	Phi (Deg.)	= -281.2
Amp	= 0.42	Hrs/Mins	= 18.45

Tangents from pole to ellipse:

Phi 1	= -269.8	Hrs/Mins	= 17.59
Phi 2	= -294.5	Hrs/Mins	= 19.38

Patients F,G,H,I (Group 2) **Phase Advance**

Phase advance was predicted by these clinical features: chronic early awakening (>90 minutes) and excessive early evening fatigue with an exceedingly short sleep onset latency.

It was not possible to complete the phase advance cell in the time frame of this study (Figure 5(v)). In fact, it took three years to attain a sample of four subjects who fit all of the criteria of the original hypothesis. Six subjects were assigned to this group based on clinical evaluation in the Sleep Disorders Clinic. Two subjects were subsequently found to have a very different pattern of core temperature disruption which was similar to that seen in other patients in Group 3 (below). However, retrospective assessment showed a much more variable clinical history than other patients in Group 2, thus they were removed from the group. This would imply that the predictive power of the clinical criteria used to assign patients to Group 2 was low. When Student's t-test was applied to the phase and amplitude data of the remaining four subjects in the group, there was a significant difference in the phase ($t = 5.28$, $p<0.01$) but not in amplitude ($t = 1.58$, $p>0.05$) as compared to the control group.

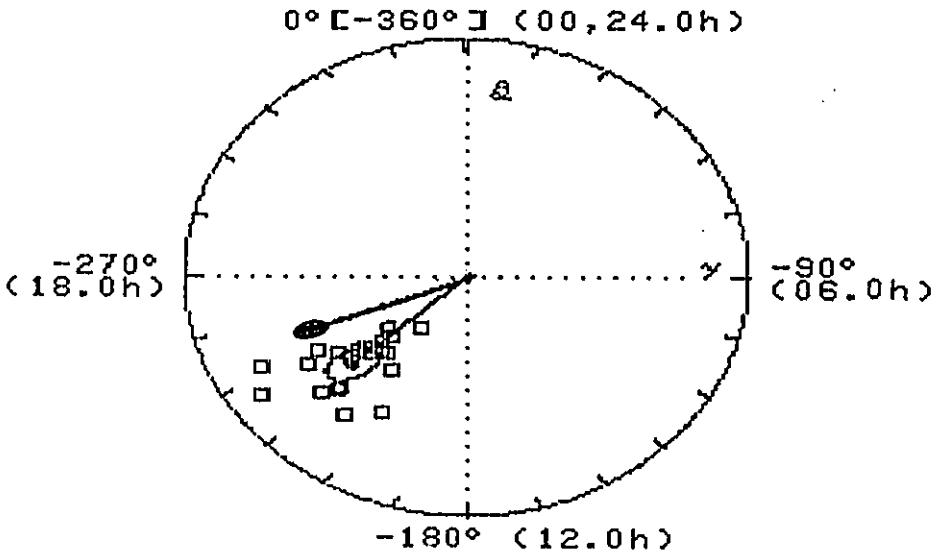
Since the individual mean acrophase could not be determined for the two patients removed from this group (K and L), it was not possible to include them in an additional analysis, before exclusion. If this had not been the case, it would be important to examine the effect of this retrospective reshuffling on the outcome measure.

Table 5(iii): Temperature Data - Patient Group 2

Patients	Day	% R	Amp	Mesor	Phi (Degrees)	Phi (Hrs/Mins)
F	1	42.0	0.67	37.09	-235.7	15.36
	2	73.7	0.37	37.20	-230.9	15.23
	3	77.3	0.55	37.36	-216.2	14.25
	4	59.4	0.32	37.25	-220.0	14.40
	5	34.4	0.20	37.40	-215.6	14.23
G	1	42.9	0.27	37.32	-231.2	15.25
	2	80.8	0.33	37.31	-223.2	14.53
	3	60.1	0.36	37.14	-230.3	15.21
	4	74.2	0.46	37.25	-239.0	15.54
	5	87.8	0.51	37.10	-236.7	15.47
H	1	76.0	0.62	36.77	-221.8	14.42
	2	74.1	0.49	36.98	-223.2	14.53
	3	81.0	0.54	36.99	-226.4	15.05
	4	56.0	0.42	36.92	-234.4	15.37
	5	52.2	0.31	36.86	-227.4	15.10
I	1	46.0	0.36	37.31	-226.7	15.06
	2	60.8	0.36	37.48	-214.8	14.19
	3	56.5	0.49	37.40	-207.2	13.49
	4	77.4	0.50	37.27	-223.7	14.55
	5	52.3	0.28	37.17	-226.5	15.06
Mean	1	51.7	0.47	37.12	-228.9	15.16
	2	72.4	0.39	37.24	-223.0	14.52
	3	68.7	0.49	37.22	-220.0	14.40
	4	66.8	0.43	37.17	-229.3	15.17
	5	56.7	0.33	37.13	-226.6	15.06

Figure 5(v): Grand Mean of Advance Group Temperature Data (N=4)

N.B. control mean and confidence interval also shown (darkened ellipse).



% R	= 67.3	Mesor	= 37.3
P-Level	= <0.001	Phi (Deg.)	= -223.0
Amp	= 0.38	Hrs/Mins	= 14.52

Tangents from pole to ellipse:

Phi 1	= -214.4	Hrs/Mins	= 14.18
Phi 2	= -231.7	Hrs/Mins	= 15.27

Patients J,K,L,M (Group 3) **Variable Phase**

It was originally hypothesized that Group 3 would be comprised of patients with severe circadian rhythm disruptions and/or arrhythmicity. What emerged instead was a group of subjects who showed a great variability in the phase angle of intact wave-forms. The general decrease in %R (variability ratio) and increase variability of rhythm amplitude were the only abberations noted (Figure 5(vi)).

This group showed a history of more variable sleep complaints and a marked increase in reported sleep deficit over Groups 1 and 2. From the clinical predictions, two of the four patients (K and L) were orginally placed in the phase advance group with some uncertainty. However, the four patients did form a distinct entity in terms of circadian data. Patients J, L and M showed insignificant individual mean cosinor results for core temperature. Patient K showed a marginal fit ($p = 0.048$), but still a substantial increase in acrophase angle variability, as compared to the matched control.

Hartley's F_{max} test for homogeneity of variance was applied to the acrophase angle of all patient core temperature data. Significant F-values were found in patients J, K, L and M, as shown below in Table 5(iv). This indicated that patients in Group 3 had a significantly greater degree of variability in their acrophase angle than the most variable controls.

Table 5(iv):

Patient	F	P-Level
J	35.2	< 0.01
K	7.6	< 0.05
L	10.8	< 0.05
M	14.5	< 0.01

It seemed reasonable to hypothesize that this acrophase angle variability could be a result of changes in circadian core temperature tau. Since the cosinor model assumed a tau of 24 hours, variations would be overlooked. Also, it was noted that the "goodness of fit" of the cosinor curves in this group was substantially less than that found in Groups 2 and 3.

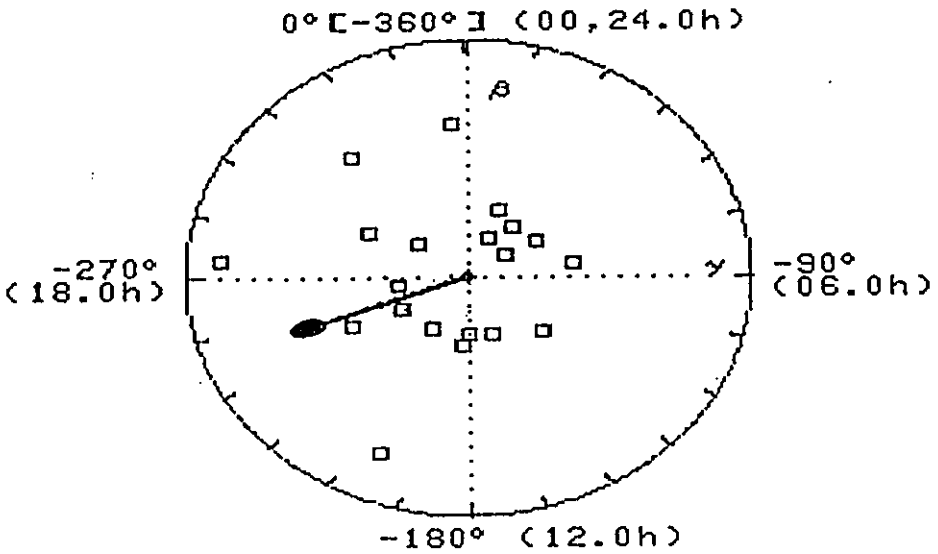
A best-fitting tau analysis was subsequently applied to all circadian core temperature data in Group 3. A range of tau lengths from 2 to 30 hours was used, with curve fitting done at 30 minute intervals. The highest %R and the lowest p-value obtained from the 56 different tau lengths was assumed to reflect the best fitting tau.

The tau length in this patient group showed a greater variability than the controls. The range for best-fitting tau was from 22 to 26 hours. In the individual patients the changes in the tau length in no way reflected the degree or direction of day to day acrophase angle variability. Furthermore, when the best-fitting tau differed from 24 hours, it was noted that the new fitted curve resulted in only a marginal increase in %R or "goodness of fit".

Table 5(v): Temperature Data - Patient Group 3

Patients	Day	% R	Amp	Mesor	Phi (Degrees)	Phi (Hrs/Mins)
J	1	22.9	0.22	37.38	- 55.2	3.41
	2	28.4	0.30	37.33	-355.7	23.56
	3	51.2	0.34	37.28	-260.1	17.20
	4	82.1	0.63	37.40	-243.3	16.13
	5	91.9	1.11	37.86	-213.0	14.12
K	1	25.5	0.52	36.82	- 82.7	5.31
	2	22.5	0.25	37.03	-307.2	20.29
	3	42.1	0.37	37.21	- 37.3	2.29
	4	82.5	0.41	37.40	- 22.0	1.28
	5	78.7	0.40	37.23	- 58.7	3.54
L	1	24.5	1.23	36.68	-274.1	18.16
	2	69.8	0.89	36.65	-321.0	21.24
	3	40.4	0.54	36.75	-297.6	19.50
	4	26.2	0.33	36.37	-177.5	11.50
	5	27.6	0.34	36.17	-209.7	13.59
M	1	30.0	0.37	36.70	- 25.9	1.44
	2	34.7	0.48	36.64	-130.5	8.42
	3	69.7	0.40	36.86	-184.0	12.16
	4	37.4	0.37	36.68	-230.5	15.54
	5	32.9	0.36	36.59	-159.3	10.37
Mean	1	25.7	0.34	37.15	---	---
	2	38.9	0.48	36.91	---	---
	3	50.9	0.41	37.03	---	---
	4	57.1	0.44	36.96	---	---
	5	57.8	0.55	36.96	---	---

Figure 5(vi): Grand Mean for Variable Phase Group Temperature Data (N=4)
N.B. control mean and confidence interval also shown (darkened ellipse).



% R	= 43.9	Mesor	= 36.9
P-Level	= 0.463 (N.S.)	Phi (Deg.)	= -274.2
Amp	= 0.12	Hrs/Mins	= 18.17

Tangents from pole to ellipse:

As the amplitude is not significantly different from zero ($p<0.05$) the ellipse extends beyond the pole and no tangents exist.

TEMPERATURE DATA (Overall):

Repeated measures ANOVA was applied to all of the temperature data of controls and patients. This analysis tested for significant differences (a) between group means; (b) over days; and (c) the group-by-day interactions. Item "c" was especially important in order to examine if there was a significant differential change over time between groups.

%R (variability ratio): There was a significant difference between group means (P=0.002), probably due to the great reduction in the %R mean in patient Group 3. There was also a significant over days interaction (P=0.002) but the group-by-day interaction was not significant. This would indicate that although there was a day effect on %R it was consistent across groups.

Amplitude: There was no significant difference between group means for amplitude. The difference over days was also nonsignificant.

Mesor: There was no significant difference between group means for mesor. The difference over days was also nonsignificant.

Phi (phase angle): There was a significant difference between group means for Phi ($p<0.001$). There was also a significant over days ($p=.006$) and a significant group-by-day interaction ($p<0.001$). This indicated a possible differential change over time between groups. However, when Group 4 (variable phase) was removed from the analysis,

the difference over days was nonsignificant. This would indicate that the highly erratic nature of Group 4 Phi values was the basis of the initial significant results, and thus it could be concluded that the differential change between groups over time was not a factor in the final analysis of these results.

CORTISOL DATA

Group 1

The mean cosinor analysis of Group 1 cortisol data showed a delayed acrophase as compared to control data. However, the increased variability of acrophase angle produced a wide confidence interval and a marginal significance of fit ($p=0.05$) of the mean cosinor curve (Figure 5(vii)).

With approximately 65% of the control confidence interval ellipse overlapping the patient confidence interval, it is apparent that there is no significant difference between patients in Group 1 and the control population in this study.

Group 2

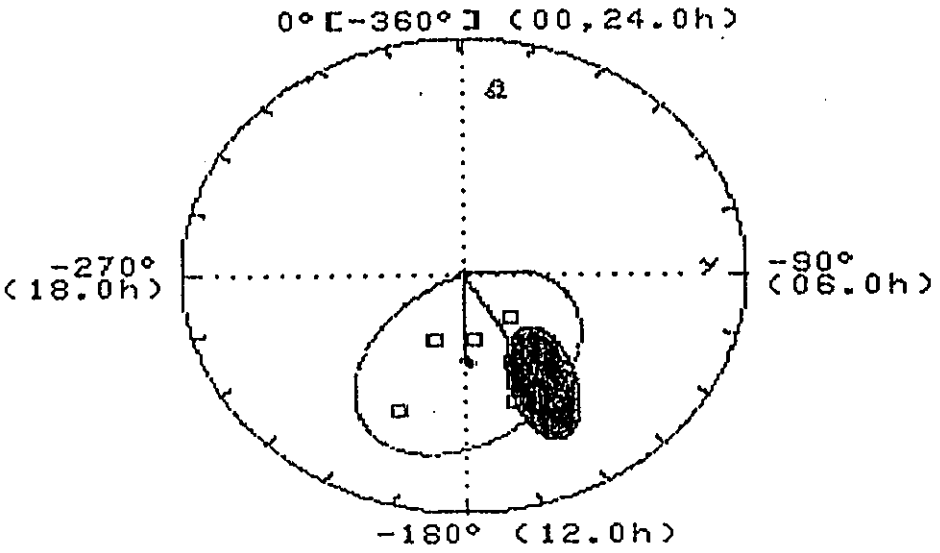
The mean cosinor analysis for Group 2 cortisol data showed a significant advance in the acrophase angle ($t = 3.23$, $p<0.05$) but not amplitude, as compared to control data (Figure 5(viii)).

Group 3

All four patients from Group 3 showed nonsignificant single cosinor curve fitting results for cortisol (Figures 5J(vii), 5K(vii), 5L(vii) and 5M(vii). Patients J and L also showed significant increases in mean cortisol levels ($p=<0.01$) and a significant elevation of daytime cortisol values ($p= <0.001$).

Figure 5(vii): Delay Group Mean of Cortisol Data (N=5)

N.B. control mean and confidence interval also shown (darkened ellipse).



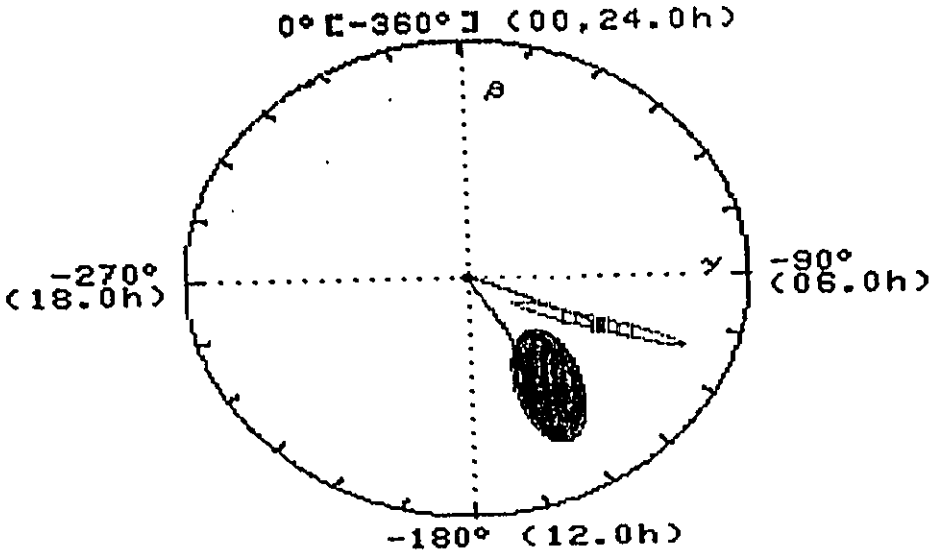
% R	= 58.6	Mesor	= 75.0
P-Level	= 0.050 (marginal)	Phi (Deg.)	= -177.8
Amp	= 40.15	Hrs/Mins	= 11.51

Tangents from pole to ellipse:

Phi 1	= - 81.3	Hrs/Mins	= 5.25
Phi 2	= -241.4	Hrs/Mins	= 16.05

Figure 5(viii): Advance Group Mean of Cortisol Data (N=4)

N.B. control mean and confidence interval also shown (darkened ellipse).



% R	= 60.2	Mesor	= 65.8
P-Level	= 0.016	Phi (Deg.)	= -112.7
Amp	= 56.92	Hrs/Mins	= 7.31

Tangents from pole to ellipse:

Phi 1	= -109.1	Hrs/Mins	= 7.17
Phi 2	= -125.5	Hrs/Mins	= 8.22

MELATONIN DATA

All Groups

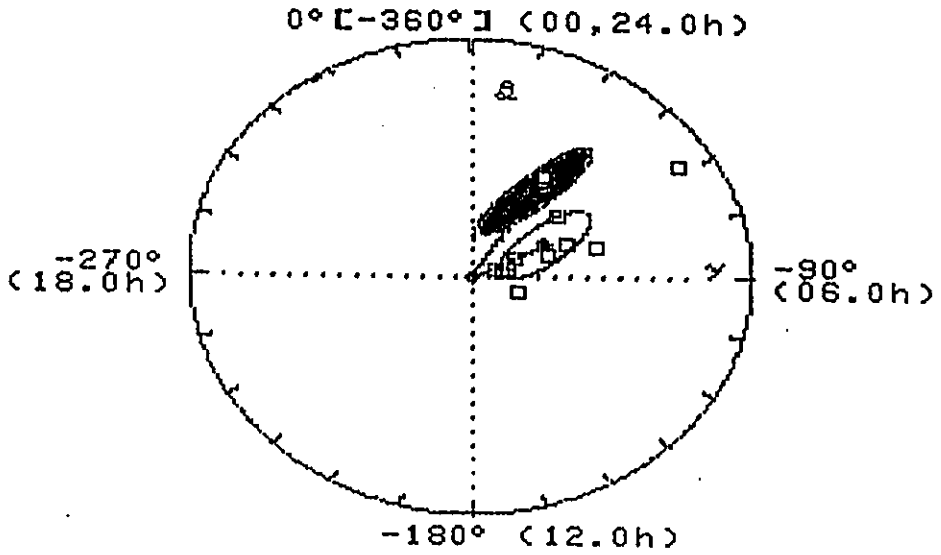
Of the 13 patients who completed this study, two (Patient G, Group 2 and Patient K, Group 3) showed nonsignificant single cosinor curve analysis of the 24 hour melatonin data. Of the 11 patients with significant circadian melatonin results, all but one (Patient D, Group 1) showed a substantial phase delay of the acrophase angle. For this reason, the significant patient data were plotted together as shown in Figure 5(ix).

The patient groups (1, 2, and 3) showed a significant delay in the acrophase angle ($t = 2.88$, $p=0.01$) but not amplitude, as compared to the control group.

An alternate method of analysis was applied using a rise of 20% above individual mean plasma levels of melatonin secretion to indicate secretory onset episodes. This also showed a significant delay in melatonin onset-times, as compared to the control ($t = 4.01$, $df = 21$, $p=<.05$).

Figure 5(ix): Significant Melatonin Data for All Patients (N=11)

N.B. control mean and confidence interval also shown (darkened ellipse).



% R	= 58.5	Mesor	= 24.8
P-Level	= 0.002	Phi (Deg.)	= - 63.4
Amp	= 17.86	Hrs/Mins	= 4.13

Tangents from pole to ellipse:

Phi 1	= - 45.1	Hrs/Mins	= 3.00
Phi 2	= - 92.2	Hrs/Mins	= 6.08

PART IV

MELATONIN TRIAL

This study was performed to test the possible therapeutic benefit of exogenous melatonin in patients with chronic insomnia. A total of 13 patients and two controls took part in a double-blind, single-crossover trial using melatonin as the active compound, and a lactose placebo.

Preliminary Study

To estimate the approximate dose of melatonin and plasma level in patients, a preliminary study was carried out. Two patients were given varying doses of melatonin over a two week period, based on previous trials with normal subjects where a range of 2.5 mg (Matthews et al., 1981) to 100 mg oral doses (Wetterberg, 1982) were given.

Dose Range

Before the trial began, two patients were given varying doses of melatonin over a 12 week period. The dose range was set-up as follows:

A = 2.5 mg (week 1)

B = 10 mg (week 3)

C = 25 mg (week 5)

D = 50 mg (week 7)

E = 75 mg (week 9)

F = 100 mg (week 11)

The patients were to take "A" for seven consecutive days,

nothing for the next seven days, then "B" for seven days, etc. They were asked to maintain sleep diaries and alertness assessment scales, and to log any benefits or side effects of the treatment for the entire period.

Both patients reported a subjective benefit at a dose of 75 mg and no benefit at 2.5, 10 or 50 mg. Both patients also reported "hangover" effects including an early morning "pressor" headache with the 100 mg dose. Based on this result, a dose of 75 mg was chosen for the treatment trial.

Plasma levels of melatonin were determined in two control subjects after taking a single 2.5 or a single 75 mg oral dose of melatonin at 2200 hours (Figure 5(x)), using the technique described in Chapter 2.

The melatonin appeared to be rapidly absorbed at either dose, with peak levels occurring within 90 minutes. The peak value after 2.5 mg dose was 2,630 pg/ml. After 75 mg plasma levels reach 64,730 pg/ml. This is in clear excess of normal physiologic concentration, which in this patient population ranged between 60-80 pg/ml.

After the 2.5 mg dose, plasma levels reached normal physiologic values within seven hours. After 75 mg, the plasma level was still at 298 pg/ml when the sampling was terminated at 0900 hours.

Results

Three important variables were assessed by the individual patients during this trial. These were: (1) subjective awareness of

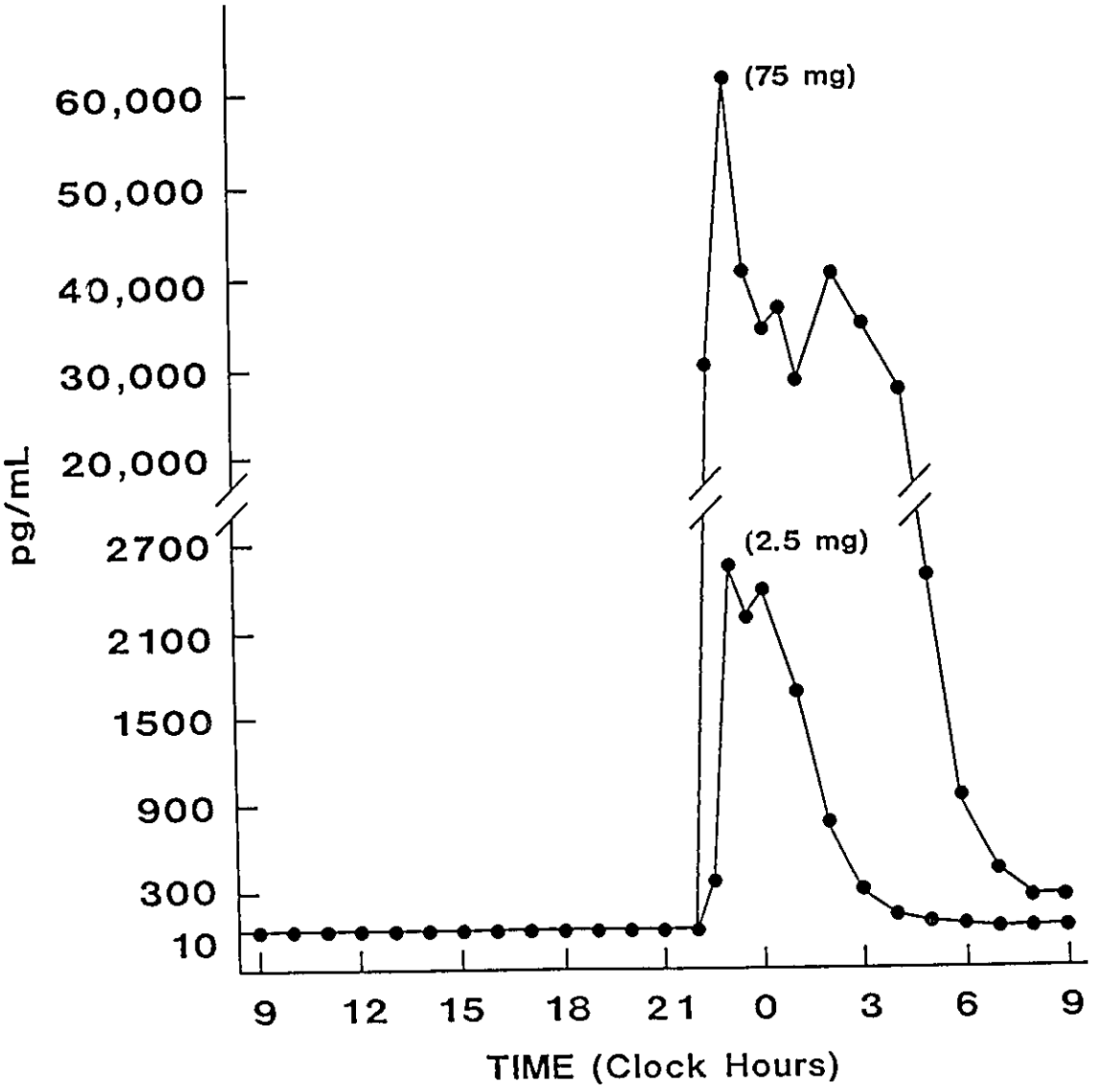


FIGURE 5(x): Serum melatonin level after a single 2.5 or 75 mg oral dose

melatonin vs. placebo; (2) subjective sleep time, and (3) subjective daytime alertness. Since the sleep time is assumed to directly affect daytime alertness in these patients, these were considered dependent variables, and therefore a multivariate analysis of variance (MANOVA)was performed.

There was a total of 182 observations (days) in this trial (91 on melatonin, 91 on placebo). Patients were able to ascertain when they were taking the active compound only 65.9% of the time. While taking placebo, patients were correct only 64.8% of the time.

PERCEIVED	ACTUAL: Melatonin	ACTUAL: Placebo
Melatonin	60	27
?	6	5
Placebo	24	59

The above data coupled with subjective reports suggest that there was not an obvious side effect that would allow patients to easily identify the melatonin.

The MANOVA was applied to all the data with one grouping factor (melatonin or placebo) and two dependent variables (sleep-time and daytime alertness). A significant Treatment effect was apparent (sleep: P=0.0019; alert: P=0.0013). The mean group statistics are shown in Table 5(vi) and plotted in Figure 5(xi). The graph shows that sleep

time and alertness covary as originally predicted. It also shows a clear trend of increased sleep time and alertness over the seven days of melatonin administration. However, this did not reach statistical significance.

Table 5(vi)

	DAY	VARIABLE	MEAN	STD. ERROR
MELATONIN	1	Sleep	4.21	0.77
		Alert	4.33	0.31
	2	Sleep	4.40	0.68
		Alert	4.00	0.33
	3	Sleep	5.28	0.48
		Alert	3.75	0.37
	4	Sleep	5.20	0.36
		Alert	3.67	0.33
	5	Sleep	4.50	0.42
		Alert	3.83	0.30
	6	Sleep	4.82	0.43
		Alert	3.33	0.28
	7	Sleep	5.02	0.39
		Alert	3.33	0.38
PLACEBO	1	Sleep	3.45	0.59
		Alert	4.08	0.38
	2	Sleep	4.05	0.65
		Alert	3.92	0.38
	3	Sleep	3.27	0.59
		Alert	4.33	0.33
	4	Sleep	3.44	0.50
		Alert	4.17	0.34
	5	Sleep	3.33	0.59
		Alert	4.50	0.29
	6	Sleep	3.62	0.56
		Alert	4.42	0.31
	7	Sleep	3.71	0.60
		Alert	4.42	0.36

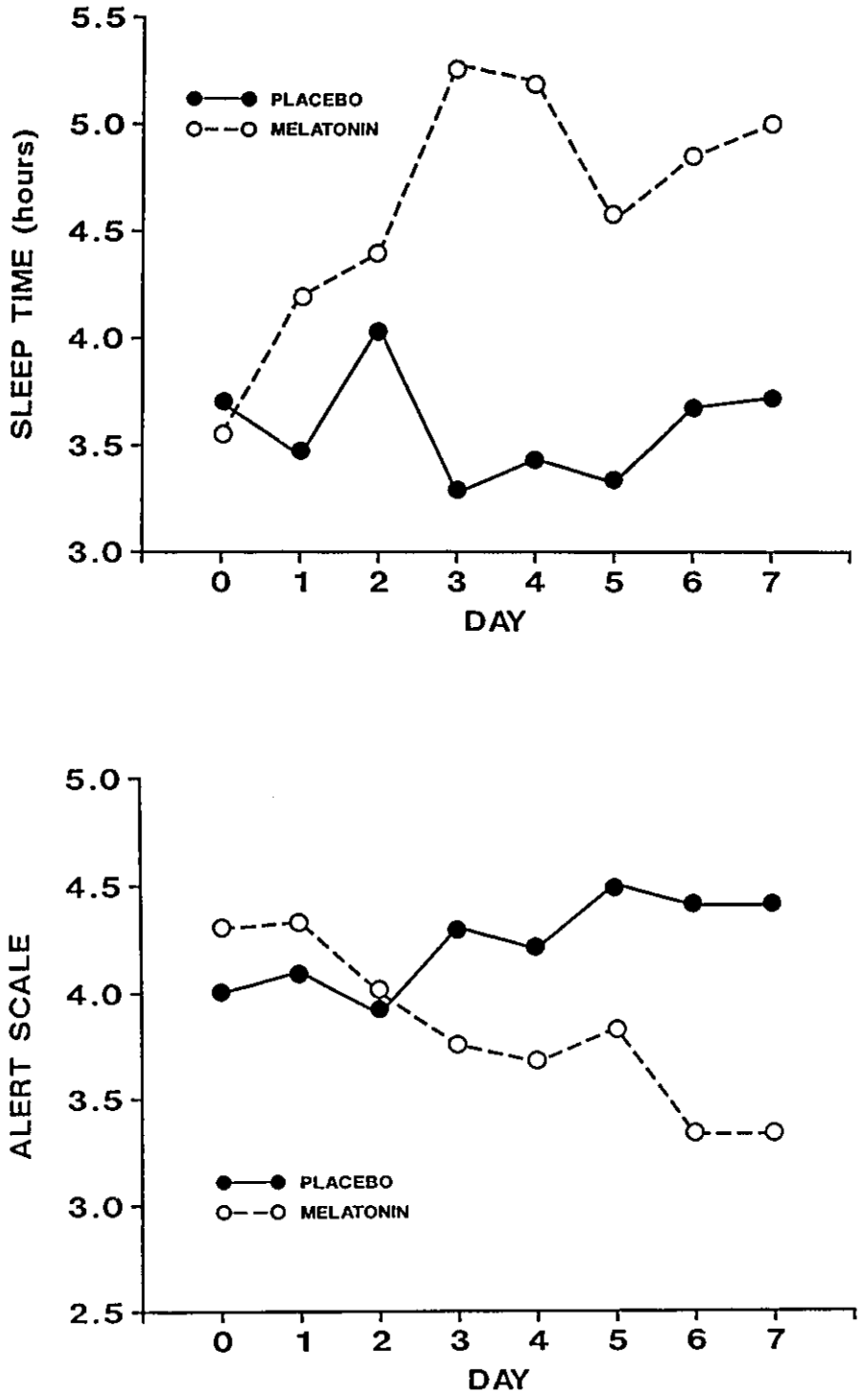


FIGURE 5(xi): Mean sleep-time and daytime alertness rating for all patients during placebo (x7 days) and melatonin (x7 days) treatment. N.B.: alertness scale: 0 = wide awake, energetic; 7 = cannot stay awake

CHAPTER 6

DISCUSSION

PART I

The main contribution of this work is that chronic insomnia has been characterized by three types of disordered of circadian phase: 1) delayed, 2) advanced, and 3) variable. While Type 1 had been clearly described by Czeisler et al. (1981b), Type 2 had been reported in only a few cases. Type 3 had not been described, although the term irregular sleep/wake cycle in the Association of Sleep Disorders Centers (ASDC, 1979) diagnostic system hinted at its existence. To date, these three cycle disorders have been considered special types of insomnia, characterized by the following clinical features: Delayed sleep phase insomnia is characterized by an intractably late sleep onset and awakening time, a consistent sleep time-frame, and an essentially normal sleep EEG. In advance phase insomnia, sleepiness becomes overpowering in the late afternoon or early evening, followed by a very short sleep onset latency and an exceedingly early awakening time. The EEG is essentially normal. In amplitude disorders and arrhythmias, sleep is marked by variable onset latencies, and severe difficulties with sleep maintenance.

This study indicates that chronic insomniac patients with a variety of presenting symptoms are very likely to demonstrate one of the three phase abnormalities. The surface characteristics are variable, with so called psychophysiological features, with dysthymic mood, with depressive disorder in remission, etc. (see clinical descriptions, Chapter 3).

All but one of these patients appeared to have a substantial phase disturbance as compared to controls. Since this disturbance is robust over five days, and since it is known that such rhythms are resistant to change, it is reasonable to infer that the prolonged chronicity of insomnia is mediated by underlying chronobiologic dysfunction.

This study also indicates that it may be possible to identify each of the three types of rhythm disturbance on the basis of clinical features that can be obtained by interview. The most important aspects appear to be: 1) chronicity of symptoms, 2) consistency of symptoms, 3) pattern of symptoms, 4) psychiatric and medical histories, and 5) recent drug histories. In addition, polysomnographic studies are required to exclude specific disorders such as nocturnal myoclonus.

PART II

Temperature Data

Group 1 (Phase Delay): It was originally hypothesized that chronic late bedtime and the desire for and/or an actual late arousal would be associated with a phase delay of core temperature. The five patients assigned to the phase delay group by the clinical criteria outlined in Chapter 4, showed a pronounced and statistically significant phase delay of their core temperature acrophase. The clinical data collected were adequate to assess and accurately predict this particular outcome variable.

A simple explanation of this phenomenon is that a consistently late bedtime would induce a phase delay. It would be expected that chronic enforced wakefulness in the late evening of control subjects would show a similar temperature acrophase delay. However, normal subjects quickly re-adapt to baseline conditions after such phase shifts. Even though all of these patients had a great desire for a much earlier sleep onset time, they were incapable of phase advancing their sleep/wake cycles.

Of anecdotal interest was the fact that three out of five patients in this group benefited from phase delay chronotherapy as originally explored by Czeisler et al. (1981b). This consisted of delaying their sleep time by two hours every night for approximately ten nights until a desired bedtime had been established. Remarkably, these patients were able to phase delay themselves clockwise halfway around the clock in a few days. Yet, they were incapable of entraining

to desired schedules by phase advancing themselves by a relatively few hours counter-clockwise, having tried for many years to do so.

Thus, chronic sleep onset insomnia appears to involve a defect in the circadian timing system. In this case the patient is incapable of entrainment by phase advance in normal living conditions where the natural light/dark cycle would presumably act as the most potent zeitgeber. These patients would likely induce phase delay rapidly after several consecutive late nights. When it came time for an earlier bedtime and awakening time (i.e., Monday morning) these patients would remain "stuck" in their old time schedule. All these patients had been stuck in a delayed phase for years. The amplitude, mesor, and the %R value are very close to that of the control data. This would imply that the timing and not the generation of this circadian rhythm is the primary defect since it involves displacement of an intact waveform.

Group 2 (Phase Advance): The second hypothesis predicted that a chronic insomnia associated with early awakening would be associated with a phase advance of core temperature acrophase. Of the six patients originally assigned to this group, only four showed a clear and statistically significant phase advance of core temperature acrophase. The failure to accurately assign two of the patients was likely secondary to the failure to ask a specific clinical question; that is "how consistent is the pattern of sleep onset and awakening?". Retrospective assessment of these two patients showed an increased variability of sleep patterns over the other four patients which

indicated inclusion with Group 3 patients. This finding will permit the inclusion of additional questions in the clinical assessment of these patients so as to test its true predictive validity again in future studies.

Unfortunately, chronotherapy for the individuals in this group was too impractical. In this group of four patients, three were unable to accommodate due to work commitments, and one was unwilling. It would have been valuable to explore the possibility of phase advancing these patients back around the clock (counter-clockwise) to an appropriate phase-angle. As with the phase delay group, these patients may be only capable of unidirectional entrainment, having been fixed for years in a pattern of early wakening regardless of bed time. In this case, susceptibility to phase advance, and inability to phase delay may be the root of their particular presenting complaint.

Phase advance of the circadian sleep/wake cycle in depressive illness has long been recognized. One of the classical symptoms of this disorder is early morning awakening (Mendels & Cochrane, 1968). Morgan and Cheadle (1976) showed that core temperature rhythms of schizophrenic patients were significantly phase advanced with respect to control subjects. Similarly, REM onset latency is much shorter (phase advanced) in depressive patients (Kupfer et al., 1978). In light of these findings, it is important to emphasize that all patients in this group had essentially normal MMPIs and unremarkable current psychiatric histories. This would then rule out the possibility that the circadian findings for this group could be secondary symptoms of occult depressive illness.

Only three cases of advanced sleep-phase syndrome have been reported in the literature (Czeisler et al., 1986; Moldofsky et al., 1986; Kamei et al., 1979). Weitzman et al. (1982) reported a shortening of the internally synchronized, free-running period of core temperature associated with advancing age. With an endogenous circadian period of less than 24 hours, the individuals would be susceptible to phase advance with associated early evening fatigue and fragmented early morning sleep. However, in the present sample of four, the eldest patient was only 54 years. The disorder is therefore not associated with normal aging, within the age range studied here. Larger samples with a wider range of age will be required to confirm Weitzman's (1982) observation.

Advanced sleep-phase insomnia appears to be a much rarer phenomenon than delayed sleep-phase. Intuitively, one would expect this as the 25-hour endogenous human circadian cycle tends to delay in situations of non-entrainment. In some individuals there may be an endogenous circadian period which is less than 24 hours (Wever, 1986). In still fewer individuals, an inability to undergo daily "adjustments" to a 24-hour light-dark cycle could leave them in a state of phase advance. Thus, phase advance and phase delay sleep-onset insomnia may represent similar clinical entities. The difference may lie in the free-running period length of the endogenous circadian pacemaker. The predominance of phase delay patients may merely reflect the fact that the endogenous period length is greater than 24 hours in a larger proportion of the general population.

Unlike the phase delay group, the circadian data did not closely parallel the subjective sleep reports in the phase advance group. The sleep diaries reported a phase advance of 120 to 415 minutes (mean = 268.75), whereas the difference between the means of the control and patient temperature acrophase data was only 97 minutes. However, all patients were required to maintain a schedule with lights-out occurring at 2300 hours. This meant that there was a period of enforced wakefulness in the evening for the phase advance group whereas enforced wakefulness in the phase delay group would occur in the morning hours with the imposition of "lights-on" by 0800 hours.

The "masking" effect of sleep on the temperature cycle is well documented (Aschoff, 1970; Mills et al., 1977). Concomitant with sleep onset is an increased rate in the downward trend (increased angular velocity) of core temperature. This rate of descent, which would have an obvious impact on cosine curve fitting, would be affected more by delaying sleep onset than by advancing awakening time. This would suggest that the timing of sleep has little or no synchronizing effect on core temperature. It may, however, affect the curve fitting by reducing the beween subject variability of the descending portion of the core temperature rhythm. Thus, although the protocol matched schedules between the patients and controls, the differences were highly significant.

Group 3 (Variable Phase): The third original hypothesis was that patients with variable sleep complaints (sleep onset and awakening)

and exceedingly short sleep episodes would display a complete arrhythmia of the circadian core temperature cycle. It was assumed that when cosinor analysis was applied to the circadian data of this group, that it would indicate a temperature pattern that was random rather than cyclic. Of the four patients in this group based on temperature data, only two were accurately assigned based on clinical assessment, as previously discussed. The key feature is that sleep is an unpredictable event and the pattern varies greatly from night to night.

The results of this group were completely unexpected. Cosinor analysis showed a significant ($p<0.05$) curve fitting for all 20 recording days on the four patients in this group. Further, the acrophase cycle displayed a huge night to night within subject variability (up to 11 hours, 14 minutes). The degree of variability of acrophase angle was statistically significant as compared to the most variable control. Patients from Groups 1 and 2 showed non-significant variability when similarly compared.

These data are not consistent with any other in the literature. Czeisler et al. (1986) report the largest shift of any, a six hour phase delay between two successive circadian cycles, but this followed critically timed exposure to bright light. Without such intervention occurring in Group 3 patients, it must be cautioned that there may be an inherent defect with the cosinor analysis. What is apparent in this group is the substantial reduction in the individual and overall %R. This would imply that a large portion of the 24-hour data was not explained by the cosinor model and was therefore due to non-sinusoidal,

possibly random variation. Also, several of the p-values for the curve fitting approached 0.05 indicating a marginal "goodness of fit".

When curve fitting was attempted over a range of period lengths (2 - 30 hours), p-values were greater than .05 except when the period approached 24 hours. There were, however, three period lengths at which the p-values were much closer to 0.05 than the others. At 3, 7 and 18 hours it appeared that the curve fitting was moving towards significant levels. In future studies, it would be valuable to apply spectral analysis, which elucidates all major frequency components, to data that defies cosinor analysis. It is possible that significant ultradian period components could exist in this group that would be of considerable interest. The limitations of the present study allow only the conclusion that there is an increased, point to point, within subject variability in Group 3.

PART III

Cortisol Data

In the original hypothesis, it was predicted, based on the two oscillator model of circadian control (Moore-Ede et al., 1982), that the circadian pattern of disruption serum cortisol levels would parallel changes in core temperature rhythm data.

Group 1 (Phase Delay): There was no significant difference in the serum cortisol acrophase angle between patients in Group 1 and controls. The apparent, but non-significant, delay in individual patient acrophase angles produced a wide confidence interval on the mean which completely overlapped the control mean and confidence interval. Examination of Figure 5(vii) shows a trend towards a phase delay in the patient cortisol data, and it is possible that an increased sample size in this group may have rendered a significant result which matched the original hypothesis.

Group 2 (Phase Advance): There was a significant difference between patients and controls in this group, even with this smaller sample size (N=4). The acrophase angle of all four patients was within a remarkable range of 19 minutes.

These data are of particular interest in light of the established endocrine circadian literature. Cortisol and its trophic hormone ACTH show a rhythm with peak concentrations at or about the normal time of waking, with a nadir occurring soon after sleep onset

(Krieger, 1979). In free-running conditions, humans showed an abrupt diminution of cortisol secretion that coincided with sleep onset (Weitzman et al., 1981b). The duration of this effect was one to three hours, always at the beginning of the sleep episode. In their analysis, they used sleep onset as a "starting or zero point" about which a time-locked response cortisol curve was obtained for several subjects. This apparent "gating" effect of sleep onset on cortisol could explain the cortisol data seen in this study. With the restriction of bedtime/lights out to 2300 hours, these patients would experience a period of enforced wakefulness in the evening hours. After lights-out, sleep onset occurred within 10 minutes for all subjects in this group, giving rise to a highly consistent sleep onset time, and a much more variable time of sleep termination. Alternately, Group 1 had a highly variable sleep onset time, and a very consistent sleep termination time with enforced awakening at 0800 hours as outlined in the protocol. These results would indicate that the circadian phase of cortisol secretion could be cued specifically by sleep onset, but not be dependent on it, as circadian rhythmicity persists even during continuous sleep deprivation (Poland et al., 1972).

However, the results from the present study may be influenced by feeding times as well as by the time of sleep onset. Sulzman et al. (1977) and Krieger (1974) showed that the timing of the first subjective daytime meal in rats could reset the phase of the cortisol rhythm. Phase advance patients in the present study would be expected to begin their daily food intake at a much earlier time than the phase delay

group. Restriction of meal times should be included in future studies so that the influence of sleep onset time on the entrainment of cortisol rhythms can be assessed in humans.

Group 3 (Variable Phase): The original hypothesis predicted that patients in this group would demonstrate a pattern of circadian cortisol secretion that would parallel changes in core temperature but could become dissociated from the timing of the sleep/wake cycle or the melatonin secretory cycle, or that no rhythm would exist. The four patients in Group 3 with variable core temperature acrophase showed a pattern of cortisol secretion that was variable and not cyclic.

Two out of the four patients also showed significant increases ($p<0.01$) of the mesor (Patient K = 202 ng/ml, Patient L = 171 ng/ml). Both showed completely random circadian secretory patterns with exceedingly high levels in the afternoon and evening, as compared to controls. Both of these patients had a history of depression, and both met the criteria for Dysthymic Disorder on the MMPI and by DSM III diagnosis. Rubin et al. (1980) showed that patients with endogenous depression had higher serum cortisol levels during most of the night and day. This was particularly evident between 2400 and 0300, when the mean cortisol level of depressed patients was twice that of control subjects. However, there are two fundamental differences in the data from the present study. First, circadian studies of Rubin et al. (1980), Carroll et al. (1976) and Carroll (1976) show a cortisol rhythm in endogenous depression with increased point-to-point mean cortisol

levels but with virtually identical period and amplitude, although, it should be noted that Sachar (1975) showed a reduction in rhythm amplitude. Secondly, these high cortisol values tended to normalize as treatment was administered and remission ensued. The two patients in the present study were in a depression free period (> 1 year) according to DSM-III criteria, and were off all antidepressant medications. Despite this, they still showed greatly elevated and highly variable cortisol secretory episodes. It is of interest that these two patients were originally assigned to the phase-advance group based on clinical assessments. Weitzman (1980) and Feinberg et al. (1982) have reported early awakenings from sleep plus an apparent redistribution of REM sleep to an earlier portion of the sleep period to be concomitant features of both bipolar and unipolar depression. Again, these anomalies tend to normalize in the free periods. It is possible that in the clinical assessment these two patients were expressing changes in sleep/wake patterns experienced during depressive episodes rather than the more general and long-term changes in the free periods.

PART IV

Melatonin Data

The original hypothesis suggested that the circadian pattern of melatonin secretion would parallel changes seen in serum cortisol, core temperature, and sleep-wake cycles in Groups 1 and 2. In the case of Group 3, the melatonin rhythm could follow the sleep/wake cycle, or may align itself with the rhythms of core temperature and cortisol, depending on the nature of the circadian control of melatonin secretion.

These were the most intriguing results of all. Eleven patients showed a substantial phase delay of an intact 24-hour melatonin wave-form. Lack of correlation between melatonin and core temperature acrophase implies that either the pineal melatonin rhythm is not coupled to the same oscillator as core temperature or that a dissociation is a part of the pathology. This becomes especially apparent in Group 3, where three patients display a completely variable pattern of both core temperature and serum cortisol secretion with an apparently normal, although phase delayed, melatonin rhythm. Two patients, one from Group 2 and another from Group 3, showed non-cyclic, variable patterns of melatonin secretion.

Since the pattern of melatonin secretion appears to be so consistent between groups, it follows that some stable between-group factor should exist that may help explain these data. That factor could be nocturnal wakefulness. When the sleep diaries for the 11 patients with significant melatonin rhythms were examined, out of the 154 nights documented, patients reported 121 nights with a waking episode of more

than 30 minutes duration between the hours of 2400 and 0300. This is the approximate confidence interval on the timing of peak melatonin values in normal control subjects. Out of the 126 nights documented by control subjects, only eight nights showed waking periods during this time frame.

Often reported with these nocturnal arousals were trips to the washroom or kitchen, or a period of reading, all of which would involve exposure to low level lighting (<300 lux). Lewy et al. (1980) discovered that bright light (2500 lux) can suppress nightime human melatonin production, but that ordinary room light cannot. Lewy et al. (1985) subsequently found that in 11 euthymic (currently well) bipolar patients suppressed twice as much as normal controls, and suppressed at much lower light intensities (<500 lux). From this they speculated that the circadian component of the depressive disorder may well derive from the patients' apparent "supersensitivity" to light.

However, the presumed exposure of the phase advance group to greater amounts of AM light specifically, should have produced a corresponding phase advance of the melatonin rhythm. Patients from all three groups showed a parallel change in the phase of core temperature, cortisol, and sleep/wake rhythms, even though -- 1) core temperature and cortisol, and 2) the sleep/wake cycle are under the control of separate mutually coupled oscillators (Moore-Ede et al., 1982). The lack of correlation in the phase shift of melatonin could imply that melatonin is not under the discrete control of either oscillator.

Illnerova and Vanecek (1982) have described a two-oscillator

control of the circadian timing of melatonin secretion in the rat. A morning (M) -- phase advance, and evening (E) -- phase delay oscillator are together responsible for the appropriate phase control of the melatonin rhythm. The relative coupling strength between the two oscillators was found to be different. That is, E has a greater impact on M than M has on E. This fits with Kronauer et al. (1982) where mathematical models of two circadian oscillators with different coupling strengths explained much of the existing circadian data. These two theories taken together implicate the SCN as having the role of the M-oscillator.

In the present study, all patients exhibited a phase delay suggesting that the E-oscillator had become somehow dominant. This may mean that a predominance of phase-delay information might impact on the pineal. It is possible that a higher threshold to the effects of light on the SCN could give rise to the consistent phase delay observed. This is supported by Lewy et al. (1985) who provided effective treatment of chronobiologic sleep disorders with bright light exposure (>2000 lux) in the early morning.

Chronic insomnia is not accompanied by a significant reduction in the mean or amplitude of circadian melatonin secretion. This supports the notion that melatonin is a coincidential rather than a causal element in sleep. However, the possibility of changes at the level of CNS melatonin receptors cannot be disregarded. Livezey et al. (1978) have demonstrated a permanent down regulation of benzodiazepine receptors in the CNS after chronic prenatal exposure to diazepam in

rats. This resulted in enduring reductions in slow wave sleep. Some individuals could have a predisposition to benzodiazepine or melatonin receptor alterations. The synchonizing effects of melatonin seem to be mediated through receptor binding at the SCN (Cassone et al., 1986). Reduced melatonin receptor density or affinity (for whatever reason) could attenuate phase information to the SCN (or M-oscillator). This would allow the E-oscillator to predominate, thereby phase delaying the circadian rhythm of melatonin.

Sharma et al. (1989) have shown a significant correlation between age and the acrophase angle of melatonin secretion. Under free-running conditions, Moore-Ede et al. (1982) have demonstrated characteristic changes in the relationship between circadian rhythms under the control of X- and Y-pacemakers. The first stage is termed "internal phase drift" where there is a drifting away of a particular rhythm parameter as compared to another. Sharma et al. (1989) have suggested that their elderly patients could be exhibiting some elements of this process with desynchrony between X- and Y-pacemakers as evidenced by a clear and consistent acrophase delay of melatonin as compared with the acrophase of cortisol secretory rhythms. They speculate that a diminished responsiveness of the Y-pacemaker to environmental zeitgebers, as a function of age, could leave the circadian system susceptible to circadian disorders.

In the present study, chronic insomnia related to chronobiologic phase disorder was associated with a similar acrophase delay in the circadian pattern of melatonin secretion. There was no difference in

the diurnal or nocturnal melatonin levels in patients as compared to controls. It is hypothesized that the pathology may lie at the level of the SCN where a hyposensitivity to light or a down regulation of melatonin receptors could reduce the influence of the SCN on the circadian timing system. However, there is no evidence for this at the present time.

PART V

Melatonin Trial

The original hypothesis predicted that an oral dose of melatonin could act to decrease sleep onset latency and/or increase total sleep time in patients with chronic insomnia. It was also suggested that the dose required for a threshold effect on these parameters might differ for patients and controls.

Arendt et al. (1984) have reported significant effects of 2.0 mg oral doses of melatonin on evening sleepiness in normal controls. In the present study, two patients reported no change in evening fatigue with 2.5, 10, 25, or 50 mg oral doses. The first subjective effect was noted at 75 mg. Although the sample is too small for statistical analysis, it does suggest that there is an increased threshold to the effects of melatonin in patients as compared to controls.

A 75 mg single oral dose given at 2200 hours was shown to have a significant effect on total sleep time ($p<0.01$) and on daytime alertness ($p<0.01$) as compared to placebo. The increase in mean total sleep time while on melatonin between day 0 and day 7 was 92 minutes, as compared to placebo where there was actually a decrease of four minutes. Mean daytime alertness increased by 0.97 units on the five point alertness scale (see Appendix B) while decreasing by 0.37 units during placebo administration.

This indicates a substantial effect of melatonin on sleep time and daytime alertness. However, seven of the 13 patients rated the effect of melatonin on their insomnia as subjectively insignificant.

MANOVA statistics showed a uniform treatment response by demonstrating a nonsignificant differential change in sleep time and alertness across days between patients. This would rule out a selective treatment effect. Also, the seven patients were able to accurately identify the active compound 58.5% of the time -- comparable to the remainder (66.7%). The most notable difference with these seven patients was found in the clinical histories. Six of the seven patients reported having met with little or no success after trying a variety of benzodiazepines to treat their insomnia. Possibly, these six patients entered the trial with a degree of pessimism as to the outcome. It is remarkable that some of these patients were experiencing sleep episodes that were substantially longer than any experienced during the baseline week, yet they still reported that this had no effect on their subjective feeling of "well being".

There was, however, a clear delayed response to melatonin but it did not reach statistical significance. This is most likely related to the inadequate power afforded by a relatively small sample size. Melatonin took two or three days to have its full effect (see Figure 5(xi)). This has been reported elsewhere in the literature (Arendt et al., 1986). It is possible that the seven patients who assessed the melatonin treatment as ineffective were convinced during the first two days that there was going to be no beneficial response to treatment. These patients were established "non-believers" in other well established and powerful pharmacologic treatment strategies. Therefore, even though seven of the 13 patients claimed not to have benefited from

the melatonin treatment, it cannot be dismissed as a nonsignificant clinical entity. Future clinical trials with melatonin should involve larger sample sizes, longer treatment intervals, and a detailed drug history which includes retrospective assessment as to the effectiveness of such treatments. This would provide three important group comparisons: Group 1 -- those who avoid drug intervention; Group 2 -- those who have had long-term treatment success with drug intervention; and Group 3 -- those who have tried many different unsuccessful drug treatments. It will be important to determine if there is a differential response to treatment between such groups.

PART VI

General Discussion

Chapter 2 reviewed the current literature regarding the general principles involved in the generation of the sleep/wake cycle, and those systems involved in the timing of the sleep episode within the 24-hour circadian cycle. Any study which measures the overt output or manipulates any part of this circadian system will provide results that may then be interpreted in terms of these extant theories. These data will either support or dispute the theories. More ambiguous data may just cloud the issues. The intent in the final section of this thesis is to relate data from the present study to existing data and theories, to summarize how these data may extend these theories, and to project how future studies may effectively confirm the new findings.

Diagnostic Criteria: The Association of Sleep Disorders Centers (ASDC) (1979) has established a nosology of sleep and arousal disorders. This study focused on disorders of the sleep-wake schedule, in the ASDC categories of delayed sleep-phase syndrome, advanced sleep-phase syndrome, and irregular sleep-wake pattern. Clinical sleep laboratory screening allowed other sleep dysfunctions to be ruled out in the present patient sample. The classification of persistent psychophysiological (learned) insomnia (PPI) was the only category with which there was a modest overlap. The ADSC nosology cites two separate but not mutually exclusive factors as the causal elements, namely

tension-anxiety and negative conditioning. This type of insomnia has been more recently investigated (Mendelson et al., 1986; Hauri and Fisher, 1985). Some of the salient features include the lack of the paradoxical first night effects[1], sleep reports that greatly underestimate the polygraphic data, increase in conversion hysteria and psychopathic deviance scales on MMPI, hyperalertness in bed or bedroom, long sleep latencies, long awakening periods during the night, and little or no early morning wake time. One patient, originally included in this study, demonstrated some of these characteristics (especially underestimation of sleep reports) and was subsequently excluded. However, we had used the term "non-restorative sleep syndrome" to describe this patient rather than PPI. He was viewed as having a very poor grasp of his relative state of consciousness, rather than a complex psychological symptom cluster. This was a slow, subdued person in whom the transition between sleeping and waking may be less demarcated than in other individuals.

The remainder of the patient population (N=13) showed none of the features outlined above despite claims by Hauri and Fisher (1985) that "almost all" insomnia of non-medical origin falls into the category of PPI. However, some patients did present with reports of frustration and arousal (possibly conditioned) associated with bedtime rituals, as is to be expected since they are aware of the negative impact that sleeplessness has on their daily lives. An important finding was that

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[1] Insomniac patients most often report an increased total sleep time on the first laboratory recording night as compared to their own baseline sleep-time.

some patients showed a moderate inability to accurately assess their total sleep time as compared to controls. However, the difference between subjective assessment and polygraph data was almost identical (for both patients and controls) when Stage 1 sleep was eliminated from the analysis. This indicated that the underestimation of total sleep time was correlated to the large increase in % Stage 1 sleep in some patients. Thus it appears that many of the features described by Hauri and Fisher (1985) characterize many patients with chronic insomnia. However, the full set of diagnostic criteria for PPI were not observed in our patients. A recent report by Schneider-Helmert (1987) supports the notion that poor sleep function in insomniacs is more related to a deficiency of sleep-controlling mechanisms than to specific state and trait psychological factors, which may be a result of the insomnia itself. Future studies should continue to examine the issue to ensure a resolution as the data accumulate.

Temperature Data: The observations made for Group 1 (phase delay) and Group 2 (phase advance) served to illustrate some interesting concepts. The significant phase delay and phase advance in the acrophase of the two groups, with no significant change in amplitude, period, or mesor as compared to the control group, supports work done by Czeisler et al. (1981b) where "phase disorders" were proposed to involve a defect in circadian rhythm timing but not in the generation of the rhythms themselves. They also hypothesized that such patients are only capable of unidirectional phase shifts during rhythm entrainment. This was

supported by the success of phase delay chronotherapy in three of five patients in Group 1. In fact, two out of the three claimed that this method was the only one that had produced desirable results in a long search (>10 years) for an effective treatment strategy. Those two patients are still using this technique 18 months after the completion of the study.

I suggest that phase delay and phase advance insomnia may reflect almost identical mechanisms. The difference may lie in the underlying endogenous circadian period length. This would relate to the work of Zulley et al. (1981) who demonstrated a relationship between changes in circadian period length and objective disruptions of sleep. Subjects entrained to an artificially lengthened day, which resulted in a phase-advanced temperature rhythm, experienced short REM latencies and difficuty with sleep maintenance. Subjects entrained to a shortened day experience phase-delayed temperature rhythms with concomitant increases in sleep onset latencies. It is possible then, that the patients in the present study exhibit increases or decreases in endogenous circadian period lengths (outside of the range of normal) which give rise to subjectively and relatively "short" and "long" days. However, it is also possible that the sleep patterns in these individuals are more dependent on phase shifting of normal circadian periods. Czeisler et al. (1980b) and Zulley et al. (1981) also demonstrate the dependence of sleep onset, total sleep time, and awakening time on the circadian phase of core temperature. Future studies should examine these two groups in free-running conditions by placing patients in an isolation facility

free of environmental time cues. In such an environment, the circadian oscillators assume their endogenous period lengths since they can no longer "depend" on external time cues for periodic information (Aschoff, 1965). After the period length had been established, entraining elements (i.e., light-dark) could be imposed in various ways to determine the range of entrainment and the phase-response curve (discussed in Chapter 2) and the threshold light intensity required for entrainment. These data could provide important clues as to the etiology of the phase related insomnias.

The mechanism involved in Group 3 (variable phase) is difficult to evaluate in terms of existing data. As previously discussed, it is unlikely that the variable phase pattern of core temperature acrophase represents a true circadian model. What is apparent is that this group has a greater degree of random variation in their circadian rhythm generation. These patients may represent a distinct clinical entity, different from Groups 1 and 2 with the defect lying at the level of rhythm timing and/or generation. The core temperature cycle can be influenced by a number of physiologic processes. These "masking effects" are most notably brought about by the impact of sleep and wakefulness, activity and rest, posture, etc. Since the activity-rest and sleep-wake cycles of these patients is so highly erratic, it is difficult to ascertain to what degree the present results reflect this interaction. Spectral analysis of these data would allow a comparison of the ultradian components of the rhythm to those behavioural elements thought to be capable of masking endogenous circadian patterns. Again,

the only way to examine this possibility is to monitor the patients in an isolation facility for a longer time interval.

One final issue with regards to the temperature data involves a vital question which has never been properly addressed in the literature: is the change in circadian core temperature pattern, in this study and in others, a reflection of the underlying circadian pathology or merely a concomitant of the sleep disturbance itself? A simple study could be expedited to clarify this important issue. Patients undergoing ambulatory core temperature monitoring could report each morning with a detailed sleep diary. An age and sex matched controls would be instructed the following night to follow the exact same sleep pattern. That is, the control must ensure that sleep onset latency, total sleep time, nocturnal awakenings, and early morning awakenings are reproduced as closely as possible to the patient's sleep diary from the previous night. This would provide a control recording period over many circadian cycles, where the sleep-wake pattern was practically identical to that of the patients. Such a protocol would not determine whether rhythm control in patients is abnormal. However, concerns regarding the degree of reliability for core temperature as an index of circadian system integrity would be adequately resolved. If controls temperature rhythm shifted in the same direction as sleep onset and wakening, then changes in circadian temperature would be secondary to sleep disturbance. If not, then abnormality of circadian rhythm regulation may underlie chronic insomnia.

Cortisol Data: The data from all three groups tended to support the hypothesis of Moore-Ede et al. (1982) that the circadian phase of core temperature and cortisol were under the control of the same circadian oscillator (X-pacemaker). This was especially apparent in Groups 2 and 3 where the degree and/or nature of the change seen in the cortisol rhythm was parallel to the core temperature pattern for the same group.

The tight confidence interval on circadian cortisol acrophase in Group 2 (phase advance) likely reflects the extremely short and consistent sleep onset latency observed with these patients. This supports the work of Weitzman et al. (1979) where sleep onset was shown to have a "masking" effect on the cortisol rhythm via a suppression of plasma levels. The present work extends this, by illustrating a possible connection between sleep-induced suppression and entrainment. This question has been examined in an isolation facility, where the rhythm of the sleep/wake cycle and that of the plasma cortisol cycle have become completely dissociated (Czeisler et al., 1980a). The present results either dispute this, or attest to the close coupling of the X-and the Y-pacemaker since the entrainment of cortisol rhythmicity seemed to be dependent on sleep onset, especially in Group 2. However, as previously mentioned, future studies should carefully control for meal times, especially when examining corticosteroid rhythms.

Melatonin Data: Eleven out of 13 patients showed a substantial and significant phase delay of circadian melatonin rhythm as compared to controls. It is not yet known which circadian pacemaker controls the

timing of the circadian melatonin rhythm or whether the pineal may represent an autonomous circadian pacemaker in itself, simply coupled to the X-and/or Y-pacemaker. The uniqueness of the melatonin data may relate to the clock-gate model of Lewy et al. (1983). Whereas sleep has significant masking and entraining influence over the circadian rhythm of core temperature and cortisol, melatonin seems to be unaffected. Rather, a possible indirect effect is observed where sleeping and waking "gate" the relative exposure of the individual to the effects of light. The effects of light involve the acute suppression and long-term entrainment of melatonin secretion. In the present study, melatonin rhythmicity dissociates from core-temperature, cortisol, and sleep-wake cycles. This does not support either hypothesis of X- or Y-pacemaker control of melatonin rhythmicity. The notion of dual oscillator control (X and Y), as implied by the work of Illnerova and Vanecek (1982) may better model the present data. If the Y-pacemaker was responsible for melatonin-offset and the phase advance portion of the PRC, then a "weakened" signal from the SCN could create a situation where a phase-delay oscillator (X) would become dominant.

One confounding issue in the present data could be eliminated by a study using identically matched patient and control sleep-wake schedules. This would show whether chronic, nocturnal wakefulness at specific times, with exposure to low-level lighting, had any influence on the acrophase of the melatonin rhythm.

Exogenous Melatonin: As early as 1971, Anton-Tay et al. had suggested

that melatonin probably affects 5-HT synthesis and increases 5-HT turnover in brain loci reported to be involved with sleeping behaviour. Axelrod (1978) suggested that the action of melatonin on 5-HT synthesis is probably mediated by its effect on brain pyridoxal kinase activity, which is essential in the conversion of pyridoxal to pyridoxal-phosphate. This cofactor forms the prosthetic group of the enzyme aromatic-L-amino acid decarboxylase, which in itself is an essential but non-specific factor for the synthesis of CNS GABA, 5-HT, DA, and NA. However, the increases in 5-HT and GABA are more pronounced (Anton-Tay et al., 1971). This mechanism of action may be one explanation of the protracted response to melatonin seen in this study. Drugs that act as neuromodulators usually have a delayed response as opposed to the action of neurotransmitters where the post-synaptic response is virtually immediate (Cooper et al., 1986).

Other studies have shown melatonin may act as an inhibitory neurotransmitter (Datta and King, 1979; Coloma and Niles, 1984; Niles et al., 1987). These studies have demonstrated that the psychopharmacologic effects of melatonin are due, in part, to its ability to bind receptor sites at the GABA-benzodiazepine-barbiturate receptor complex and allosterically modulate central GABAergic transmission by increasing GABA receptor affinity (Coloma and Niles, 1988). GABA is the major inhibitory neurotransmitter of the CNS (deFeudis, 1984). Non-selective binding of melatonin to receptors of the GABA complex throughout the CNS would enhance general inhibition. The pharmacologic mechanism of action of supraphysiologic doses of melatonin, in this case, would be obvious.

Acute sleep-induction as with the benzodiazepines would be expected. However, the delayed onset of increased evening tiredness, as was the case in this study, would suggest that melatonin may act selectively on sleep-timing mechanisms as well as in a more general way on sleep-inducing mechanisms, which may only be activated to a lesser degree, at very high doses.

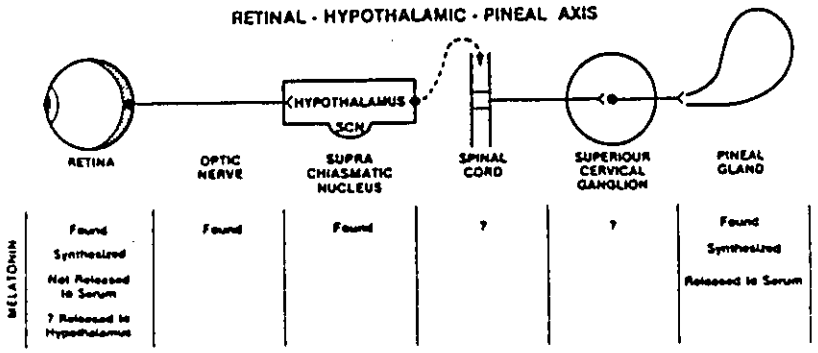
Another observation not previously reported in the literature, is the apparent increased dose threshold to melatonin in two patients. Several possible explanations exist. Six of the patients had used various benzodiazepines (as required) for a prolonged period (>5 years). This may have produced consequential changes in benzodiazepine receptor function, with a possible concomitant decrease in melatonin receptor affinity. Such alterations would require that melatonin and/or benzodiazepines would be present in increased concentrations to produce the desired effects. Since sleep-timing mechanisms are selectively affected at lower doses (Redman et al., 1983) it is possible that receptor density or affinity changes at the melatonin-benzodiazepine receptors have the most profound effect at the hypothetical high affinity binding sites of the circadian timing system. However, this may not be due exclusively to chronic benzodiazepine treatment. Patients may have a predisposition to GABA receptor alterations. Pharmacokinetic evaluation, which showed a decrease in brain benzodiazepine receptor binding in rat thalamus, forebrain, and cerebellum after prenatal exposure to diazepam possibly represents the first true animal model for insomnia and chronic anxiety (Livezey et

al., 1985). It would be worthwhile to examine changes in the synchronizing capacity of melatonin using this animal model.

Some fascinating future prospectives exist. 24-hour peripheralbenzodiazepine receptor assays on blood samples drawn each hour should be carried out on patient and controls. Changes in receptor density and affinity from both a homeostatic and circadian standpoint could then be compared. However, low-affinity binding sites on serum albumin have a substrate affinity and specificity that are quite distinct from those found in the CNS (Mohler and Okada, 1977). It may be too large a step to infer CNS benzodiazepine receptor status based on peripheral binding studies. Perhaps central melatonin and benzodiazepine receptors (especially hypothalamic) could be examined using positron emission tomography (Brown, 1987). This would allow a "first hand" examination of central receptor function. Receptor alterations could explain why insomniacs may be relatively insensitive to the synchronizing effects of melatonin.

A most interesting hypothesis based on the present data is that phase related insomnias may represent changes in the sensitivity of the circadian timing system to ambient light. This extends on the work of Lewy et al. (1985) with depressive patients. Future studies should examine the threshold light intensities required for melatonin suppression in phase-shift insomniacs as compared to controls. Changes in the physiological milieu of the photoreceptors in transmission along the retinohypothalamic tract, or in receptor affinity at the SCN itself may alter the impact of the light/dark cycle on the circadian system. In

lower vertebrates, Quay (1983) has demonstrated that melatonin may act as a modulator of photoreceptor processes by reducing retinal sensitivity to light. This may implicate retinal changes as an important component of chronobiologic disorders as recently proposed by Steiner et al. (1988). Melatonin has been localized at several sites along the retinal-hypothalamic-pineal axis (RHPA) (Seggie et al., 1987).



(from Seggie et al., 1987)

Thus, the light-dark cycle could possibly be transduced into a physiological, regulatory signal by virtue of its modulating effect on the RHPA. Possible alterations in this axis, possibly at the receptor level, could precipate circadian rhythm aberrations (Seggie, 1987).

CHAPTER 7

SUMMARY

CONCLUSION

This study has demonstrated some important relationships between certain circadian variables in situations of non-entrainment. Advanced, delayed and variable sleep-phase insomnia are three instances which afford an opportunity to scrutinize certain properties of circadian rhythms. Continuous monitoring of core temperature, serum cortisol, serum melatonin, and sleep-wake scheduling provided the variables with which to assess circadian status.

Core temperature and serum cortisol showed congruent phase patterns which were highly correlated to the pattern of the sleep-wake disturbance. Serum melatonin secretory patterns showed an independent phase relationship to all other circadian variables. This resembled the previously described concept of "internal phase drift". These data support the notion that melatonin is not under the discrete control of either the X-or Y-oscillator. Rather, the Y-oscillator could provide "melatonin offset" information to the pineal, while "melatonin onset" information could be provided by a separate oscillator (possibly X) Reduced sensitivity of the SCN to the effects of light, possibly as a result of down-regulation of melatonin receptors, could allow a predominance of phase delay input to the pineal thereby inducing secretory acrophase delay.

The reduced entrainment capacity of light might also allow the emergence of endogenous period lengths in the circadian pattern of other variables. If this endogenous period was greater than 24 hours, then a one could expect non-entrainment to present as a circadian phase delay.

These situations would be the result of a reduced sensitivity to the transduction of environmental light stimuli. This would reduce the corrective capacity of the light-dark cycle on the endogenous period length, which is usually greater (and sometimes less) than 24 hours. Such individuals would most often present with Delayed Sleep Phase Insomnia, whereas Advanced Sleep Phase Insomnia would be a more rare phenomenon.

The variable phase pattern may represent an even more rare circumstance, where inadequate phase information may give rise to an endogenous period that is either more random, or contains ultradian elements that could not be adequately characterized in this study. All three phenomena may arise from identical pathologic mechanisms.

Endogenous melatonin administered in a 75 mg oral dose significantly increased total sleep time, and increased daytime alertness in patients within this study. Of anecdotal interest was the fact that there appeared to be an increased threshold to the effects of melatonin in patients as compared to controls.

Future studies should examine two important details: (1) the endogenous circadian rhythm characteristics in a similar patient population, during extended recording periods in an isolation facility; and (2) a rigorous assessment of the use of bright-light therapy for the treatment of these disorders.

REFERENCES

Adam, K. Sleep as a restorative process and a theory to explain why. Progress in Brain Research, 53:289-305, 1979.

Akerstedt, T. Inversion of the sleep wakefulness pattern: Aging and tolerance to shift work. Ergonomics, 23:55-64, 1980.

Akerstedt, T., Gillberg, M. and Wetterberg, L. The circadian covariation of fatigue and urinary melatonin. Biological Psychiatry, 17:547-554, 1982.

Anton-Tay, F. and Wurtman, R.J. Regional uptake of ^{3}H-melatonin from the blood or cerebrospinal fluid by rat brain. Nature, 221:474-475, 1969.

Anton-Tay, F., Diaz, J.L. and Fernandez-Guardiola, G. On the effect of melatonin upon human brains: Its possible therapeutic implications. Life Science, 10:841-850, 1971.

Arendt, J. Personal communication. McMaster University, 1988.

Arendt, J., Aldhous, M. and Marks, V. Alleviation of jet-lag by melatonin: Preliminary results of controlled double blind trial. British Medical Journal, 292:1170, 1986.

Arendt, J., Bojkowski, C., Franey, C., Wright, J. and Marks, V. Immunoassay of 6-hydroxy melatonin sulphate in human plasma and urine: Abolition of the urinary rhythm with atenolol. Journal of Clinical Endocrinology and Metabolism, 60:1166-1172, 1985.

Arendt, J., Borbely, A.A., Franey, C. and Wright, J. The effects of chronic, small doses of melatonin given in the late afternoon on fatigue in man: A preliminary study. Neuroscience Letters, 45:317-321, 1984.

Arendt, J., Hampton, S., English, J., Kwasowski, P. and Marks, V. 24-hour profiles of melatonin, cortisol, insulin, C-peptide, and GIP following a meal and subsequent fasting. Clinical Endocrinology, 16:89-95, 1982.

Armstrong, S.M., Cassone, V.M., Chesworth, M.J., Redman, J.R. and Short, R.V. Synchronization of mammalian circadian rhythms by melatonin. Journal of Neural Transmission, Suppl 21:375-394, 1986.

Aschoff, J. Circadian rhythms in man: A self sustained oscillator with an inherent frequency underlies human 24-hour periodicity. Science, 148:1427-1432, 1965.

Aschoff, J. Circadian rhythm of activity and body temperature. In: J.D. Hardy, A.P. Gagge, and J. Stolwijk (eds.), Physiological and Behavioral Temperature Regulation. Springfield, Ill.: Thomas, 1970.

Aschoff, J. Problems of re-entrainment of circadian rhythms: Asymmetry effect, dissociation, and partition. In: I. Assenmacher, D.S. Furner (eds.), Environmental Endocrinology. New York: Springer-Verlag, 1978.

Aschoff, J. Circadian rhythms: General features and endocrinological aspects. In: D.J. Krieger (ed.), Endocrine Rhythms. New York: Raven Press, 1979.

Aschoff, J., Gerecke, U., and Wever, R. Desynchronization of human circadian rhythms. Journal of Physiology, 17:450-457, 1967.

Association of Sleep Disorders Centers. Diagnostic classification of sleep and arousal disorders. Sleep, 2:1-137, 1979.

Axelrod, J. Introductory remarks on regulation of pineal indoleamine synthesis. Journal of Neural Transmission (Suppl)13:73-79, 1978.

Barchas, J., DaCosta, F. and Spector, S. Acute pharmacology of melatonin. Nature, 214:919-920, 1967.

Benzinger, T.H., Kitzinger, C. and Pratt, A.W. The human thermostat. In: Temperature -- Its Measurement and Control in Science and Industry. New York: Reinhold Publishing, 1963.

Berger, R.J. Bioenergetic functions of sleep and activity of rhythms and their possible relevance to aging. Proceedings of the Federation of American Society for Experimental Biology, 34:97-102, 1975.

Birkland, A.J. Plasma melatonin levels and nocturnal transitions between sleep and wakefulness. Neuroendocrinology, 34:126-131, 1982.

Borbely, A.A. A two process model of sleep regulation. Human Neurobiology, 1:195-204, 1982.

Borbely, A.A. Sleep regulation: Outline of a model and its implications for depression. Experimental Brain Research, Suppl 8:272-284, 1984.

Borbely, A.A., Achermann, P., Trachsel, L. and Tobler, I. Sleep initiation and initial sleep intensity: Interactions of homeostatic and circadian mechanisms. Journal of Biological Rhythms, 4:149-160, 1989.

Brainard, G.C., Richardson, B.A., Petterborg, L.J. and Reiter, R.J. The effects of different light intensities on pineal melatonin content. Brain Research, 233:75-81, 1982.

Brown, G.M., Grota, L.J., Penney, D., Reichlin, S. Pituitary-adrenal function in the squirrel monkey. Endocrinology, 86:519-529, 1970.

Brown, G.M., Grota, L.J., Pulido, O., Burns, T.G., Niles, L.P. and Snieckus, V. Application of immunology techniques to the study of pineal indoles. In: Reiter, R.J. (ed.), Annual Pineal Reviews. New York: Allan R. Liss, New York, 1983.

Brown, G.M. Personal communication. McMaster University, 1987.

Brown, G.M., Young, S.N., Gauthier, S., Tsui, H. and Grota, L.J. Melatonin in human cerebrospinal fluid in daytime; its origin and variation with age. Life Sciences, 25:929-936, 1979.

Carpenter, M.B. Core Text of Neuroanatomy. 2nd Edition. Baltimore: Williams and Wilkins, 1985.

Carroll, B.J. Limbic system-adrenal cortex regulation in depression and schizophrenia. Psychosomatic Medicine, 38:106-121, 1976.

Carroll, B.J., Curtis, G.C. and Mendels, J. Neuroendocrine regulation in depression. Archives of General Psychiatry, 33:1039-1044, 1976.

Cassone, V.M., Chesworth, M.J. and Armstrong, S.M. Entrainment of rat circadian rhythms by daily injection of melatonin depends upon the hypothalamic suprachiasmatic nuclei. Physiology and Behavior, 36:1111-1121, 1986.

Castroviejo, D.A., Rosenstein, R.E., Romeo, H.E. and Cardidnali, D.P. Changes in Gamma-amino butyric acid high affinity binding to cerebral cortex membranes after pinealectomy or melatonin administration to rats. Neuroendocrinology, 43:24-31, 1986.

Cheung, P.W. and McCormack, C.E. Failure of pinealectomy or melatonin to alter circadian rhythmicity of the rat. American Journal of Physiology, 242:R261-R264, 1982.

Chik, C.L., Ho, A.K. and Brown, G.M. Effect of food restriction on 24h serum and pineal melatonin content in male rats. Acta Endocrnologica, 115: 507-513, 1987.

Cleghorn, J.M., Kaplan, R.D., Bellissimo, A., Szatmari, P. Insomnia: I. Classification, assessment and pharmaceutical treatment. Canadian Journal of Psychiatry, 28:339-346, 1983.

Coloma, F.M. and Niles, L.P. In vitro effects of melatonin on [^{3}H]-Muscimol binding in rat brain. Progress in Neuropsychopharmacology and Biological Psychiatry, 8:669-672, 1984.

Coloma, F.M. and Niles, L.P. Melatonin enhancement of [^{3}H] gamma-aminobutyric acid and [^{3}H] muscimol binding in rat brain. Biochemical Pharmacology, 37:1271-1274, 1988.

Cooper, J.R., Bloom, F.E. and Roth, R.H. (eds). The Biochemical Basis of Pharmacology (2nd Edition). New York: Oxford University Press, 1986.

Cornelissen, G., Halberg, F., Stebbings, J., Halberg, E., Carandente, F. and Hsi, B. Chronobiometry with pocket calculators and computer systems. La Ricerca Clin Lab, 10:333-385, 1980.

Craft, C.M., Morgan, W.W. and Reiter, R.J. 24-hour changes in catecholamine synthesis in rat and hamster pineal glands. Neuroendocrinology, 38:193-198, 1984.

Craft, C.M., Morgan, W.W., Jones, D.J. and Reiter, R.J. Hamster and rat pineal gland Beta-receptor characterization with iodocyanopindol and the effect of decreased catecholamine synthesis on the receptor. Journal of Pineal Research, 2:51-66, 1985.

Cramer, H., Rudolph, J., Consbruch, U., et al. On the effects of melatonin on sleep and behavior in man. Advances in Biochemistry and Psychopharmacology, 11:187-191, 1974.

Czeisler, C.A., Allan, J.S., Strogatz, S.H., et al. Bright light resets the human circadian pacemaker independent of the timing of the sleep-wake cycle. Science, 233:667-671, 1986.

Czeisler, C.A., Kronauer, R.E., Allan, J.S., et al. Bright light induction of strong (type 0) resetting of the human circadian pacemaker. Science, 244:1328-1333, 1989.

Czeisler, C.A., Richardson, G.S., Coleman, R.M., Zimmerman, J.C., Moore-Ede, M.C., Dement, W.C. and Weitzman, E.D. Chronotherapy: Resetting the circadian clocks of patients with delay sleep phase insomnia. Sleep, 4:1-21, 1981b.

Czeisler, C.A., Richardson, G.S., Zimmerman, J.C., Moore-Ede, M.C. and Weitzman, E.D. Entrainment of human circadian rhythms by light-dark cycles: A reassessment. Photochemistry and Photobiology, 34:239-247, 1981a.

Czeisler, C.A., Weitzman, E.D., Moore-Ede, M.C. and Krause, A.L. Relationship of the circadian rhythms of skin and core body temperature under entrained and free-running conditions in man. Federation Proceedings, 36:423, 1977.

Czeisler, C.A., Weitzman, E.D., Moore-Ede, M.C., Zimmerman, J.C. and Knauer, R.C. Human sleep: Its duration and organization depend on its circadian phase. Science, 210:1264-1267, 1980a.

Czeisler, C.A., Zimmerman, J.C., Ronda, J., Moore-Ede, M.C. and Weitzman, E.D. Timing of REM sleep is coupled to the body temperature of man. Sleep, 2:329-346, 1980b.

Daan, S. and Beersma, D. Circadian gating of human sleep and wakefulness. In: Mathematical Modeling of Circadian Systems. New York: Raven Press, 1983.

Daan, S. and Pittendrigh, C.S. Functional-analysis of circadian pacemakers in nocturnal rodents. 2. Variability of phase response curves. Journal of Comparative Physiology, 106:253-266, 1976.

Daan, S., Beersma, D.G.M. and Borberly, A.A. The timing of human sleep: Recovery process gated by a circadian pacemaker. American Journal of Physiology, 246:R161-R178, 1984.

Datta, P.C. and King, M.G. Effects of MIF-1 and melatonin on novelty induced defecation, plasma 11-OHCS and central catecholamines. Pharmacology, Biochemistry, and Behavior, 11:172-182, 1979.

DeCoursey, P.J. Daily light sensitivity rhythms in a rodent. Science, 131:33-35, 1960.

defeudis, F.V. GABA receptors in the vertebrate CNS. In: P.J. Marangos, I.C. Campbell and R.M. Cohen (eds), Brain Receptor Methodologies. Toronto: Academic Press, 1984.

Dement, W.C. Some must watch while some must sleep. Stanford, California: Stanford Alumni Association, 1972.

Dement, W.C. and Carskadon, M.A. Current perspectives on daytime sleepiness: The issues. Sleep, 5:S56-S66, 1982.

Dubocovich, M.L. Characterization of retinal melatonin receptors. Journal of Experimental Therapeutics, 234:395-401, 1985.

Enright, J.T. Studies of Brain Function (3): The Timing of Sleep and Wakefulness. New York: Springer-Verlag, 1980.

Enright, J.T. The parallactic view, statistical testing, and circular reasoning. Journal of Biological Rhythms, 4:295-304, 1989.

Feinberg, M., Gillin, J.C., Carroll, B.J., Greden, J.F. and Zis, A.P. EEG studies of sleep in the diagnosis of depression. Biological Psychiatry, 17:305-316, 1982.

Gillberg, M. and Akerstedt, T. Body temperature and sleep at different times of the day. Sleep, 5:378-388, 1982.

Halberg, E. and Halberg, F. Chronobiologic study design in everyday life, clinic, and laboratory. Chronobiologia, 7:95-113, 1980.

Halberg, F.A., Johnson, E.A., Nelson, W., Runge, W. and Southern, R. Autorhythmometry - procedures for physiologic self-measurements and their analysis. Physiology Teacher, 1:1, 1972.

Hariharasubramanian, N., Nair, N.P.V. and Pilapil, C. Circadian rhythm of plasma melatonin and cortisol during the menstrual cycle. Advances in Biosciences, 53: 31-36, 1985.

Hartmann, E. The Functions of Sleep. New Haven, Connecticut: Yale University Press, 1973.

Hartmann, E. The Sleeping Pill. New Haven, Connecticut: Yale University Press, 1978.

Hauri, P. and Fisher, J. Persistent psychophysiologic (learned) insomnia. Sleep, 9:38-53, 1985.

Hildebrandt, G., Rohmert, W. and Rutenfranz, J. 12 and 24 hour rhythms in error frequency of locomotive drivers and the influence of tiredness. International Journal of Chronobiology, 2:175-180, 1974.

Hobson, J.A., Lydic, R. and Baghodoyan, H.A. Evolving concepts of sleep cycle generation: From brain centers to neuronal populations. The Behavior and Brain Sciences, 9:374-448, 1986.

Holmes, S.W. and Sugden, D. Effects of melatonin on sleep and neurochemistry in the rat. British Journal of Pharmacology, 76:95-101, 1982.

Ibuka, N., Inouye, S.T. and Kawamura, H. Analysis of sleep-wakefulness rhythms in male rats after suprachiasmatic nucleus lesions and ocular enucleation. Brain Research, 122:33-47, 1977.

Illnerova, A. and Vanecek, J. Two-oscillator structure of the pacemaker controlling the circadian rhythm of N-acetyltransferase in the rat pineal gland. Journal of Comparative Physiology, 145:539-548, 1982.

Johansson, G.E.K., Ho, A.K., Chik, C.L. and Brown, G.M. Interference in melatonin radioimmunoassay by heparin preparations. Advances in Biosciences, 53:47-52, 1985.

Johnsson, A., Pfug, B., Engelmann, W. and Klemke, W. Effects of lithium carbonate on circadian periodicity in humans. Pharmacophsychiatry and Neuropsychopharmacology, 12:423-425, 1979.

Jones, B.E., Harper, S.T. and Halaris, A.E. Effects of locus coeruleus lesions upon cerebral monoamine content, sleep-wakefulness states, and the response to amphetamine in the cat. Brain Research, 124:473-496, 1977.

Kafka, M.S., Wirz-Justice, A., Naber, D., Moore, R.Y. and Benedito, M.A. Circadian rhythms in rat brain neurotransmitter receptors. Federation Proceedings, 42:2796-2801, 1983.

Kamei, R., Hughs, L., Miles, L. and Dement, W.C. Advanced sleep phase syndrome studied in a time isolation facility. Chronobiologia, 6:115-121, 1979.

Kapen, S., Fleming, P.D. and Drachman, D.A. Cholinergic enhancement and REM sleep latency in the aged: Lecithin does not reproduce physostigmine effect. Neurology, 36:1079-1083, 1986.

Kawato, M., Fujita, K., Suzuki, R., and Winfree, A.T. A three oscillator model of the human circadian system controlling the core temperature rhythm and the sleep-wake cycle. Journal of Theoretical Biology, 98:369-392, 1982.

Klein, K.E., Wegmann, H.M. and Hunt, B.I. Desynchronization of body temperature and performance circadian rhythm as a result of outgoing and homegoing transmeridian flights. Aviation and Space Environmental Medicine, 43:119-132, 1972.

Koella, W.P. Serotonin and sleep. Experimental Medicine and Surgery, 27:157-169, 1969.

Koella, W.P., Feldstein, A. and Czicman, J.S. The effect of para-chlorophenylalanine on the sleep of cats. Electroencephalography and Clinical Neurophysiology, 25:481-490, 1968.

Krieger, D.T. Rhythms in CRF, ACTH and corticosteroids. In: D.T. Krieger (ed.), Comprehensive Endocrinology: Endocrine Rhythms. New York: Raven Press, 1979.

Krieger, D.T. Ventromedial hypothalamic lesions abolish food shifted circadian adrenal and temperature rhythmicity. Endocrinology, 106:649-654, 1980.

Krieger, D.T., Hauser, H. and Krey, L.C. Suprachiasmatic nuclear lesions do not abolish food shifted circadian adrenal and temperature rhythmicity. Science, 197:398-399, 1977.

Kronauer, R., Czeisler, C.A., Pilato, S.F., Moore-Ede, M.C. and Weitzman, E.D. Mathematical model of the human circadian system with two interacting oscillators. American Journal of Physiology, 242:R3-R17, 1982.

Krueger, J.M., Pappenheimer, J.R. and Karnovsky, M.L. The composition of sleep promoting factor isolated from human urine. Journal of Biological Chemistry, 257:1664-1669, 1982a.

Krueger, J.M., Papenheimer, J.R. and Karnovsky, M.L. Sleep promoting effects of muramyl peptides. Proceedings of the National Academy of Science, 79:6102-6106, 1982b.

Kupfer, D.J., Foster, F.G., Coble, P., McPartland, R.J. and Ulrich, R.F. The application of EEG sleep for the differential diagnosis of affective disorders. American Journal of Psychiatry, 135:64-74, 1978.

Lavie, P. Ultra short sleep-waking schedules. III. 'Gates' and 'forbidden zones' for sleep. Electroencephalography and Clinical Neurophysiology, 63:414-425, 1986.

Legendre, R. and Pieron, H. Le probleme des facteurs de sommeil. Resultats d'injections vasculaires et intracerebrales de liquides insomniques. CR Social Biology (Paris), 68:1077-1078, 1910.

Lewy, A.J., Nurnberger, J.I., Wehr, T.A., Pack, B., et al. Supersensitivity to light: Possible trait marker for manic-depressive illness. American Journal of Psychiatry, 142:725-727, 1985.

Lewy, A.J., Siever, L.J., Uhde, T.W. and Markey, S.P. Clonidine reduces plasma melatonin levels. Journal of Pharmacy and Pharmacology, 38:555-556, 1986.

Lewy, A.J., Sack, R.L. and Singer, C.L. Assessment and treatment of chronobiologic disorders using plasma melatonin levels and bright light exposure: A clock-gate and phase response curve. Psychopharmacology Bulletin, 20:561-565, 1983.

Lewy, A.J., Sack, R.L. and Singer, C.L. Bright light, melatonin, and biological rhythms: Implications for affective disorders. Psychopharmacology Bulletin, 21:365-372, 1985a.

Lewy, A.J., Wehr, T.A., Goodwin, F.K., Newsome, D.A. and Markey, S.P. Light suppresses melatonin production in humans. Science, 210:1267-1269, 1980.

Lieberman, H.R. Behavior, sleep and melatonin. Journal of Neural Transmission, 21:233-241, 1986.

Livezey, G.T., Radulovacki, M., Isaac, L. and Marczynski, T.J. Prenatal exposure to diazepam results in enduring reductions in brain receptors and deep slow-wave sleep. Brain Research, 334:361-365, 1985.

Lobban, M.C. Seasonal variations in daily patterns of urinary excretion by Eskimo subjects. In: R.J. Shephard and S. Itoh (eds.), Circumpolar Health. Toronto: University of Toronto Press, 1976.

MacFarlane, J.G. Circadian Rhythms and Insomnia. Master's Thesis. McMaster University, Hamilton, Ontario, 1983.

MacFarlane, J.G. and Wu, C. Vitanalysis time-base file program. Computer Services Unit, McMaster University, Hamilton, Ontario, 1983.

Marczynski, T.J., Yamaguchi, N., Ling, G.M. and Grodzinska, L. Sleep induced by the administration of melatonin (5-methoxy-N-acetyltryptamine) to the hypothalamus of unrestrained cats. Experimentia, 20:435-437, 1964.

Martin, C.R. Endocrine Physiology. New York: Oxford University Press, 1985.

Matthews, C.D., Kennaway, D.J., Fellengerg, A.J.G., Phillipou, G., Cox, L.W. and Seamark, R.F. Melatonin in man. Advances in Biosciences, 29:371-381, 1981.

McGinty, D.J. Encephalization of the neural control of behavior. In: Sternman, M.S., McGinty, D.J. and Adinolfi, A.M. (eds.), Brain Development and Behavior. New York: Academic Press, 1971.

Meddis, R. The functions of sleep. Animal Behavior, 23: 676-691, 1975.

Mendels, J. and Cochrane, C. The nosology of depression: The endogenous-reactive concept. American Journal of Psychiatry, 124:1-11, 1968.

Mills, J.N., Minors, D.S. and Waterhouse, J.M. The physiological rhythms of subjects living on a day of abnormal length. Journal of Physiology, 268:803-826, 1977.

Mistelberger, R.F., Bergmann, B.M., Waldenar, W. and Rechtschaffen, A. Recovery sleep following sleep deprivation in intact and SCN lesioned rats. Sleep 6:217-233, 1983.

Mohler, H. and Okada, T. Benzodiazepine receptor: Demonstration in central nervous system. Science, 198:845-851, 1977.

Moldofsky, H., Lue, F.A., Eisen, J., Keystone, E. and Gorczynski, R.M. The relationship of interleukin-1 and immune functions to sleep in humans. Psychosomatic Medicine, 48:309-318, 1986.

Moldofsky, H., Musisi, S. and Phillipson, E.A. Treatment of a case of advanced sleep phase syndrome by phase advance chronotherapy. Sleep, 9:61-65, 1986.

Moore, D.C., Paunier, L. and Sizonenko, P.C. Effect of adrenergic stimulation and blockade on melatonin production in humans. In: J.A. Kappers and P. Pevet (eds.), The Pineal Gland of Vertebrates Including Man. Amsterdam: Elsevier, 1979.

Moore, R.Y. Suprachiasmatic nucleus, secondary synchronizing stimuli and the central neural control of circadian rhythms. Brain Research, 183:13-28, 1980.

Moore, R.Y. Organization and function of a central nervous system circadian oscillator: The suprachiasmatic hypothalamic nucleus. Federation Proceedings, 42:2783-2789, 1983.

Moore, R.Y. and Lenn, N.J. A retinohypothalamic projection in the rat. Journal of Comprehensive Neurology, 146:1-14, 1972.

Moore, R.Y. and Eichler, V.B. Loss of an adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Research, 42:201-206, 1972.

Moore, R.Y. and Klein, C.C. Visual pathways and the central neural control of a circadian rhythm in pineal serotonin N-acetyltransferase activity. Brain Research, 71:17-33, 1974.

Moore, R.Y. and Rapport, R.L. Pineal and gonadal function in the rat following cervical sympathectomy. Neuroendocrinology, 7:361-374, 1970.

Moore-Ede, M.C., Sulzman, F.M. and Fuller, C.A. The Clocks That Time Us: Physiology of the Circadian Timing System. Massachusetts: Harvard University Press, 1982.

Morgan, R. and Cheadle, A.J. Circadian body temperature in chronic schizophrenia. British Journal of Psychiatry, 129:350-354, 1976.

Moruzzi, G. The functional significance of sleep with particular regard to the brain mechanisms underlying consciousness. In: Eccles, J.C. (ed.), Brain and Conscious Experience. New York: Springer, 1966.

Moses, J., Lubin, A., Naitoh, P. and Johnson, L.C. Circadian variation in performance subjective sleepiness, and oral temperature during an altered sleep-wake schedule. Biological Psychiatry, 6:301-308, 1978.

Mrosovsky, N. Phase response curves for social entrainment. Journal of Comparative Physiology, A162:35-46, 1988.

Nagasaki, H., Iriki, M., Inouye, S. and Uchizono, K. The presence of sleep promoting material in the brain of sleep deprived rats. Proceedings of the Japanese Academy, 50:241-246, 1974.

Nelson, N., Tong, Y.L., Lee, J-L. and Halberg, F. Methods for cosinor rhythmometry. Chronobiologia, 6:305-323, 1979.

Niles, L.P., Pickering, D.S. and Arciszewski, M.A. Effects of chronic melatonin administration on GABA and diazepine binding in rat brain. Journal of Neural Transmission, 70:117-124, 1987.

Oswald, I. Sleep the great restorer. New Scientist, 46:170-172, 1970.

Pickard, G.E. and Turek, F.W. The suprachiasmatic nuclei: Two circadian clocks? Brain Research, 268:201-210, 1983.

Page, R.B., Galacich, J.H. and Grunt, J.A. Alteration of circadian temperature rhythm with third ventricle obstruction. Journal of Neurosurgery, 38:309-319, 1973.

Pang, S.F., Brown, G.M., Grota, L.J., Chambers, J.W. and Rodman, R.L. Determination of N-acetylserotonin and melatonin activities in the pineal gland, retina, Harderian gland, brain and serum of rats and chickens. Neuroendocrinology, 23:1-13, 1977.

Poland, R.E., Rubin, R.T., Clark, B.R. and Gouin, P.R. Circadian patterns of 17-OHCS and VMH excretion during sleep deprivation. Diseases of the Nervous System, 33:456-458, 1972.

Quay, W.B. Individuation and lack of pineal effect in the rat's circadian locomotor rhythm. Physiology and Behavior, 3:109-118, 1968.

Quay, W.B. Pineal homeostatic regulation of shifts in circadian activity rhythm during maturation and aging. New York Academy of Science, 34:239-254, 1972.

Quay, W.B. Humoral interrelations of the pineal gland with lateral eyes and orbital glands. In: J. Axelrod, F. Fraschini and G.P. Velo (eds.), The Pineal Gland and its Endocrine Role. New York: Plenum Press, 1983.

Rechtschaffen, A., Gilliland, M.A., Bergman, B.M. and Winter, J.B. Physiological correlates of prolonged sleep deprivation in rats. Sciences, 221:182-184, 1983.

Rechtschaffen, A. and Kales, A. A Manual of Standardized Terminology, Techniques, and Scoring System for Sleep Stages of Human Subjects. Public Health Service, U.S. Government Printing Office, Washington, D.C., 1968.

Redman, J., Armstrong, S. and Ng, K.T. Free running activity rhythms in the rat: Entrainment by melatonin. Science, 219:1089-1091, 1983.

Reinberg, A., Vieux, N., Ghata, J., Chaumont, A.J. and LaPorte, A. Circadian rhythm amplitude and individual ability to adjust to shift work. Ergonomics, 21:763-766, 1978.

Reinberg, A. Oral temperature, circadian rhythm amplitude, ageing, and tolerance to shift work. Ergonomics, 23:55-64, 1980.

Reiter, R.J. and Peters, J.F. Non-suppressibility by room light of pineal NAT activity and melatonin levels in two diurnally active rodents. Endocrine Research, 10:113-121, 1984.

Reiter, R.J., Sorrentino, S., Ralph, C.L., Lynch, H.L., Mull, D. and Jarrow, E. Some endocrine effects of blinding and anosmia in adult male rats with observations on pineal melatonin. Endocrinology, 88:895-900, 1971.

Rivest, R.W. and Wurtman, R.J. Relationship between light intensity and drinking rhythms in rats. Neuroendocrinology, 37:155-160, 1983.

Rodbard, D. Statistical quality control and routine data processing for radioimmunoassays and immunoradiometric assays. Clinical Chemistry, 20:R55-R63, 1974.

Rollag, M.D. and Niswender, G.D. Radioimmunoassay of serum concentrations of melatonin in sheep exposed to different lighting regimens. Endocrinology, 98:482-489, 1976.

Romijn, H.J. The pineal, a tranquillizing organ. Life Sciences, 23: 225-227, 1978.

Rubin, R.T., Poland, R.E., Blodgett, A.L.N., Winston, R.A., Forster, B. and Carroll, B.J. Adrenal and gonadal function in affective disorder. In: F. Brambilla, G. Racagni and deWied (eds.), Progress in Psychoneuroendocrinology. Amsterdam: Elsevier/North, 1980.

Sachar, E.J. Twenty-four-hour cortisol secretory patterns in depressed and manic patients. Progress in Brain Research, 42:81-91, 1975.

Sakai, K., Sastre, J.P., Kanamori, N. and Jouvet, M. State specific neurons in the ponto-medullary reticular formation with special reference to postural atonia during paradoxical sleep in the cat. In:

O. Pompeiano and C. Ajmone-Marsan (eds.), Brain Mechanisms and Perceptual Awareness and Purposeful Behavior. New York: Raven Press, 1981.

Sastre, J.P., Sakai, K. and Jouvet, M. Are gigantocellular tegmental field neurons responsible for paradoxical sleep? Brain Research, 229:147-161, 1981.

Schneider-Helmert, D. Twenty-four-hour sleep-wake function and personality patterns in chronic insomniacs and healthy controls. Sleep, 10:452-462, 1987.

Schneider-Helmert, D. and Spinweber, C.L. Evaluation of L-tryptophan for the treatment of insomnia: A review. Psychopharmacology, 89:1-7, 1986.

Seggie, J. Melatonin, the retinal-hypothalamic-pineal axis, and circadian rhythm regulation. In: F.N. Johnson (ed.), Lithium Therapy Monographs, Vol. 2, Lithium and the Endocrine System. Basel: Karger Press, 1988.

Seggie, J., Werstiuk, E.S. and Grota, L. Lithium and circadian patterns of melatonin in the retina, hypothalamus, pineal and serum. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 11:325-334, 1987.

Seybert, D.W., Lancaster, J.R., Lambeth, J.D. and Kamin, H. Participation of the membrane in the side chain cleavage of cholesterol. Journal of Biological Chemistry, 254:12088-12098, 1980.

Sharma, M., Placios-Bois, J., Schwartz, G., Iskandar, H., Thakur, M., Quirion, R. and Nair, N.P.V. Circadian rhythms of melatonin and cortisol in aging. Biological Psychiatry, 25:305-319, 1989.

Silverman, A.K., Hoffman, D.S. and Zimmerman, E.A. The descending afferent connections of the paraventricular nucleus of the hypothalamus. Brain Research Bulletin, 6:47-61, 1981.

Steiner, M., Werstiuk, E.S. and Seggie, J. Dysregulation of neuroendocrine crossroads: Depression, circadian rhythms and the retina -- A hypothesis. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 11:267-278, 1987.

Strogatz, S.H., Kronauer, R.E. and Czeisler, C.A. Circadian pacemaker interferes with sleep onset at specific times each day: Role in insomnia. American Journal of Physiology, 253:R172-178, 1987.

Sugden, D., Weller, J.L., Klein, D.C., Kirk, K.L. and Creveling, C.R. Alpha-adrenergic potentiation of beta-adrenergic stimulation of rat pineal N-acetyltransferase: Studies using ciragotine and flourine analogs of norepinephrine. Biochemical Pharmacology, 33:3947-3951, 1984.

Sulzman, F.M., Fuller, C.A. and Moore-Ede, M.C. Feeding time synchronizes primate circadian rhythms. Physiology and Behavior, 18:775-779, 1977.

Swanson, L.W. and Cowan, W.M. The efferent connections of the suprachiasmatic nucleus of the hypothalamus. Journal of Comprehensive Neurology, 160:1-12, 1975.

Takahashi, J.S. and Zatz, M. Regulation of circadian rhythmicity. Science, 217:1104-1111, 1982.

Tamarkin, L., Baird, C.J. and Almeida, O.F.X. Melatonin: A coordinating signal for mammalian reproduction. Science, 227:714-720, 1985.

Tobler, I. Evolution of sleep processes: A phylogenic approach.Experimental Brain Research, Suppl 8:207-226, 1984.

Tobler, I., Borberly, A.A. and Groos, G. The effect of sleep deprivation on sleep in rats with suprachiasmatic lesions. Neuroscience Letters, 42:49-54, 1983.

Turek, F.W. and Losee-Olsen, S.H. A benzodiazepine used in the treatment of insomnia phase shifts the mammalian circadian clock. Nature, 321:167-168, 1986.

Turek, F.W. and Losee-Olsen, S.H. Dose response curve for the phase shifting effect of triagolam on the mammalian circadian clock. Life Science, 40:1037-1038, 1987.

Underwood, H. The pineal and circadian rhythms. In: R.J. Reiter (ed.), The Pineal Gland. New York: Raven Press, 1984.

Vakkuri, O., Leppalusto, J. and Vuolteenh, O. Development and validation of a melatonin radioimmunoassay using radio-iodinated melatonin as a tracer. Acta Endocrinology, 106:152-157, 1984.

Vale, W., Spiess, J., Rivier, C. and Rivier, J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science, 213:1394-1397, 1981.

Van den Pol, A.N. and Tsujimoto, K.L. Neurotransmitters of the hypothalamic nucleus: Immunocytochemical analysis of 25 neuronal antigens. Neuroscience, 15:1049-1086, 1985.

Van Reeth, O. and Turek, F. Simulated activity mediates phase shifts in the hamster circadian clock induced by dark pulses or benzodiazepines. Nature, 339:49-51, 1989.

Vaughan, G.M. Melatonin in humans. In: R.J. Reiter (ed.), Pineal Research Reviews. New York: Alan R. Liss, 1984.

Vollrath, L., Semm, P. and Gammel, G. Sleep induction by intranasal application of melatonin. Advances in the Biosciences, 29:327-329, 1981.

Vokac, M. A comprehensive cosinor system program written for the Apple II microcomputer. Oslo, Norway, 1983.

Waldhauser, F., Waldhauser, M., Lieberman, H.R., Deny, M., Lynch, H.T. and Wurtman, R.J. Bioavailability of oral melatonin in humans. Neuroendocrinology, 39:307-313, 1984.

Walker, W.H.C. An approach to immunoassay. Clinical Chemistry, 23:384-402, 1977.

Webb, W.B. The adaptive functions of sleep patterns. In: P. Levin and W.P. Koella (eds.), Sleep. Basel: Karger Press, 1974.

Wehr, T.A., Wirz-Justice, A., Goodwin, F.K., Duncan, W. and Gillan, J.C. Phase advance of the circadian sleep-wake cycle as an antidepressant. Science, 206:710-713, 1979.

Weitzman, E.D. Sleep and its disorders. Annual Review of Neuroscience, 4:381-417, 1981.

Weitzman, E.D., Czeisler, C.A. and Moore-Ede, M.C. Sleep-wake, neuroendocrine and body temperature circadian rhythms under entrained and non-entrained (free-running) conditions in man. In: M. Suda, O. Hayaishi and H. Nakagawa (eds.), Biologic Rhythms and Their Central Mechanisms. New York: Elsevier North-Holland, 1979.

Weitzman, E.D., Czeisler, C.A., Coleman, R.M., Spielman, A.J., Zimmerman, J.C., Dement, W.C., Richardson, G.S. and Pollak, C.P. Delayed sleep phase syndrome: A chronobiologic disorder with sleep-onset insomnia. Archives of General Psychiatry, 38:737-746, 1981a.

Weitzman, E.D., Czeisler, C.A., Zimmerman, J.C. and Moore-Ede, M.C. Biological rhythms in man: Relationship of sleep-wake, cortisol, growth hormone and temperature during temporal isolation. In: J.B. Martin, S. Reichlin and K.L. Bick (eds.), Neurosecretions and Brain Peptides. New York: Raven Press, 1981b.

Weitzman, E.D., Czeisler, C.A., Zimmerman, J.C. and Ronda, J.M. Timing of REM and stages 3 and 4 sleep during temporal isolation in man. Sleep, 2:391-407, 1980.

Weitzman, E.D., Fukushima, D.K., Nogeire, C., et al. Twenty-four hour patterns of the episodic secretion of cortisol in normal subjects. Journal of Clinical Endocrinology and Metabolism, 33:14-22, 1971.

Weitzman, E.D., Moline, M.L., Czeisler, C.A. and Zimmerman, J.C. Chronobiology of ageing: Temperature, sleep-wake rhythms and entrainment. Neurobiology and Ageing, 3:299-309, 1982.

Wetterberg, L. Clinical importance of melatonin. Progress in Brain Research, 52:539-547, 1979.

Wever, R.A. Properties of human circadian rhythms: Free-running rhythms in deep body temperature.

Wever, R. The circadian system of man: Results of experiments under temporal isolation. In: E. Shaefer (ed.), Topics in Environmental Physiology and Medicine. New York: Springer-Verlag, 1979.

Wever, R.A. Phase shifts of human circadian rhythms due to shifts of artificial zeitgebers. Chronobiologia, 7:303-326, 1980.

Wever, R.A. Fractional desynchronization of human circadian rhythms: A method for evaluating entrainment limit and functional interdependencies. Pfluegers Archiv, 396:128-137, 1983.

Wever, R.A. Characteristics of circadian rhythms in human functions. Journal of Neural Transmission, 21:323-373, 1986.

Winer, B.J. Statistical Principles in Experimental Design. (2nd Edition) New York: McGraw-Hill, 1971.

Winfree, A.T. Human body clocks and the timing of sleep. Nature, 297:23-27, 1982.

Winfree, A.T. Impact of a circadian clock on the timing of human sleep. American Journal of Physiology, 245:R-497-R504, 1983.

Winfree, A.T. Circadian rhythms: Benzodiazepines set the clock. Nature, 321:114-115, 1986.

Wurtman, R.J. and Lieberman, H.R. Melatonin secretion as a mediator of circadian variations in sleep and sleepiness. Journal of Pineal Research, 2:301-303, 1985.

Zepelin, H. Normal age related changes in sleep. In: M. Chase and E.D. Weitzman (eds.), Sleep Disorders: Basic and Clinical Research. New York: SP and Scientific Books, 1983.

Zepelin, H. and Rechtshaffen, A. Mammalian sleep, longevity, and energy metabolism. Brain, Behavior, and Evolution, 10:425, 1974.

Zulley, J.R., Wever, R. and Aschoff, J. The dependence of onset and duration of sleep on the circadian rhythm of rectal temperature. Pfluegers Archiv, 391:314-318, 1981.

A P P E N D I X A

CERTIFICATION FOR VENIPUNCTURE AND I.V. MAINTENANCE

McMASTER UNIVERSITY MEDICAL CENTRE

CERTIFICATE

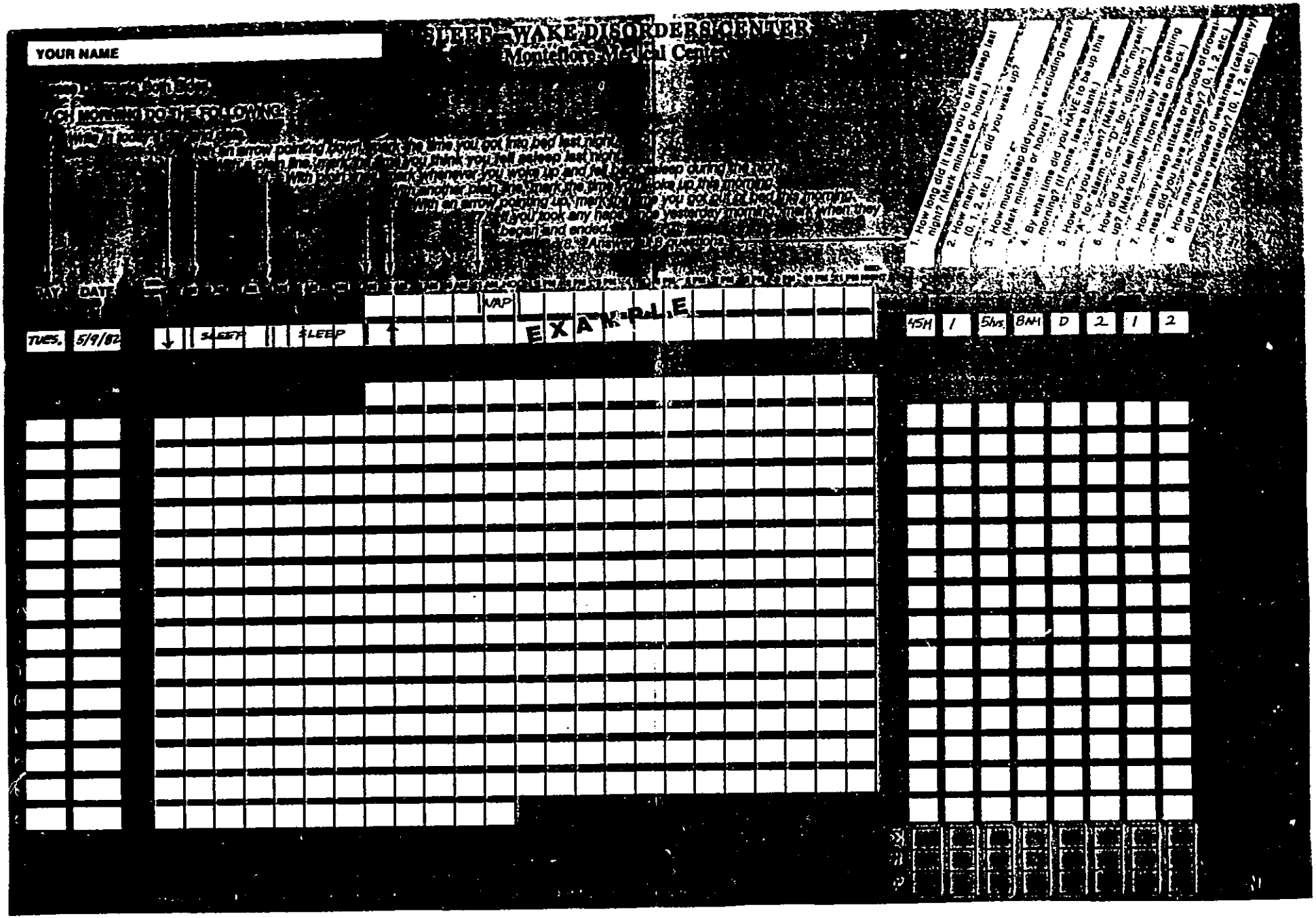
This is to certify that _____ has been examined and found to be competent to perform the following procedure(s):

SPECIAL PROCEDURE (According to Official List)	PHYSICIAN ASSESSING COMPETENCE	DATE
Lumbar puncture & needle and manometer	[illegible]	
Intravenous therapy needle		

To be retained on each unit and transferred to Personnel upon resignation.

A P P E N D I X B

SLEEP DIARIES AND ALERTNESS ASSESSMENT SCALES


YOUR NAME
SLEEP-WAKE DISORDERS CENTER
Montefiore Medical Center
1. How long did it take you to fall asleep last night? (Mark minutes or hours.)
2. How many times did you wake up? (0, 1, 2, etc.)
3. How much sleep did you get, excluding naps? (Mark minutes or hours.)
4. By what time did you HAVE to be up this morning? (If none, leave blank.)
5. How did you awaken? (Mark "M" for "myself," "A" for "alarm," or "D" for "disturbed.")
6. How did you feel immediately after getting up? (Mark number from scale on back.)
7. How many sleep attacks or periods of drowsiness did you have yesterday? (0, 1, 2, etc.)
8. How many episodes of weakness (cataplexy) did you have yesterday? (0, 1, 2, etc.)
DATE
TUES. 5/9/82
SLEEP
SLEEP
NAP
EXAMPLE
45M 1 5hrs. 8AM D 2 1 2

APPENDIX C

CONSENT FORM

CONSENT FORM FOR SLEEP STUDY II - J.G. MacFarlane and Dr. J.M. Cleghorn

I agree to participate in a study that will measure certain body biorhythms over a period of 7 days.

I understand that a small rectal probe will be used so that my body temperature can be measured easily and accurately. This electrode will be connected to a portable, battery-operated recorder which will be worn on a belt around my waist. The electrode will remain in place for the entire period, although it may be removed for bathing, sports activities, etc.

I understand that I will carry on with my normal daily routines for the first 5 days of the study, and keep a detailed record of my sleep periods. The 6th and 7th nights, and the intervening day of the study, will be spent in the Sleep Lab.

I understand that a total of 24 blood samples will be withdrawn during the last 24 hours of the study from an intravenous needle (I.V.). Each sample will be approximately 10cc (2 teaspoons).

I understand that an electroencephalograph (EEG) will be recorded during my sleep on the 5th and 6th nights, and that the electrodes used in this recording are placed on the scalp, and remain there throughout each night.

I understand that for an additional 14 nights, I will maintain a detailed record of my sleeping and waking schedules and that diaries will be provided. I also understand that at 10 pm each night I will be taking an oral dose of either 75 mg of Melatonin (a natural hormone of the body) or a placebo, and that Melatonin will be administered on at least 7 of the 14 days. This hormone, normally produced within the brain, has no known side effects when administered in this fashion, except that it may increase fatigue.

I understand that I may withdraw from this study at any time and I understand that this will in no way influence or prejudice my future health care. All information leading up to, or arising from this study, will be treated in a confidential manner.

SUBJECT'S NAME _____ _____
(Print) (Signature)

WITNESS _____ _____
(Print) (Signature)

DATE _____

I, Dr. _____, hereby certify that I have fully explained the nature of this study to the patient.

(10/85)

PAID: _____ _____
(Signature)

A P P E N D I X D

HPB APPROVAL AND ANALYTICAL INFORMATION FOR MELATONIN

Health and Welfare Canada
Health Protection Branch

Santé et Bien-être social Canada
Direction générale de la protection de la santé

FILE NO. — N° DE DOSSIER
9427-M2105-43C

SUBMISSION OF DATA AND INFORMATION ON DRUGS FOR CLINICAL INVESTIGATION IN CANADA
ÉSENTATION DES DONNÉES ET AUTRES RENSEIGNEMENTS SUR LES DROGUES DESTINÉES AUX INVESTIGATIONS CLINIQUES AU CANADA

DATE

IN COMPLIANCE WITH THE PROVISIONS OF SECTION C.08.005 OF THE FOOD AND DRUG REGULATIONS, THE UNDERSIGNED SUBMITS A PRECLINICAL SUBMISSION, AS DESCRIBED IN SECTION C.08.005, ON BEHALF OF

PAR APPLICATION DE L'ARTICLE C.08.005 DES RÈGLEMENTS SUR LES ALIMENTS ET DROGUES LE SOUSSIGNÉ PRÉSENTE UNE PRÉSENTATION PRÉCLINIQUE CONFORME À LA DESCRIPTION DONNÉE À L'ARTICLE C.08.005 au nom de

NAME AND ADDRESS OF COMPANY — NOM ET ADRESSE DE LA SOCIÉTÉ

McMaster University
Medical Centre
1200 Main Street West
Hamilton, Ontario
L8S 4J9

1a (i) OBJECTIVES OF PROPOSED CLINICAL TESTING — OBJECTIFS DES ÉPREUVES CLINIQUES PROPOSÉES

please see attached protocol and addendum

(ii) IDENTIFYING NAME OR MARK OF NEW DRUG — NOM OU MARQUE D'IDENTITÉ DE LA DROGUE NOUVELLE

MELATONIN

(iii) CHEMICAL STRUCTURE OR OTHER SPECIFIC INFORMATION OF THE COMPOSITION OF NEW DRUG
STRUCTURE CHIMIQUE (OU TOUT AUTRE DÉTAIL SPÉCIFIQUE) QUI PERMET D'IDENTIFIER LA COMPOSITION DE LA DROGUE NOUVELLE

(iv) SOURCE OF NEW DRUG — SOURCE DE LA DROGUE NOUVELLE

THE ADDITIONAL DATA REQUIRED BY SECTION C.08.005 1a (v) TO (ix) ACCOMPANIES THIS FORM

LES AUTRES DONNÉES REQUISES PAR L'ARTICLE C.08.005 (1) (a) SOUS-ALINÉAS (v) À (ix) SONT JOINTES À CETTE FORMULE

SIGNATURE

Executive Director
of Hospital :

DATE

Mar 4/85

This preclinical submission has been received and has been found to comply with the requirements of Section C.08.005 (I) (a). All supplies of this investigational new drug must be labelled in accordance with the requirements of Section C.08.005 (I) (b) and may only be distributed for clinical investigation after compliance with the provisions of Section C.08.005 (I) (c) and providing the clinical investigator has agreed to comply with the requirements of Section C.08.005 (I) (d).

Please use the assigned file number in any further correspondence on preclinical submission.

Cette présentation pré-clinique a été dûment reçue et trouvée conforme aux dispositions de l'article C.08.005 (I) a). Tous les stocks de cette nouvelle drogue de recherche doivent être étiquetés en conformité des dispositions de l'article C.08.005 (I) b), et la drogue ne peut être distribuée que pour investigation clinique, en conformité des dispositions de l'article C.08.005 (I) c), et pourvu que l'investigateur clinique ait convenu de se conformer aux dispositions de l'article C.08.005 (I) d).

Prière d'inscrire le numéro de dossier assigné ci-dessus dans toute correspondance ultérieure relative à cette présentation pré-clinique.

EXECUTIVE DIRECTOR GENERAL — DIRECTEUR GÉNÉRAL EXÉCUTIF

ASSISTANT DEPUTY MINISTER-LE SOUS-MINISTRE-ADJOINT

DATE

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Mr. J. G. MacFarlane
McMaster University
Department of Psychiatry
Health Sciences Center
1200 Main Street, West
Hamilton, Ontario
CANADA L8N 325

ANALYTICAL INFORMATION

PRODUCT NAME	Melatonin		
PRODUCT NUMBER	M 5250 FD	LOT NUMBER	12-6-12
FORMULA	$C_{13}H_{16}N_2O_2$	FORMULA WEIGHT	232.3
CAS	[73-31-4]		

APPEARANCE	White powder
APPEARANCE OF SOLUTION	Clear, faint yellow at 50 mg/mL in 95% ethanol
ULTRAVIOLET SPECTROSCOPY	λmax 279 nm, E^{mM} 6.3 95% ethanol
IDENTITY	Infrared spectrum consistent with structure
ELEMENTAL ANALYSIS	67.4% Carbon 6.9% Hydrogen 11.9% Nitrogen
PURITY	Greater than 99% by thin layer chromatography

Sigma warrants that its products conform to the information contained in this and other Sigma publications. See reverse side of invoice or packing slip for additional terms and conditions of sale.

By Carl Tenpas CT

Date August 12, 1985

CT/ec

APPENDIX E

DEVELOPMENT OF TYMPANIC THERMISTER

The tympanic thermocouple used in our pilot study (MacFarlane et al., 1984) was an accurate reflection of core temperature and more asthetically acceptable than a rectal thermister. The thermocouple operated on the principal that a differential resistance occurs at the junction of two dissimilar metals which is directly proportioned to the ambient temperature (Webster, 1978). When a reference resistance is provided by a recording device (LeBarge, Model 6000) then the differential resistance divided by the reference resistance is equal to the temperature value at the junction. However, the circuitry for generating and calculating reference resistances adds considerable weight and size to the recording device.

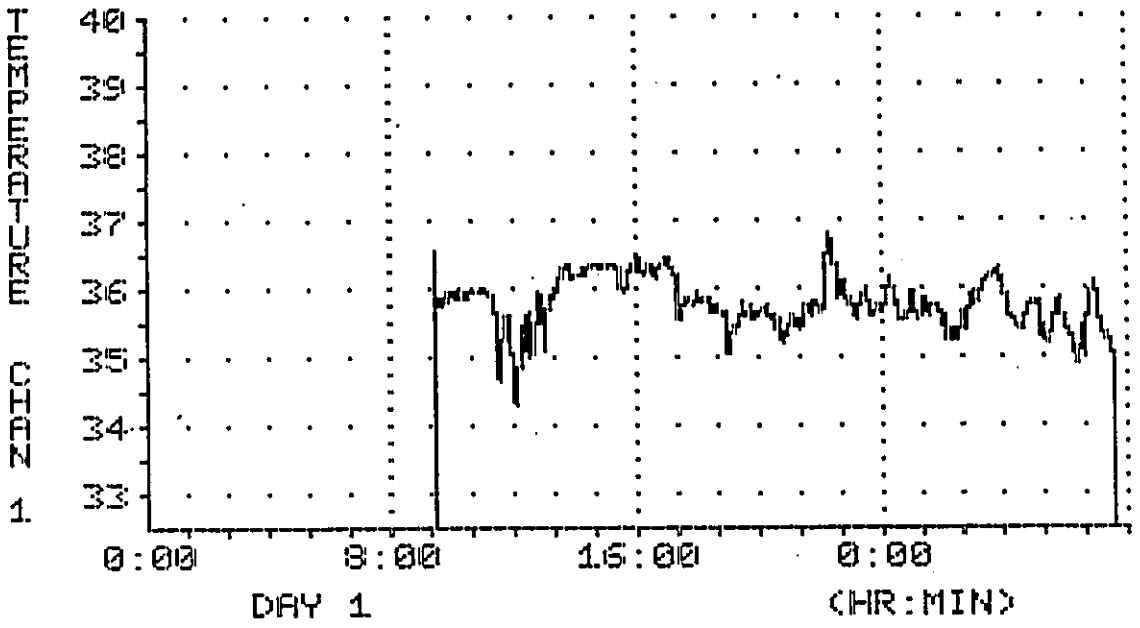
Thermisters, on the other hand, are semiconductors made of ceramic materials that require no reference temperature. These thermal resistors have a high negative temperature coefficient such that the resistance decreases as the temperature increases (Webster, 1978). Although thermisters have a slower response time than thermocouples, they have the advantage of greatly increased long term stability and sensitivity (Sapoff, 1971).

An attempt was made to develop a thermister for measuring tympanic temperature which, according to YSI (Ohio), had not yet been produced or tested. YSI supplied the ceramic bead which matched the resistance specifications of the Vitalog PMS-8 recorder. After affixing the wires, casing, and appropriate accessories to hold the probe firmly in the external auditory meatus, the electrical characteristics were

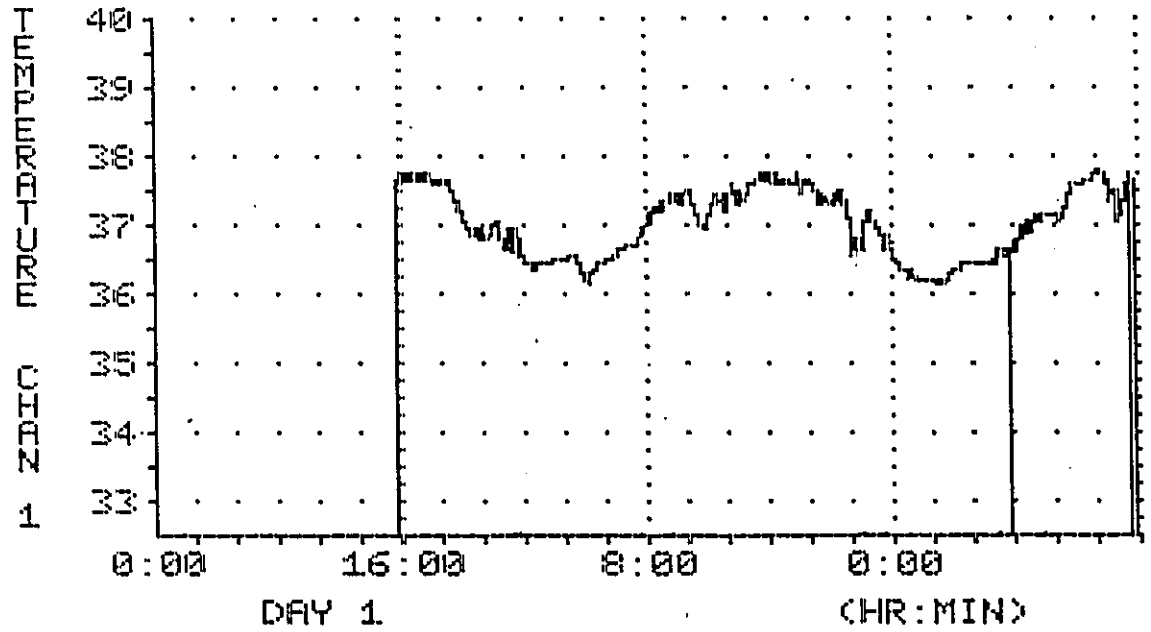
shown to be identical to the YSI series 400 rectal probe.

When rectal and tympanic temperatures were compared for two consecutive 24 hour recording periods, the tympanic thermister was shown to be influenced by ambient air temperature to a degree that obliterated the circadian temperature pattern. Based on these data, it was decided that the rectal thermister was the obvious choice for temperature procurement in this study. (See results on following page.)

TYMPANIC



RECTAL



APPENDIX F

TESTING FOR ACCURACY AND DRIFT OF THE VITALOG TEMPERATURE MONITORING SYSTEM

The results shown below are a sampling of the temperature data collected at 1 minute intervals for 168 hours (7 days) by the Vitalog System, and at 1 hour intervals from 0800 hours to 1700 hours each day by the Beckman Variance Mercury thermometer.

DAY	TIME	VITALOG	BECKMAN
1	0800	36.58	36.55
	0900	36.07	36.10
	1000	37.89	37.90
	1100	38.34	38.35
	1200	38.01	38.00
	1300	36.89	36.90
	1400	36.45	36.47
	1500	36.50	36.50
	1600	37.33	37.35
	1700	37.95	38.00
7	0800	37.05	37.00
	0900	36.95	36.95
	1000	37.90	37.90
	1100	38.05	38.00
	1200	37.55	37.57
	1300	37.33	37.30
	1400	38.45	38.45
	1500	38.50	38.50
	1600	37.33	37.40
	1700	36.75	36.75

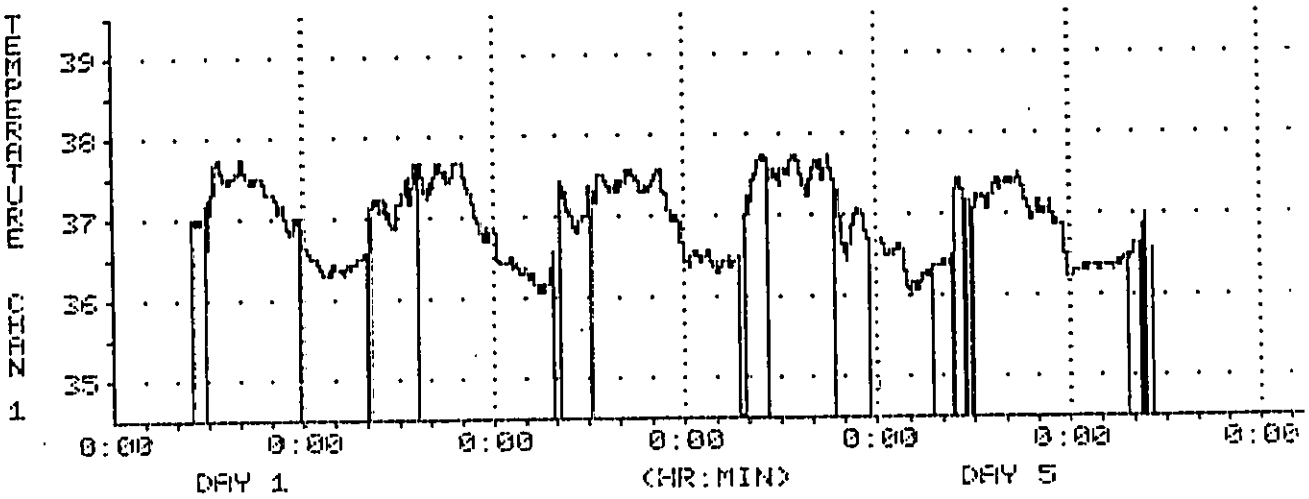
The results indicated 0% drift over the 7 day recording period and showed a maximum temperature discrepancy of only 0.05°C.

A P P E N D I X G

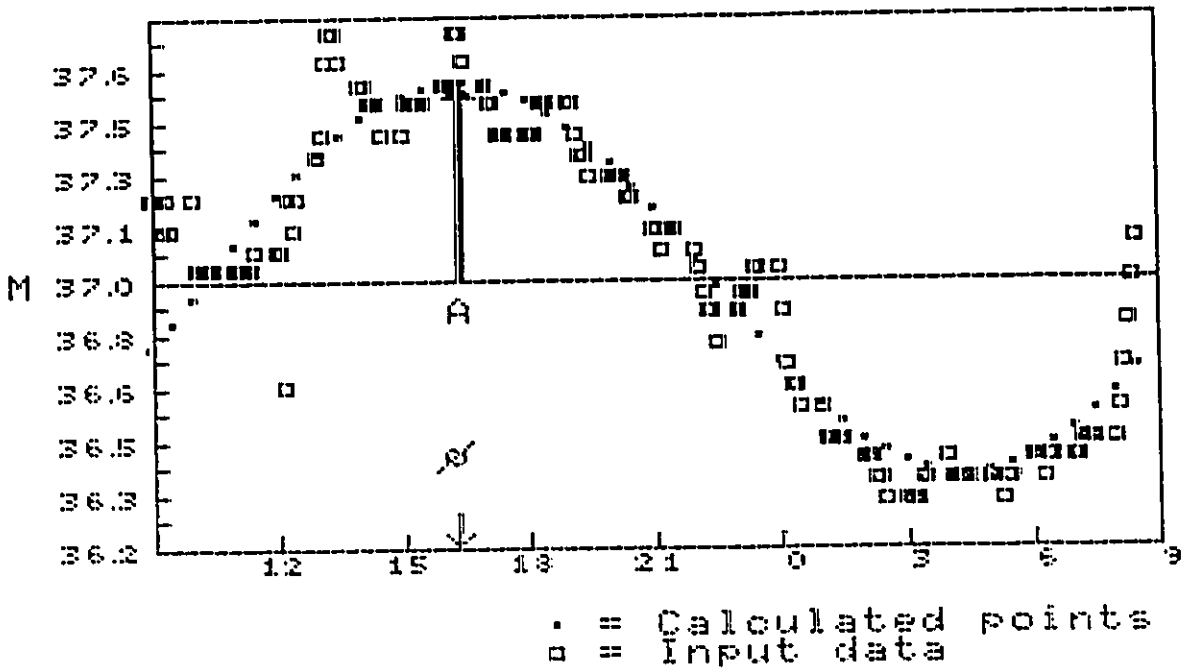
COMPLETE RAW DATA SAMPLES (1 PATIENT/GROUP) WITH

WITH COSINOR CURVE FITTING DISPLAYED FOR 1 OF THE 5 DAYS

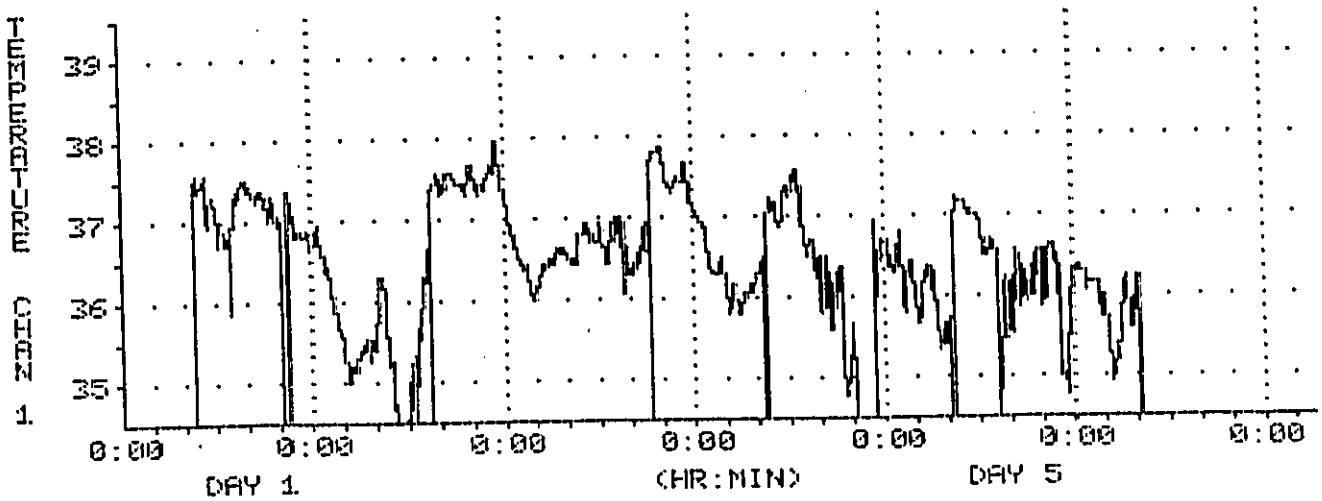
Control Subject



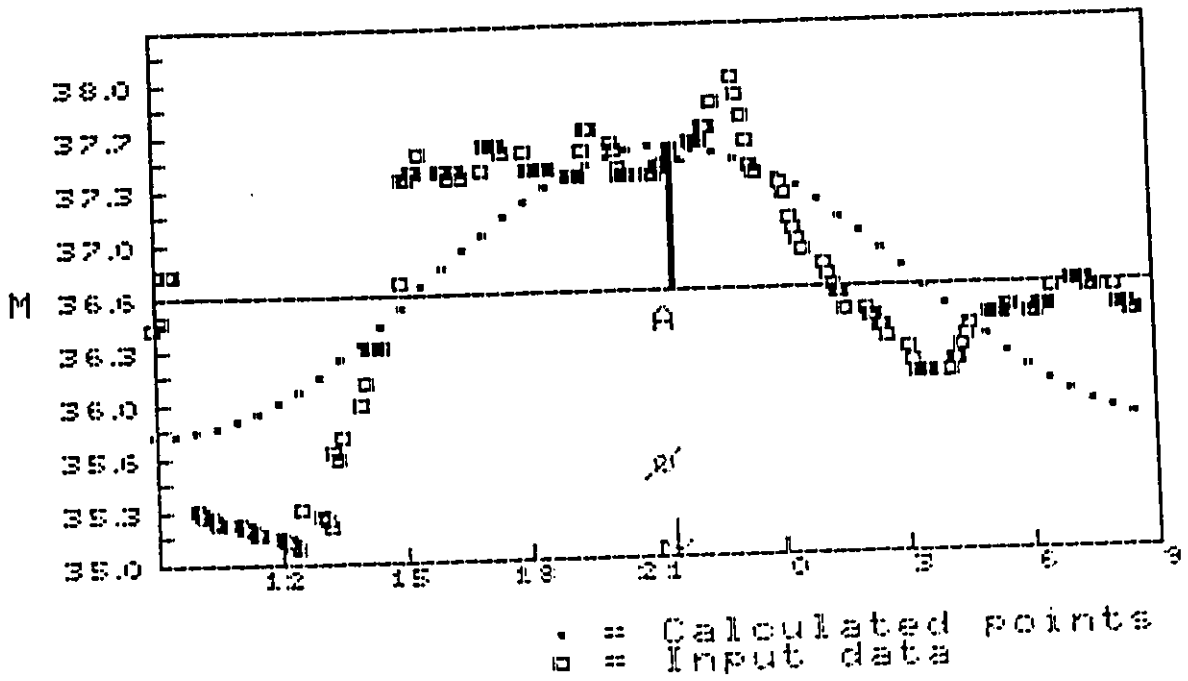
Cosinor Sample (day 1)



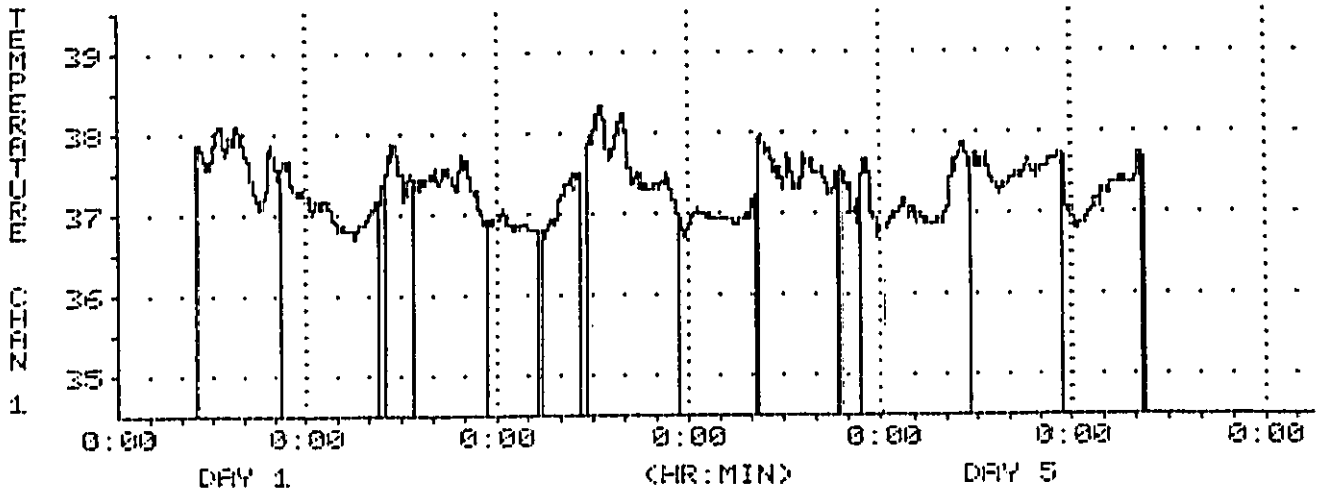
Variable Phase (Patient L)



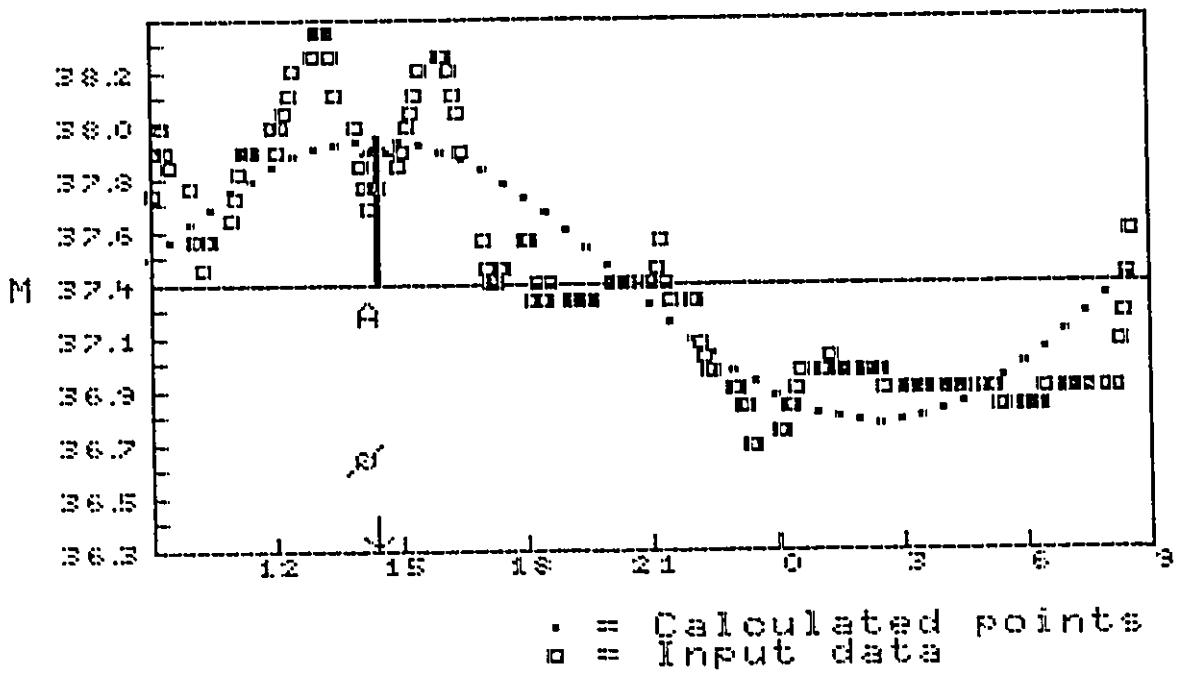
Cosinor Sample (Day 2)



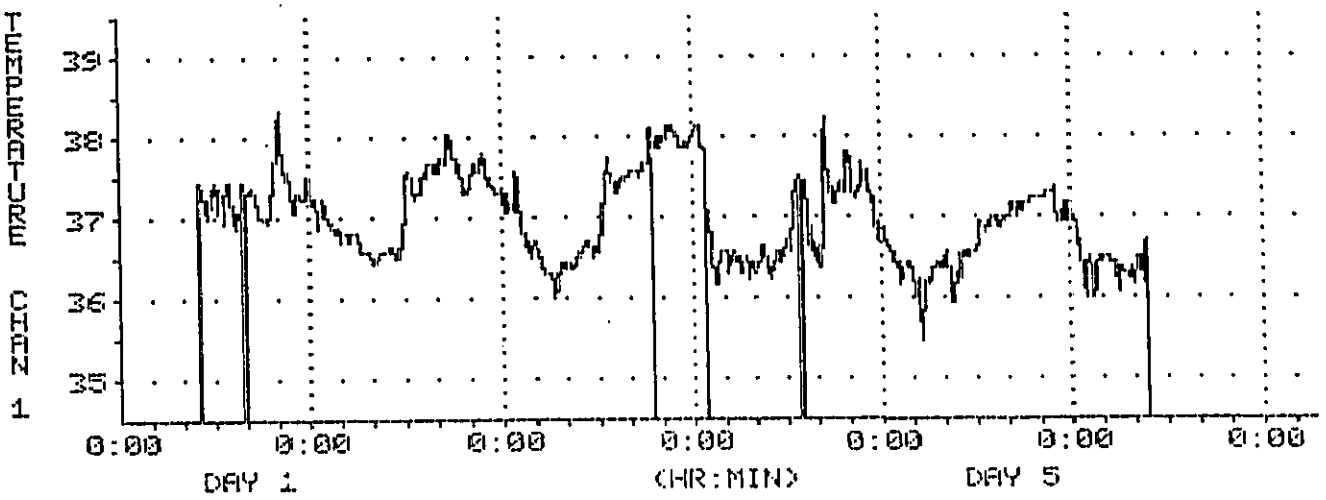
Advance Phase (Patient F)



Cosinor Sample (Day 3)



Delayed Phase (Patient C)



Cosinor Sample (Day 4)