Resetting the Melatonin Rhythm with Light in Humans

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Abstract The endogenous circadian rhythm of melatonin in humans provides information regarding the resetting response of the human circadian timing system to changes in the light-dark (LD) cycle. Alterations in the LD cycle have both acute and chronic effects on the observed melatonin rhythm. Investigations to date have firmly established that the melatonin rhythm can be reentrained following an inversion of the LD cycle. Exposure to bright light and darkness given over a series of days can rapidly induce large-magnitude phase shifts of the melatonin rhythm. Even single pulses of bright light can shift the timing of the melatonin rhythm. Recent data have demonstrated that lower light intensities than originally believed are capable of resetting the melatonin rhythm and that stimulation of photopically sensitive photoreceptors (i.e., cones) is sufficient to reset the endogenous circadian melatonin rhythm. In addition to phase resetting, exposure to light of critical timing, strength, and duration can attenuate the amplitude of the endogenous circadian rhythm of melatonin. Measurement of melatonin throughout resetting trials provides a dynamic view of the resetting response of the human circadian pacemaker to light. Future studies of the melatonin rhythm in humans may further characterize the resetting response of the human circadian timing system to light.

Key words melatonin, circadian, light, phase, amplitude, resetting, human, review

Introduction

Circadian rhythms, endogenous self-sustaining biological oscillations occurring with a period of approximately 24 h, are present at all levels of biological complexity from unicellular organisms, including both prokaryotes and eukaryotes, to humans. The phase, amplitude, and period are the most salient characteristics of a circadian oscillation; as such, they often are described in studies using measurements of output variables believed to reflect the state of the internal circadian pacemaker. The light-dark (LD) cycle is the most powerful synchronizing stimulus acting on the human circadian timing system (Czeisler, 1995). Light is responsible for entrainment of the pacemaker to the 24-h day, and exposure to light and darkness can acutely reset the timing of the circadian pacemaker. The molecular mechanisms by which the LD cycle resets the human circadian pacemaker, and by which the circadian oscillator communicates temporal information to generate output rhythms, remain to be defined.

When studied under constant conditions, a number of physiological parameters exhibit circadian rhythmicity in humans: core body temperature, the sleep-wake cycle, circulating hormones (e.g., cortisol,

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gonadotropin, growth hormone, melatonin, parathyroid hormone, prolactin, thyroid-stimulating hormone), blood pressure, cognitive performance, urine volume, and electrolyte levels (see Czeisler, 1995, for references). Because direct studies of the mechanisms of the hypothalamic circadian pacemaker in humans have not been technically possible, investigators rely heavily on measurements of overt rhythms downstream from the clock. As such, there is much debate over which characteristics of the output variables (e.g., phase, period, amplitude) represent the status of the clock itself versus measures that initially were generated by the clock but have been modulated at some point along the signal transduction pathway between the clock and the observed output rhythm.

An observed rhythm may reflect a combination of the endogenous component of the rhythm with exogenous or masking effects superimposed. To use a marker variable to represent the output of the circadian pacemaker, it is important to isolate the underlying endogenous component of the rhythm by minimizing confounding influences of environmental or evoked effects. One method by which to assess the endogenous components of the output rhythms is to monitor circadian rhythms under constant environmental and behavioral conditions for a minimum of one circadian cycle, as initially described by Mills et al. (1978) and later modified by Czeisler (1995). This technique either eliminates or evenly distributes throughout the circadian cycle masking effects associated with environmental (e.g., ambient light exposure) or behavioral (e.g., posture, state of consciousness) influences. The multiple output rhythms that have been measured in the study of human circadian physiology have varying degrees to which a rhythm is susceptible to masking effects. In addition, each measured variable may have a different series of stimuli that produce evoked effects on an endogenous rhythm. Therefore, in the study of human circadian physiology, it has been desirable to find a marker of the circadian timing system that is relatively resistant to external masking effects while at the same time convenient to measure.

Use of the Melatonin Rhythm as a Marker of the Human Circadian Timing System

Previous studies under constant routine conditions have indicated that the fitted minimum of the body temperature cycle, the most widely characterized circadian marker in humans, occurs an average (± *SEM*) of 1.4 ± 0.3 h before the habitual wake time in healthy young men under entrained conditions (Czeisler et al., 1992). Use of a harmonic regression model to determine the fitted maximum of the plasma melatonin rhythm in 8 young men studied under constant routine conditions indicated that the fitted maximum occurs an average of 1.9 ± 0.3 h prior to the fitted temperature minimum (Shanahan and Czeisler, 1991). Recently, a two-dimensional linear differential equation model of the melatonin rhythm by Brown et al. (1997) indicated that the onset of melatonin secretion occurs an average of just over 7 h before the endogenous circadian temperature nadir and that the offset of melatonin secretion occurs about 40 min after the temperature nadir in healthy young men.

As methods of melatonin collection and measurement have been refined, the melatonin rhythm has emerged as an important marker of the human circadian timing system. Neuroanatomical and neurophysiological evidence indicates that the mammalian circadian pacemaker, located in the suprachiasmatic nuclei (SCN) of the hypothalamus, governs the timing of melatonin production and release into the peripheral circulation via sympathetic innervation of the pineal gland (Watts, 1991). This neural link between the circadian pacemaker and the pineal gland, which secretes circulating melatonin, has strengthened its use as one of the only circadian markers with a well-defined neuroanatomical link. The role melatonin plays in modulating the human circadian timing system, as well as the physiological mechanisms underlying the response of the circadian pacemaker to light, can be better elucidated through investigations of the response of the melatonin rhythm to a variety of circadian interventions.

When reviewing the effects of exogenous stimuli on the melatonin rhythm in humans, it is important to remember that light exposure, including photoperiod, can alter the shape of the observed melatonin rhythm. Light influences melatonin synthesis in three ways in humans. First, the LD cycle entrains and can reset the circadian pacemaker governing the timing of the melatonin rhythm. Second, bright light exposure acutely suppresses elevated melatonin levels. Third, changes in photoperiod associated with seasonal variations can alter the duration and amplitude of the melatonin peak, as demonstrated by Wehr (1991). The latter two phenomena are described in greater detail in other review articles within this issue.

It initially was claimed that melatonin was an ideal circadian marker because it was free of nearly all

nonphotic masking effects (Lewy, 1984), including state of consciousness (i.e., sleep or wake) (Morris et al., 1990), which has been shown to influence most other circadian variables in humans. But recent evidence suggests that posture significantly affects the profile of the melatonin rhythm (Deacon and Arendt, 1994b; Shanahan, 1995). About 70% of circulating plasma melatonin is bound to albumin, preventing its free diffusion across capillary membranes. As such, the postural changes in melatonin concentrations have been attributed to the hemoconcentration effect that occurs when an individual changes from a supine to an upright posture. These findings stress the importance of controlling for posture in attempts to assess the endogenous melatonin rhythm, especially when estimating phase shifts.

This review of the effects of light on the endogenous circadian rhythm of circulating melatonin focuses on the following topics: (1) resetting of the phase of the endogenous circadian melatonin rhythm by light, (2) attenuation of the amplitude of the endogenous circadian melatonin rhythm by light, (3) effect of light intensity on the resetting response, and (4) effect of light wavelength on the resetting response. Literature describing the effect of light on the melatonin rhythm in psychiatric disorders or in patients with underlying medical conditions is outside the scope of this review, which is limited to studies of normal physiology.

Resetting the Melatonin Rhythm by Light in Humans

Studies reporting the resetting of the melatonin rhythm by light in humans can be divided into categories based on the interval over which the melatonin assessment occurred: (1) entrainment studies in which the interval between the prestimulus and poststimulus assessment of the melatonin rhythm was about 1 week or longer, (2) resetting trials in which the response of the melatonin rhythm was assessed before and after exposure to multiple pulses of light occurring within 1 week, (3) resetting trials in which the response of the melatonin rhythm was assessed before and after exposure to a single pulse of light, and (4) resetting trials in which the response of the melatonin rhythm was recorded continuously throughout a resetting trial. A representative group of such studies is summarized in Table 1.

Entrainment Studies Reporting Phase Shifting of the Melatonin Rhythm That Occurred over a Time Interval of 1 Week or More

Many of the early studies of phase shifting of the melatonin rhythm in humans involved entrainment studies in which the melatonin rhythm was assessed before and after about a week or more of an LD cycle intervention. These studies were critical in assessing whether or not the melatonin rhythm could be entrained by a periodic photic stimulus. Broadway et al. (1987) reported that the entire melatonin profile shifted following 6 weeks of periodic exposure to bright light in men living in the Antarctic winter. This study was important in demonstrating that the melatonin rhythm was indeed responsive to changes in the LD cycle. However, the photoperiod also changed significantly during the course of such studies conducted over long time intervals, making the observed changes in the melatonin profile, now known to be sensitive to the effects of changing day length (Wehr, 1991), more difficult to interpret. Broadway et al. (1987), in fact, reported that in addition to observing a phase shift of the melatonin rhythm, seasonal changes were observed in the melatonin rhythm. Daily tracking of melatonin metabolites in the urine led Lynch et al. (1978) to conclude that the melatonin rhythm required 5 to 10 days to reentrain following a 12-h shift in the timing of the LD cycle associated with a change in the sleep-wake cycle, consistent with results of a similar, more recent study (Whitson et al., 1995). Koller et al. (1994) measured light intensity and assessed the phase of the melatonin rhythm in a field study of shift workers. Data indicated that the varying degrees of adaptation of the melatonin rhythm to the shifted sleep-wake cycle observed was dependent on the dose and phase of the daily light exposure. Lewy et al. (1987) reported that 1 week of daily exposure for 2 hours to 2500 lux of artificial light produced a 1-h phase advance (morning light stimulus), confirmed by Lemmer et al. (1994), and a 3-h phase delay (evening light stimulus) of the onset of melatonin secretion. These studies provided further evidence of the resetting effect of light on the phase of the melatonin rhythm and that the timing of melatonin secretion was not simply dependent on the timing of the sleep-wake cycle.

Exposure to bright light during night shift hours subsequently was found to reset the phase of the

Table 1. Representative group of studies reporting the resetting of the melatonin rhythm by light in humans.

Author	Year	Subjects	Intensity of Light Stimulus (lux)	Duration of Light Stimulus (hours)	Cycles of Light Exposure	Was Exposure to Nonstimulus Light Controlled?	Specimen Type	Sampling Interval (minutes)	Lighting Conditions during Phase Assessment (lux)	Was Posture Controlled during Phase Assessment?	Phase Shift (hours)	Prestimulus Assessment	Assessment throughout Trial	Poststimulus Assessment
More than 1 week														
Broadway et al.	1987	5	2500	2	6 weeks	No	Blood	60	Unspecified	Unspecified	~ 2	Yes	No	Yes
Koller et al.	1994	14	Variable	Unspecified	Unspecified	No	Saliva	120	Unspecified	Unspecified	> 6	No	No	Yes
Lemmer et al.	1994	18	2500	3	6 days	Unspecified	Blood	180	Unspecified	Unspecified	~ 1.5	Yes	No	Yes
Lewy et al.	1985	4	Unspecified	Unspecified	2 weeks	No	Blood	30-120	0-300	Unspecified	1.0-2.5	Yes	Partial	Yes
Lewy et al.	1987	7	2500	2	1 week	Partial	Blood	30	Unspecified	Unspecified	0.50-1.75	Yes	Partial	Yes
Lynch et al.	1978	4	Unspecified	180° shift	13 days	Unspecified	Blood, urine	240-720	830	Unspecified	~ 12	Yes	Yes	Yes
Ross et al.	1995	14	2500-3000	2	1 week	No	Blood, urine	60-240	Dim red	Unspecified	~ 7	Yes	Partial	Yes
Neri and Czeisler	1996	12	5000	8	1 week	No	Blood	60	0-5000	Yes	~ 8	Yes	No	Yes
Whitson et al.	1995	15	7000-12,000	2-6	1 week	No	Saliva, urine	120	Unspecified	Unspecified	9-12	Yes	Yes	Yes
Multiple pulses														
Boivin et al.	1995	8	180	5	3 days	Yes	Blood	20-60	< 15	Yes	1.6 ± 0.7	Yes	No	Yes
Deacon and Arendt	1994a	6	1200	6	3 days	No	Blood, urine, saliva	60-240	150-300	Partial	2.67 ± 0.30	Yes	No	Yes
Dijk et al.	1989	8	2000	3	3 days	No	Blood	30	1	Yes	~ 1	Yes	No	Yes
Illnerová et al.	1993	4	3000	3 in morning, 3 in night	4 days	No	Blood	60-120	5-50	Unspecified	1-3	Yes	Partial	Yes
Shanahan	1995	7	10,000	5-9	2-3 days	Yes	Blood	20-60	150	Yes	4.3-9.5	Yes	No	Yes
Shanahan	1995	23	10,000	5	3 days	Yes	Blood	60	< 15	Yes/partial	3.7-9.5	Yes	Yes	Yes
Shanahan and Czeisler	1991	8	10,000	5	3 days	Yes	Blood	20-60	150	Yes	4-10	Yes	No	Yes
Touitou et al.	1992	6	2000	2	3 days	No	Urine	120	50-2000	Unspecified	n.s.	Yes	No	Partial
Zeitzer et al.	1997	7	220	5	3 days	Yes	Blood	20-60	< 15	Yes	0.9 ± 0.5	Yes	No	Yes
Single pulse														
Burešová et al.	1991	6	3000	6	1 day	No	Blood	60-120	0-50	Unspecified	0.6-2.6	Yes	Yes	Yes
Hashimoto et al.	1996	7	200-10,000	3	1 day	No	Blood	60	< 200	Unspecified	~ 1.5	Yes	No	Yes
Kennaway et al.	1987	6	> 3000	7	1 day	No	Urine	Unspecified	Unspecified	Unspecified	~ 1	Yes	No	Yes
Laakso et al.	1993	12	500	1	1 day	No	Saliva	60	< 100	Unspecified	~ 0.5	Yes	Yes	Yes
Van Cauter et al.	1994	17	5000	3	1 day	Partial	Blood	20	< 300	Yes	1-2	Yes	Yes	Partial

melatonin rhythm (Fig. 1), as measured on a weekly basis during a 3-month simulation of rotating shift work (Shanahan et al., 1994b; Neri and Czeisler, 1996). The shifted melatonin rhythm in workers receiving bright light intervention during the work hours peaked during the scheduled day sleep episode, whereas the melatonin rhythm in workers remaining in room lighting conditions (~ 150 lux) during the scheduled overnight work hours failed to shift from the usual nocturnal profile. Therefore, melatonin continued to peak during the nighttime hours (approximately 2200-0800 h) in the control group, independent of the alteration in the timing of the daily sleep episodes necessitated by night shift work (Shanahan et al., 1994b; Neri and Czeisler, 1996). Ross et al. (1995) also reported that weekly assessment of the melatonin rhythms over a 4-week interval demonstrated that the rhythm could be shifted with daily exposure to 2 h of light (2500-3000 lux) in a series of shift workers. Over a 2-week light intervention protocol involving 4 subjects, Lewy et al. (1985) measured the entire melatonin waveform at various intervals and detected initial phase shifts of the melatonin rhythm early in the course of study.

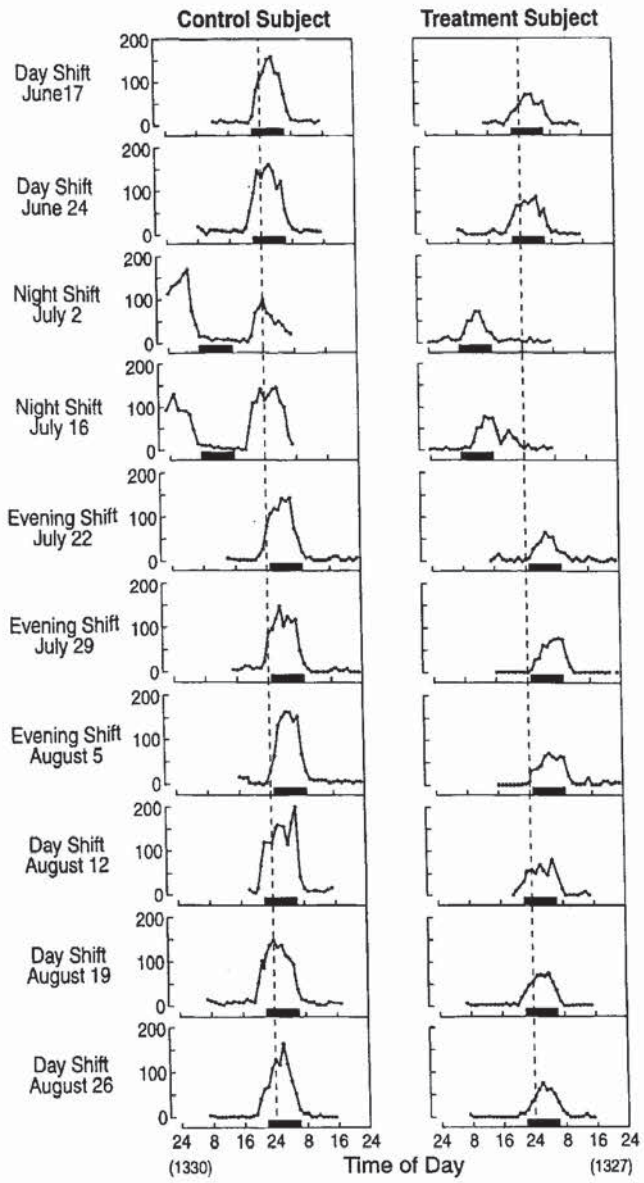

Figure 1. Melatonin rhythm observed at weekly intervals in 2 subjects during a 12-week shift rotation work schedule study. After 2 weeks of baseline evaluation (sleep 2200-0600 h, work 0700-1500 h), subjects worked 3 weeks of the night shift (sleep 0800-1600 h, work 2300-0700 h), 3 weeks of the evening shift (sleep 0100-0900 h, work 1500-2300 h), and 3 weeks of the day shift (same as baseline). The treatment subject (right) received bright light (up to 5000 lux) during the work shift; the control subject (left) remained in room light (~ 150 lux) during the work shift. Vertical dashed line represents midnight; solid horizontal bars represent sleep episodes. The melatonin rhythm in the control subject continued to peak during the hours of 2200 to 1000 h, despite multiple shifts of his sleep-wake cycle; therefore, the rhythm failed to adapt to the rotating shift work schedule and often peaked during the work-wake episode. The melatonin rhythm in the light-treated subject peaked during the sleep episode on all work rotations, and this adaptation to rotating shifts was evident during the 1st week of a new work schedule.
SOURCE: Adapted from Shanahan et al. (1994b) and Neri and Czeisler (1996), with permission.

A number of conclusions can be drawn from these laboratory entrainment and field studies. First, the entrained phase of the melatonin rhythm is sensitive to the timing of the LD cycle. Changes in the daily light exposure pattern can shift the phase of the melatonin rhythm. Second, habitual timing of exposure to the LD cycle exerts greater influence than does timing of the sleep-wake cycle on the phase of the melatonin rhythm.

However, interpretations from the preceding data are limited by the following issues. The exact strength of the resetting stimulus was not known in any of these studies, as subjects experienced uncontrolled light exposure during intervals in which they were allowed to leave the laboratory (Table 1). Also, the posture of the subjects during the melatonin assessments often was unclear, making it likely that both endogenous and evoked components influenced the observed melatonin rhythms. Finally, given that phase assessments in such studies often were conducted before and after a stimulus interval of many days or using a melatonin phase marker with limited temporal precision (e.g., urinary metabolites), the precise temporal dynamics of the resetting response cannot be derived from such studies.

Multiple Pulses of Light Resetting the Melatonin Rhythm within 1 Week

As data became available indicating that the melatonin rhythm responded fairly rapidly to changes in the LD cycle, studies were conducted over shorter time intervals, making it possible to conduct human studies restricted to laboratory conditions that can allow for more precise control of the LD cycle. A study by Dijk et al. (1989) reported a consistent advance in the onset of melatonin secretion by approximately 1 h following 3 days of morning bright light exposure. Touitou et al. (1992) failed to detect a phase shift of the melatonin rhythm following three cycles of a 2-h exposure to bright light (~ 2000 lux). However, subjects were allowed to leave the laboratory during the daytime, and this uncontrolled exposure to other light may have been a contributing factor. In a skeleton photoperiod protocol with 4 days of morning and evening bright light (~ 3000 lux for 3 h), Illnerová et al. (1993) phase advanced the melatonin rhythm by 1 to 3 h. Deacon and Arendt (1994a) measured plasma and salivary melatonin and 6-sulphatoxymelatonin in a resetting trial using three cycles of 1200 lux of light and were able to detect phase shifts in the circadian rhythms of circulating plasma melatonin levels, sali-

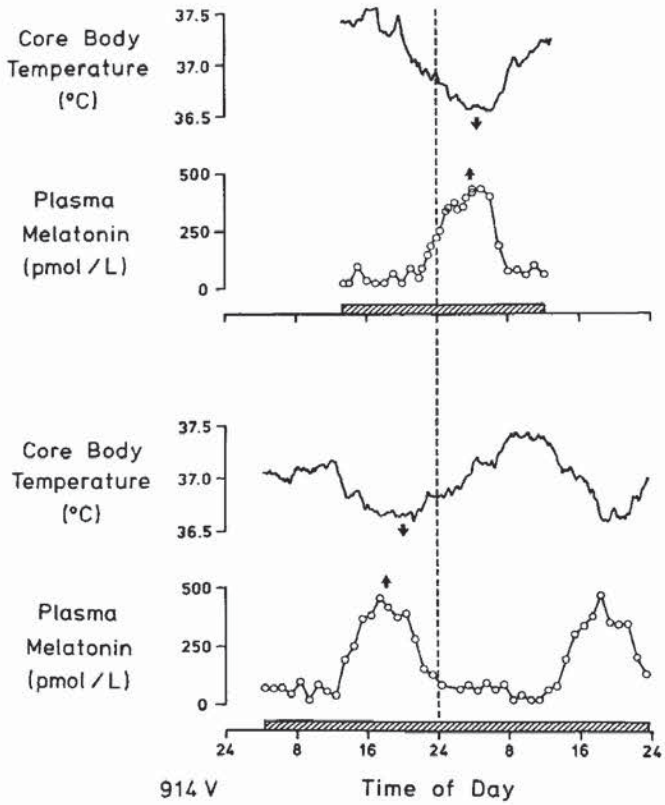

Figure 2. Melatonin and temperature rhythms from a 22-year-old man observed under constant routine conditions (hatched bars) before (top panel) and after (bottom panel) 3 days of a 5-h bright light (~ 10,000 lux) resetting stimulus. Arrows indicate the timing of the fitted maximum and minimum of the melatonin and temperature rhythms, respectively. The melatonin and temperature rhythms phase advanced 9.8 and 8.4 h, respectively.
SOURCE: Reproduced from Shanahan and Czeisler (1991) with permission from The Endocrine Society.

vary melatonin, and urinary melatonin metabolites of similar magnitude.

A study of the melatonin rhythm observed under constant routine conditions demonstrated that the endogenous plasma melatonin and core body temperature rhythms both can exhibit very large magnitude shifts (Fig. 2) after only three cycles of daily exposure to 5 h of bright light (~ 10,000 lux) and darkness (Shanahan and Czeisler, 1991). In this study, use of the constant routine procedure before and after the resetting stimulus allowed the endogenous components of both the melatonin and temperature rhythms to be used as markers of circadian phase. The constant routine eliminated changes in posture that are well known to produce evoked effects on the endogenous temperature rhythm and, more recently, have been demonstrated to also affect circulating levels of melatonin (Deacon and Arendt, 1994b; Czeisler, 1995; Shanahan, 1995). Both phase advances and phase delays of up to 10 h were achieved within 3 days. Previous studies of the resetting of the melatonin rhythm reported phase shifts of much smaller magnitude. Importantly, in the Shanahan and Czeisler (1991) study, two markers of circadian phase were measured. Both the temperature and melatonin rhythms maintained a fixed-phase relationship to one another throughout the rapid phase-resetting trial, suggesting that a central circadian pacemaker responsible for the timing of both observed rhythms was reset by exposure to the LD cycle. This result, obtained in constant routine conditions, contrasts with data from many prior studies suggesting a transient dissociation of rhythms after a phase shift. In fact, measured under constant routine conditions, the endogenous circadian components of the melatonin, cortisol, thyroid-stimulating hormone, urine volume, alertness, and performance rhythms have been found to maintain a fixed-phase relationship to the endogenous circadian temperature rhythm after an acute phase shift (see Czeisler, 1995, for references), supporting the conclusion that a central circadian pacemaker drives the endogenous components of these various circadian rhythms. The lack of dissociation between the endogenous components of these rhythms following a phase shift suggests that differential sensitivity to the masking effects of sleep and activity may be responsible for the dissociation of rhythmic variables often reported following acute phase shifts.

As has been widely reported in the literature, the amplitude of the melatonin profile varied greatly between subjects. However, within a subject, the amplitude of the melatonin rhythm after the resetting light stimulus was unchanged compared to that observed before the intervention, indicating that these interindividual differences in melatonin amplitude were robust traits (Shanahan and Czeisler, 1991). Results from other studies are consistent with the conclusion that bright light can shift the phase of the melatonin rhythm in a short time interval (Dijk et al., 1989; Illnerová et al., 1993; Deacon and Arendt, 1994a; Boivin et al., 1995; Zeitzer et al., 1997), although no other study has documented such large phase shifts within 3 days as those observed by our group (Shanahan and Czeisler, 1991; Shanahan et al., 1994a; Shanahan, 1995).

Taken together, these multiple-pulse studies demonstrate that the melatonin rhythm can be shifted up to 10 h by only 2 to 3 days of light exposure. The magnitude and direction of the phase shift are de-

pendent on the timing, duration, and intensity of the light exposure. As mentioned previously, the exact timing, strength, and duration of the stimulus cannot be defined precisely in studies in which subjects have had access to varying amounts of light exposure at times other than the planned resetting stimulus, making comparison of study results difficult. Based on these findings, study designs should carefully control for those factors now known to influence circulating levels of melatonin, especially when attempts to detect small changes in the timing of the rhythms are undertaken. Finally, most of these studies relied on phase assessments before and after several daily light exposures, again limiting evaluation of the temporal dynamics of the resetting response.

Response of the Melatonin Rhythm to a Single Pulse of Bright Light

A series of studies have now been conducted to evaluate the response of the melatonin rhythm to single pulses of bright light exposure of varying intensities and durations. In 1987, Kennaway et al. (1987) reported a 1-h phase delay of the rhythm of 6-sulphatoxymelatonin, the major metabolite of melatonin excreted into the urine, following a single 6-h pulse of bright light (> 3000 lux). However, reliance on urinary metabolites limits the precision of such an evaluation. Several years later, Burešová et al. (1991) reported a similar finding that a single 6-h pulse of bright light (~ 3000 lux) phase advanced the serum melatonin rhythm 1.0 to 2.5 h. However, subjects in that study were allowed to leave the laboratory during the 3 days of the experiment and, therefore, experienced additional light exposure of unknown intensity and duration. Likewise, in a study by Hashimoto et al. (1996), subjects were allowed exposure to natural daylight following a single light pulse, and no significant phase shifts were observed. It is quite possible that such extraneous light exposure had significant effects on the melatonin rhythm, making interpretation difficult. Laakso et al. (1993) reported that the salivary melatonin rhythm was phase delayed 30 min following a 1-h pulse of evening light (~ 500 lux), but again, extraneous light exposure was uncontrolled. A study by Van Cauter et al. (1994) reported that a single 3-h pulse of bright light (~ 5000 lux) either phase advanced or phase delayed the melatonin rhythm by approximately 1 h, depending on the timing of the light exposure. This study had the advantage of reporting multiple markers of the human circadian timing system under constant routine conditions, as temperature and thyrotropin also were measured; however, ambient light levels were not strictly controlled in that study.

The preceding data, nonetheless, indicate that even a single pulse of bright light exposure can shift the phase of the melatonin rhythm in humans by approximately 0.5 to 2.5 h. Changes in the timing of the melatonin rhythm can be detected within the same circadian cycle, indicating a very rapid response of the rhythm to the light stimuli.

Response of the Melatonin Rhythm Measured throughout a Resetting Trial

Previous studies by our group reporting large-magnitude phase shifts of circadian rhythms in humans using three cycles of bright light have reported phase assessments of the temperature rhythm observed under constant routine conditions before and after the resetting intervention. However, day-by-day phase information throughout the resetting trial has not been available secondary to masking effects because it is not possible to extend constant routine conditions over the entire interval of the resetting trial (Czeisler et al., 1989). We hypothesized that the melatonin rhythm observed throughout the three-cycle resetting trial could provide a dynamic view of the daily phase-resetting characteristics of the human circadian timing system, notwithstanding the fact that only partial control of posture could be maintained in between the initial and final constant routines. Shanahan and colleagues studied a series of 23 healthy young men, 12 of whom were randomly assigned to receive three cycles of bright light (~ 10,000 lux) exposure, timed to either phase advance (centered 1.5 h after the fitted temperature minimum) or phase delay (centered 1.5 h before the fitted temperature minimum) the human circadian pacemaker (Shanahan et al., 1994a; Shanahan, 1995). The other 11 subjects were assigned to a control group and received three cycles of exposure to darkness similarly timed. The bright light stimuli were timed to occur outside the critical region of phase resetting. Initial and final constant routine phase assessments were conducted surrounding the three-cycle bright light intervention, and hourly melatonin samples were collected throughout the protocol.

Both temperature and melatonin rhythms observed during the phase assessments before and after the resetting intervention responded to resetting stimuli in equivalent manners, consistent with other three-cycle bright light studies (Shanahan and Czeisler, 1991; Boivin et al., 1995). The melatonin rhythm ob-

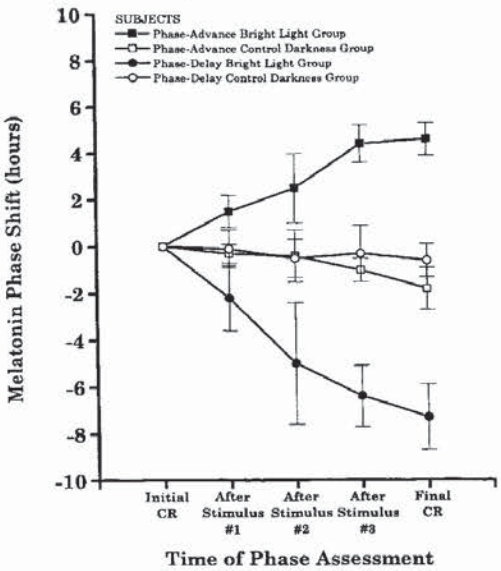

Figure 3. Phase shifts of the melatonin rhythm observed throughout a three-cycle stimulus. The average ±*SD* daily phase shift of the melatonin rhythm is plotted for the phase-delay light and control subjects (closed and open circles, respectively) and phase-advance light and control subjects (closed and open squares, respectively). For each subject, the melatonin phase assessment from the initial constant routine (CR) was defined as zero; all subsequent phase assessments were referenced to this phase to determine phase shifts. In the phase-delay light subjects ($n = 6$), the overall average phase delay (~ 8 h) of the melatonin rhythm was achieved by daily incremental phase shifts of 1 to 3 h. In the phase-advance light subjects ($n = 6$), the overall average phase advance (~ 5 h) of the melatonin rhythm was achieved by daily incremental phase shifts of 1 to 2 h in 5 subjects shown here. Phase assessments from a 6th subject who demonstrated amplitude attenuation were not possible. Subjects in both control groups ($n = 11$) demonstrated a small daily phase delay in their melatonin phase of –0.1 to –0.3 h per day. This is consistent with the delay expected due to the intrinsic period the melatonin rhythm observed under dim lighting conditions (Czeisler et al., 1995a; Shanahan, 1995).
SOURCE: Adapted from Shanahan (1995), with permission.

served throughout the resetting trial showed daily phase shifts on the average of 1 to 3 h following each cycle of bright light exposure (Fig. 3), consistent with other one-pulse bright light studies (Kennaway et al., 1987; Burešová et al., 1991; Laakso et al., 1993; Van Cauter et al., 1994; Hashimoto et al., 1996). The melatonin data from control subjects, all of whom remained in dim lighting and darkness, delayed on the average of –0.2 h per day, consistent with the daily delay expected due to the intrinsic period of the human circadian pacemaker observed in dim lighting conditions (Czeisler et al., 1995a). These results from resetting trials conducted outside the critical region are consistent with predictions from Kronauer's phase and amplitude model of the resetting effect of light on the human circadian pacemaker (Kronauer and Czeisler, 1993; Shanahan, 1995).

Attenuation of the Amplitude of the Melatonin Rhythm by Light

In 1991, Jewett et al. reported the attenuation of endogenous circadian amplitude in humans in response to near-critical bright light stimuli, describing the temperature and cortisol rhythms observed under constant routine conditions. However, 2 years later, Beersma and Daan (1993) challenged the conclusion of Jewett et al. (1991) that light exposure could suppress the amplitude of the human circadian pacemaker, arguing that the dissimilar responses of the cortisol and temperature rhythms displayed in some of the subjects in that study illustrated that changes in the output of slave oscillations may not reflect pacemaker amplitude. In fact, Beersma and Daan (1993) argued that a phase-only model was more parsimonious than Kronauer's phase-amplitude model of the human circadian pacemaker (Kronauer and Czeisler, 1993).

Melatonin data of 7 subjects from the original Jewett et al. (1991) study are now available (Shanahan, 1995) to address independently the question of pacemaker amplitude using a variable known to be driven by the SCN via a defined neural pathway. Subjects from that study underwent a protocol consisting of (1) an initial constant routine phase assessment, (2) one or two cycles of a bright light stimulus designed to drive the human circadian timing system to the singular region, (3) an intermediate constant routine phase assessment, (4) an additional light stimulus designed to drive the circadian timing system from the singular region to a shifted phase, and (5) a final constant routine phase assessment. Following exposure to one or two cycles of the critical light stimulus, all subjects demonstrated at least a 50% attenuation of the amplitude of the melatonin rhythm observed during the intermediate constant routine; in 2 of the subjects, the melatonin rhythm was absent (Shanahan, 1995). Following an additional single light stimulus (interposed between the intermediate and final constant routines), all subjects demonstrated a recovered amplitude and shifted phase (by 0.6 to 8.0 h) of the endogenous component of the melatonin rhythm in response to a single pulse of light (Shanahan, 1995). As illustrated in Fig. 4, both the phase and amplitude of the temperature and melatonin rhythms remained highly correlated throughout the resetting trial. As light drives the circadian oscillation toward the singularity, circadian

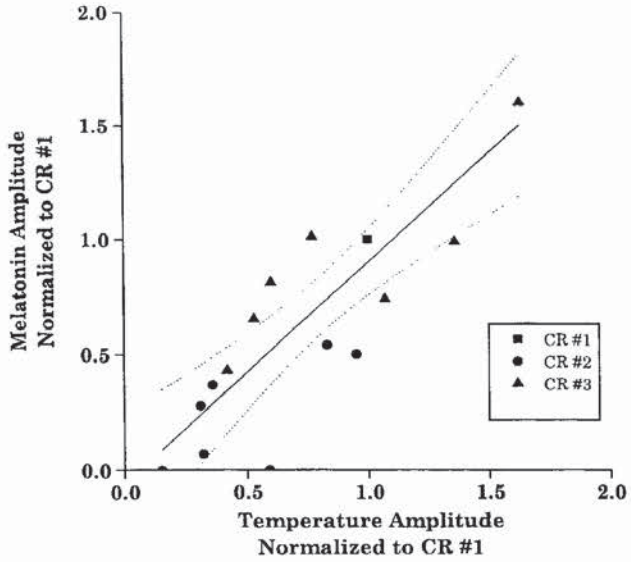

Figure 4. Correlation between the normalized amplitude of the temperature and melatonin rhythms during a circadian amplitude attenuation protocol (Jewett et al., 1991). The amplitudes of the temperature and melatonin rhythms observed during the initial constant routine (CR #1) were defined as 1; all other amplitude measurements were referenced to the initial CR (closed squares). The normalized amplitudes of the temperature and melatonin rhythms were correlated ($R = .86$, $p < .0001$) in the reduced amplitude state (CR #2, closed circles) and after amplitude recovery (CR #3, closed triangles). There was a one-to-one relationship between the two variables, as indicated by the regression line ($y = -0.06 + 0.96x$); the 95% confidence interval is indicated by dashed lines.
SOURCE: Reproduced from Shanahan (1995), with permission.

amplitude decreases. This study demonstrated that, as occurs with body temperature, there is amplitude attenuation of the melatonin rhythm with near-critical resetting and a subsequent recovery when an additional light stimulus is applied. These data provide strong evidence that it is the amplitude of the clock itself, not just its output variables, that can be decreased with critically timed bright light exposure. We thus concur with Kronauer and Czeisler (1993) that both phase and amplitude should be considered when describing the resetting effects of light on the human circadian pacemaker, consistent with Kronauer's phase-amplitude model (Kronauer and Czeisler, 1993; Lakin-Thomas, 1993).

Effect of Light Intensity on Resetting of the Melatonin Rhythm

Melatonin concentrations are suppressed by light in a dose dependent manner, and light intensities as low as 100 to 300 lux have been reported to suppress melatonin concentrations in subjects with freely constricting pupils (McIntyre et al., 1989; Brainard et al., 1988; Gaddy et al., 1993). In subjects with pharmacologically dilated pupils, as little as 5 lux of monochromatic light has been reported to induce melatonin suppression (Brainard et al., 1988). In addition to the suppressant effect of light on the melatonin rhythm, the phase-shifting effect of varying light intensities also has been investigated. As mentioned previously, Laakso et al. (1993) reported a study of 5 subjects in which a 1-h pulse of 500 lux of light shifted the salivary melatonin rhythm approximately 30 min; however, there were several methodological issues making interpretation of this study difficult. More recently, Boivin et al. (1995) reported that the melatonin rhythm observed under constant routine conditions phase advanced an average ($\pm SEM$) of 1.6 ± 0.7 h when subjects were exposed to 3 consecutive days of 5-h pulses of indoor room light (~ 180 lux). Further data also have indicated that the phase of the melatonin rhythm may be affected by intensities lower than room light. These data suggest, as predicted by Kronauer's model (Kronauer and Czeisler, 1993), that the human circadian pacemaker is more sensitive to lower light intensities than was originally believed.

Effect of Light Wavelength on Resetting of the Melatonin Rhythm

Although there has been extensive investigation of the wavelength sensitivity of the circadian system of nonhuman mammals, including assessments of the action spectrum of phase shifting, melatonin suppression, and modulation of seasonal reproductive function, there have been few studies that have examined the wavelength sensitivity of the human circadian timing system. Such investigations in nonhuman mammals have proven useful in the pursuit of the identity of the photoreceptive retinal elements responsible for transducing photic information from the environment to the SCN.

In humans, the majority of investigations into the wavelength sensitivity of the circadian timing system have evaluated light-induced melatonin suppression in response to various wavelengths (Brainard et al., 1985). Studies have indicated that light-induced melatonin suppression is more sensitive to the middle wavelengths of light (green) as opposed to the longer (red) and, possibly, shorter (blue) wavelengths. Melatonin suppression also was studied in humans with color-deficient vision, and it was concluded that the

photoreceptors used for conscious sight may not be necessary for circadian photoreception (Ruberg et al., 1996). Although this conclusion is consistent with results of our recent studies of blind people (Czeisler et al., 1995b), these studies have not had the sensitivity to discriminate the underlying photoreceptive mechanism responsible for the suppression of melatonin concentrations. Through the examination of the phase shifting of light on both the melatonin and core body temperature rhythms, a recent study on the wavelength sensitivity of the circadian pacemaker indicated that photopic photoreceptive elements (i.e., cones) are sufficient to mediate circadian photoreception (Zeitzer et al., 1997).

Summary

In summary, investigations into the effects of light on the human circadian timing system demonstrate that the melatonin rhythm is an important marker of the output of the circadian pacemaker. Melatonin can be measured to observe both chronic and acute effects of changes in the LD cycle on the human circadian system. The findings consistently reported in the studies reviewed in this article include the following. First, exposure to bright light can rapidly induce large-magnitude (> 10-h) phase-advance and phase-delay shifts of the melatonin rhythm in humans, supporting the hypothesis that the human circadian pacemaker is reset by altered LD cycles. Second, the melatonin rhythm is shifted by an amount equivalent to other endogenous circadian rhythms, such as the endogenous component of the cortisol and body temperature rhythms, supporting the hypothesis that a central circadian pacemaker, probably the SCN of the hypothalamus, generates the observed output rhythms. Third, a single pulse of light can shift the melatonin phase 1 to 3 h, suggesting that phase resetting in humans may occur within one cycle of light exposure. Fourth, a light stimulus of critical timing, duration, and intensity can attenuate the amplitude of the melatonin rhythm, indicating that both phase and amplitude need to be considered when describing the pacemaker driving the endogenous circadian melatonin rhythm. Fifth, a single light pulse can shift melatonin phase by up to 8 h when the melatonin rhythm is in a state of reduced amplitude, providing further evidence of Type 0 phase resetting in humans. Sixth, indoor light exposure (~ 180 lux) can phase shift the endogenous circadian melatonin rhythm in humans, suggesting that the human circadian pacemaker is much more sensitive to light than was originally believed. Seventh, the spectral sensitivity of phase shifting the melatonin rhythm supports the hypothesis that photopic photoreceptive elements are sufficient to mediate circadian photoreception in humans.

The utility of melatonin as a marker of endogenous circadian phase is greatly enhanced when evoked components, which are superimposed on the endogenous component of the rhythm, are minimized. Many early studies lacked strict control of environmental conditions and behavioral factors now known to exert masking effects on the endogenous melatonin waveform, as the melatonin rhythm initially was thought to be resistant to nearly all masking effects. As more data became available, it became clear that even low light levels, activity, posture, and photoperiod may alter the melatonin waveform. There remain many experimental conditions in which measurement of melatonin as a circadian marker continues to have advantages over other circadian variables. The measurement and correlation of multiple circadian variables during experiments designed to assess circadian resetting responses strengthens the interpretation of results. The studies described in this review confirm that when possible, the entire melatonin waveform should be monitored because changes in the amplitude, duration, or shape of the melatonin waveform may provide valuable information about the resetting response of the human circadian pacemaker to light intervention beyond the most commonly reported change in phase.

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References

Beersma DGM and Daan S (1993) Strong or weak phase resetting by light pulses in humans? J Biol Rhythms 8:340-347.

Boivin DB, Zeitzer JM, and Czeisler CA (1995) Resetting of the endogenous circadian rhythm of plasma melatonin and body temperature by ordinary room light in humans. J Sleep Res 5:S20.

Brainard GC, Lewy AJ, Menaker M, Fredrickson RH, Miller LS, Weleber RG, Cassone V, and Hudson D (1985) Effect of light wavelength on the suppression of nocturnal

plasma melatonin in normal volunteers. Ann NY Acad Sci 453:376-378.

Brainard GC, Lewy AJ, Menaker M, Fredrickson RH, Miller LS, Weleber RG, Cassone V, and Hudson D (1988) Dose response relationship between light irradiance and the suppression of plasma melatonin in human volunteers. Brain Res 454:212-218.

Broadway J, Arendt J, and Folkard S (1987) Bright light phase shifts the human melatonin rhythm during the Antarctic winter. Neurosci Lett 79:185-189.

Brown EN, Choe Y, Shanahan TL, and Czeisler CA (1997) A mathematical model of diurnal variations in human plasma melatonin levels. Am J Physiol 272:E506-E516.

Burešová M, Dvoráková M, Zvolsky P, and Illnerová H (1991) Early morning bright light phase advances the human circadian pacemaker within one day. Neurosci Lett 121:47-50.

Czeisler CA (1995) The effect of light on the human circadian pacemaker. In *Circadian Clocks and Their Adjustment*, JM Waterhouse, ed, pp 254-302, Wiley, Chichester, UK.

Czeisler CA, Duffy JF, Shanahan TL, Brown EN, Mitchell JF, Dijk D-J, Rimmer DW, Ronda JM, Allan JS, Emens JS, and Kronauer RE (1995a) Reassessment of the intrinsic period (τ) of the human circadian pacemaker in young and older subjects. Sleep Res 24A:505.

Czeisler CA, Dumont M, Duffy JF, Steinberg JD, Richardson GS, Brown EN, Sánchez R, Ríos CD, and Ronda JM (1992) Association of sleep-wake habits in older people with changes in output of circadian pacemaker. Lancet 340:933-936.

Czeisler CA, Kronauer RE, Allan JS, Duffy JF, Jewett ME, Brown EN, and Ronda JM (1989) Bright light induction of strong (Type 0) resetting of the human circadian pacemaker. Science 244:1328-1333.

Czeisler CA, Shanahan TL, Klerman EB, Martens H, Brotman DJ, Emens JS, Klein T, and Rizzo JF III (1995b) Suppression of melatonin secretion in some blind patients by exposure to bright light. N Engl J Med 332:6-11.

Deacon SJ and Arendt J (1994a) Phase-shifts in melatonin, 6-sulphatoxymelatonin and alertness rhythms after treatment with moderately bright light at night. Clin Endocrinol 40:413-420.

Deacon SJ and Arendt J (1994b) Posture influences melatonin concentrations in plasma and saliva in humans. Neurosci Lett 167:191-194.

Dijk D-J, Beersma DGM, Daan S, and Lewy AJ (1989) Bright morning light advances the human circadian system without affecting NREM sleep homeostasis. Am J Physiol 256:R106-R111.

Gaddy JR, Rollag MD, and Brainard GC (1993) Pupil size regulation of threshold of light-induced melatonin suppression. J Clin Endocrinol Metab 77:1398-1401.

Hashimoto S, Nakamura K, Honma S, Tokura H, and Honma K-I (1996) Melatonin rhythm is not shifted by lights that suppress nocturnal melatonin in humans under entrainment. Am J Physiol 270:R1073-R1077.

Illnerová H, Burešová M, Nedvídková J, Dvoráková M, and Zvolsky P (1993) Maintenance of a circadian phase adjustment of the human melatonin rhythm following artificial long days. Brain Res 626:322-326.

Jewett ME, Kronauer RE, and Czeisler CA (1991) Light-induced suppression of endogenous circadian amplitude in humans. Nature 350:59-62.

Kennaway DJ, Earl CR, Shaw PF, Royles P, Carbone F, and Webb H (1987) Phase delay of the rhythm of 6-sulphatoxy melatonin excretion by artificial light. J Pineal Res 4:315-320.

Koller M, Härma M, Laitinen JT, Kundi M, Piegler B, and Haider M (1994) Different patterns of light exposure in relation to melatonin and cortisol rhythms and sleep of night workers. J Pineal Res 16:127-135.

Kronauer RE and Czeisler CA (1993) Understanding the use of light to control the circadian pacemaker in humans. In *Light and Biological Rhythms in Man*, L Wetterberg, ed, pp 217-236, Pergamon, Oxford, UK.

Laakso M, Hätönen T, Stenberg D, Alila A, and Smith S (1993) One-hour exposure to moderate illuminance (500 lux) shifts the human melatonin rhythm. J Pineal Res 15:21-26.

Lakin-Thomas PL (1993) Commentary: Strong or weak phase resetting by light pulses in humans? J Biol Rhythms 8:348-350.

Lemmer B, Bruhl T, Witte K, Pflug B, Kohler W, and Touitou Y (1994) Effects of bright light on circadian patterns of cyclic adenosine monophosphate, melatonin and cortisol in healthy subjects. Eur J Endocrinol 130:472-477.

Lewy AJ (1984) Human melatonin secretion (II): A marker for the circadian system and the effects of light. In *Frontiers of Clinical Neuroscience: Neurobiology of Mood Disorders*, RM Post and JC Ballenger, eds, pp 215-226, Williams & Wilkins, Baltimore, MD.

Lewy AJ, Sack RL, Miller LS, and Hoban TM (1987) Antidepressant and circadian phase-shifting effects of light. Science 235:352-354.

Lewy AJ, Sack RL, and Singer CM (1985) Immediate and delayed effects of bright light on human melatonin production: Shifting "dawn" and "dusk" shifts the dim light melatonin onset (DLMO). Ann NY Acad Sci 453:253-259.

Lynch HJ, Jimerson DC, Ozaki Y, Post RM, Bunney WE Jr, and Wurtman RJ (1978) Entrainment of rhythmic melatonin secretion in man to a 12-hour phase shift in the light/dark cycle. Life Sci 23:1557-1564.

McIntyre IM, Norman TR, Burrows GD, and Armstrong SM (1989) Human melatonin suppression by light is intensity dependent. J Pineal Res 6:149-156.

Mills JN, Minors DS, and Waterhouse JM (1978) Adaptation to abrupt time shifts of the oscillator(s) controlling human circadian rhythms. J Physiol 285:455-470.

Morris M, Lack L, and Barrett J (1990) The effect of sleep/wake state on nocturnal melatonin excretion. J Pineal Res 9:133-138.

Neri DF and Czeisler CA (1996) Effect of bright light on vigilance and subjective sleepiness during a simulated shiftwork rotation. Sleep Res 25:144.

Ross JK, Arendt J, Horne J, and Haston W (1995) Night-shift work in Antarctica: Sleep characteristics and bright light treatment. Physiol Behav 57:1169-1174.

Ruberg FL, Skene DJ, Hanifin JP, Rollag MD, English J, Arendt J, and Brainard GC (1996) Melatonin regulation in humans with color vision deficiencies. J Clin Endocrinol Metab 81:2980-2985.

Shanahan TL (1995) Circadian physiology and the plasma melatonin rhythm in humans. Doctoral thesis, Harvard Medical School.

Shanahan TL and Czeisler CA (1991) Light exposure induces equivalent phase shifts of the endogenous circadian rhythms of circulating plasma melatonin and core body temperature in men. J Clin Endocrinol Metab 73:227-235.

Shanahan TL, Emens JS, Kronauer RE, Duffy JF, and Czeisler CA (1994a) The melatonin rhythm observed throughout a 3-cycle resetting stimulus. Society for Research on Biological Rhythms, Amelia Island, FL.

Shanahan TL, Neri D, and Czeisler CA (1994b) The melatonin rhythm observed during three months of rotating shift work. In *Stress Research Reports*, T Åkerstedt and G Kecklund, eds, p 119, Karolinska Institute, Stockholm, Sweden.

Touitou Y, Benoit O, Foret J, Aguirre A, Bogdan A, Clodoré M, and Touitou C (1992) Effects of a two-hour early awakening and of bright light exposure on plasma patterns of cortisol, melatonin, prolactin and testosterone in man. Acta Endocrinol 126:201-205.

Van Cauter E, Sturis J, Byrne MM, Blackman JD, Leproult R, Ofek G, L'Hermite-Balériaux M, Refetoff S, Turek FW, and Van Reeth O (1994) Demonstration of rapid light-induced advances and delays of the human circadian clock using hormonal phase markers. Am J Physiol 266:E953-E963.

Watts AG (1991) The efferent projections of the suprachiasmatic nucleus: Anatomical insights into the control of circadian rhythms. In *Suprachiasmatic Nucleus: The Mind's Clock*, DC Klein, RY Moore, and SM Reppert, eds, pp 77-106, Oxford University Press, Oxford, UK.

Wehr TA (1991) The durations of human melatonin secretion and sleep respond to changes in daylength (photoperiod). J Clin Endocrinol Metab 73:1276-1280.

Whitson PA, Putcha L, Chen Y-M, and Baker E (1995) Melatonin and cortisol assessment of circadian shifts in astronauts before flight. J Pineal Res 18:141-147.

Zeitzer JM, Kronauer RE, Czeisler CA (1997) Photopic transduction implicated in human circadian entrainment. Neurosci Lett 232:135-138.