The Human Circadian Pacemaker Can See by the Dawn's Early Light

Konstantin V. Danilenko,*[,2] Anna Wirz-Justice,[†,1]
Kurt Kräuchi,[†] Jakob M. Weber,[‡] and Michael Terman[§]

*Institute of Physiology, Siberian Branch of the Russian Academy of Medical Sciences, Novosibirsk, Russia, [†]Centre for Chronobiology, Psychiatric University Clinic, CH-4025 Basel, Switzerland, [‡]Bühlmann Laboratories, CH-4124 Schönenbuch, Switzerland, [§]Columbia University & New York State Psychiatric Institute, New York, NY 10032, USA

Abstract The authors' previous experiments have shown that dawn simulation at low light intensities can phase advance the circadian rhythm of melatonin in humans. The aim of this study was to compare the effect of repeated dawn signals on the phase position of circadian rhythms in healthy participants kept under controlled light conditions. Nine men participated in two 9-day laboratory sessions under an LD cycle 17.5:6.5 h, < 30:0 lux, receiving 6 consecutive daily dawn (average illuminance 155 lux) or control light (0.1 lux) signals from 0600 to 0730 h (crossover, random-order design). Two modified constant routine protocols before and after the light stimuli measured salivary melatonin (dim light melatonin onset DLMOn and offset DLMOff) and rectal temperature rhythms (midrange crossing time [MRCT]). Compared with initial values, participants significantly phase delayed after 6 days under control light conditions (at least –42 min DLMOn, –54 min DLMOff, –41 min MRCT) in spite of constant bedtimes. This delay was not observed with dawn signals (+10 min DLMOn, +2 min DLMOff, 0 min MRCT). Given that the endogenous circadian period of the human circadian pacemaker is slightly longer than 24 h, the findings suggest that a naturalistic dawn signal is sufficient to forestall this natural delay drift. Zeitgeber transduction and circadian system response are hypothesized to be tuned to the time-rate-of-change of naturalistic twilight signals.

Key words dawn simulation, human circadian rhythms, constant routine, rectal temperature, melatonin

Circadian rhythms are driven by an innate program that evolved to adapt the organism to the most reliable and predictable of environmental changes, the solar cycle of day and night (Pittendrigh, 1960). Light, the major zeitgeber synchronizing endogenous rhythms to the 24-h day in all species from fungi to fish to flying foxes, is also the major synchronizing agent in humans. The history of light and entrainment in humans is complex, with primary evidence coming from phase response curves to light (e.g., Honma et al., 1987; Czeisler et al., 1989; Minors et al., 1991) or from blind persons (e.g., Sack et al., 1992; Lockley et al., 1997). Initially, high light intensities were required to be effective: 2500 lux to suppress nocturnal melatonin

1. To whom all correspondence should be addressed.
2. Present address: Institute of Internal Medicine, Siberian Branch of the Russian Academy of Medical Sciences, 2a, Vladimirovsky spusk, Novosibirsk 630003, Russia.

JOURNAL OF BIOLOGICAL RHYTHMS, Vol. 15 No. 5, October 2000 437-446
© 2000 Sage Publications, Inc.

secretion (Lewy et al., 1980) and 1 to 3 consecutive pulses of 6000 to 10,000 lux for several hours to phase advance or delay the circadian core body temperature rhythm (Honma et al., 1987; Czeisler et al., 1989; Minors et al., 1991). Only recently have the effects of lower light intensities on the human circadian system been demonstrated (e.g., Laakso et al., 1993; Boivin et al., 1996; Samkova et al., 1997).

In nature, the dynamic light-dark cycle spans an approximate 9-log unit range of illuminance from starlight (approx. 0.0001 lux) to midday maxima (approx. 100,000 lux). The timing and shape of this illuminance profile is determined by season and latitude. The seasonal differences are most pronounced during the twilight transitions of dawn and dusk. The natural zeitgeber, the dawn and dusk signal, is an obvious but little investigated paradigm given the standard laboratory procedure of rectangular wave (on/off) illumination. A comprehensive algorithm predicts the illuminance on a horizontal surface of the earth, from direct sunlight and skylight sources, at any latitude, longitude, day of the year, and time of the day (Terman et al., 1994). The twilight segment of the algorithm has been validated against outdoor measurements (Terman et al., 1989a) and a version with computational refinements (Terman et al., 1996) used to drive interior lighting devices with constant color temperature (light-emitting diode, shutter-attenuated flourescent) or variable color temperature (incandescent). Although incandescent dimming produces a spectral shift to long wavelengths, these are not veridical mimics of outdoor twilight red shifts, which vary geographically and with time of year, weather conditions, and the density and composition of particulate matter in the atmosphere.

In a recent laboratory application with hamsters, artificial dawn simulation successfully mimicked the zeitgeber effect of light at much lower total illuminance (Boulos et al., 1996 a,c). When a complete day-night cycle is presented with dawn and dusk transitions, and rats are given recourse to a darkened area, they self-select an individual balance of twilight exposures that compensates for the free-running period and achieves entrainment (Terman et al., 1991).

Daily exposure to high-intensity artificial light has been shown to be effective for treatment of seasonal affective disorder (SAD; reviewed in Lam and Levitt, 1999). In patients with SAD, 1 week's presentation of simulated dawn signals in the bedroom during the final period of sleep is not only antidepressant but also phase advances melatonin secretion (Terman et al., 1989b), which suggests that humans are responsive to the natural twilight transition preceding full daylight.

Endogenous circadian rhythms in humans are masked both by the organism's own behavior and external environmental stimuli, thus hindering precise determination of the circadian characteristics of amplitude and phase. For this reason, constant routine protocols (CR) provide the necessary controlled conditions to assess circadian parameters under reduced masking conditions (e.g., Mills et al., 1978; Czeisler et al., 1989). We have shortened and modified the "gold standard" 40-h CR to permit sleep but still reduce masking (Kräuchi et al., 1997). Phase markers other than the classic core body temperature minimum or melatonin peak during sleep deprivation are measured during the wake phase. In two such CR protocols, we have shown that a single or triple dawn signal could phase advance dim light melatonin onset in healthy participants (Danilenko et al., 1997, 2000; Wirz-Justice et al., 1998). That study lacked a control group to assess the specific effect of the dawn signal. The present study used a truncated dawn signal as a control in which constant dim light exposure (0.1 lux) was substituted for the gradual progression toward sunrise. We hypothesized that phase advances would be obtained only with the complete dawn signal. In broader terms, we hypothesize that the human circadian system is tuned to gradual increments of low-level illumination during naturalistic dawns, characterized by an accelerating time-rate-of-change that ends before sunrise.

MATERIALS AND METHODS

Participants

The study was carried out in Novosibirsk (55 °N) between December 1997 and September 1998. The study participants were screened for general medical and psychological health, were nonsmokers, had no sleep disorders, did not use medications or recreational drugs, and did not engage in shift work or transmeridian travel in the prior 2 months. Nine young men (age 24.0 ± 4.8 SD) completed both arms of the study. The protocol was approved by the Ethics Committee of the Department of Medicine, University of Basel, and participants gave written informed consent.

Experimental Protocol

Participants took part in a set of two 9-day laboratory sessions (random-order crossover of dawn and control conditions) separated by a 9-day washout period outside the laboratory under their normal routine. In the week before each session, bedtime was between 2300 and 0000 h, whereas wakeup time could vary individually within a 1-h range; compliance was monitored by sleep logs. Other than for the morning light signal, the laboratory protocol was identical in the two sessions (Fig. 1). The schedule of sleep, daytime activity, meals, and constant routine snacks was kept consistent across participants. They were permitted morning naps (under 8 lux illumination) if recovery sleep was needed on Days 4, 6, and 7. Daytime illumination in the laboratory was < 30 lux, with no light from outdoors. Participants had access to clock time of day except during the constant routines. They were studied singly, not in groups, under continuous staff supervision.

Participants remained supine in bed for two modified 42.5-h CR protocols (for methods, see Kräuchi et al., 1997) at the beginning (initial CR) and after the last light signal (final CR). The CRs were carried out in a sound-proof and time-free room (8 lux at eye level, constant comfortable ambient temperature), taking isocaloric snacks and water at hourly intervals, with sleep permitted from 2330 to 0730 (0 lux). Rectal temperature was monitored continuously every 2 min throughout the CRs by a portable minilogger (ARM production, Novosibirsk; resolution 0.022 °C). Saliva for melatonin assay was collected at 30-min intervals from wake-up time to 1100 h and from 1730 to 2330 h during the CRs, and additionally on days of the first (20-min intervals to measure melatonin suppression kinetics), third, and sixth signal presentations for which the participants were also supine, under 8 lux lighting, to avoid effects of posture change or light on the melatonin profile. A battery of self-assessment scales was administered every 30 min during the CRs and on days when saliva was collected. Instruments included 100 mm visual analogue scales (VAS) for mood, alertness, tension, and interest; the Karolinska Sleepiness Scale (KSS); and the Karolinska Sleepiness Symptoms Checklist (KSSCL; Gillberg et al., 1994).

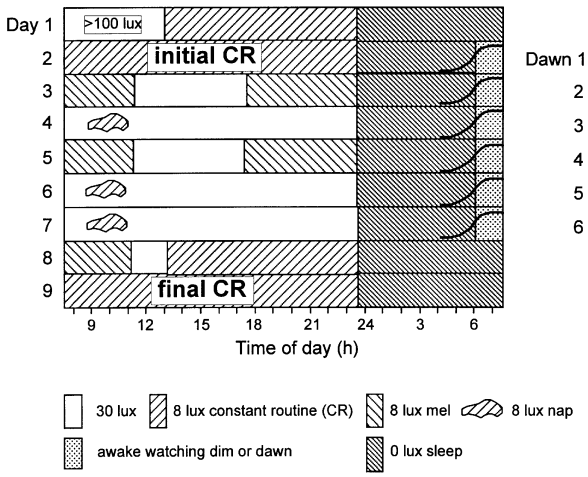

Figure 1. Schematic protocol of a 9-day dawn session during which a subject was assigned to receive six consecutive dawn stimuli in the morning. The control light session differed only in that a 0.1 lux signal was presented as a truncated control instead of dawn (crossover, random-order design). The session began and ended with a 42.5-h modified constant routine (CR) permitting sleep (see Kräuchi et al., 1997) during which the initial and final phases of rectal temperature and melatonin rhythms were measured. CR-like conditions (supine, 8 lux) were additionally implemented on the days of the first, third, and sixth stimulus presentations to collect saliva for melatonin assay. When not in the CR, light intensity in the laboratory was < 30 lux (0 lux during sleep). A recovery nap was permitted on the morning of the second, fourth, and fifth stimulus presentations, from 0830 h, under 8 lux, equally in control or dawn sessions.

Dawn Simulation

The dawn simulation system (SphereOne, Inc., Silver Plume CO, USA; Fairhurst et al., 1999) included a computer algorithm (MacLite), which drove an electronic controller connected to an overhead halogen lamp reflector/diffuser fixture (Wafer) that delivered an intensity range of 0.001 to 1000 lux at the location of the participant's head while in bed (confirmed by measurement of illuminance with a digital photometer). The signal is designed to represent the rate of change of illuminance with time. We chose the dawn waveform for 50 °N, 21 June to produce a slowly rising dawn, by comparison with faster transitions that occur closer to the equator and the equinoxes (cf. Danilenko et al., 2000; Fig. 1). This natural sunrise was displaced to coincide with wake-up time, that is, so that 1000 lux was attained a few minutes postsunrise (775 lux) at 0730 h (Fig. 2). The dawn was programmed to begin at 0330 h (0.003 lux during sleep) and reached 0.1 lux at 0600 h. The participant was awakened to watch the dawn from 0600 h while in bed, and after 0730 h, light was reduced to < 30 lux. For the control light session, the signal also began at 0330 h during sleep but was truncated at 0.1 lux from 0600 h for the

same 1.5-h period after waking (this level was sufficient for staff members to verify that participants' eyes remained open). The daytime laboratory light level (< 30 lux) commenced at 0730 h. Each session comprised 6 consecutive dawn or control signal presentations.

Circadian Phase Markers

The first phase marker was dim light melatonin onset (DLMOn), previously validated under identical conditions (Kräuchi et al., 1997). The morning melatonin offset (DLMOff) was also measured (but is a somewhat more problematic circadian phase marker; Lewy et al., 1999). Salivary samples were assayed for melatonin using a highly specific direct double-antibody RIA, validated by GCMS, with an analytical least detectable dose of 0.15 pg/ml and a functional least detectable dose of 0.65 pg/ml (Weber et al., 1997). For each participant, the DLMOff and DLMOn were determined by linear interpolation as the time when melatonin levels crossed a 3 pg/ml threshold. In 3 participants, the morning melatonin level did not always fall below 3 pg/ml by the end of the sampling interval at 1100 h; a reasonable higher threshold was chosen for calculation of DLMOff (5.0, 8.0, and 8.5 pg/ml). In these 3 participants and 1 further case, DLMOn could not be determined at the end of the 9-day session because none of the evening melatonin values had reached 3 pg/ml by lights out at 2330 h (n = 5 for this calculation).

The second circadian phase marker was the midrange crossing time (MRCT) of the morning rise and nocturnal decline of temperature (T) during the CRs. The first 2 h of temperature data were excluded from the analysis due to postural effects after lying down. Thereafter, each curve was smoothed by 1-h moving averages. The adjacent minimum and maximum values (°C) were averaged (midrange value, °C). This value was taken to determine the MRCT (for details, see Kräuchi et al., 1997). During the 1.5 days of each CR, the temperature declined, rose, and declined again, that is, three MRCT times could be derived from each curve.

Statistical evaluation of the data was performed by analysis of variance (ANOVA) for repeated measures. Huynh-Feldt statistics were used to adjust the covariance matrix for violations of sphericity. When the F ratio was significant, a post-hoc analysis was performed to locate significant differences (Fisher-PLSD test). Where no multiple comparisons could be made, Student's paired t-test was applied to ascertain significant differences between the means. Linear correlation analysis was used to determine a link between the parameters.

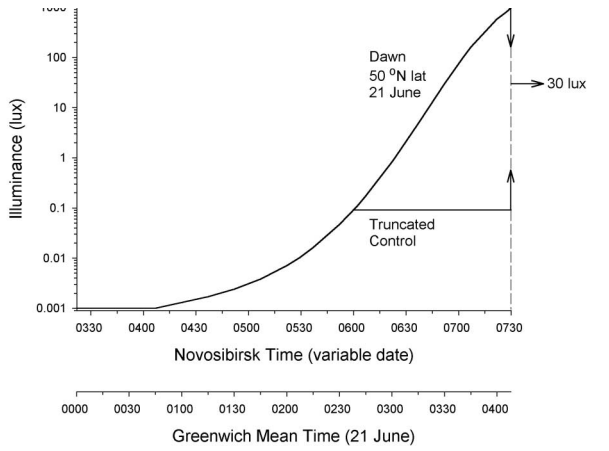

Figure 2. The dawn signal simulation for 50 °N latitude on 21 June (Greenwich Mean Time) was shifted in local time (Novosibirsk) so that 1000 lux was reached at 0730 h for all experiments regardless of date. Illuminance is plotted on a log lux scale, and the signal was set to begin at 0330 h (Novosibirsk time). Participants slept until awakened at 0600 h when the signal reached 0.1 lux. They watched this signal grow to 1000 lux (a few minutes postsunrise, 775 lux). The control signal was truncated and maintained at 0.1 lux. After 0730 h, both groups were kept under laboratory lighting that never exceeded 30 lux (a level within the civil twilight range).

RESULTS

Phase Shifts

The day-by-day time course of melatonin concentration throughout the experiment is presented in Figure 3, averaged over the 9 participants. During the control light condition, a gradual phase delay occurred across the 9 days of the experiment. A three-way ANOVA for repeated measures using only the initial and final CRs yielded a significant three-way interaction term for the evening melatonin values ($F_{12,96}$ = 3.03, $p < 0.05$) and a significant main effect for the morning melatonin values (control/dawn session vs. initial/final CR: $F_{1,8}$ = 7.97, $p < 0.03$). A post-hoc analysis with Fisher-PLSD revealed that the significant changes occurred after control light exposure: The amount of melatonin secreted was decreased in the evening (t = 2.37, $p < 0.05$) and increased in the morning (t = 6.00, $p < 0.001$), which

can be interpreted as a phase delay. The dawn group did not change over days ($p > 0.05$).

A similar analysis was performed with DLMOff and DLMOn times, even though the DLMOn group was reduced in number because 4 participants did not always attain the 3 pg/ml threshold. The evolution of DLMOff and DLMOn is illustrated in Figure 4. Again, only the initial and final CRs were entered into a two-way ANOVA and yielded a significant two-way interaction term both for DLMOff ($F_{1,8} = 6.79$, $p = 0.03$) and for DLMOn ($F_{1,4} = 11.37$, $p < 0.03$). A post-hoc analysis with Fisher-PLSD revealed a significant phase delay after the control session (DLMOff: –54 ± 15 min, $t = 3.56$, $p < 0.01$; DLMOn: –34 ± 9 min, $t = 3.65$, $p < 0.03$). No significant phase shift was found after the dawn session. The differences between initial and final CR in phase position were significantly different for the control session compared with the dawn session, both by DLMOff (–54 vs. +2 min, $t = 2.61$, $p = 0.03$) and DLMOn (–34 vs. +10 min, $t = 3.69$, $p = 0.02$). At the final CR, DLMOn differed significantly between control and dawn ($n = 5$, 34 ± 9 min, $t = 3.69$, $p = 0.02$).

All but 1 participant delayed under control light. For DLMOff, this delay averaged –8 min per day. For DLMOn, if we add to the analysis the 4 participants whose DLMOn occurred later than the last sampling time at 2330 h by estimating a conservative value of 2400 h, the average phase delay of DLMOn for the whole group was at least –42 min, that is, –6 min per day. Thus, both DLMOff and DLMOn drifted to later by similar rates.

Figure 5 summarizes the dynamic of the temperature phase markers during the control and dawn sessions. A three-way ANOVA for repeated measures of the MRCT times (factors: control/dawn session, initial/final CR, time of day) revealed a significant two-way interaction between control/dawn session and initial/final CR ($F_{1,8} = 5.55$, $p < 0.05$; all other interaction terms including the factor control/dawn session were not significant). A post-hoc analysis with Fisher-PLSD indicated a significant phase delay in the control session (–41 ± 15 min, $t = 3.34$, $p = 0.01$) but not in the dawn session (0 ± 12 min, n.s.). The phase delay in the final control session was significantly larger than in the dawn session (–40 ± 17 min, $t = 2.36$, $p < 0.05$).

Both DLMOff and MRCT times appeared to be earlier in the initial CR of the control light session compared with the initial CR of the dawn session; the difference reached significance for DLMOff (0846 h ± 22 min vs. 0928 h ± 23 min; $t = 2.35$, $p < 0.05$; Fig. 4). Analysis of the participants' sleep diaries over the 5 days

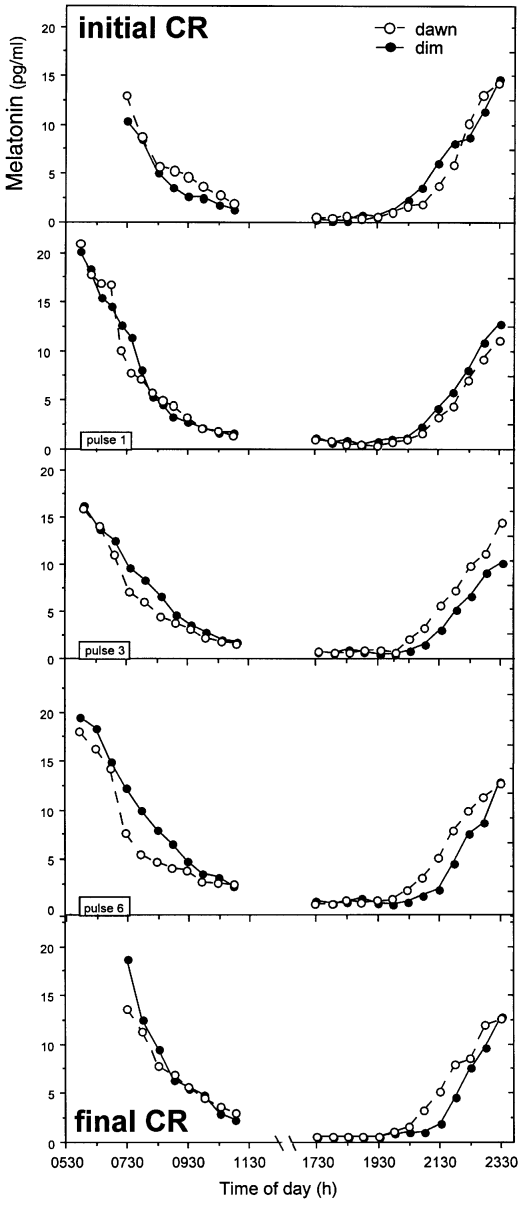

Figure 3. Time course of average salivary melatonin concentrations over the dawn (open circles) or control 0.1 lux light (closed circles) sessions in 9 healthy young men (crossover). Open bars represent the time of the light signal (dawn or control). During the constant routines (CRs), melatonin sampling began after wake-up time at 0730 h; on days 3, 5, and 8 at the beginning of dawn or control exposure at 0600 h. Saliva was collected every 30 min, except for day 3 when it was collected at 20-min intervals to measure suppression kinetics. The standard errors of the mean are omitted for clarity but were of similar order of magnitude for both groups and depended on time of day. For the dawn and control curves combined, selected standard errors of the mean are ($n = 10$, in pg/ml) 2.31 at 0730 h, 0.76 at 1100 h, 0.17 at 1730 h, 4.10 at 2330 h. Statistics for the comparison initial/final CRs are reported in the text. When all morning melatonin profiles were analyzed by a two-way ANOVA for repeated measures (factors: days, time of day), only the control session revealed a significant effect of days of the experiment.

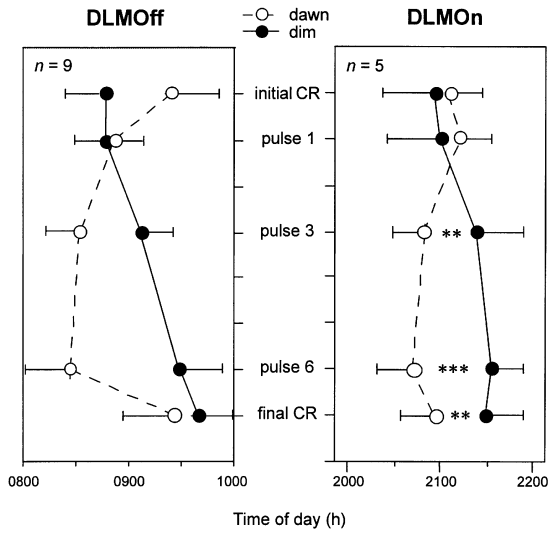

Figure 4. Evolution of salivary melatonin offset and onset times (mean ± SEM) in participants receiving dawn or control light (crossover). Dim light melatonin offset DLMOff and onset DLMOn times were determined using primarily a 3 pg/ml threshold (see Methods for details). DLMOff (left-hand figure) was averaged for 9 participants; DLMOn (right-hand figure) was averaged for the 5 participants in whom it could be always determined. The dawn session was masked by the direct effect of the light signal ("earlier" phase during signals 1, 3, and 6), and thus these DLMOff values were not analyzed. A gradual phase delay of both DLMOff and DLMOn was observed during the control light session (seen in the complete curves in Fig. 3) but not during the dawn session. A one-way ANOVA for repeated measures of DLMOff during the control session revealed a significant effect of days ($F_{4,32} = 5.87$, $p < 0.01$). A two-way ANOVA for repeated measures of DLMOn (factors: control/dawn session, days) revealed a significant two-way interaction term ($F_{4,16} = 6.96$, $p < 0.01$). Significant differences between the control and dawn sessions are indicated by $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (PLSD test). There were also differences when comparing with initial values: DLMOff control signal 6 ($p < 0.05$) and final CR ($p < 0.01$); DLMOn control signal 3 ($p < 0.05$), signal 6 ($p < 0.01$), final CR ($p < 0.01$), and dawn signal 6 ($p < 0.05$) (all PLSD test).

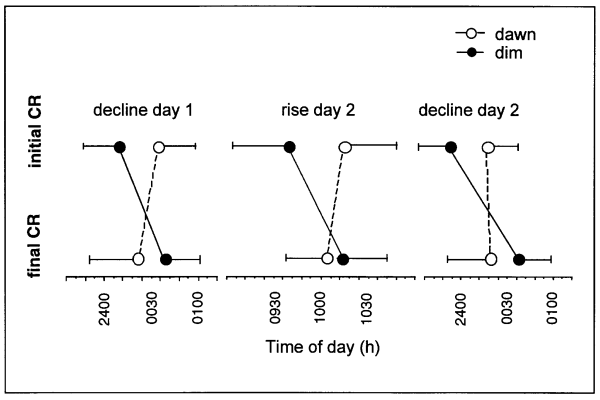

Figure 5. Phase position of the rectal temperature rhythm as measured by three midrange crossing times (nocturnal decline day 1, morning rise day 2, nocturnal decline day 2) during 1.5 days of a modified CR before (initial) and after (final) six consecutive dawn and control light exposures ($n = 9$, mean ± SEM). The overall circadian phase (averaged over three midrange crossing times) significantly delayed after the control session by –41 ± 15 min. This delay was significantly larger than in the dawn session, indicating entrainment under dawn light conditions.

preceding the CRs indeed revealed a somewhat earlier sleep phase (a sampling error) before the control light session as revealed by bedtime (2326 h ± 5 min vs. 2343 h ± 9 min; $t = 2.33$, $p < 0.05$) or sleep midpoint (0347 h ± 15 min vs. 0403 h ± 14 min; $t = 2.46$, $p < 0.05$), even though sleep duration was very similar (8 h 41 min vs. 8 h 40 min).

Suppressive Effect of Dawn on Melatonin

Investigation of melatonin kinetics during the dawn signal was one of the points of interest in the study, which was the reason for collecting saliva every 20 min during the first dawn presentation (Fig. 3). Small decrements (as compared with the control light curve) could be observed at 0720 h ($t = 1.99$, $p < 0.09$) and 0740 h ($t = 1.38$, $p = 0.20$). When the DLMOff curves during the first, third, and sixth signal presentations were averaged (Fig. 6), there was a significant difference at 0730 h (31%; $t = 2.83$, $p = 0.02$), gradually diminishing at 0800 h (29%; $t = 2.45$, $p = 0.04$) and 0830 h (25%; $t = 1.87$, $p < 0.1$), whereas the time course from 0600 to 0700 h and from 0900 to 1100 h was similar. These data suggest that suppression began after 0700 h (given some delay between melatonin suppression and its detection in saliva), when the light intensity of the dawn signal had reached ~100 lux.

Subjective Ratings

VAS for mood, alertness, tension, or interest, and the KSSCL did not vary significantly across the day, nor did they differ between groups. More detailed analyses were carried out with the KSS, which showed significant diurnal variations (the F ratios ranged between 1.84 and 2.80 for the factor Time of Day; $F_{31,246}$ $p = 0.1 – 0.0005$). The significant interaction term permitted focus on the most interesting times for sleepiness ratings: early morning and late evening. When the difference between the averaged first three and last three ratings was calculated, participants were more "advanced" after both dawn and control ses-

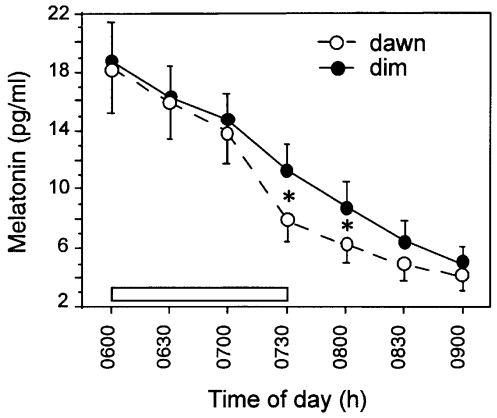

Figure 6. The kinetics of melatonin suppression in the morning is illustrated by averaging the individual values at each time point on days when the first, third, and sixth dawn light signals were given, and then averaging over subjects (n = 9, mean ± SEM). The open bar represents the time of wakefulness watching the light stimulus (dawn or control 0.1 lux). Statistics in text.

sions (ANOVA factors initial/final CR, $F_{1,8} = 5.59$, $p < 0.05$), probably as a result of the protocol itself, in which participants were woken at 0600 h each day.

A direct effect of the light signal on sleepiness was analyzed analogous to the melatonin suppression curves in Figure 6. The sleepiness ratings from 0630 to 1100 h were averaged over days 1, 3, and 6 of dawn or control light exposure. There was a significant difference ($F_{1,8} = 11.51$, $p < 0.01$) beginning at 0630 h ($t = 1.89$, $p < 0.1$), 0700 h ($t = 3.09$, $p = 0.01$), and 0730 h ($t = 2.58$, $p = 0.03$), those exposed to dawn light being more alert. There were no subjective complaints of disturbed sleep during the initial part of the dawn or control signal from 0330 to 0600 h (0.003 to 0.1 lux). Both groups had a similar timing and duration of naps on the permitted days (average nap duration 2 h 32 ± 28 SD min vs. 2 h 35 ± 24 min, respectively).

Phase Relationships

In terms of synchronization of different phase markers, the initial temperature phase was positively correlated with all other phase markers studied at baseline: DLMOff (n = 16 [those with 3.0 pg/ml crossing], $r = 0.72$, $p < 0.01$), DLMOn ($n = 16$, $r = 0.58$, $p < 0.05$), sleep midpoint ($n = 18$, $r = 0.72$, $p < 0.001$), and KSS "sleepiness phase" ($n = 18$, $r = 0.60$, $p < 0.01$). DLMOn was correlated with sleep midpoint ($n = 16$, $r = 0.54$, $p < 0.05$) and KSS sleepiness phase ($n = 16$, $r = 0.56$, $p < 0.05$), but not with DLMOff ($n = 16$, $r = 0.34$, n.s.). DLMOff was additionally correlated with KSS sleepiness phase ($n = 16$, $r = 0.50$, $p < 0.05$). The phase shift in the temperature rhythm was correlated with the phase shift in both DLMOn ($n = 10$, $r = 0.67$, $p < 0.05$) and DLMOff ($n = 18$, $r = 0.61$, $p < 0.01$).

DISCUSSION

In a 9-day experiment carried out under controlled environmental conditions, including a modified CR protocol before and after the light stimuli to measure phase markers, we were able to detect a significant difference between exposure to control light and six consecutive dawn simulations. The dawn signal prevented the phase delay in melatonin and temperature rhythms occurring under control light conditions: the circadian system remained entrained to 24 h. Given that the underlying intrinsic period of the human circadian pacemaker is slightly longer than the solar cycle (Campbell et al., 1993; Middleton et al., 1996; Hiddinga et al., 1997; Carskadon et al., 1999; Czeisler et al., 1999), we provide initial evidence for its entrainment by the naturalistic zeitgeber to which it evolved, the dawn's early light.

The maintenance of similar phase relationships between rectal temperature and melatonin rhythms in all sessions and interventions also supports our interpretations and is consistent with results from controlled studies after many days of dim or bright light (Shanahan et al., 1999).

Phase Shift: Control

Control participants remained under very dim light conditions for 9 days. The LD cycle with alternating intensities of < 30:0 lux appeared insufficient to entrain the circadian system—or may have entrained it at a later phase, as would be expected when the strength of the zeitgeber cycle is reduced. Indeed, there was a gradual phase delay even though sleep timing (at least, bedtime) was set to be constant. The only behavioral factor in the protocol that might have added a "darkness pulse" was the recovery nap permitted in the morning on days 4, 6, and 7 (for both control and dawn). We used this nap timing because in our previous experiment it did not block the DLMOn advance after dawn stimuli (Danilenko et al., 1997, 2000). However, there is evidence that a longer sleep duration may phase delay the melatonin rhythm (Samkova et al., 1997). A counterargument is that the

phase delay had already begun during the first 3 days of the experiment when no recovery nap had yet been scheduled. The delay as measured by DLMOn was –5 ± 5 min (day 1 vs. day 2, $n = 14$) and –14 ± 7 min (day 2 vs. day 3, $n = 14$), which was significant (one-way ANOVA: $F_{2,26} = 3.81$, $p < 0.04$). Therefore, the morning nap was probably not a relevant factor.

Phase Shift: Dawn

In our previous two experiments (without a control light group), we demonstrated a 20-min phase advance of DLMOn following a single and a 34-min phase advance following a triple dawn signal (Danilenko et al., 1997, 2000). In the present experiment, there was no phase advance after a single, triple, or six dawn signals. Certain differences in the earlier protocols may explain this. First, even though time in bed was similarly scheduled, dawn was administered 20 to 30 min earlier, which may have been crucial. Additionally, in the single signal experiment the participants spent the morning before the final CR in a brighter laboratory environment (~100 lux rather than < 30 lux), which may have provided an additional phase advancing signal. In the triple signal experiment, the final dawn signal was also brighter. If the three experiments are compared in terms of illuminance, this is on average 125, 317, and 155 lux over 1.5 to 1.7 h, or in total 11,250, 32,334, and 13,950 lux · min, respectively. The dawn signal we used in the present study is 4 times lower than that previously reported to induce a phase advance (180 lux pulse for 5 h, or 54,000 lux · min; Boivin et al. 1996).

Phase Shift: Threshold Light Intensity

Our study touches the important question, What is the minimal light intensity level needed to maintain entrained circadian phase throughout daily LD cycles (which may not be the same as that required to induce a phase shift)? In the present study, the LD < 30:0 lux cycle led to a delay of approx. –6 min/day. The replacement of the last 1.5 h of darkness by a dawn stimulus (averaging 155 lux) was sufficient to maintain an entrained phase position. Two studies have demonstrated that morning light of 100 lux or less is superior to darkness (Samkova et al., 1997) or < 10 lux (Laakso et al., 1993) to maintain entrainment of the melatonin rhythm. We are not aware of any further studies using a similar protocol to ours in terms of fixed sleep times and dim daytime lighting for many days. Simulation of our protocol with a new mathematical model (Kronauer et al., 1999), using approximated parameters for the dawn signal (that is, geometric mean of light intensity during nine 10-min square wave signals) yielded phase differences between the initial and final CRs of +14 min for the dawn condition and –47 min for the control condition, which was similar to our results (C. Cajochen, personal communication, March 2000). Our experiments thus provide new data to tease out a threshold light intensity between 10 and 100 lux as necessary and sufficient to entrain the human circadian pacemaker.

Acute Effect of a Dawn Signal

Earlier studies had shown that white light of intensity 100 lux (Gaddy et al., 1993) or 80 lux (G. C. Brainard, personal communication, November 1998) but not 11 lux (K. Thapan and D. J. Skene, personal communication, June 1999) was sufficient to suppress melatonin secretion when administered under pupillary dilation. Our finding that melatonin was significantly lower after light intensity had reached approximately 100 lux is similar to these previously described thresholds, though we used another paradigm, that of the dawn light signal in a nocturnally dilated pupil waking up from darkness. The similarity of ranges suggests that suppressive and entrainment properties of light may be physiologically coupled. The behavioral ratings indicate that participants were more alert during the early morning under dawn than under the control light session. Reduction of fatigue upon waking has recently been demonstrated with simple dawn ramp devices (Arakawa et al., 1999; Meesters et al., 1999). Thus, even such low light intensities may have direct alerting effects (Campbell et al., 1995; Cajochen et al., 2000).

Perspectives: Dawn versus Square Wave Light Pulse

The experiment does not answer the question, Is the naturalistic signal more efficient than a square wave light pulse providing identical illuminance? In animal studies, simulated twilights do enhance entrainment (e.g., Boulos et al., 1996 a-c; Cooper et al., 1998; Wirz-Justice et al., 1998). To provide an "equal illuminance" rectangular control light pulse is complicated, in that it would have far higher intensity at the

corresponding early dawn hour, which might well potentiate phase shifts. However, we hypothesize that indeed the temporal pattern of the naturalistic dawn function is related to optimum zeitgeber strength.

In summary, our study in healthy participants demonstrates how the circadian system may free-run or phase-delay under control light conditions even though bedtime is held constant, and it shows for the first time that a low illuminance dawn signal in combination with daytime illuminance no higher than 30 lux is sufficient to entrain the human circadian system.

ACKNOWLEDGMENTS

This study was supported by Swiss National Science Foundation Grant #7SUPJ048549 (Joint Research Projects: Cooperation in Science and Research with Central and Eastern European Countries and Newly Independent States). We thank Stephen Fairhurst for his work in development of the dawn simulation system, Dr. Jiuan S. Terman and the anonymous reviewers for comments on the manuscript, and Tatjana Karpova, Sergei Kondratov, and Claudia Renz for their help in data acquisition. Rights to U.S. Patents 5,343,121 and 5,589,741 are assigned to the Research Foundation for Mental Hygiene, Inc., New York State Psychiatric Institution Division.

REFERENCES

Arakawa K, Shirakawa S, Kobayashi T, Oguri M, Kamei Y, and Tumura T (1999) Effects of the gradually increasing dawn light stimulation on sleep feeling. Psychiatry Clin Neurosci 53:247-248.

Boivin DB, Duffy JF, Kronauer RE, and Czeisler CA (1996) Dose-response relationships for resetting of human circadian clock by light. Nature 379:540-542.

Boulos Z, Macchi M, and Terman M (1996a) Twilight transitions promote circadian entrainment to lengthening light-dark cycles. Am J Physiol 40:R813-R818.

Boulos Z, Macchi M, and Terman M (1996b) Effects of twilights on circadian entrainment patterns and re-entrainment rates in squirrel monkeys. J Comp Physiol [A] 179:687-694.

Boulos Z, Terman JS, and Terman M (1996c) Circadian phase response curves for simulated dawn and dusk twilights in hamsters. Physiol Behav 60:1269-1275.

Cajochen C, Zeitzer JM, Czeisler CA, and Dijk DJ (in press) Dose-response relationship for light intensity and ocular and electroencephalographic correlates of human alertness. Behav Brain Res.

Campbell SS, Dawson D, and Zulley J (1993) When the human circadian system is caught napping: Evidence for endogenous rhythms close to 24 hours. Sleep 16:638-640.

Campbell SS, Dijk DJ, Boulos Z, Eastman CI, Lewy AJ, and Terman M (1995) Light treatment for sleep disorders: Consensus report: III. Alerting and activating effects. J Biol Rhythms 10:129-132.

Carskadon MA, Labyak SE, Acebo C, and Seifer R (1999) Intrinsic circadian period of adolescent humans measured in conditions of forced desynchrony. Neurosci Lett 260:129-132.

Cooper HM, Dkhissi O, Sicard B, and Groscarret H (1998) Light evoked c-fos expression in the SCN is different under on/off and twilight conditions. In *Biological Clocks. Mechanisms and Applications*, Y Touitou, ed, pp 181-188, Elsevier Science, Amsterdam.

Czeisler CA, Duffy JF, Shanahan TL, Brown EN, Mitchell JF, Rimmer DW, Ronda JM, Silva EJ, Allan JS, Emens JS, Dijk DJ, and Kronauer RE (1999) Stability, precision, and near-24-hour period of the human circadian pacemaker. Science 284:2177-2181.

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.

Danilenko KV, Terman M, Kräuchi K, Cajochen C, Weber JM, and Wirz-Justice A (1997) Dawn simulation phase advances human dim-light melatonin onset. Sleep Res 26:708.

Danilenko KV, Wirz-Justice A, Kräuchi K, Cajochen C, Weber JM, Fairhurst S, and Terman M (2000) Phase advance after one or three simulated dawns in humans. Chronobiol Int 17:659-668.

Fairhurst S, Levitt J, and Terman M (1999) *MacLite™ Operations Manual* (Ver. 0.8.0), SphereOne, Silver Plume, CO.

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.

Gillberg M, Kecklund G, and Akerstedt T (1994) Relations between performance and subjective ratings of sleepiness during a night awake. Sleep 17:236-241.

Hiddinga AE, Beersma DG, and Van den Hoofdakker RH (1997) Endogenous and exogenous components in the circadian variation of core body temperature in humans. J Sleep Res 6:156-163.

Honma K, Honma S, and Wada T (1987) Phase-dependent shift of free-running human circadian rhythms in response to a single bright light pulse. Experientia 43:1205-1207.

Kräuchi K, Cajochen C, Möri D, Graw P, and Wirz-Justice A (1997) Early evening melatonin and S-20098 advance circadian phase and nocturnal regulation of core body temperature. Am J Physiol 272:R1178-R1188.

Kronauer RE, Forger DB, and Jewett ME (1999) Quantifying human circadian pacemaker response to brief, extended, and repeated light stimuli over the photopic range. J Biol Rhythms 14:500-515.

Laakso M, Hatonen 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.

Lam R and Levitt AJ, eds (1999) *Canadian Consensus Guidelines for the Treatment of Seasonal Affective Disorder*, Clinical and Academic Publishing, Vancouver, BC.

Lewy AJ, Cutler NL, and Sack RL (1999) The endogenous melatonin profile as a marker for circadian phase position. J Biol Rhythms 14:227-236.

Lewy AJ, Wehr TA, Goodwin FK, Newsome DA, and Markey SP (1980) Light suppresses melatonin secretion in humans. Science 210:1267-1269.

Lockley SW, Skene DJ, Arendt J, Tabandeh H, Bird AC, and Defrance R (1997) Relationship between melatonin rhythms and visual loss in the blind. J Clin Endocrinol Metab 82:3763-3770.

Meesters Y, Beersma DGM, and Partonen TT (1999) Dawn simulation for easy wake-up. Soc Light Treatment Biol Rhythms Abstr 11:32.

Middleton B, Arendt J, and Stone BM (1996) Human circadian rhythms in constant dim light (8 lux) with knowledge of clock time. J Sleep Res 5:69-76.

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.

Minors DS, Waterhouse JM, and Wirz-Justice A (1991) A human phase-response curve to light. Neurosci Lett 133:36-40.

Pittendrigh C (1960) Circadian rhythms and the circadian organisation of living systems. Cold Spring Harb Symp Quant Biol 25:159-184.

Sack RL, Lewy AJ, Blood ML, Keith LD, and Nakagawa H (1992) Circadian rhythm abnormalities in totally blind people: Incidence and clinical significance. J Clin Endocrinol Metab 75:127-134.

Samkova L, Vondrasova D, Hajek I, and Illnerova H (1997) A fixed morning awakening coupled with a low intensity light maintains a phase advance of the human circadian system. Neurosci Lett 224:21-24.

Shanahan TL, Kronauer RE, Duffy JF, Williams GH, and Czeisler CA (1999) Melatonin rhythm observed throughout a three-cycle bright-light stimulus designed to reset the human circadian pacemaker. J Biol Rhythms 14:237-253.

Terman M, Fairhurst S, Hughes P, and Levitt J (1996) *System for Creating Naturalistic Illumination Cycles*. Washington, DC, U.S. Patent and Trademark Office (No. 5,589,741).

Terman M, Fairhurst S, Perlman B, and McCluney R (1989a) Daylight deprivation and replenishment: A psycho-biological problem with a naturalistic solution. In *Architecture and Natural Light* (American Society of Heating, Refrigeration and Air Conditioning Engineers), E Bales and R McCluney, eds, pp 438-445, Atlanta, GA.

Terman M, Perlman B, and Fairhurst S (1994) *Naturalistic Illumination System*. Washington, DC, U.S. Patent and Trademark Office (No. 5,343,121).

Terman M, Remé CE, and Wirz-Justice A (1991) The visual input stage of the mammalian circadian pacemaking system: II. The effect of light and drugs on retinal function. J Biol Rhythms 6:31-48.

Terman M, Schlager D, Fairhurst S, and Perlman B (1989b) Dawn and dusk simulation as a therapeutic intervention. Biol Psychiatry 25:966-970.

Weber JM, Schwander JC, Unger I, and Meier D (1997) A direct ultrasensitive RIA for the determination of melatonin in human saliva: Comparison with serum levels. Sleep Res 26:757.

Wirz-Justice A, Terman M, Terman JS, Boulos Z, Remé CE, and Danilenko KV (1998) Circadian functions and clinical applications of dawn simulation. In *Biological Clocks. Mechanisms and Applications*, Y Touitou, ed, pp 189-194, Elsevier Science, Amsterdam.