Neuroendocrinology Letters ISSN 0172–780X

Neuroendocrinology Letters 2000; **21**:9–15 pii: NEL210100ER1
Reprinted with permission: Ciencia e Cultura 1998; **50**:172–177

The human body circadian: How the biologic clock influences sleep and emotion

by Daniel P. Cardinali
Departamento de Fisiología, Facultad de Medicina,
Universidad de Buenos Aires, Argentina.

Diurnal, nocturnal or seasonal modes of behavior are not passive responses to changes in the environment; rather, they are generated by an endogenous circadian pacemaker, entrained by a few environmental cues like light-dark cycles. Circadian clock mechanisms involve periodic gene expression, synchronized by a hierarchically superior structure located in mammals in the hypothalamic suprachiasmatic nuclei. Cycles of sleep and wakefulness are the most conspicuous circadian rhythm. Since modern humans use artificial light to extend their period of wakefulness and activity into the evening hours, they adhere to a short-night sleep schedule with a highly consolidated and efficient sleep. As shown by studies in artificial long nights, modern humans may be sleep-deprived. Humans have also increasingly insulated themselves from the natural cycles of light and darkness. Still, the human circadian pacemaker has conserved a capacity to detect seasonal changes in day length. A mood disorder involving a recurring autumn or winter depression (seasonal affective disorder, SAD) is related to latitude, with the number of cases increasing with distance from the equator. SAD is ameliorated by using brilliant light. In nonseasonal depression, mood typically fluctuates daily, with improvement over the course of the day, and various physiological functions exhibit an altered circadian pattern, suggesting a link with circadian disruption. Treatment of circadian rhythm disorders, whether precipitated by intrinsic factors (e.g., sleep disorders, blindness, mental disorders, aging) or by extrinsic factors (e.g., jet lag, shift work) has led to the development of a new type of agents called "chronobiotics," among which melatonin is the prototype.

Introduction

Many biological functions wax and wane in cycles that repeat each day, month, or year. Such patterns do not reflect simply organism's passive response to environmental changes. Rather, they reflect the organism's biological rhythms, that is, its ability to keep track of time and to direct changes in function accordingly [1–5].

Because the Earth rotates on its axis, it presents two environments, i.e. light and darkness; because the Earth's axis of rotation is tilted, durations of daily periods of darkness and light vary systematically during the course of the year. Through Evolution, animals responded to these environmental changes by preferentially adapting to them. This is the origin of biological rhythms that repeat approximately every 24 hours, called circadian rhythms (from the Latin *circa,* for around, and *dies,* for day), and of rhythms that oscillate annually, following the recursive appearance of the seasons [1–6]. Thus when animals switch between diurnal, nocturnal or seasonal modes of their behavior, they are not simply responding passively

to changes in external lighting conditions. They are responding to signals generated by a circadian pacemaker that is synchronized with the cycles of the Earth's rotation, anticipating the transitions between day and night, and triggering appropriate changes in behavioral state and physiological substrates. In this way, the circadian pacemaker creates a day and night within the organism that mirrors approximately the world outside [6–8].

The rationale for a circadian structure is linked to homeostasis, that is to the mechanisms that enable the body to keep equilibrium in response to variations of the environment. Such a variation may disturb the physiological balance of the organism, a situation that engenders adaptive responses in the whole biologic range, from gene expression to behavior. The full repertory of strategies mediating adaptation to unpredictable variations in the environment is called "reactive homeostasis." Besides such unpredictable challenges, organisms that are subjected to environmental cues arising at regular intervals anticipate periodic environmental challenges, rather than adapt to them every time they appear. Such homeostatic mechanisms generate endogenous signals that act on cellular and physiological systems and prepare the organism to anticipated changes in external conditions ("predictive homeostasis") [9].

Research in animals and humans has shown that only a few such environmental cues, such as light-dark cycles, are effective entraining agents for the circadian oscillator ("Zeitgebers") [10, 11]. In addition, the sleep wake schedule and social cues may also be important entraining agents in humans. An entraining agent can actually reset, or phase shift, the internal clock. Variable shifting of the internal clock is illustrated in the phase response curve: depending on when an organism is exposed to such an entraining agent, circadian rhythms may be advanced, delayed, or not shifted at all [1]. Therefore, involved in adjusting the daily activity pattern to the appropriate time of day is a rhythmic variation in the influence of the Zeitgeber as a resetting factor. In humans, light exposure during the first part of the night delays the phase of the cycle; a comparable light change near the end of the night, advances it [11, 12]. At other times during the day light exposure has no phase-shifting influence. Melatonin, the endogenous chemical code of the night, showed an opposite phase response curve to light, producing phase advances during the first half of the night and phase delays during the second [13].

Among the innumerable periodic changes that underlie and support the overt physiologic rhythms, the peak values occur in a characteristic sequence over the day ("phase map"). Such a sequence and spacing reflects the order and temporal relationships of cause-effect in the normal interactions of the various bodily processes and is very indicative of the organism's health [1–5]. Phase maps may undergo transitory disruptions when an organism is compelled to make a rapid phase adjustment as, for example, after a rapid move to a new geographic longitude or as a consequence of shift work. Under such circumstances the various individual 24-hour components comprising the circadian phase map do not reset their phases to the new environmental times at the same rate, and they become somewhat displaced in their relations to one another. To reset them to the new local time requires several days of exposure to the local phase setters. This phenomenon is quite familiar to persons who have traveled long distances rapidly. The resultant rhythmic dislocation and the need for gradual adjustment over two to ten days at the end of such a trip is often referred to as "jet lag."

The Clock

Based upon work in many laboratories it is now established that the mechanism of the circadian clock involves periodic gene expression [14–17]. Circadian rhythms have been documented throughout the plant and animal kingdom at every level of eukaryotic organization and in various species *(Drosophila melanogaster, Neurospora, mouse, golden hamster)* the genes controlling circadian rhythms have been identified (genes: *per, frq, clock, tau).* However the products of these clock genes and their biochemical roles are not yet known. Clock genes appear not to work in an enzymatic manner but rather display stoichiometric behavior and effects. Presumably they are a universal property of all cell types and organisms, such as the universality of the cell cycle [14–17].

In pluricellular organisms, circadian phenomenology of every cell requires being synchronized by a hierarchically superior structure to build up a circadian rhythm. In mammals, considerable experimental evidence indicates that a region of the hypothalamus, the suprachiasmatic nuclei (SCN), is a major circadian pacemaker [4, 18, 19]. The SCN, composed of a cluster of thousands of small nerve cells, can generate circadian rhythms when isolated from other areas of the brain. The integrity of the SCN is necessary for the generation of circadian rhythms as well as for synchronization of rhythms with light-dark cycles. Compelling evidence that the SCN functions as the primary circadian pacemaker comes from animal studies of SCN transplantation. In these experiments, the SCN is destroyed, abolishing cir-

cadian rhythms. When fetal brain tissue containing SCN nerve cells is transplanted into the brains of these animals, circadian rhythms are restored [4, 18, 19].

Light in the environment activates cells in the eye, which in turn activate early genes, e.g. the c-fos gene, within cells in the SCN. The output is principally transmitted down the axons of SCN neurons to specific targets in the central nervous system and hence to the rest of the body through neuronal and endocrine pathways. Indeed, like human oligarchies, the SCN "neuronal oligarchy" controls the circadian clocks of trillions of cells in the body monopolizing the two major networks of communication: the endocrine and the autonomic nervous systems. The clock can be entrained not only by photic stimuli. principally through neuronal signals from the retina, but also by non-photic stimuli like the pineal hormone melatonin or physical activity, as well as by associative learning processes [10].

Sleep-Wakefulness Cycle

Daily cycles of sleep and wakefulness form the most conspicuous circadian rhythm among humans. Sleep is a necessary component of life. When synchronized to the 24-hour day, sleep typically occurs during the night hours and bears a constant, if complex, relationship to other circadian rhythms [20–22]. The circadian clock governs particular components of sleep, including total amount of sleep and sleepiness, are governed by circadian clock. Even a modest reduction in sleep leads to decrements in performance, especially at night. Furthermore, when deprived of a night or more of sleep, individuals can find sleep impossible to resist, especially in monotonous situations, and they experience brief episodes of sleep, called microsleeps. Therefore, periods of sleep are not readily rescheduled, deferred, or resisted. The requirement for sleep and the control of its timing have important implications for work schedules and shift work [23].

Sleep is not a homogeneous state [21, 24]. Polysomnography, the measurement of electrical activity in the brain, eye movement, and muscle tone, has revealed distinct stages of sleep. During stages 1 through 4, sleep becomes progressively deeper. In stages 3 and 4, which constitute slow-wave sleep, the eyes do not move, heart rate and respiration are slow and steady, and muscles retain their tone but show little movement; dreams are infrequent. As sleep continues, dramatic changes occur: brain activity appears similar to that seen during wakefulness, heart rate and respiration increase and become erratic, dreams are vivid and frequently reported, and the eyes move rapidly. This stage of sleep is rapid-eye-movement (REM) sleep. Typically, cycles of non-REM sleep (stages 1 through 4) and REM sleep repeat every 90 to 100 min throughout the course of a night's sleep [21].

Since modern humans use artificial light to extend their period of wakefulness and activity into the evening hours, they adhere to a short-night sleep schedule throughout the year for most of their lives [24]. In these circumstances, individuals fall asleep shortly after lying down and sleep without interruption until they arise in the morning. This type of sleep, which we tend to regard as our only normal type of sleep, is highly consolidated and efficient, occupying almost all of the nightly period of bed rest. However, modern humans probably obtain less than their full quota of nightly sleep. At steady state in artificial long nights, men sleep an average of 8.25 h/night, which is more than most men obtain in modern life. This finding raises the possibility that modern humans are sleep-deprived and less fully awake in the daytime than would otherwise be the case.

Differing from man, the sleep of most other animals is polyphasic, exhibiting multiple bouts per day. In fact, most people would probably regard sleep in polyphasic bouts that alternate with periods of quiet wakefulness as abnormal and undesirable if it occurred. However, slee p studies of voluntaries in long nights indicate that human sleep may also be polyphasic [25–27]. The periods of quiet rest that may have once occurred in association with this type of sleep in the past are nearly extinct in modern times. In long nights, periods of quiet rest and contemplation often begin after transitions to wakefulness from periods of REM sleep (and dreaming) that are particularly intense. It is tempting to speculate that in prehistoric times this arrangement provided a channel of communication between dreams and waking life that has gradually been closed off as humans have compressed and consolidated their sleep. If so, then this alteration might provide a physiological explanation for the observation that modern humans seem to have lost touch with the wellspring of myths and fantasies.

Chronobiology and Mood Disorders

Our hominid ancestor, *Homo erectus,* used caves as shelters and may have used fire as early as 1.5 million years ago. *Homo sapiens* began to construct artificial dwellings (which could block out the rays of the sun) as early as 45,000 years ago, and to make lamps (which could be used to extend the daily period of illumination into nighttime hours) as early as 28,000 years ago [28]. In the past 200 years,

humans have developed increasingly efficient lamps and inexpensive sources of energy to power them. At the same time, they have increasingly moved their activities from countryside to city and from outdoors to indoors, where natural light may not penetrate. Consequently, humans have increasingly insulated themselves from the natural cycles of light and darkness that have shaped the endogenous rhythms of life on this planet for billions of years.

However, the human circadian pacemaker has conserved a capacity, like that in other animals, to detect seasonal changes in the length of the day and to make corresponding adjustments in the durations of the biological day and night within [6–8, 27]. One of these seasonal rhythms is that of mood. While seasonal tendencies in mood disorders have been noted for hundreds of years, only in the 1980s researchers documented a mood disorder involving a recurring autumn or winter depression. Currently, this form of seasonal affective disorder (SAD) is the subject of extensive study [29–31].

Each fall or winter, individuals suffering from SAD may tire easily, crave carbohydrates, gain weight, experience increased anxiety or sadness, and exhibit a marked decrease in energy. With protracted daylight in the spring, patients emerge from their depression and sometimes even display modest manic symptoms. Epidemiological studies indicate that SAD is related to latitude, with the number of cases increasing with distance from the equator. Data from several studies suggest that light therapy is a useful treatment for SAD. The recommended protocol for treatment involves exposure to light in the morning with an intensity of 2,500 lux (which is equivalent in intensity to outdoor light at dawn) for 2 h per day [29–31].

The cause of SAD and the way in which light therapy alleviates it are not known. It has been hypothesized that circadian rhythms are delayed or possibly that the amplitude is dampened. Patients with SAD have been reported to show phase-delayed circadian temperature and melatonin rhythms. Hypotheses to account for SAD pathophysiology include [29]: (i) SAD is triggered by the decrease in light availability during autumn and winter, while light therapy increases total daily light exposure; (ii) SAD is triggered by the shortening of day lengths in autumn and winter, while light therapy lengthens the effective photoperiod; and (iii) SAD is triggered by a seasonally dependent abnormally delayed circadian phase position, while light therapy acts to phase advance the circadian pacemaker and thus correct the phase abnormality.

In the case of nonseasonal depression, several observations suggest a link with altered circadian rhythm [29]. Among persons suffering from depression, mood typically fluctuates daily, with improvement over the course of the day. Persons also demonstrate seasonal patterns, with an apparent increase in the incidence of depression, as indicated by hospital admissions, electroconvulsive therapy and suicide records, in the spring and autumn. Various physiological functions may exhibit an altered circadian pattern in depression, notably the timing of REM sleep. In people suffering from depression, the first REM episode occurs earlier after sleep begins, and REM sleep is abnormally frequent during the early hours of sleep. Rhythms of body temperature, hormone and brain chemical secretion, and sleep wake cycles deviate during episodes of depression, peaking earlier than normal or, more commonly, exhibiting dampened amplitude. The action of various antidepressant drugs and therapies provides further evidence for a link between circadian rhythm disruption and depression. In animal studies several classes of antidepressant drugs either lengthen the circadian cycle, delay distinct circadian rhythms, or influence synchronization with environmental cues.

Studies showing that late-night sleep deprivation temporarily alleviates depression also suggest, but do not prove, a link between circadian rhythms and nonseasonal depression. Missing one night's sleep halted depression immediately in about 60% of the patients (the next sleep episode usually resulted in the return of depression, and in about 30% of the patients a manic episode was triggered) [27, 28]. According to the phase-advance hypothesis, one might predict that experimentally imposed phase-delays of sleep timing should induce depression-like symptoms in healthy normal subjects, and, indeed, modest but reliable mood decrements have been reported. Sudden circadian phase-shifts can induce depressive symptoms in predisposed individuals, as documented for subjects with a history of affective illness becoming depressed after a westbound flight or manic after an eastbound flight across several time zones.

It has been suggested that blunted amplitude of circadian rhythms may be the main chronobiological abnormality associated with affective disorder [29]. In addition, it has been suggested that the pathophysiology of depression be primarily characterized by intra- or intersubject instability of rhythms, rather than by a particular rhythm abnormality. Phase instability could arise through any of several mechanisms, including an endogenous circadian period either very close to or very far from 24 h, a low-amplitude pacemaker or weak coupling between the pacemaker and its photic and nonphotic entraining stimuli.

While light is traditionally considered the primary synchronizing agent, humans are responsive to nonphotic cues including social stimulation, food availability, and behaviorally derived feedback. Therefore social Zeitgebers—defined as personal relationships, jobs, or interpersonal demands that serve to entrain biological rhythms—are playing an increasingly role in circadian hypotheses of affective disorders. Psychosocial precipitants such as stressful life events or lack of appropriate social support systems can destabilize social Zeitgebers, leading to disrupted circadian rhythms. Sudden changes in habits or duties could precipitate depressive episodes, e.g., in recently widowed subjects; individuals with most highly disrupted social rhythms had the highest depression scores. Indeed, photic and social Zeitgebers can be quite difficult to disentangle, since social behavior and cognitive factors influence light exposure, and since people may respond to imposed changes in lighting as informational signals, rather than as photic cues. Depressed patients have been reported to show increased light sensitivity and blunting of light sensitivity induced by antidepressants [26, 27, 29].

Control of Circadian Rhythms in Humans

Interest in manipulating the internal clock has grown with improved understanding of human circadian rhythms and increasing awareness of circadian rhythm disruption, whether precipitated by intrinsic factors (e.g., sleep disorders, blindness, mental disorders, or aging) or extrinsic factors (e.g., air travel across time zones and shift work). Several agents are under investigation or have been proposed for use in manipulating circadian rhythms. They are generically called "chronobiotics," to define its activity on the circadian clock [32]. In order to assert that an agent's primary action is on the circadian system, several questions must be addressed, including: How are circadian rhythms modified? Are all circadian rhythms altered or only certain rhythms, such as the sleep-wake cycle? How is the circadian pacemaker influenced? Does the effect vary at different times during the circadian cycle? Does the agent have any side effects or drawbacks?

Besides light, one prototype chronobiotic agent is melatonin [13, 33–35]. Melatonin is a hormone produced by the pineal gland. In mammals, including humans, information about light in the environment is transmitted from the eye through multiple nerve cells to the pineal gland. Melatonin secretion is normally limited to nighttime hours, a pattern of secretion that is regulated in two ways: i) light suppresses the pineal gland's production of melatonin, and ii) melatonin secretion is regulated by the circadian pacemaker, exhibiting a circadian rhythm even in the absence of environmental cues. For this reason, melatonin can be used as a marker for circadian rhythms [34].

Melatonin plays an established role in controlling reproduction and is involved in sexual maturation. It has a major influence on the circadian organization of vertebrates including human beings as well as it exhibits diverse behavioral effects. Photoperiodic time measurement in adult and fetal mammals is critically dependent upon the melatonin signal. In the fetus, strong evidence exists for a physiological role of the maternal melatonin signal as a synchronizer [13, 33–35].

The mechanisms sensitive to melatonin that mediate the clock message sent by the pineal gland in the form of a melatonin cycle may reside in the brain. There are also data indicating that the melatonin receptors located in different other organs can convey circadian-meaningful information to every cell in the organism, i.e., melatonin can play the role of an "internal Zeitgeber." It is also clear that melatonin has a number of versatile functions that far transcend its actions on photoperiodic time measurement and circadian entrainment, like its free radical scavenger and immunomodulatory properties [36–38].

The effect of melatonin on circadian rhythms has been examined in humans. In several controlled studies, melatonin succeeded to act as a synchronizing agent. Other studies reported that melatonin counteracted subjective feelings of jet lag [34]. Several studies have used blind persons, who are often not synchronized with the environment (i.e. in a free running of its endogenous clock), to assess melatonin's effect on the circadian pacemaker. The administration of melatonin synchronized the disturbed sleep-wake cycle of blind individuals and caused phase advances in blind subjects with free-running rhythms [34].

Another major biologic situation in which circadian rhythm alteration can be improved by melatonin is aging [39–41]. Studies confirm that many physiological and behavioral functions that typically display circadian rhythms are altered with advancing age in humans. For example, there are usually conspicuous changes in sleep habits, such as earlier onset of sleepiness, early morning awakening, and increased daytime napping. During sleep, there is an increase in the number and duration of waking episodes, there is a reduction in the nondreaming phases of sleep, the first REM phase occurs earlier in the night, and the tendency to fall asleep is increased during the day. While factors such as changing social habits, medication, and disease pro-

cesses can impinge on functions that exhibit circadian rhythms, such as sleep, activity, alertness, and hormone secretion, research suggests that aging affects the circadian system itself [39–41]. A decrease in the amplitude and length of various cycles with age has been observed in animal studies, including longitudinal studies.

From an epidemiological point of view, complaints of difficulty in initiating and maintaining sleep and daytime drowsiness are more common in the elderly than in any other age group. Evidence indicates that impaired melatonin secretion is associated with sleep disorders in old age [34, 40]. Therefore, melatonin therapy can be useful to augment the amplitude of circadian rhythms, as well as to phase shift or entrain the rhythms in elderly people. In a recent study [42] we assessed the sleep-promoting action of melatonin (3 mg p.o.) in elderly subjects with psychophysiological insomnia. Melatonin was taken at bedtime for a 3-week period. The results indicate that melatonin therapy was beneficial in the initiation and maintenance of sleep in patients suffering from insomnia alone or associated with depression symptoms. Self-rated estimates of next-day function (i.e., alertness in the morning and during the day) improved significantly after melatonin in elderly patients with sleep disturbances alone, but not in insomnia patients showing depression. Hence, melatonin efficacy to improve sleep did not correlate with a similar beneficial effect on mood in depressed patients.

Benzodiazepines are widely used in the elderly population for the initiation of sleep. However, very frequently, complaints about poor sleep maintenance persist despite benzodiazepine treatment. In our study, 62% of elderly patients suffering insomnia and taking melatonin reduced or suppressed benzodiazepine use [42]. The results are in agreement with the efficacy of melatonin treatment to increase sleep efficiency and total sleep time and to decrease wake after sleep onset, sleep latency and number of awakenings in elderly subjects who have been taking benzodiazepines and had low melatonin output [43]. Since we previously reported in animal studies that melatonin and benzodiazepines shared several neurochemical and behavioral properties [33], melatonin therapy could be postulated as an effective tool to decrease the dose of benzodiazepine needed in patients. Indeed, this was recently shown to be true in clinical studies [44].

Concluding Remarks

Consciousness, defined by the English philosopher John Locke as "the perception of what passes in a man's own mind," depends heavily on the levels of alertness and therefore exhibits significant circadian fluctuations. Particular types of performance peak at different times during the circadian cycle, depending on perceptual involvement, the use of memory, and the amount of logical reasoning required. Performance of tasks involving manual dexterity, simple recognition, and reaction time parallels the circadian rhythm of body temperature, peaking when body temperature is highest, in the late afternoon. Verbal reasoning peaks earlier in the circadian cycle and may adjust more quickly than other types of performance to such disruptions as jet lag or shift work. In addition, when subjects are asked to indicate their level of alertness, weariness, happiness, or other moods on a visual scale at regular times throughout the course of the day, consistent circadian patterns emerge.

Many aspects of human performance decline to minimal levels at night, reflecting not only the influence of the circadian pacemaker, but also the lack of sleep. Sleep deprivation, even for one night, is one of the most important disrupting factors of human mental and physical function. The circadian clock also leads to a nighttime minimum in many types of performance. Thus, sleep deprivation combined with the influence of the circadian pacemaker can severely curtail performance at night. These factors have important implications for our "24-Hour Society," that superimposes an increasing demand for night work on our grossly inadequate physiological design, unable to keep unmodified levels of alertness regardless of time of day.

Acknowledgments

Studies in author's laboratory were supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina, the University of Buenos Aires and the Community of the European Countries, Brussels.

Correspondence to:
Dr. D. P. Cardinali,
Departamento de Fisiología,
Facultad de Medicina, UBA, CC 243.
1425 Buenos Aires, ARGENTINA
TEL: 54-1-9619866; FAX: 54-1-9636287
E-mail: cardinal@mail.retina.ar

REFERENCES

1 Aschoff J. Circadian clocks. Amsterdam: North Holland, 1965.
2 Minors DS, Waterhouse JM. Circadian rhythms and the human. Bristol, London, Boston: Wright; 1981.
3 Cardinali DP, Golombek DA, Bonanni Rey RA. Relojes y Calendarios Biológicos. Sincronía del Hombre con su Medio Ambiente. Buenos Aires: Fondo de Cultura Económica; 1992.
4 Rusak B. The mammalian circadian system: models and physiology. J Biol Rhythms 1989; **4**:121–134.
5 Marques N, Menna-Barreto L, editors. Cronobiologia: Princípios e Aplicações. EDUSP, São Paulo; 1997.
6 Pittendrigh CS. The photoperiodic phenomena: Seasonal modulation of the 'day within'. J Biol Rhythms 1988; **3**:173–188.
7 Roenneberg T, Aschoff J. Annual rhythm of human reproduction: 1. Biology, sociology, or both? J Biol Rhythms 1990; **5**:195–216.
8 Roenneberg T, Aschoff J. Annual rhythm of human reproduction: 11. Environmental correlations. J Biol Rhythms 1990; **5**:217–239.
9 Moore-Ede M. Physiology of the circadian timing system: predictive versus reactive homeostasis. Am J Physiol 1986; **250**: R737–R752.
10 Hastings MH, Best JD,Ebling FJ, Maywood ES, McNulty S, Schurov I, et al. Entrainment of the circadian clock. Prog Brain Res 1996; **111**: 147–174.
11 Murphy PJ, Campbell SS. Physiology of the circadian system in animals and humans. J Clin Neurophysiol 1996; **13**:2–16.
12 Duffy JF, Kronauer RE, Czeisler CA. Phase-shifting human circadian rhythms: Influence of sleep timing, social contact and light exposure. J Physiol London 1996; **495**:289–297.
13 Cagnacci A. Influences of melatonin on human circadian rhythms. Chronobiol Int 1997; **14**:205–220.
14 Young MW, editor. Molecular genetics of biological rhythms. New York: Marcel Dekker; 1993.
15 Gekakis N, Saez L, Delahaye-Brown A, Myers MP, Sehgal A, Young MW, Weitz CJ. Isolation of timeless by PER protein interaction: defective interaction between timeless protein and long-period mutant PERL. Science 1995; **270**:811-5.
16 Emery IF, Noveral JM, Jamison CF, Siwicki KK. Rhythms of Drosophila period gene expression in culture. Proc Natl Acad Sci USA 1997; **94**:4092–4096.
17 Menaker M, Moreira LF, Tosini G. Evolution of circadian organization in vertebrates. Braz J Med Biol Res 1997; **30**:305–313.
18 Sumova A, Travnickova Z, Peters R, Schwartz WJ, Illnerova H. The rat suprachiasmatic nucleus is a clock for all seasons. Proc Natl Acad Sci 1995; **92**:7754-7758.
19 Hofman MA, Zhou JN, Swaab DF. Suprachiasmatic nucleus of the human brain: an immunocytochemical and morphometric analysis. Anat Rec 1996; **244**:552–562.
20 Dijk D-J, Czeisler CA. Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity in humans. J Neurosci 1995; **15**:3526-3538.
21 Billiard M, Carlander B, Besset A. Circadian rhythms in normal and disordered sleep. Pathologie Biologie 1996; **44**:509–517.
22 Lack LC, Lushington K. The rhythms of human sleep propensity and core body temperature. J Sleep Res 1996; **5**:1–11.
23 Sack R, Blood M, Lewy A. Melatonin rhythms in night shift workers. Sleep 1997; **15**:434–441.
24 Lavie P, Zvuluni A. The 24-h sleep propensity function: Experimental bases for somnotypology. Psychophysiol 1992; **29**:566–575.
25 Wehr TA. In short photoperiods, human sleep is biphasic. J Sleep Res 1992; **1**:103-107.
26 Wehr TA, Moul DE, Barbato G, Giesen HA, Seidel JA, Barker C, et al. Conservation of photoperiod-responsive mechanisms in humans. Am J Physiol 1993; **265**:R846–R857.
27 Wehr TA, Sack DA, Duncan WC, Rosenthal NE, Mendelson WB, Gillin JC, et al. Sleep and circadian rhythms in affective patients isolated from external time cues. Psychiatry Research 1985; **15**:327–339.
28 de Beaune SA, White R. Ice age lamps. Sci Amer 1993; **268**:108–113.
29 Duncan WC. Circadian rhythms and the pharmacology of affective illness. Pharmacology & Therapeutics 1996; **71**:253–312.
30 Monk TH, Buysse DJ, Reynolds CF, Berga SL, Jarrett DB, Begley AE, et al. Circadian rhythms in human performance and mood under constant conditions. J Sleep Res 1997; **6**:9–18.
31 Thompson C, Childs PA, Martin NJ, Rodin I, Smythe PJ. Effects of morning phototherapy on circadian markers in seasonal affective disorder. Brit J Psychiatry 1997; **170**:431–435.
32 Dawson D, Armstrong SM. Chronobiotics—drugs that shift rhythms. Pharmacol Ther 1996; **69**:15–36.
33 Golombek D, Pevet P, Cardinal D. Melatonin effect on behavior: Possible mediation by the central GABAergic system. Neurosci Biobehav Rev 1996; **20**:403–412.
34 Arendt J, Deacon S. Treatment of circadian rhythm disorders—Melatonin. Chronobiol Int 1997; **14**:185–204.
35 Brzezinski A. Melatonin in humans. N Engl J Med 1997; **336**:186–195.
36 Cardinali DP, Golombek DA, Rosenstein RE, Cutrera RA, Esquifino AI. Melatonin site and mechanism of action: Single or multiple? J Pineal Res 1997; **23**:32–39 .
37 Cardinali DP, Brusco LI, Selgas L, Esquifino AI. Melatonin: A synchronizing signal for the immune system. Neuroendocrinol Lett 1997; **18**:73–84.
38 Reiter RJ. Antioxidant actions of melatonin. Adv Pharmacol 1997; **38**:103–117.
39 Touitou Y, Bogdan A, Haus E, Touitou C. Chronobiological approach to ageing. Pathol Biol 1996; **44**:534–546.
40 Haimov I, Lavie P. Melatonin—A chronobiotic and soporific hormone. Arch Gerontol Geriatr 1997; **24**:167–173.
41 Vitiello MV. Minireview: Sleep disorders and aging: Understanding the causes. J Gerontol Series A—Biol Sci Med Sci 1997; **52**:M189–M191.
42 Fainstein I, Bonetto A, Brusco I, Cardinali DP. Melatonin effects in sleep-disturbed elderly patients. Curr Ther Res 1997; in press.
43 Garfinkel D, Laudon M, Zisapel N. Improvement of sleep quality by controlled-release melatonin in benzodiazepine-treated elderly insomniacs. Arch Gerontol Geriatr 1997; **24**:223–231.
44 Dagan Y, Zisapel N, Nof D, Laudon M, Atsmon J. Rapid reversal of tolerance to benzodiazepine hypnotics by treatment with oral melatonin: A case report. Europ Neuropsychopharmacol 1997; **7**:157–160.