Human circadian melatonin rhythm phase delay during a fixed sleep–wake schedule interspersed with nights of sleep deprivation

Abstract: The human circadian pacemaker, with an intrinsic period between 23.9 and 24.5 hr, can be reset by low levels of light. Biomathematical models of the human clock predict that light–dark cycles consisting of only ∼3.5 lux during 16 hr of wakefulness and 0 lux during 8 hr of sleep should entrain ∼45% of the population. However, under real-life conditions, sleep–wake schedules and the associated light–dark exposures are often irregular. It remains unclear whether the phase of the pacemaker would remain stable under such conditions. We investigated the stability of the circadian phase in dim light by assessing the plasma melatonin rhythm during nine consecutive circadian cycles. Ten subjects were scheduled to sleep for 8 hr (0.03 lux) and to be awake for 16 hr (5–13 lux) during all days except on days 4 and 8, during which the subjects were sleep deprived for 40 hr (5–13 lux), either in a sitting/standing or supine body posture. In all subjects, the phase of the melatonin rhythm occurred at a later clock time on day 9 than on day 2 (average delay: 1.4 hr). Largest delays in the melatonin onset were observed in subjects with low amplitude melatonin rhythms. The area under the curve during active melatonin secretion was significantly reduced when subjects were sleep deprived in the 40-hr supine body posture condition compared with either the 40-hr sitting/standing sleep deprivation (SD) or the ambulatory condition under non-SD conditions. Posture differences did not significantly affect the relative phase position of the melatonin profiles. The data indicate that under conditions of reduced zeitgeber strength, the phase of the human circadian pacemaker, using plasma melatonin as a marker, can be phase delayed by one night of SD and the associated dim light exposure.

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Introduction

The accuracy and stability with which the circadian system adopts a distinct phase relationship with the 24-hr day–night cycle is thought to be of crucial importance for the temporal organization of physiology and behavior [1]. Accuracy and stability of entrainment can be accomplished by resetting mechanisms that correct the deviations (T−tau) of the endogenous circadian period (tau) from the solar day (T). These resetting mechanisms include circadian phase-dependent shifts of the pacemaker and potentially circadian phase-dependent changes in the period (velocity) of the pacemaker in response to resetting stimuli (zeitgebers) such as the light–dark cycle [2–6]. It is now well established that the human circadian pacemaker, as assayed by the rhythms of melatonin, core body temperature and cortisol, can be reset by light exposure [6–9]; for a review see [10]. Research during the past 2 decades has revealed that (1) the human circadian pacemaker is sensitive to light for a major part of the circadian cycle [5, 11]; (2) the response to light varies systematically throughout the endogenous cycle and can be summarized in phase response curves (PRCs) [4, 6, 9]; and (3) the pacemaker is sensitive to low light levels and the

illuminance–response curve is non-linear such that, after 3 days under dim light conditions, ∼100 lux may exert 50% of the effect obtained with ∼9000 lux [7, 12]. In the above-mentioned PRC studies, the sleep–wake, activity and social interaction schedules have been shifted in synchrony with the light stimulus. It is therefore possible that the phase shifts observed in these studies include a contribution from non-photic cues.

It has also been recognized that the intrinsic period of the human circadian pacemaker is not 25 hr, as was once thought, but is closer to 24 hr, ranging from ≈23.9 to 24.5 (for a review see [13]). This implies that a daily phase advance of ∼20 min only will suffice for stable entrainment in most people. Such an advance could conceivably be induced by very little light. Indeed, Wright et al. [14] reported that five of six subjects were entrained to a regular 24-hr cycle consisting of ∼1.5 lux of light during 16 hr of scheduled wakefulness and 8 hr of scheduled sleep in darkness.

The data on the light sensitivity of the human circadian pacemaker as well as the recent estimates of the intrinsic circadian period have been incorporated into mathematical models of the effects of light on the human circadian

pacemaker. These models have successfully described the results of various protocols [15, 16] including the dynamics of melatonin phase in the course of a 3-day resetting trial [17]. However, these models do not incorporate the putative effects of zeitgebers other than light (non-photic) on the human circadian pacemaker. Several lines of evidence now suggest that non-photic zeitgebers may contribute to entrainment of human circadian rhythms. Exogenous melatonin (for a review see [18]), physical activity [19–21], food intake [22], and the duration and timing of sleep episodes [23–25], or sleep per se [26] have been reported to affect the phase of the human circadian pacemaker. In fact, the entrainment observed during a 24-hr schedule of sleep and wakefulness in the presence of a weak light–dark cycle in carefully controlled laboratory studies (e.g. as in [14]) could be mediated by either the effects of light, the scheduled regular sleep–wake cycle, or a combination of both.

In non-laboratory conditions, such as for shift workers, people may be exposed to low light levels during wakefulness and sleep–wake cycles are often irregular. Such schedules could conceivably compromise stability of entrainment. We therefore investigated the stability of the phase of the plasma melatonin rhythm, which is considered to be a reliable marker of the human circadian pacemaker, during exposure to a regular dim light/dark and wake/sleep cycle, interrupted by two nights of sleep deprivation (SD) in dim light. The data were compared with predictions of a mathematical model in which only photic stimuli exert a drive onto the human circadian pacemaker [15, 16].

Methods

Subjects

Potential volunteers were recruited via poster advertisement in the Greater Boston area. After passing a telephone screening interview, potential subjects gave informed consent and completed the following screening questionnaires: the Beck Depression Inventory-II [27], the Horne–Östberg Morningness–Eveningness Questionnaire [28], and a questionnaire covering sleep habits and physical health. Subjects showing no evidence of psychopathology or symptoms of a sleep disorder on these screening instruments were scheduled for a physical examination, routine blood and urine chemistries, and a 12-lead electrocardiogram. They also received a screening interview with a licensed psychologist to rule out personal or familial history of major psychopathology and to determine their comprehension and ability to comply with the investigational procedures. Finally, potential subjects were interviewed by an investigator, and written informed consent was obtained for the protocol, which was approved by the Brigham and Women's Hospital's Human Research Committee.

Seven men and five women (mean age: 22.1 years; range: 19–28 years) were studied. Women were studied in the follicular phase of their menstrual cycle. Subjects were instructed to abstain from caffeine, nicotine, alcohol, and drugs for 3 wk before the study; their compliance was verified on the day of admission to the laboratory with urinary toxicologic analysis. During the 3 wk before their admission to the laboratory, subjects were asked to keep a regular sleep–wake schedule (bedtimes and wake times within ± 30 min of self-selected target times scheduled 8 hr apart). Adherence to this regular schedule during the week immediately prior to admission was verified with a wrist actigraph (Mini Motionlogger, AMI, Ardsley, NY, USA). All subjects gave their written informed consent. The protocol, consent form, and advertisements were approved by the Human Research Committee of the Brigham and Women's Hospital.

Protocol

The inpatient portion of the protocol (Fig. 1) consisted of a 12-day stay in the General Clinical Research Center (GCRC) of the Brigham and Women's Hospital. All suites in the facility were constructed to enable the study of subjects in an environment shielded from external time cues (windowless, sound proofing, double door entry, etc.).

Subjects were admitted to individual suites in the GCRC between 15:00 and 18:00 hours on day 1 of the protocol and discharged between 10:00 and 14:00 hours on day 12. The timing of the subjects' sleep–wake cycle in the laboratory was scheduled according to the midpoint of their typical 8-hr sleep episodes and were assessed by averaging their bedtimes and wake times as reported in their diaries. Each day, the subject arose at her/his typical wake time and went to sleep at her/his typical bedtime with the exception of day
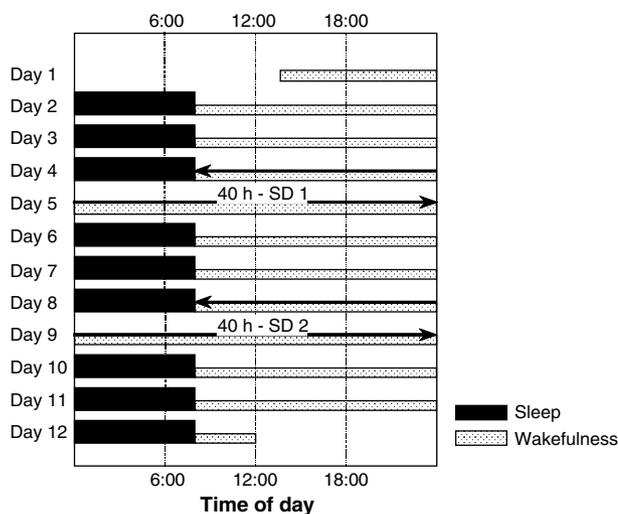

Fig. 1. Experimental protocol. After 3 days of baseline recordings (days 1–3) under a light–dark cycle of 16:8 hr (approximately 5–13 lux during wakefulness, tippled bars and approximately < 0.03 lux during sleep, black bars), subjects underwent a sleep deprivation (SD, 40 hr of prolonged wakefulness in 5–13 lux). The first SD (SD 1) was followed by two recovery days (days 6 and 7) under a 16:8 light–dark (LD) cycle. On habitual wake time on day 8, the second 40-hr SD (SD 2) in 5–13 lux started, followed by three recovery days under a 16:8 LD cycle (days 10–12). Subjects were scheduled according to their habitual bedtimes and wake times. The only difference between the two SDs was body posture, which was either supine or sitting/standing throughout the entire SD 1 or SD 2 (counterbalanced). The timing of bedtimes presented in the plot, represents the average subjects' bedtime.

4 and day 8. Upon awakening on these days (day 4 and day 8) subjects began a 40-hr SD protocol. During one of these SDs, subjects were in a constant supine posture throughout the 40-hr episode. In the other SD, subjects alternated between sitting (40 min) and standing (20 min). During the standing portion of the protocol, subjects stood in front of a laptop mounted on a high table, and they could walk around in an area of 1–3 m. The order of these two SD protocols was balanced. During the entire 12-day period, light levels were maintained at < 50 lux during wakefulness and at < 0.03 lux during scheduled sleep episodes. Ceiling-mounted fluorescent lamps (T8 and T80, Phillips, Eindhoven, the Netherlands) with a 4100 K color temperature produced a spectrum of white light. Clear polycarbonate lenses filtered 99.9% of the light in the UV range. During scheduled wakefulness the highest possible illuminance was 50 lux. Technicians measured the light levels in the angle of gaze during scheduled wakefulness on each day of the protocol. Post hoc analyses of these values revealed that the ambient light levels varied between 5 and 13 lux.

Core body temperature was measured every minute via a rectal thermistor (YSI, Yellow Springs, OH, USA) throughout the experiment, and blood samples were taken through an indwelling, intravenous catheter every 30 min from day 2 to day 9. During the sitting/standing CR, the blood samples were drawn during the middle of the sitting (40 min) and standing episodes (20 min), respectively, which resulted in a 30-min sampling interval. An attached 12-foot line allowed for frequent blood collection during both episodes of wake and sleep, with minimal disturbance of the subject. Collected samples were centrifuged at 2–4°C and a portion of the plasma was taken and frozen for the estimation of melatonin concentration by radio-immunoassay (assay sensitivity of 2.5 pg/mL; intra- and inter-assay coefficients of variation, 8 and 13%, respectively: Diagnos-Tech, Osceola, WI, USA).

Assessment of circadian phase and statistical analysis

The circadian phase was estimated from plasma melatonin data. Comparative analysis has shown that melatonin phase is a more reliable and accurate measure of circadian phase than the core body temperature rhythm [17, 29]. Difficulties with blood sampling procedures during sleep episodes prevented continuous melatonin phase assessment in two subjects. A complete melatonin data train was available in the remaining 10 subjects. For each subject, the period during which melatonin was collected (days 2–9), the upward and downward crossing times of the 24-hr mean (15:00–15:00 hours the next day) was calculated in addition to the timing of the midpoint between (Fig. 2, for details on this method see [12]). Melatonin secretion, defined as the average melatonin level between the upward and downward mean crossing, and the width of melatonin secretion, defined as the duration between the upward and downward mean crossing, were calculated (Fig. 2). In addition, the area under curve (AUC) between the upward and downward mean crossing time was calculated on z-scored melatonin values using the trapezoidal method. For each subject, the values were z-scored over the entire data train
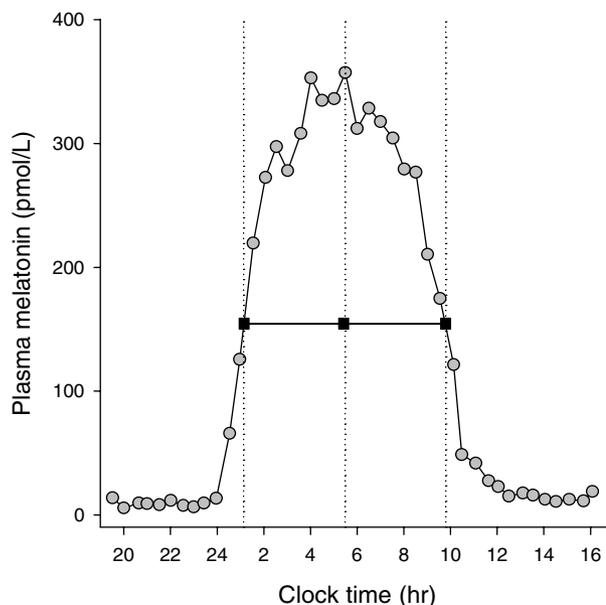

Fig. 2. Example of an individual plasma melatonin profile. Vertical stippled lines delineate the upward mean crossing time, the midpoint of the melatonin peak and the downward mean crossing time. The horizontal line indicates the mean 24-hr level of plasma melatonin in this subject.

and then averaged across the subjects. In order to evaluate time-dependent changes in the circadian phase, repeated measures ANOVAS (rANOVA) were used with the repeated factor 'day'. All P values derived from rANOVAs were based on Huynh–Feldt's (H–F) corrected degrees of freedom, but the original degrees of freedom are reported. When the F-ratio proved significant, post hoc comparisons using the Duncan's multiple range test were performed. For single comparisons, the paired t-test or the Wilcoxon matched pairs test were used (e.g. whether the observed phase drift was significantly different from 0 to 24 hr, respectively). Pearson's correlation coefficients were computed to compare the secretion, the width and AUC of melatonin secretion with the observed drift. Statistics were performed using the statistical packages SAS® (version 6.0, SAS® Institute Inc., Cary, NC, USA) and Statistica® (version 5.0, StatSoft, Inc., Tulsa, OK, USA).

The protocol was simulated using Kronauer's light model [15, 16] using the Circadian Performance Simulation Software (version 1.2, 2002, CPSS ®, Brigham and Women's Hospital, Boston, MA, USA). This model predicts the phase and amplitude of the human circadian pacemaker during exposure to photic stimuli, with a temporal resolution of 0.1 hr (simulation step size).

Results

Fig. 2 illustrates the average (n = 10) time course of plasma melatonin concentration (z-scores) during days 2–9. Data were plotted with respect to the average scheduled wake time on day 2, which occurred at 8:00 hours. Visual inspection of the daily melatonin profiles indicated a progressive drift to a later clock time (Fig. 3).
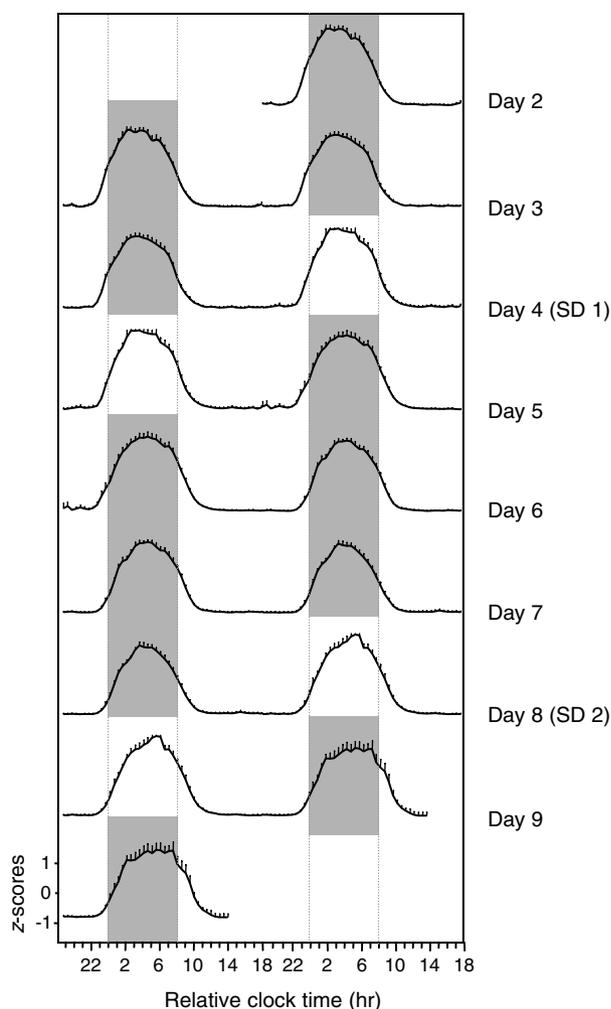
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Fig. 3. Double plot of average daily profiles of plasma melatonin secretion throughout the protocol (mean values n = 10; +1 S.E.M.). Data are double plotted relative to the average wake time on day 2 (08:00 hours). Melatonin samples were not taken on day 1, and the end of blood collection was in the morning on day 9 after SD 2. Gray areas illustrate the timing of the scheduled sleep episodes throughout the study protocol. An overall delay drift from days 2 to 9 in the melatonin profile is discernible.

The daily drift of the melatonin midpoint was on average 12.0 ± 1.6 min (Table 1). This was significantly different from zero ($P < 0.00008$, paired t-test) and corresponds to an overall drift of 84.2 ± 10.8 min in 7 days. The temporal relationship between upward and downward crossings, the midpoint, and the imposed light–dark cycle is illustrated in Fig. 4 for each day during the experiment. The midpoint of plasma melatonin secretion occurred at 03:24 hours on day 2, whereas at 04:48 hours on day 9. Comparisons of the drifts of the upward and downward mean crossing times revealed a significant greater day-to-day variability in the downward mean crossing times compared with the upward mean crossing times (downward variance: $\sigma^2 = 0.61$ hr; upward variance: $\sigma^2 = 0.39$ hr, $z = 2.2$; $P < 0.03$, Wilcoxon matched pairs test).

Correlational analyses revealed that the amount of daily drift in the onset of melatonin secretion was negatively

correlated with the AUC of melatonin secretion (Table 1 and Fig. 5; $r = -0.74$; $P < 0.02$). Correlations between the offset of melatonin secretion and AUC were not significant (Table 1 and Fig. 5).

The day-to-day variability in the width of melatonin secretion yielded significance (rANOVA: $F_{7,63} = 3.1$, $P < 0.02$). Post hoc comparisons revealed that the width of melatonin secretion was significantly longer during night that immediately followed the two 40-hr SDs ($P < 0.05$ in all cases, Fig. 6). The width of secretion during the other nights did not differ significantly ($P > 0.5$).

In order to test the influence of body posture on the melatonin profile an one-way rANOVA with the factor 'posture' (ambulatory, sitting–standing and supine) was performed for all the measures (secretion, width, onset, offset, and AUC). For none of the measures, except for AUC, a significant effect of the factor 'posture' was found. AUC was significantly reduced during the 40-hr supine SD condition (rANOVA: $F_{2,18} = 4.3$, $P < 0.03$; post hoc comparisons: $P < 0.02$ versus sitting/standing and $P < 0.01$ versus ambulatory, Duncan's multiple range test). Further, the time course of plasma melatonin during the 40-hr supine SD condition was compared with the time course during the 40-h sitting/standing SD condition, which was further separated in sitting and standing melatonin levels (Fig. 7). A two-way rANOVA with the factors 'posture' (supine versus sitting versus standing) and time (4 hr before and after the melatonin midpoint) yielded a significant main factor 'posture' ($F_{2,18} = 3.8$, $P < 0.05$) and time ($F_{4,36} = 73.3$, $P < 0.0001$) without a significant interaction between them. The mean secretion during this time interval was significantly higher in the standing position compared with the supine and sitting position (post hoc comparisons: $P < 0.05$ for standing versus sitting and $P < 0.005$ for standing versus supine, Duncan's multiple range test).

Close inspection of the phase of the melatonin rhythm on consecutive days suggests that the drift of phase during SD was larger than on non-SD days. This could be related to the continuous light exposure during SD. Alternatively this may reflect an effect on SD conditions per se or a combination of light exposure and SD conditions. To further investigate this, we simulated the progression of phase on the basis of Kronauer's light model [15, 16]. For the simulations, the light–dark cycles were approximated (i.e. the two 40-hr SDs were included) with an average light level of 1, 5 and 13 lux during scheduled wakefulness and 0 lux during scheduled sleep episodes (5–13 lux range of actual measured ambient light levels at the angle of gaze). In comparison with our experimental data (melatonin midpoint drift), the simulated drifts, achieved after 7 days, differed significantly (13:0 lux condition: 35.4 min versus 84.2 min; $P < 0.009$; 5:0 lux condition: 49.2 min versus 84.2 min; $P < 0.05$, paired t-test, Fig. 8). The overall observed phase drift and the simulated 'drift' with 1 lux during wakefulness and 0 lux during sleep (66.0 min versus 84.2 min) did not differ significantly. Day-to-day comparisons between the experimental data and the model predictions indicated significant differences starting from day 5, which corresponds to the day after the first SD. Melatonin phase occurred significantly later in the data as was predicted by the light model (Fig. 8).

Table 1. Parameters of mean daily plasma melatonin secretion profile per subject (n = 8 days, ±1 S.E.M.) as well as averaged over subjects (n = 10, ±1 S.E.M.). Plasma melatonin secretion (pmol/L) was defined as the mean melatonin secretion between the upward and downward mean crossing times, the area under the curve (AUC) was calculated on *z*-scores between the upward and downward mean crossing times using the trapezoidal method, the width of the profile comprised the time (hr) between the upward and downward mean crossing time; and average daily drift was expressed in minutes relative to the circadian phase position of the upward mean crossing, the midpoint and downward mean crossing on day 2 (see text)

| Subject no. | Secretion (pmol/L) | AUC (on *z*-scores) | Width (hr) | Average drift per day (min) | | |
				Onset	Mid	Offset
1833y	242 ± 12.0	710.3 ± 25.4	8.9 ± 0.1	17.1 ± 7.6	16.4 ± 9.0	14.3 ± 11.5
1861y	243 ± 17.7	777.5 ± 55.4	8.8 ± 0.1	12.0 ± 5.8	8.4 ± 4.4	4.9 ± 9.9
1864y	346 ± 15.8	793.9 ± 44.7	9.5 ± 0.3	6.1 ± 15.2	6.7 ± 11.8	7.3 ± 16.7
1878y	288 ± 8.4	718.5 ± 8.5	9.5 ± 0.1	5.6 ± 5.5	5.1 ± 4.3	4.7 ± 4.6
18a5y	374 ± 12.9	816.3 ± 64.2	8.5 ± 0.1	7.0 ± 7.4	9.5 ± 8.4	12.0 ± 13.0
18d7y	300 ± 10.4	762.2 ± 21.7	9.8 ± 0.2	6.4 ± 4.4	8.2 ± 6.4	9.7 ± 12.3
1939y	303 ± 11.9	702.2 ± 30.3	9.1 ± 0.1	16.7 ± 6.2	17.4 ± 6.2	18.1 ± 8.7
1987y	219 ± 20.2	778.9 ± 51.5	8.8 ± 0.3	8.1 ± 4.3	19.1 ± 3.9	30.0 ± 9.5
1992y	200 ± 14.5	751.2 ± 77.3	9.4 ± 0.2	9.7 ± 9.3	14.0 ± 8.4	18.3 ± 13.6
19g5y	97 ± 6.8	670.3 ± 14.1	8.9 ± 0.2	18.4 ± 6.1	15.3 ± 10.0	12.1 ± 16.4
Mean ± S.E.M.	261.0 ± 25.1	748.1 ± 14.6	9.1 ± 0.1	10.8 ± 1.6	12.0 ± 1.6	13.1 ± 2.4
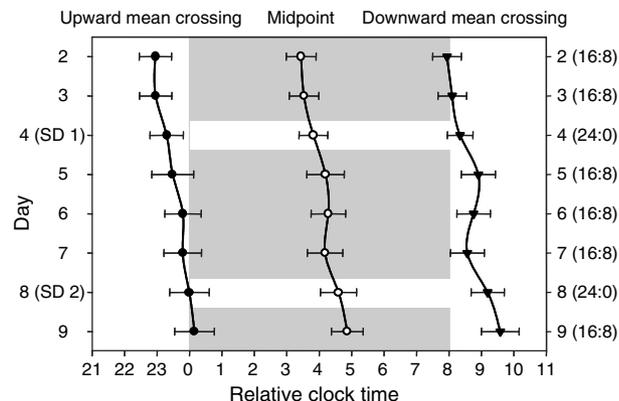
Fig. 4. Day-to-day variability in the upward mean crossing time (filled circles), the midpoint (open circles) and the downward mean crossing time (filled triangles) of the melatonin profile (n = 10; mean values ±1 S.E.M.). Gray areas delineate the timing of scheduled sleep episodes [light–dark (LD) cycle; for sleep < 0.03 lux, white areas for wakefulness: 5–13 lux]. Daily L:D ratios are indicated on the right-hand side ordinate.

Discussion

The circadian phase of plasma melatonin in subjects exposed to light–dark cycles consisting of 16 hr of 5–13 lux and 8 hr of < 0.03 lux interrupted by two 40-hr episodes of sustained wakefulness under 5–13 lux, delayed more than 80 min during a 7-day episode. Nighttime melatonin secretion (AUC) and the phase delay observed in the course of the experiment were such that lower melatonin levels were correlated with larger phase delays in the melatonin onset. The duration of plasma melatonin secretion (the width of melatonin profile) was longer during night following the 40-hr SD. The delay of the plasma melatonin rhythm appeared modulated by the presence or absence of sleep and darkness such that following the 40-hr SD, melatonin phase delayed more than during the 24-hr episodes of sleep and darkness. In addition, changes in

posture did alter the melatonin profile. The area under the melatonin curve was reduced during the supine condition of the 40-hr SD compared with 40-hr SD sitting/standing and ambulatory conditions. Phase markers such as the upward and downward mean crossing times were not significantly altered by posture. Combining these data indicate that stable entrainment of the human circadian pacemaker may be compromised by one single night of SD and associated dim light exposure in subjects who are otherwise exposed to a weak photic synchronizer. To conclusively answer this question, SD protocols in complete darkness are required.

The observed drift to a later clock time in all circadian phase markers of the melatonin rhythm can be interpreted as a delay of the light sensitive pacemaker. The magnitude of the delay is similar to the delay that may be expected if the pacemaker, with an intrinsic period of 24:12 min, was free-running under these circumstances [3]. Such a delay, of approximately 0.2–0.8 hr per day, has been observed in many protocols in which subjects were exposed to low light levels [3, 30–33]. In addition, a similar delay has been observed in protocols in which the sleep–wake cycle was inverted and subjects were exposed to three 5-hr dark pulses during their biologic nights and dim light during the remainder of the wake episode [34, 35]. This could indicate that under circumstances of reduced zeitgeber strength the pacemaker effects a delay until a new stable phase angle of entrainment is reached. Alternatively, it may indicate that under these circumstances the human circadian pacemaker cannot be synchronized with the zeitgeber cycle. The current protocol does not allow us to distinguish between these possibilities but allows the conclusion that the pacemaker does not reach stable entrainment when subjects are exposed to days of a light–dark cycle with < 13 lux during wakefulness interrupted by two 40-hr SD in dim light. Furthermore, the modulation of the magnitude of the phase shift on consecutive 24-hr periods by the presence or absence of sleep and associated light exposure demonstrates conclusively that these behavioral and/or environmental factors indeed exert a significant drive onto the human circadian pacemaker.
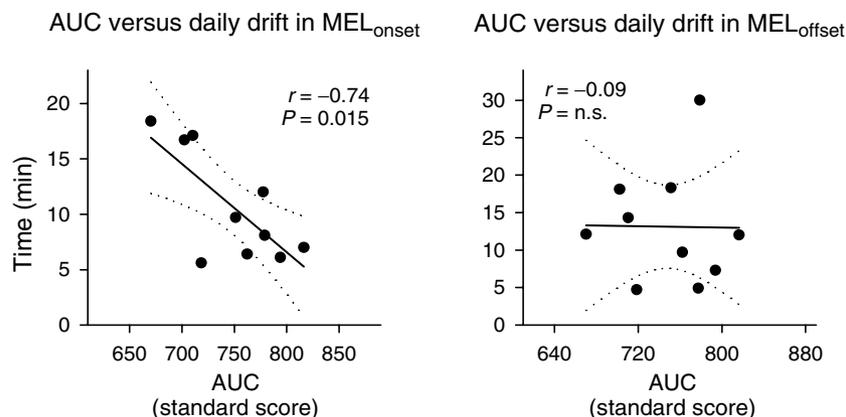
AUC versus daily drift in MEL$_{onset}$ AUC versus daily drift in MEL$_{offset}$

Fig. 5. Pearson's correlations between the area under the curve (AUC; for methods see text) and the observed drift in either the melatonin upward mean crossing time (Melon) or the downward mean crossing time (Meloff) of the melatonin curve. Each point represents one subject (n = 10). Before calculating the correlation, for each subject AUC and the observed Melon and Meloff drift over 8 days (days 2–9) was averaged. A significant positive correlation between the AUC and the observed Melon was found. The correlation between AUC and the observed drift in Meloff was not significant. Stippled lines delineate the 95% confidence interval.
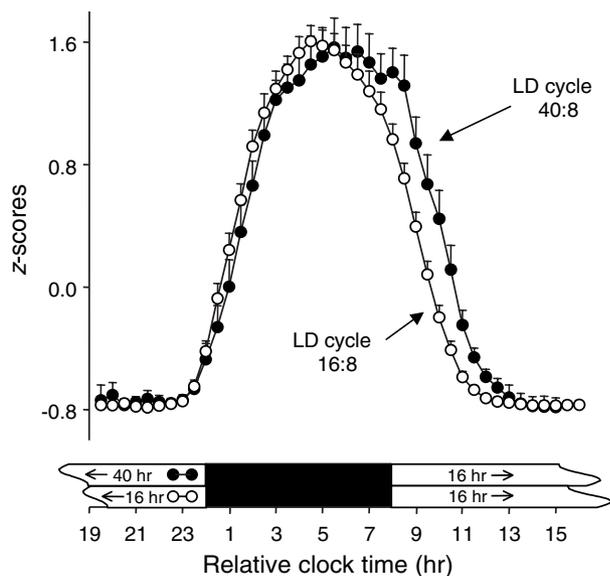

Fig. 6. Average plasma melatonin profile after a 16:8 hr light–dark (LD) cycle and after a 40:8 hr LD cycle (*z*-scores; mean values + S.E.M., n = 10). The 16:8 plasma melatonin profile represents the daily average over days 2, 3, 6 and 7, whereas the 40:8 plasma melatonin profile represents the daily average over days 5 and 9 (post-SD days). The profile during the sleep deprivation episodes (SD 1 and SD 2) were not included in the present figure as this profile represents an intermediate LD cycle and did not significantly differ from the 16:8 profile. The duration of plasma melatonin secretion (the width of the profile) was significantly longer after the 40:8 LD cycle and the downward mean crossing time occurred at a significantly later clock time ($P < 0.05$ in all cases, Duncan's multiple range test). No significant differences were found in the timing of the upward mean crossing time.

The negative correlation between the magnitude of the observed delays and the nighttime level of melatonin secretion may indicate that low amplitude melatonin rhythms reflect reduced robustness of the circadian oscillation, which theoretically implies increased sensitivity to

perturbations. Indeed, it has been shown that subjects are more susceptible to the phase shifting effect of light when their circadian amplitude was reduced by light [36–38], which is in agreement with the concept of Wever that the duration of re-entrainment correlates with the circadian amplitude such that a smaller amplitude is associated with more rapid re-entrainment ([39]). This suggests that circadian amplitude is a determinant of rapidity and/or phase of entrainment as well as phase resetting in human beings [34, 37, 38, 40]. It remains to be determined whether these changes in circadian amplitude are also related to tau.

Application of Kronauer's model to simulate the effects of the light–dark schedule used in this protocol on circadian phase yielded a good relationship between data and simulations. In particular, both data and simulations showed a progressive delay of circadian phase. The relationship between data and simulations was high when the model was exposed to 1 lux during scheduled wakefulness and 0 lux during scheduled sleep.

The main discrepancy between the data and the model prediction occurred after the first SD. In contrast to the model, which predicted a phase advance of circadian phase, circadian phase in the experimental data drifted to a later clock time. During the 3 days following the first SD, circadian phase did not fully advance to the phase predicted by the model and then further delayed during the second SD. Possible explanations for these discrepancies could be that the current version of the model underestimated the response in the phase delay portion of the PRC to light, generally overestimated the response to dim light (< 13 lux) or misestimated the direct effect of light (5–13 lux) during the CR on the circadian period. Alternatively this discrepancy may have occurred because the model does not incorporate the putative effects of SD on the circadian phase. Such effects have been reported in animal studies in which light exposure and physical activity were carefully controlled [41–43], and in blind individuals [44] and more recently also in sighted subjects [45]. Our data confirm previous findings that posture affects plasma melatonin
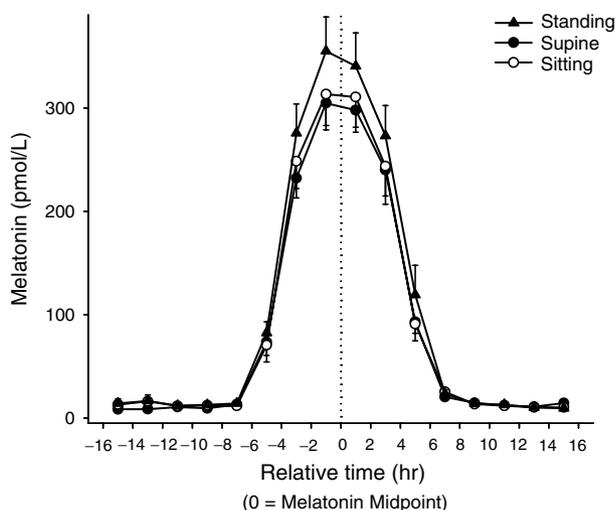
Fig. 7. Average plasma melatonin profile during the supine and the sitting/standing SD-protocol (mean values ± S.E.M., n = 10). During the sitting/standing protocol, separate curves were drawn for melatonin samples taken while in sitting and standing body posture, respectively, and later collapsed into 2-hr bins. Data are plotted relative to the melatonin midpoint (0).

concentrations with lower melatonin levels during supine positions [46, 47]. A standing position results in a redistribution of the blood onto the lower parts of the body, which includes the dependent arms and legs. This produces an increase in hydrostatic pressure in the lower extremities leading to a decrease in plasma volume. This change is associated with an increase in plasma constituents, particularly that of proteins and blood constituents bound to them [48]. Hence, hormones, such as melatonin bound to plasma proteins, may be affected by a posture change. However, we have no evidence from the present study that our phase markers (upward and downward mean crossing times) were affected by posture. It is likely that the present data indicate that SD indeed may effect phase of the human

circadian pacemaker and that these effects are greater than those predicted from the light exposure which accompanies the SD.

The significant day-to-day variation in the duration of melatonin secretion (width of melatonin profile) indicates variation in the waveform in response to the imposed light–dark and sleep–wake cycle. The significantly greater day-to-day variability in the downward mean crossing is in accordance with previous reports of greater variability in changes in the dim light melatonin offset (DLMoff) than in the dim light melatonin onset [49]. The increase in the duration of the interval between upward and downward mean crossing was related to a significantly greater phase delay in the downward mean crossing time than in the upward mean crossing, in particular during the sleep episodes following SD. This could reflect an acute non-photic effect of sleep structure after total SD or an effect of the imposed 40:8 light–dark cycle in dim light on the timing of the melatonin offset. Changes in the waveform of the melatonin rhythm could reflect changes in the *X* variable of the Van der Pol Oscillator. Alternatively, the upward and downward crossings may be controlled by two separate oscillators: an evening and a morning oscillator (E–M oscillator), as shown in rats [50]. Wehr has argued that changes in the waveform of melatonin in humans reflects changes in the phase relationship between M and E [51]. If the two-oscillator models can be applied to humans, the onset and offset oscillators are tightly coupled and maintain a constant phase relationship under most conditions (e.g. 16:8 light–dark cycle) [52] and is not markedly changed in blind individuals [53]. The acute change in the light–dark cycle from 16:8 to 40:8 in our study could have induced a slight modification in the phase relationship between melatonin onset and offset, reflected in a transient looser coupling between these oscillators.

Phase resetting experiments have previously demonstrated that the human circadian pacemaker is very sensitive to dim light [7, 12] and can be entrained by a regular dim light–dark cycle (16:8) and the associated sleep–wake cycle
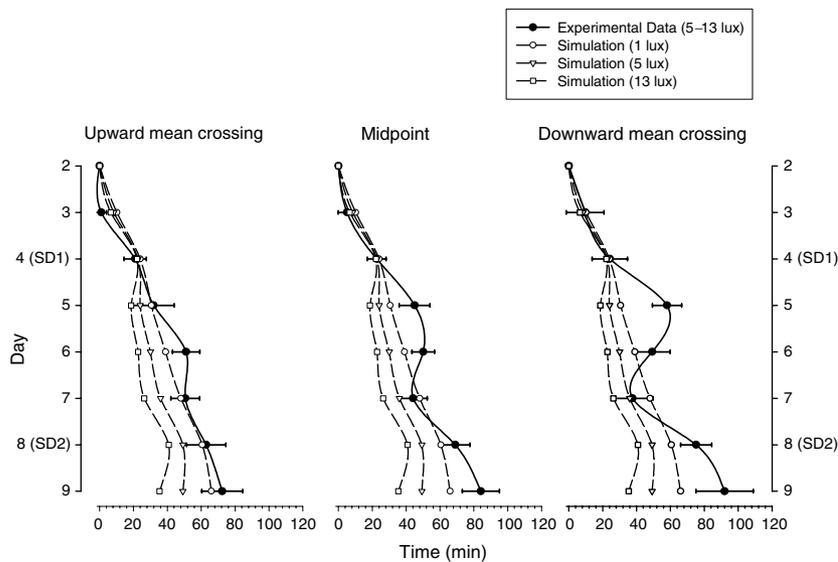

Fig. 8. Comparison of the day-to-day variability of the phase markers (upward, downward, and midpoint) with predicted values using Kronauer's light model [15]. For the simulations, three light intensities were used during scheduled wakefulness (1, 5, and 13 lux) with a light–dark cycle of 16:8 during days 2, 3, 6, and 7 and 40:8 light–dark cycle from days 4 to 5 and from days 8 to 9, respectively. Experimental data represent mean values (n = 10; ±1 S.E.M.).

[14]. The current data demonstrate that the human circadian pacemaker is so sensitive to dim light and/or the absence or presence of sleep that substituting one 8 hr sleep/dark episode by wakefulness in 5–13 lux can significantly disturb the stability of circadian phase – even under lighting conditions that are a magnitude above the predicted illuminance levels for human circadian entrainment. Our data indicate that short-term SD influences the process of clock adjustment in humans.

Acknowledgments

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