

**Elizabeth B. Klerman, David W. Rimmer, Derk-Jan Dijk, Richard E. Kronauer, Joseph F. Rizzo, III and Charles A. Czeisler**  
*Am J Physiol Regulatory Integrative Comp Physiol* 274:991-996, 1998.

**You might find this additional information useful...**

---

A **corrigendum** for this article has been published. It can be found at:

<http://ajpregu.physiology.org/cgi/content/full/275/2/Ra1>

This article **cites** 37 articles, 13 of which you can access free at:

<http://ajpregu.physiology.org/cgi/content/full/274/4/R991#BIBL>

This article **has been cited by** 10 other HighWire hosted articles, the first 5 are:

**An arousing, musically enhanced bird song stimulus mediates circadian rhythm phase advances in dim light**

N. Goel

*Am J Physiol Regulatory Integrative Comp Physiol*, September 1, 2006; 291 (3): R822-R827.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

**Late-night presentation of an auditory stimulus phase delays human circadian rhythms**

N. Goel

*Am J Physiol Regulatory Integrative Comp Physiol*, July 1, 2005; 289 (1): R209-R216.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

**Efficacy of a single sequence of intermittent bright light pulses for delaying circadian phase in humans**

C. Gronfier, K. P. Wright Jr., R. E. Kronauer, M. E. Jewett and C. A. Czeisler

*Am J Physiol Endocrinol Metab*, July 1, 2004; 287 (1): E174-E181.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

**Effects of VDT tasks with a bright display at night on melatonin, core temperature, heart rate, and sleepiness**

S. Higuchi, Y. Motohashi, Y. Liu, M. Ahara and Y. Kaneko

*J Appl Physiol*, May 1, 2003; 94 (5): 1773-1776.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

**Exercise elicits phase shifts and acute alterations of melatonin that vary with circadian phase**

O. M. Buxton, C. W. Lee, M. L'Hermite-Baleriaux, F. W. Turek and E. Van Cauter

*Am J Physiol Regulatory Integrative Comp Physiol*, March 1, 2003; 284 (3): R714-R724.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

**Medline items on this article's topics** can be found at <http://highwire.stanford.edu/lists/artbytopic.dtl> on the following topics:

Oncology .. Melatonin  
Physiology .. Photoreception  
Physiology .. Chronobiology  
Medicine .. Visual Disability  
Medicine .. Pacemaker  
Physiology .. Humans

**Updated information and services** including high-resolution figures, can be found at:

<http://ajpregu.physiology.org/cgi/content/full/274/4/R991>

**Additional material and information** about *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* can be found at:

<http://www.the-aps.org/publications/ajpregu>

---

This information is current as of November 23, 2009 .

*The American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* publishes original investigations that illuminate normal or abnormal regulation and integration of physiological mechanisms at all levels of biological organization, ranging from molecules to humans, including clinical investigations. It is published 12 times a year (monthly) by the American Physiological Society, 9650 Rockville Pike, Bethesda MD 20814-3991. Copyright © 2005 by the American Physiological Society. ISSN: 0363-6119, ESSN: 1522-1490. Visit our website at <http://www.the-aps.org/>.

# Nonphotic entrainment of the human circadian pacemaker

ELIZABETH B. KLERMAN,<sup>1</sup> DAVID W. RIMMER,<sup>1</sup> DERK-JAN DIJK,<sup>1</sup>  
RICHARD E. KRONAUER,<sup>2</sup> JOSEPH F. RIZZO III,<sup>3</sup> AND CHARLES A. CZEISLER<sup>1</sup>  
<sup>1</sup>*Circadian, Neuroendocrine and Sleep Disorders Section, Endocrinology-Hypertension  
Division, Department of Medicine, Brigham and Women's Hospital and Harvard  
Medical School, Boston 02115;* <sup>2</sup>*Division of Applied Sciences, Harvard University,  
Cambridge 02138;* and <sup>3</sup>*Department of Ophthalmology, Harvard Medical School  
and Massachusetts Eye and Ear Infirmary, Boston, Massachusetts 02114*

**Klerman, Elizabeth B., David W. Rimmer, Derk-Jan Dijk, Richard E. Kronauer, Joseph F. Rizzo III, and Charles A. Czeisler.** Nonphotic entrainment of the human circadian pacemaker. *Am. J. Physiol.* 274 (*Regulatory Integrative Comp. Physiol.* 43): R991–R996, 1998.—In organisms as diverse as single-celled algae and humans, light is the primary stimulus mediating entrainment of the circadian biological clock. Reports that some totally blind individuals appear entrained to the 24-h day have suggested that nonphotic stimuli may also be effective circadian synchronizers in humans, although the nonphotic stimuli are probably comparatively weak synchronizers, because the circadian rhythms of many totally blind individuals “free run” even when they maintain a 24-h activity-rest schedule. To investigate entrainment by nonphotic synchronizers, we studied the endogenous circadian melatonin and core body temperature rhythms of 15 totally blind subjects who lacked conscious light perception and exhibited no suppression of plasma melatonin in response to ocular bright-light exposure. Nine of these fifteen blind individuals were able to maintain synchronization to the 24-h day, albeit often at an atypical phase angle of entrainment. Nonphotic stimuli also synchronized the endogenous circadian rhythms of a totally blind individual to a non-24-h schedule while living in constant near darkness. We conclude that nonphotic stimuli can entrain the human circadian pacemaker in some individuals lacking ocular circadian photoreception.

activity; circadian rhythms; light; blindness

MANY CRITICAL PHYSIOLOGICAL functions in animals and humans have near 24-h rhythms. Those that persist in conditions free from environmental time cues are described as circadian (from the Latin *circa* = near, *dies* = day). Because the neural pacemaker generating these rhythms oscillates at a cycle length that is not exactly 24 h, it must be synchronized to the periodic external environment. Light is the principal environmental synchronizer of the circadian pacemaker in nearly all species studied, from unicellular organisms to humans (8, 36).

When healthy, normal, sighted subjects are shielded from a 24-h light-dark cycle in an environment free of time cues (4, 26, 39), their endogenous circadian rhythms, such as core body temperature (CBT), plasma melatonin, cortisol, and sleep propensity, “free run” with a period different from 24 h. In view of the pivotal role of light in entraining the human circadian pacemaker driving these observed rhythms, it is not surprising that many blind humans also have free-running circadian rhythms. Analyses of data from such individuals reveal that variables such as melatonin and cortisol

free run despite the individuals' attempts to maintain a 24-h sleep-wake schedule (2, 11, 15, 17, 19–21, 24, 25, 29, 32–34, 37, 38, 43), and the quality of their sleep is modulated by the phase of the endogenous circadian pacemaker, resulting in a recurring cyclic sleep disturbance.

Some blind individuals, despite their lack of conscious light perception, are able to maintain their sleep-wake cycle on a 24-h schedule without such difficulties. In those individuals, circadian variables such as melatonin, cortisol, and CBT appear to be synchronized to the 24-h day (11, 20, 38). In some of these entrained blind persons, appropriately timed exposure of the eye to bright light suppresses plasma melatonin levels [positive melatonin suppression test (MST)], indicating that light information is reaching their circadian pacemaker (11), enabling photic entrainment. Other blind persons, however, remain entrained despite evidence suggesting loss of ocular circadian photoreception, i.e., a negative MST (11) or bilateral enucleation (20, 38).

Four parsimonious explanations for the apparent entrainment of these other blind persons' circadian clocks are 1) photic synchronization of the circadian clock despite absence of detectable light-induced melatonin suppression; 2) an intrinsic circadian period indistinguishable from that of the entraining stimulus (i.e., very close to 24 h); 3) circadian synchronization via extraocular photoreceptors; and 4) circadian synchronization via nonphotic stimuli. We tested these explanations in a subset of our entrained blind individuals and herein report conclusive evidence that nonphotic stimuli can affect the human circadian pacemaker based on long-term experiments not confounded by concurrent light exposure.

## MATERIALS AND METHODS

### Subjects

Fifteen totally blind individuals (i.e., without conscious vision) met our criteria of no conscious light perception, no pupillary light reflex, a negative electroretinogram, and negative or abnormal visual evoked-potential testing. In addition, the pathway relaying information of environmental lighting from the retina through the site of the circadian clock to the pineal appeared to be nonfunctional in these individuals, because light-induced suppression of melatonin could not be elicited [negative MST, described below (11)]. All these subjects were otherwise healthy as defined by history, physical exam, chest radiography, electrocardiography, psychological questionnaires, and biochemical and toxicological screen-

ing tests. They were not using prescription or nonprescription medications on a regular basis.

### Protocols

**Outpatient protocol.** This protocol was designed to determine whether an individual had entrained or free-running circadian rhythms while living in society. Details of the outpatient protocol have been previously reported (11); each subject lived at home but returned to the laboratory for phase assessments on three or more occasions, ~3 wk apart. On each of these laboratory visits, the subject was scheduled to one or more 24-h day(s) based on their prestudy activity-rest schedule, followed by a 40- to 50-h constant routine (CR; described below), during which circadian phase and amplitude were assessed. For the first visit only, the CR included an MST. The core body temperature (CBT) and melatonin phase markers on the first three or four visits plus the initial (prestimuli) value on the visit for the phase-shift protocol (in subjects 1415 and 1451 only) were fit using linear regression to determine whether the circadian rhythms were free running or synchronized to the 24-h day.

**Phase-shift protocol.** This protocol was designed to determine whether the exposure of the eye to bright light would affect the circadian pacemaker. Details of the phase shift protocol have been previously reported (12). The subject was scheduled to three 24-h days followed by a ~30-h CR. After the CR, the subject's schedule included three 24-h days with an activity-rest schedule shifted ~6.5 h earlier. Each day included 8 h of bright-light (~10,000 lx) exposure scheduled so the midpoint of the light exposure was both 1.5 h after CBT minimum assessed during the preceding CR and in the middle of each wake episode. The subject then underwent a second ~40-h CR, followed by three 24-h days with 8 h of dark (<0.01 lx) exposure that was scheduled so the midpoint of the dark exposure was both 1.5 h after CBT minimum, assessed during the preceding CR and in the middle of each wake episode. After a third ~45-h CR, the subject slept at his original sleep time and then went home. Light levels were 150 lx during the wake episodes of the initial inpatient scheduled days, <0.01 lx whenever the subject was asleep or during the three 8-h dark stimuli, ~10,000 lx during the three 8-h bright-light stimuli, and 10 lx at all other times.

**Forced desynchrony and nonphotic protocols.** The forced desynchrony protocol was designed to evaluate the intrinsic period of the human circadian pacemaker. Details of the forced desynchrony protocol have been previously reported (9, 18a). The subject's schedule included three 24-h days followed by a ~40-h CR. After the CR, the subject was scheduled to 24 28.0-h "days" (9.3 h scheduled sleep, 18.7 h scheduled awake), a second ~40-h CR, and a final sleep episode (see Fig. 2). Light levels were 150 lx when the subject was awake in the first 3 inpatient days, <0.01 lx whenever the subject was asleep, and 10 lx at all other times.

The nonphotic protocol was designed to assess whether nonphotic stimuli could affect the human circadian pacemaker. For the nonphotic stimuli protocol, the subject's schedule included three 24-h days followed by a ~40-h CR. After the CR ended, the subject was scheduled to 24 23.8-h days (7.9 h scheduled sleep, 15.9 h scheduled awake) until the activity-rest schedule of the subject had been advanced a total of 4.8 h (arrow in Fig. 2). The subject was then scheduled to 14 24.0-h days, followed by a ~45-h CR. After the CR ended, he had a final sleep episode. The subject had one 10-min bike riding bout daily at ~6 h after wake time at a pace chosen by the subject. His heart rate at the end of the bike riding sessions averaged 66% of maximum heart rate (defined as 220 beats/min minus the age of the subject). During the first 3 scheduled days, the subject was in ambient light of 150 lx

when lights were on and <0.01 lx when lights were off. From the beginning of CR1 through the end of CR2, during both scheduled sleep and wake times, the subject's light exposure was <0.03 lx in the angle of gaze, which was achieved by placing blue acrylic filters (Roscolux 74, Rosco, Port Chester, NY) over the light source. This created a constant, very dim short wavelength (blue) band-pass filtered light (with a maximum light intensity anywhere in the room of <0.4 photopic lx and <6.8 scotopic lx). These lighting intensities are far below the levels shown to elicit significant effects on the circadian pacemaker in sighted individuals (5). Whenever auxiliary light was required by the technicians (to check blood samples, intravenous catheter, etc.) or was present from any source (computer or television screen, etc.), the subject wore blackout goggles or the screen was covered. At the end of CR2, light levels were <0.01 lx for the sleep episode.

### Inpatient Conditions, General Procedures, and Constant Routines

During laboratory visits, subjects lived in an environment free of time cues in the Intensive Physiological Monitoring Unit of Brigham and Women's Hospital's General Clinical Research Center. Trained technicians were available at all times to ensure subject safety, to carry out the protocol, and to participate in social interaction with the subject.

During these inpatient studies, subjects followed the activity-rest schedules determined by the protocol. Subjects remained in bed in the dark and were to sleep only during scheduled rest times. During scheduled activity times, activities normally associated with wakefulness, including meals, upright posture, locomotor activity, and social contacts were permitted. The activity-rest schedule for the initial three 24-h days (pre-CR) of each inpatient study was based on the subject's schedule of the previous 7 days of activity and rest at home.

CRs consisted of enforced semirecumbent wakefulness with multiple small meals (10). These procedures were designed to minimize the evoked effects of sleep or wake states or transitions, large meals, activity, and posture changes on CBT and plasma melatonin. CRs began immediately after a subject awakened from a scheduled rest episode.

### Physiological Measures

CBT was assessed every minute using a rectal temperature thermistor (Yellow Springs Instruments, Yellow Springs, OH). After the CBT data were edited to remove obvious artifacts, the CBT data from each CR were analyzed using a two-harmonic regression method (6) to find the CBT phase, defined as the time of the fitted CBT minimum for that CR.

Plasma samples for melatonin were collected at least once per hour from the beginning of CR1 until the end of the last sleep episode after CR2 for all studies. Melatonin was assayed by radioimmunoassay (Elias USA, Osceola, WI). The melatonin phase marker was defined by the midpoint between the upward and downward crossing of the 24-h mean of the melatonin data during the CR and for the upward crossing of this level during ambulatory portions of the study.

Phase shifts were defined as the difference in phase during the CRs before and after the stimulus for CBT and melatonin data. The observed period in each of the protocols was calculated using the phase shift of CBT and melatonin data and the number of days between CRs.

The MST consisted of exposure to a ~90-min bright-light (~10,000 lx) stimulus at the time of expected maximum melatonin levels. It was performed during each subject's first CR only. A positive MST was defined as a plasma melatonin concentration during the final 60 min of the bright-light

exposure  $\leq 33\%$  of the concentration during the corresponding 60-min interval 24 h earlier (11).

All protocols were approved by Brigham and Women's Hospital's Human Research Committee. All subjects gave informed consent before participating in the protocols.

## RESULTS

Fifteen totally blind individuals had negative MST results. While living in society, six of these individuals had free-running circadian rhythms. The circadian rhythms of the remaining nine (60%) appeared synchronized to the 24-h day (i.e., the observed period was 24.0 h). The phase relationship between the CBT minimum and wake time was within the normal range for only five of the nine individuals (Table 1, Fig. 1). In the remaining four individuals, the CBT minimum was located either very early in the sleep episode or several hours after habitual wake time.

We decided to evaluate the possible causes of apparent circadian entrainment in a subset of these blind subjects within our laboratory. To evaluate the hypothesis of continued photic entrainment despite negative MST results, we exposed individuals (*subjects 1415 and 1451*) to a bright-light stimulus that had elicited a phase shift in sighted individuals (12). Three consecutive days with 8-h exposures to bright light centered 1.5 h after CBT minimum induced neither a significant suppression of plasma melatonin nor a significant phase shift of the endogenous circadian CBT or melatonin rhythms in these blind subjects. This is consistent with the previously obtained negative MST results for these subjects and indicates that photic stimuli or circadian photoreception (ocular or extraocular) could

Table 1. Characteristics of blind subjects with negative MST results

Case No.	Age, yr/Gender	Eye Status (Presence or Absence of At Least 1 Nonprosthetic Eye)	Observed Period, h	Relationship Between CBT Phase and Wake Time, h
1138	70/M	Present	24.5	Free running
1261	38/F	Present	24.2	Free running
1268	60/M	Absent	24.0	2.7*
1337	32/M	Absent	24.0	-2.3
1415	41/M	Present	24.0	-1.9
1451	32/M	Present	24.0	-1.4
1473	23/M	Present	24.0	-7.2*
14B0	41/M	Present	24.0	0.3
1516	45/F	Present (evisceration)	24.0	3.6*
1531	23/M	Absent	24.4	Free running
1573	36/F	Absent	24.0	-7.8*
1605	64/F	Absent	24.2	Free running
1610	27/F	Absent	24.1	Free running
1611	47/M	Absent	24.4	Free running
1612	25/M	Present	24.0	-0.5

In this outpatient protocol, observed period was determined by linear regression through the filled core body temperature (CBT) minima during constant routines. Subjects with observed periods of 24.0 h were considered entrained; all others were considered free running. MST, melatonin suppression test. \*Subjects with relationships between CBT minimum and wake time outside the range for sighted subjects [-4.9 h (CBT before wake time) to +0.6 h (CBT phase after wake time), 95% confidence interval; J. F. Duffy, personal communication]. Information about *subjects 1138-1451* was previously reported (11).

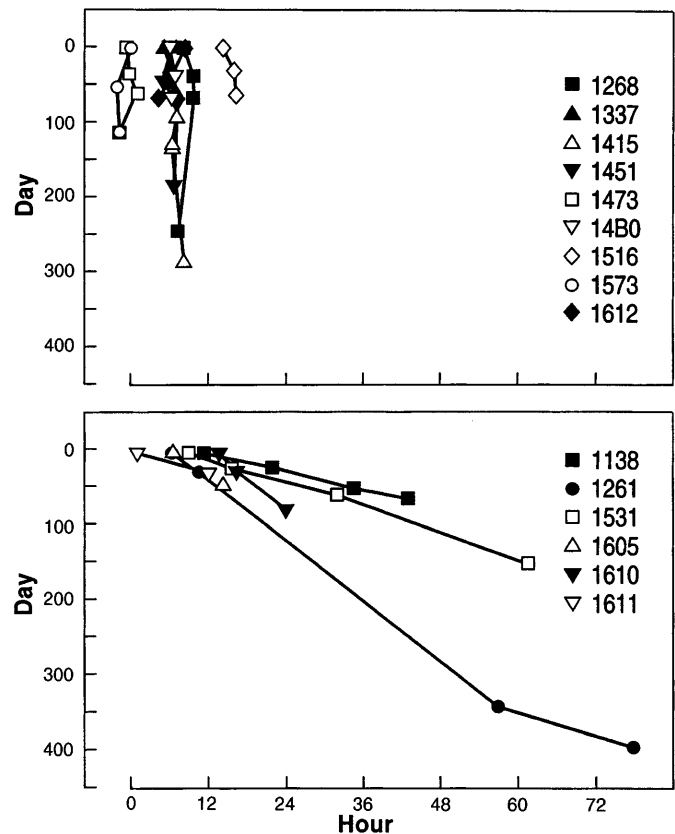


Fig. 1. Results of the outpatient protocol in 15 subjects. Each symbol represents a separate subject within each portion of the graph. Subjects with entrained circadian rhythms are plotted at *top* and those with free-running rhythms at *bottom*. Each point on the graph represents the time of a core body temperature (CBT) minimum assessed during a constant routine (CR) on that relative day of study, where the date of CBT minimum during the first CR for all subjects was defined as *day 1*. All data are reported in standard time for the subject's home time zone.

not be responsible for the entrainment in these blind subjects.

We continued to intensively study one individual (*subject 1451*) to test the remaining hypotheses concerning apparent entrainment of the circadian clock in these blind individuals. To test the hypothesis that the intrinsic period of this subject was indistinguishable from 24.0 h, we assessed the subject's intrinsic period by imposing a 28-h activity-rest schedule (Fig. 2). This forced period of 28-h is outside the range of entrainment of the circadian clock even for a proven synchronizer such as moderately bright light in sighted individuals (18a). During the 1-mo protocol, CBT and melatonin data both exhibited a cumulative phase delay of 2.6 h, consistent with an intrinsic period of 24.1 h, which is within the range of intrinsic periods observed in sighted humans on such a protocol (9). Given the 24.1-h intrinsic period of this subject assessed by the forced desynchrony protocol, his circadian phase would have been expected to demonstrate a cumulative phase delay of  $\sim 8$  h during the 2 $\frac{3}{4}$  mo of outpatient study (rather than remaining stable, as was observed).

To test the hypothesis that entrainment of the circadian clock was achieved by nonphotic stimuli, the subject was scheduled to 24 cycles of a 23.8-h activity-

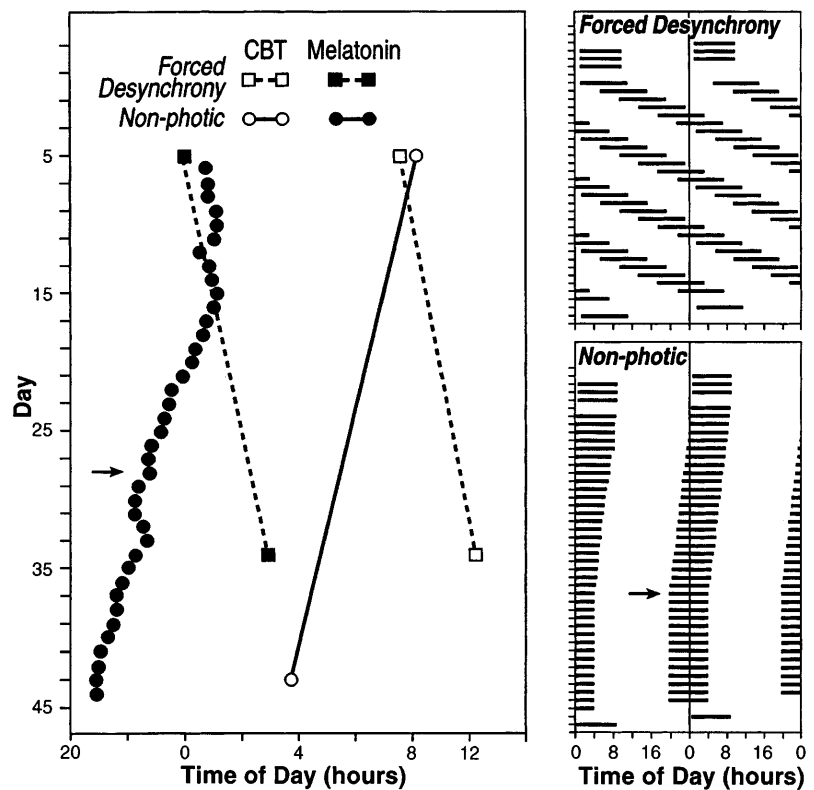


Fig. 2. A plot of time of melatonin upward crossing of 24-h mean levels during in-bed time (filled symbols) and CBT minima (open symbols) during CRs during the forced desynchrony (28.0-h "day" schedule, squares) and nonphotic stimuli (23.8-h day and 24.0-h day schedule, circles) protocols in 1 subject. Scheduled rest times for these protocols are shown double plotted in raster format. Arrow indicates when the schedule changed from a 23.8-h day to a 24.0-h day.

rest episode followed by 14 cycles of stabilization on a 24.0-h activity-rest schedule (Fig. 2) in constant very dim light levels ( $<0.03$  lx). In contrast to the results of the 28-h forced desynchrony experiment, the observed period of both CBT and plasma melatonin was shorter than 24-h when computed over the 5½ wk of this experiment. The endogenous circadian rhythm of CBT advanced 4.5 h, rather than delaying 3.8 h as would have been expected in the absence of exposure to an entraining stimulus, given the subject's 24.1-h intrinsic period. The endogenous circadian rhythm of melatonin also advanced 2.5 h between the two CRs. Inspection of the daily pattern of melatonin secretion suggested that melatonin began advancing only after ~10 days of the 23.8-h days and continued advancing through the 14 24.0-h days until the phase relationship between melatonin and the rest-activity schedule was the same as at the beginning of the protocol.

## DISCUSSION

These studies demonstrate that the human circadian pacemaker can be entrained to the 24-h day despite absence of photic input via the eye to the circadian pacemaker. In 9 of 15 blind people studied, the observed entrainment of their circadian pacemaker was unlikely to be mediated by light, because light did not induce suppression of plasma melatonin nor did light induce significant phase shifts of the rhythms of melatonin and CBT (in the 2 subjects studied). Although previous studies have reported entrainment of the circadian pacemaker in blind persons, the absence of ocular circadian photoreception was not documented in those studies.

The high proportion of individuals (44%) with an abnormal phase relationship between the phase of their CBT temperature and melatonin rhythms and their rest-activity schedule can be interpreted in several ways. In sighted subjects, the light-dark cycle is the most powerful synchronizer of the circadian clock and therefore the phase relationship between the rest-activity cycle (which determines light input to the pacemaker) and the endogenous circadian rhythms, such as melatonin and CBT, are primarily determined by the phase response curve (PRC) to light. In blind subjects without light input to the circadian clock, putative nonphotic synchronizers (such as the rest-activity cycle) become the dominant synchronizers and therefore the phase relationship between the rest-activity cycle and the endogenous circadian rhythms of melatonin and CBT is primarily determined by the PRC to the nonphotic stimuli. Animal research has demonstrated that the PRC to light is very different from the PRC to nonphotic stimuli (30). On the assumption that similar differences exist in humans, entrainment to a nonphotic synchronizer should result in a different (i.e., abnormal) phase relationship between CBT and wake time compared with that observed in sighted individuals. That the melatonin rhythm began advancing in *subject 1451* after ~10 days of the 23.8-h schedule would thus be expected, because it would take a number of cycles for the appropriate phase relationship to be reached.

Whereas previous studies in humans have only induced phase delays of the circadian timing system with scheduled regular activity (7, 13, 16, 35, 41), the present studies demonstrate that nonphotic synchroniz-

ers can induce the phase advances required for entrainment of the >24-h period of the human circadian pacemaker to the 24.0-h day. Previous human studies could not provide conclusive evidence for nonphotic entrainment because the nonphotic stimulus was often associated with concurrent light exposure or the experiments were of short duration (3, 35, 41, 44). Extraocular photoreception, demonstrated in reptiles and birds, cannot explain the present findings, because the nonphotic protocol was conducted in near darkness (<0.03 lx).

The nonphotic aspect of the rest-activity cycle responsible for entrainment remains to be elucidated. Our observations are in accordance with studies in which nonphotic stimuli such as wheel running or vigorous activity affected the circadian rhythms of rodents and other animals (14, 22, 27, 31, 40–42). Meals have also been reported to affect the circadian clock of rodents (28). However, neither social contacts, once thought to be powerful synchronizers in humans, nor knowledge of time of day is sufficient to entrain the human circadian pacemaker (18, 23, 26). Other nonphotic stimuli may affect the clock indirectly, by affecting exposure to light, e.g., a medication or event that causes an individual to go to sleep (turn off the lights) or become more active (resulting in increased light exposure).

Regardless of which nonphotic stimulus affects the circadian clock, the strength of the resetting response to that nonphotic stimulus in an individual who never had ocular circadian photoreception may differ from that of a sighted individual. In congenitally blind individuals, the retinohypothalamic tract may not develop normally and/or other input pathways to the circadian clock may exert a stronger influence. Because some of our entrained subjects were congenitally blind, it is unlikely that the success of the nonphotic stimuli in entraining some individuals is due to a conditioned response (1).

The synchronizing effect of nonphotic stimuli appears to be of sufficient strength to entrain the circadian rhythms of only some blind individuals to the 24-h day. Most previous reports focused on the many blind individuals whose circadian rhythms free ran despite a regular rest-activity schedule (11, 17, 20, 38). In the present study, 6 of the 15 blind patients with a negative MST were not entrained to the 24-h day, despite maintenance of a regular rest-activity cycle. This is consistent with the recent demonstration that nonphotic stimuli are weaker than light stimuli in affecting the human circadian pacemaker (12). Sighted humans were exposed to an inversion of their rest-activity schedule with and without bright-light exposure. Only those subjects who were exposed to bright light had significant phase shifts of the circadian rhythms; the inversion of the rest-activity cycle alone was not sufficient to cause a phase shift. Slow, gradual, and relatively small phase shifts may be all that nonphotic stimuli can elicit, and those stimuli may only be effective if they are applied at a specific circadian phase. In addition, small phase shifts caused by nonphotic stimuli may not be detectable in short-term (<20 day) experiments using experimental measures with

significant day-to-day variability. However, only a small phase shift is required for entrainment of a circadian clock to environmental time if the intrinsic period is only a few minutes different from the 24.0-h period of environmental time.

The crucial difference between totally blind individuals who are entrained and those who are free running may be whether their intrinsic period is close enough to 24.0 h that the relatively weak synchronizing effect of nonphotic stimuli can be effective in entraining their intrinsic circadian pacemaker to the 24.0-h day. Even in totally blind individuals whose circadian rhythms appear free running, relative coordination or other interaction with a synchronizer of insufficient strength to entrain may still affect the observed circadian period so that it is different from that of the endogenous circadian pacemaker.

We conclude from the results obtained in these blind individuals that nonphotic stimuli can indeed exert a small but significant synchronizing or resetting effect on the human circadian pacemaker, as reflected in the CBT and plasma melatonin rhythms. Given that these individuals lack ocular circadian photoreception, we are confident that this synchronizing influence is not mediated via an indirect effect of ocular light exposure. Although photic stimuli remain far stronger influences on the circadian pacemaker in that they are capable of inducing phase shifts of up to 12 h in 3 days, nonphotic stimuli elicit more modest but still significant resetting responses from the pacemaker. Further experiments to define the active nonphotic stimulus responsible for eliciting the observed phase shifts, the optimal timing of such a stimulus, and the interaction of photic and nonphotic stimuli are required and may aid in the treatment of blind persons with free running rhythms.

#### NOTE ADDED IN PROOF

We studied *subject 1415* under a nonphotic entrainment evaluation protocol similar to that used for *subject 1451*, which included 3 24-h days followed by an ~40-h CR, 16 28-h (forced desynchrony) cycles, an ~48-h CR, 26 23.8-h (nonphotic) cycles, and a final ~40-h CR. The lighting and exercise conditions for each of these segments were similar to those described for *subject 1451*. In contrast to his previous studies reported above, *subject 1415* was no longer entrained at the time of admission to the laboratory and was complaining of a recent 3-wk episode of disturbed sleep. His free-running period, as assessed during the forced desynchrony portion of the protocol, was 24.1 h. His circadian rhythms of CBT and melatonin did not appear to be affected by the nonphotic protocol; they continued to oscillate with a 24.1-h period, notwithstanding an enforced 24.8-h sleep-wake schedule. These results suggest interindividual differences as well as intraindividual variation in response to nonphotic stimuli.

We are grateful to the following individuals for support: Dr. G. H. Williams, Dr. M. Sowell, G. Jayne, J. Zeitzer, J. Kao, and the staff of the General Clinical Research Center of Brigham and Women's Hospital.

This work was supported by the National Institute of Mental Health, the National Institute on Aging, the National Aeronautics and Space Administration, the National Heart, Lung, and Blood Institute, and the General Clinical Research Program of the National Center for Research Resources. E. B. Klerman is supported by an award from the National Institute on Aging.

Address for reprint requests: E. B. Klerman, Circadian, Neuroendocrine and Sleep Disorders Section, Endocrinology-Hypertension Division, Dept. of Medicine, Brigham and Women's Hospital & Harvard Medical School, 221 Longwood Ave., Boston, MA 02115.

Received 3 October 1997; accepted in final form 16 December 1997.

#### REFERENCES

- Amir, S., and J. Stewart. Resetting of the circadian clock by a conditioned stimulus. *Nature* 379: 542–545, 1996.
- Arendt, J., M. Aldhous, and J. Wright. Synchronisation of a disturbed sleep-wake cycle in a blind man by melatonin treatment. *Lancet* 1: 772–773, 1988.
- Aschoff, J., M. Fatranská, H. Giedke, P. Doerr, D. Stamm, and H. Wisser. Human circadian rhythms in continuous darkness: entrainment by social cues. *Science* 171: 213–215, 1971.
- Aschoff, J., and R. Wever. Spontanperiodik des Menschen bei Ausschuss aller Zeitgeber. *Naturwissenschaften* 49: 337–342, 1962.
- Boivin, D. B., J. F. Duffy, R. E. Kronauer, and C. A. Czeisler. Dose-response relationships for resetting of human circadian clock by light. *Nature* 379: 540–542, 1996.
- Brown, E. N., and C. A. Czeisler. The statistical analysis of circadian phase and amplitude in constant-routine core-temperature data. *J. Biol. Rhythms* 7: 177–202, 1992.
- Buxton, O. M., S. A. Frank, M. L'Hermite-Balériaux, R. Leproult, F. W. Turek, and E. Van Cauter. Roles of intensity and duration of nocturnal exercise in causing phase delays of human circadian rhythms. *Am. J. Physiol.* 273 (Endocrinol. Metab. 36): E536–E542, 1997.
- Czeisler, C. A. The effect of light on the human circadian pacemaker. In: *Circadian Clocks and Their Adjustment*. Chichester, UK: Wiley, 1995, p. 254–302. (Ciba Foundation Symp. 183)
- Czeisler, C. A., J. F. Duffy, T. L. Shanahan, E. N. Brown, J. F. Mitchell, D.-J. Dijk, D. W. Rimmer, J. M. Ronda, J. S. Allan, J. S. Emens, and R. E. Kronauer. Reassessment of the intrinsic period ( $\tau$ ) of the human circadian pacemaker in young and older subjects (Abstract). *Sleep Res.* 24A: 505, 1995.
- Czeisler, C. A., R. E. Kronauer, J. S. Allan, J. F. Duffy, M. E. Jewett, E. N. Brown, and J. M. Ronda. Bright light induction of strong (Type 0) resetting of the human circadian pacemaker. *Science* 244: 1328–1333, 1989.
- Czeisler, C. A., T. L. Shanahan, E. B. Klerman, H. Martens, D. J. Brotman, J. S. Emens, T. Klein, and J. F. Rizzo III. Suppression of melatonin secretion in some blind patients by exposure to bright light. *N. Engl. J. Med.* 332: 6–11, 1995.
- Duffy, J. F., R. E. Kronauer, and C. A. Czeisler. Phase-shifting human circadian rhythms: influence of sleep timing, social contact and light exposure. *J. Physiol. (Lond.)* 495: 289–297, 1996.
- Eastman, C. I., E. K. Hoese, S. D. Youngstedt, and L. Liu. Phase-shifting human circadian rhythms with exercise during the night shift. *Physiol. Behav.* 58: 1287–1291, 1995.
- Edgar, D. M., and W. C. Dement. Regularly scheduled voluntary exercise synchronizes the mouse circadian clock. *Am. J. Physiol.* 261 (Regulatory Integrative Comp. Physiol. 30): R928–R933, 1991.
- Hollwich, F., and B. Dieckhues. Circadian rhythm in the blind. *J. Interdiscip. Cycle Res.* 2: 291–302, 1971.
- Honma, K.-I., S. Hashimoto, K. Nakamura, and S. Honma. Entrainment of human sleep-wake rhythm by forced sleep-wake schedule (Abstract). *Soc. Res. Biol. Rhythms* 5: 205, 1996.
- Klein, T., H. Martens, D.-J. Dijk, R. E. Kronauer, E. W. Seely, and C. A. Czeisler. Chronic non-24-hour circadian rhythm sleep disorder in a blind man with a regular 24-hour sleep-wake schedule. *Sleep* 16: 333–343, 1993.
- Kleitman, N. *Sleep and Wakefulness*. Chicago, IL: Univ. of Chicago Press, 1963.
- Klerman, E. B., D. J. Dijk, R. E. Kronauer, and C. A. Czeisler. Simulations of the effect of light on the human circadian pacemaker: implication for assessment of intrinsic period. *Am. J. Physiol.* 270 (Regulatory Integrative Comp. Physiol. 39): R271–R282, 1996.
- Lewy, A. J., and D. A. Newsome. Different types of melatonin circadian secretory rhythms in some blind subjects. *J. Clin. Endocrinol. Metab.* 56: 1103–1107, 1983.
- Lockley, S. W., D. J. Skene, H. Tabandeh, A. C. Bird, R. Defrance, and J. Arendt. Relationship between napping and melatonin in the blind. *J. Biol. Rhythms* 12: 16–25, 1997.
- Lund, R. *Circadiane Periodik physiologischer und psychologischer Variablen bei 7 blinden Versuchspersonen mit und ohne Zeitgeber* (PhD dissertation). Munich: Technical University of Munich, 1974.
- Marchant, E. G., and R. E. Mistlberger. Entrainment and phase shifting of circadian rhythms in mice by forced treadmill running. *Physiol. Behav.* 60: 657–663, 1996.
- Middleton, B., J. Arendt, and B. M. Stone. Human circadian rhythms in constant dim light (8 lux) with knowledge of clock time. *Sleep Res.* 5: 69–76, 1996.
- Miles, L. E. M., and M. A. Wilson. High incidence of cyclic sleep/wake disorders in the blind (Abstract). *Sleep Res.* 6: 192, 1977.
- Miles, L. E. M., D. M. Raynal, and M. A. Wilson. Blind man living in normal society has circadian rhythms of 24.9 hours. *Science* 198: 421–423, 1977.
- Mills, J. N. Circadian rhythms during and after three months in solitude underground. *J. Physiol. (Lond.)* 174: 217–231, 1964.
- Mistlberger, R. E. Nonphotic entrainment of circadian activity rhythms in suprachiasmatic nuclei-ablated hamsters. *Behav. Neurosci.* 106: 192–202, 1992.
- Mistlberger, R. E., and E. G. Marchant. Computational and entrainment models of circadian food-anticipatory activity: evidence from non-24-hr feeding schedules. *Behav. Neurosci.* 109: 790–798, 1995.
- Moog, R., H. Endlich, G. Hildebrandt, and H. Martens. Circadian rhythms in blind persons. *J. Interdiscip. Cycle Res.* 16: 295, 1985.
- Mrosovsky, N., S. G. Reeb, G. I. Honrado, and P. A. Salmon. Behavioural entrainment of circadian rhythms. *Experientia* 45: 696–702, 1989.
- Mrosovsky, N., and P. A. Salmon. A behavioural method for accelerating re-entrainment of rhythms to new light-dark cycles. *Nature* 330: 372–373, 1987.
- Nakagawa, H., R. L. Sack, and A. J. Lewy. Sleep propensity free-runs with the temperature, melatonin and cortisol rhythms in a totally blind person. *Sleep* 15: 330–336, 1992.
- Okawa, M., T. Nanami, S. Wada, T. Shimizu, Y. Hishikawa, H. Sasaki, H. Nagamine, and K. Takahashi. Four congenitally blind children with circadian sleep-wake rhythm disorder. *Sleep* 10: 101–110, 1987.
- Orth, D. N., G. M. Besser, P. H. King, and W. E. Nicholson. Free-running circadian plasma cortisol rhythm in a blind human subject. *Clin. Endocrinol. (Oxf.)* 10: 603–617, 1979.
- Piercy, J., and L. Lack. Daily exercise can shift the endogenous circadian phase (Abstract). *Sleep Res.* 17: 393, 1988.
- Rusak, B. Neural mechanisms for entrainment and generation of mammalian circadian rhythms. *Federation Proc.* 38: 2589–2595, 1979.
- Sack, R. L., T. M. Hoban, and A. J. Lewy. Free-running melatonin rhythms in totally blind people (Abstract). *Sleep Res.* 16: 636, 1987.
- Sack, R. L., A. J. Lewy, M. L. Blood, L. D. Keith, and H. Nakagawa. Circadian rhythm abnormalities in totally blind people: incidence and clinical significance. *J. Clin. Endocrinol. Metab.* 75: 127–134, 1992.
- Siffre, M. *Beyond Time*. New York: McGraw Hill, 1964.
- Turek, F. W. Effects of stimulated physical activity on the circadian pacemaker of vertebrates. *J. Biol. Rhythms* 4: 135–147, 1989.
- Van Reeth, O., J. Sturis, M. M. Byrne, J. D. Blackman, M. L'Hermite-Balériaux, R. Leproult, C. Oliner, S. Refetoff, F. W. Turek, and E. Van Cauter. Nocturnal exercise phase delays circadian rhythms of melatonin and thyrotropin secretion in normal men. *Am. J. Physiol.* 266 (Endocrinol. Metab. 29): E964–E974, 1994.
- Van Reeth, O., and F. W. Turek. Stimulated activity mediates phase shifts in the hamster circadian clock induced by dark pulses or benzodiazepines. *Nature* 339: 49–51, 1989.
- Weitzman, E. D., M. Perlow, J. F. Sassin, D. Fukushima, B. Burack, and L. Hellman. Persistence of the twenty-four hour pattern of episodic cortisol secretion and growth hormone release in blind subjects. *Trans. Am. Neurol. Assoc.* 97: 197–199, 1973.
- Wever, R. A. *The Circadian System of Man: Results of Experiments Under Temporal Isolation*. New York: Springer-Verlag, 1979.