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ABBREVIATIONS:
AG: Angle of gaze; MLE: modulated light exposure; T: T-cycle, light:dark cycle; ψ: phase angle; τ: circadian period, tau; MEL_{on}: Melatonin Onset; MEL_{off}: Melatonin Offset.

ABSTRACT

Entrainment of the circadian pacemaker to the light-dark cycle is necessary for rhythmic physiological functions to be appropriately timed over the 24 h day. Non-entrainment results in sleep, endocrine, and neurobehavioral impairments. Exposures to intermittent bright light pulses have been reported to phase shift the circadian pacemaker with great efficacy. Therefore, we tested the hypothesis that a modulated light exposure (MLE) with bright light pulses in the evening would entrain subjects to a light-dark cycle 1 h longer than their own circadian period (τ). Twelve subjects underwent a 65-day inpatient study. Individual subject circadian period was determined in a forced-desynchrony protocol. Subsequently, subjects were released into thirty longer-than-24-h days (daylength of τ+1 h) in one of three light-dark conditions: 1) ~25 lux; 2) ~100 lux; 3) MLE: ~25 followed by ~100 lux, plus two 45-min bright light pulses of ~9,500 lux near the end of scheduled wakefulness. We found that lighting levels of ~25 lux were insufficient to entrain all subjects tested, and that exposure to ~100 lux was sufficient to entrain all subjects although at a significantly wider phase angle compared to baseline. Exposure to MLE was able to entrain all subjects to the imposed sleep-wake cycles at a phase angle comparable to baseline. These results suggest that MLE can be used to entrain the circadian pacemaker to non-24 h days. The implications of these findings are important, as they could be used to treat circadian misalignment associated with spaceflight, shiftwork, and circadian rhythm sleep disorders.

"Entrainment of a circadian rhythm by a zeitgeber fulfills biological purpose in providing a very distinct phase relationship between the periodicity of the organism and that of the environment". J. Aschoff (1)

INTRODUCTION

To be of functional significance for the organism, circadian rhythms must be entrained to the 24-h day. For nearly all species studied, the light-dark cycle is the most powerful circadian synchronizer. The resetting capacity of light depends on its intensity, timing, duration, temporal pattern and spectral composition (2-7). In totally blind people, the circadian timekeeping system often looses synchrony with the earth's 24-hour light-dark cycle (8). Well described in animals (9-11) and only recently confirmed in humans (12), entrainment of the circadian system depends on 1) its intrinsic period (τ), 2) on the light-dark cycle to which it is exposed (T; T-cycle), and 3) on the strength of the entraining stimulus (zeitgeber). The generally accepted non-parametric model on circadian entrainment predicts immediate phase shifting in response to light, according to a phase response curve (PRC) (3, 9, 13). In humans, for whom the intrinsic period is on average ~24.2 h (14), entrainment to the solar day of earth (T=24 h) requires that the biological clock be "reset" by on average of ~0.2-h per day in the advance direction. At the individual level, persons with τ shorter-than-24 h require a daily phase delay ($-\Delta\phi$) whereas individuals with τ longer-than-24 h require a daily advance ($+\Delta\phi$) to synchronize to T= 24 h. Entrainment is achieved when $T = \tau - \Delta\phi$ with a stable phase relationship (or phase angle, Ψ) between a phase marker for the synchronizing cycle (e.g. light offset) and a phase marker of the driven rhythm (e.g., melatonin onset). In animals, there is a well known quantitative relationship between ψ and T, such that ψ generally increases as T lengthens, and Ψ decreases when the strength of the zeitgeber increases (13). Additionally, the response to a resetting stimulus is correlated with τ, such that animals with short τ (fast pacemakers) tend to be more phase delayed and less phase advanced by light than animals with longer τ (slow pacemakers) (9).

In the present study, we evaluated entrainment of the human circadian system to longer-than-24h entraining T-cycles in response to three zeitgebers of different strengths: dim light of 25 lux, room light of 100 lux, and a modulated light exposure (MLE) protocol consisting of dim light of 25 lux for the first 10h of the waking day, room light of 100 lux for the remainder the waking day plus exposure to two bright light pulses near the end of the waking day (Fig.1). Based on the mathematical model of the effect of light on the human circadian pacemaker developed by Kronauer et al. (15-17), we hypothesized that the resetting effect of the MLE would entrain the biological clock of subjects to a τ +

1 h T-cycle, whereas the resetting effect of 25 and 100 lux would be insufficient to entrain the biological clock to a τ + 1 h T-cycle.

RESULTS AND DISCUSSION

Range of circadian periods observed

A 1-h range of circadian periods was observed in our subjects (from 23.47 h to 24.48 h, Fig. 2 and Table 1) consistent with that of prior forced desynchrony studies (14, 18). This confirms the importance of having customized the circadian entrainment challenge to which each subject was exposed (τ+1h), particularly since there was a difference ($p<0.05$) in circadian periods between groups to which subjects were assigned (Table 1). The average composite period was 24.07 ± 0.33 h, and 4 out of 12 subjects had periods shorter than 24 h (Table 1). Although there was a statistical difference between the average temperature period (τ_t) and melatonin period (τ_m) (24.04 ± 0.32 h vs. 24.10 ± 0.34 h respectively, Student-t = -3.5, $p< 0.005$), the two estimates were highly correlated ($r_{pearson}$ =0.99; $p<0.0001$) in this cohort, and no difference on average was found in other cohorts (19). The scalloping of the melatonin onsets observed across the forced desynchrony protocol (Fig. 2) is consistent with relative coordination (20, 21).

Figure 3 reveals that the phase angle of entrainment, as determined by the relationship between melatonin onset and habitual bedtime (ψ_{MELon}), is strongly correlated with τ such that participants with shorter circadian periods have an earlier ψ_{MELon} than those with longer periods. This is consistent with weaker correlations from data collected immediately after entrainment to a 24h day (22), and even after entrainment to a variety of daylength and lighting conditions (23). The very high correlation demonstrated in Fig. 3 indicates that the phase estimates collected on a single day may provide a tool for estimating intrinsic circadian period. Further research will be needed to define the specific conditions that are required in order to maintain the high correlation between circadian phase observed and the measure of circadian period. It should be noted that in the present cohort, this high correlation was observed in subjects who maintained a wake:sleep schedule with a light:dark ratio of 2:1, for at least 3 weeks prior to entry in the study. In addition, once they entered the laboratory, stringent control of lighting conditions were maintained for 3 consecutive days, and the transition from ordinary room light (~90 lux) to dim light (~1.5 lux) was made on day 3, 10 hours after habitual waketime, at a phase which is on average of minimal sensitivity for photic resetting.

Re-establishment of a normal ψ prior to the entrainment trial to T = τ + 1h

Figure 4 shows the timing between lights off and melatonin onset (ψ_{MELon}) and between lights on and melatonin offset (ψ_{MELoff}) at different times during the protocol. From day 2 to CR1, subjects showed a consistent ψ_{MELoff} (no significant change in ψ_{MELoff} from day 3 (D3) to the second day of the first CR [CR1b], ANOVA $p>0.05$). MEL_{on} occurred later on day 2 (D2), when subjects were exposed to ~90 lux in the angle of gaze, than on D3 and CR1, when light levels were ~1.5 lux ($p<0.0001$ for both days). This is consistent with the masking effect of room light on melatonin (MEL_{on}). As illustrated in Fig. 4, re-establishment of ψ after forced-desynchrony within a normal range was largely successful in all of our subjects. That is, the wide distribution of ψ is compressed into a normal range of ψ on CR3, after five 24.0-h days scheduled at a normal phase angle under 450 lux background light. Note that both MEL_{on} and MEL_{off} phases were slightly delayed on CR3 compared to Days 2, 3 and CR1 (ANOVA, Student-Newman-Keuls $p<0.0001$). This change, which likely occurred in response to the phase delaying effect of 5 days in 450 lux, is consistent with previous finding in animals (9, 13) and in humans (23), showing that phase angle shortens with increased light intensities.

Entrainment to T = τ + 1 h with zeitgebers of different strengths

Figure 5 illustrates the change in phase angle of entrainment between MEL_{off} and the T-cycle (waketime) for subjects in the three zeitgeber light conditions. Due to the shorter phase angles that occurred after re-entrainment under a strong zeitgeber (~450 lux), changes in phase angle throughout the T-cycle were expressed relative to the phase angle on Day 3. As shown in Table 1, three of four subjects in the 25 lux condition were not entrained to the τ+1h T-cycle. In the three non-entrained subjects, melatonin offset gradually drifted to an earlier time relative to the light-dark cycle (Fig. 5, left panel). These subjects were classified as not entrained because the 95% confidence interval (CI) of their observed period (τ_{obs}) did not include the period of the T-cycle (Table 1). Strikingly, one subject (2195) remained entrained to the τ+1h T-cycle under the same low light levels of 25 lux. This subject had the shortest τ of all subjects (23.47 h). Subjects with short τ require a daily phase delay to entrain to the 24-h LD cycle of Earth. By contrast, subjects with a longer-than-24h τ require a daily advance shift. One might expect that after years of producing required delays an individual with a short τ might develop enhanced delay sensitivity. This would be in agreement with the Pittendrigh and Daan entrainment model (24), which, in order to explain stable phase angle of entrainment despite

photoperiodic changes across the year, requires that night active species need a short period and an asymmetric PRC with higher sensitivity in the delay region (larger range and higher amplitude), whereas day-active animals optimally need a period greater than 24 h and higher sensitivity in the advance region (24). Therefore, one could hypothesize that the subject with the shortest period could present a PRC asymmetry with a very sensitive delay region. The two other subjects with a short τ were misaligned (subject 2209, τ = 23.58 h, average phase delay during the T-cycle = 0.73h per cycle; subject 22T1, τ = 23.75 h, average phase delay during the T-cycle = 0 h). This does not necessarily contradict the hypothesis of PRC asymmetry. Indeed, the amplitude and shape of the PRC varies between species and individuals, as well as does the range of entrainment (9). In two of the three subjects who did not entrain to T = τ + 1 h, the imposed T-cycle still exerted an effect on the circadian clock (τ_{obs}) but the synchronizing stimulus was of insufficient strength to entrain it (Table 1).

All four subjects exposed to 16 hours of 100 lux showed a transitory drift in MEL_{off} to an earlier time. Following the first week in the T-cycle, the timing of melatonin offsets was stable for all subjects (Fig. 5). These subjects were classified as entrained to the T-cycle (Table 1). On average, by the end of the T-cycle, ψ had widened by -1.26 ± 0.36 h in DL100 condition compared to baseline (paired t-test: p=0.038; Wilcoxon matched pairs test: p=0.0678, marginal effect). All 4 subjects showed a widened ψ compared to baseline (binomial test: p<0.0001).

Subjects exposed to MLE also showed a transitory drift in MEL_{off} to an earlier time (Fig. 5). This segment was however shorter than in the two other groups, as melatonin offsets appeared to reach a stable ψ after only ~5 days in the T-cycle. All four subjects were classified as entrained to the T-cycle (Table 1). On average for this group of subjects, ψ was not significantly different at the end of the T-cycle from that measured at the beginning of the study (mean change: -0.19 ± 0.41 h, paired t-test: p=0.67; Wilcoxon matched pairs test, p=0.47). Interestingly, even subject 2082, who was mistakenly scheduled to τ + 1.14 h, successfully entrained (τ_{obs}=T) at a normal ψ despite the additional phase delay challenge of 0.14 h (8 minutes) per day.

Figure 6 (*Supporting information [SI]*) illustrates the dynamics of the melatonin rhythm in 3 individuals exposed to either 25 lux, 100 lux, or MLE.

Entrainment or masking?

The last CR of our protocol (CR4) was carried out to distinguish entrainment of the circadian pacemaker from masking of the circadian phase marker by light. Once released into constant routine (CR4), the phase of the circadian system corresponded to that expected from the previous cycle (Fig. 5), and did not show an abrupt change in phase ("jump") that is characteristic of masking. In addition, our choice of using MEL_{off} as an appropriate unmasked phase marker of the circadian system is supported by the result that the change in phase (MEL_{off}) from the last day in the T-cycle to CR4 was not significantly different among the 3 groups (-1.08 ± 0.40 in 25 lux, -1.04 ± 0.87 in 100 lux and -0.45 ± 1.17 in MLE, Kruskall Wallis: p=0.69) despite different lighting conditions during daytime under the entraining T-cycle (25 or 100 lux).

Phase angle and strength of the zeitgeber

As shown both in non-human species (13, 25) and humans (22, 23), the phase angle of entrainment between the imposed T-cycle and the phase marker of an entrained rhythm is a function of the difference in their period [$\psi=f(T-\tau)$] and of the strength of the zeitgeber. Phase angle increases as T lengthens, and ψ decreases as zeitgeber strength increases (13). In our study, the difference in the period of T and τ was equal for each subject (1 h by protocol design). Therefore, the difference in phase angle of entrainment between conditions is likely due to difference in the respective zeitgeber strength, i.e. the light intensity. As predicted by entrainment theory, a larger phase angle was observed with a decrease in zeitgeber strength in our human subjects, as achieved in other mammals (26) (see also expanded discussion in *Supporting Information*).

High sensitivity to moderate light intensities of 25 and 100 lux

The mathematical model developed by Kronauer et al. (15-17) was used as a guide to the design of these experiments. The present results imply a higher sensitivity to 100 lux than could have been inferred from previous data. The model has been modified to incorporate these and other recent findings on non-photic stimuli (27). It can be considered as a “continuous” model, designed to accommodate even brief (few minutes) stimuli.

Physiologically, the higher-than-expected sensitivity to 25 and 100 lux light could be related to the effects of prior light history in that the response to light may be enhanced after background light

exposure of low intensity (28, 29). The mechanisms explaining the effects of prior light history are unknown, but they could involve the recently described modulation of photosensitive retinal ganglion cells (ipRGC) sensitivity by background light levels (30). When exposed to a constant bright background, the background evoked response of ipRGC decay, and their responses to superimposed flashes suggest light adaptation of those photosensitive cells. Additionally, after extinction of a light-adapting background, sensitivity recovered progressively, indicating dark adaptation. On the other hand, it has been recently shown than the intrinsic light-responsive RGCs adapt their expression of the photopigment melanopsin to environmental light and darkness in such a way that prolonged exposure to darkness increases melanopsin mRNA level, whereas exposure to constant light decreases melanopsin mRNA levels (31). Therefore, the increase in ipRGC sensitivity during the course of the T-cycle in 25 and 100 lux light conditions could explain, at least in part, that the response drive be greater in relatively low light levels than expected.

Conclusions and perspectives

Our findings demonstrate that the human circadian system shares the basic properties of entrainment biology that have been described in other species: the phase angle of entrainment 1) increases with increased period of the T-cycle (11), and 2) decreases as the strength of the zeitgeber increases (13).

Non-photic stimuli have been reported to exert a small but significant synchronizing effect on the human circadian system, both in sighted (32, 33) and blind subjects (34, 35). Therefore, in our protocol we cannot exclude that non-photic cues, such as showers (pulses of temperature), meals, and sleep-wake schedule, exerted a weak effect on the clock.

Only outputs of the central circadian clock (core body temperature, melatonin) were evaluated to assess entrainment in our study. The concept of multiple oscillators normally synchronized with each other but which can become desynchronized under “free-running” conditions was developed 30 years ago (36). In recent studies, it has been shown that the central circadian pacemaker (SCN) acts as a master clock, synchronizing a multitude of peripheral clocks (37). Therefore, we may have classified as entrained, subjects whose peripheral clocks were misaligned with each other and/or with the central clock. Such a misalignment between peripheral and central clock has been shown to occur

in aged rodents (38). The occurrence of partial entrainment and its impact in humans remain to be investigated.

Failure to entrain to the required sleep-wakefulness schedule occurs in numerous situations in real life conditions. Jet-lag, shift work, circadian disorders such as advanced- and delayed sleep phase syndromes, non-24 sleep-wake syndrome, are all associated, to different extents, to a condition where the circadian system is out of synchrony with the light-dark/rest-activity cycle. The generally associated symptoms range from cognitive and psychomotor impairment, to sleep disorders (18, 39-42) and endocrine disturbances (43). The resulting inappropriate functioning, consistent with the hypothesized circadian regulation of brain metabolism (44), may be an important factor contributing to an increased risk of accident associated with circadian misalignment (45). Evidence of significant sleep loss and disruption of circadian rhythms in astronauts (42), and associated performance decrements are also reported during space missions. In these situations, sleep and circadian disruptions could have serious consequences on the effectiveness, health and safety of astronaut crews (46, 47). Moreover, long-duration exploration class space missions may require astronauts to be scheduled to non-24-h light dark periods for extended periods of time, in conditions in which gravity could additionally impact circadian physiology (48). The above issues emphasize the importance of developing effective countermeasures to maintain circadian entrainment. Our findings suggest that appropriately timed light exposure can be used as an effective means to maintain the circadian clock in synchrony with a rest-activity cycle different from 24h or under insufficient light conditions. A lighting protocol such as the one tested in the current study would enable astronauts to entrain to the 24.65 h Martian day while caring for crops in a brightly-lit greenhouse module (49, 50) provided that those duties were performed at the appropriate circadian phase.

METHODS

Subjects

Twelve healthy young subjects participated in the study (22-33 years old, average 28.8 ± 4.1 [SD] years old, 9 males and 3 females) after a rigorous screening procedure (see *Supporting Information*). Subjects were required to maintain a regular 8:16 h sleep: wakefulness schedule at

home for at least three weeks prior to laboratory admission, verified by wrist activity and light exposure recordings.

Protocol

Subjects were maintained in individual rooms free from external time cues during the entire 65-day study (Fig. 1). Timing of light exposure, sleep opportunities, meals, and showers were scheduled. Subjects were maintained on a 24.0-h schedule for 3 days, followed by a 40-h constant routine (CR) protocol that was used to assess circadian phase (51). Subsequently, participants were scheduled to a forced-desynchrony protocol (FD) using a 28-h dim light-dark cycle. This FD protocol was used to estimate their intrinsic circadian period (τ) (14). Then, a second CR was used to re-assess circadian phase. Participants were then scheduled for five 24.0 h days with sleep scheduled at the same phase of the endogenous circadian temperature cycle as that observed on the first CR. Successful re-establishment of phase was verified by a third CR. Participants were then scheduled for 30 days to a T- cycle of τ + 1 h in one of three assigned light conditions (below and SI Fig. 8). For example, a subject with a τ of 24.24 h as determined during FD would be scheduled to a 25.24 h day. A fourth CR was used to assess circadian phase after the τ + 1 h schedule, and followed by three 24.0 h days.

The rationale for scheduling participants to a T-cycle based on their own intrinsic circadian period (τ + 1h) rather than to a fixed T-cycle was to evaluate the effectiveness of three light:dark cycles of different zeitgeber strength on the circadian system challenged to a similar T-cycle relative to their circadian period. Figure 2 and Table 1 show the range of circadian periods of the 12 subjects studied. A fixed T-cycle of, for example, 24.5 h would have required a phase delay shift of ~1 hour per day for the subject with the shortest period (2195, τ =23.47 h) whereas subject 2110 would virtually not necessitate any phase adjustment to remain entrained to the T-cycle given its τ of 24.48 h. Therefore, in the case of a fixed T-cycle, it would be difficult, if not impossible, to assess the relative efficacy of different zeitgeber strength.

All experimental procedures were carried out in accordance with the principles of the Declaration of Helsinki (revised in 2000), and the protocol was approved by the Human Research Committee at the Brigham and Women's Hospital. Subjects provided written informed consent.

Lighting conditions

Experimental suites were equipped with ceiling-mounted cool-white fluorescent lamps (see *Supporting Information*). A light:dark ratio of 2:1 was maintained for all day lengths. Light intensities and irradiances were measured at a height of ~1.37 m to approximate the intensity received in the average angle of gaze [AG] during wakefulness (see supporting information). Light intensities were: ~90 lux during the first 2.5 baseline days (D1-3); ~1.5 lux during the last 6 hours of day 3, during constant routines (CR1-4) and the forced-desynchrony segment; ~450 lux during the re-entrainment segment between CR2 and CR3; and depending on the lighting condition, subjects were exposed to either ~25 lux, ~100 lux or a modulated light exposure (MLE) protocol consisting of dim light of ~25 lux for the first 10h of the waking day, room light of ~100 lux for the remainder the waking day plus exposure to two bright light pulses of ~9,500 lux near the end of the waking day (*SI*, Fig. 8) during the entrainment segment of the protocol. Scheduled sleep occurred in total darkness. The timing, duration, intensity and pattern of light exposure stimuli were based on the dynamic resetting model developed by Kronauer et al. (15-17). The model predicted a successful circadian entrainment to $T=\tau+1h$ for the MLE condition, and failure to entrain to $T=\tau+1h$ for the 25 and 100 lux light conditions.

Data collection and analyses

Temperature was recorded every minute via a rectal thermistor (Yellow Springs Instrument, Yellow Spring, OH, USA). Blood samples were collected every 10-60 min during baseline days, CR, and FD segments, and every 60 min during 5 time-windows of the entrainment segment. Plasma melatonin concentrations were assayed using RIA techniques (ALPCO Diagnostics assay, NH, USA). The assay sensitivity was ~0.7 pg/ml. Average intra- and inter-assay CVs were below 8% and 12% respectively.

A dual-harmonic regression model (52) was used to assess the phase of the temperature minimum (CBT_{min}) during CR1 and CR2. The phase angle between CBT_{min} and habitual bedtime measured on CR1 ($\psi_{CBTminCR1}$) was used as an estimate of the baseline phase angle of entrainment. After CR2, subjects were scheduled to a sleep-wake cycle at the same ψ between CBT_{min} and habitual bedtime as that measured in CR1, such that $\psi_{CBTminCR1} = \psi_{CBTminCR2}$.

Melatonin Onset (MEL_{on}) and Melatonin Offset (MEL_{off}) were calculated using a least-square regression analysis and a threshold of 25% of the peak-to-trough amplitude (4). Phase angle of

entrainment was calculated as the difference in time between lights off (bedtime) and melatonin onset (ψ_{MELon}) and between lights on (waketime) and melatonin offset (ψ_{MELoff}). Based on prior findings that melatonin levels are acutely suppressed by light (53), even at relatively low light levels (2), ψ_{MELoff} was chosen to assess entrainment of the circadian system during the entrainment segment of our study because we exposed subjects to bright light in the evening hours in the MLE condition.

Intrinsic circadian period was estimated on temperature (τ_t) and melatonin (τ_m) data collected during FD using a non-orthogonal spectral analysis procedure (14). A composite estimate of the intrinsic period for each subject (τ) was computed by averaging τ_t and τ_m. Subjects were classified as entrained to the T-cycle when the 95% CI of their observed period (τ_{obs}) included the period of the T-cycle (12).

Unless otherwise indicated, results are reported as means ± SEM. Statistical significance is ascribed for $p<0.05$. We also report as marginal effects with a *p* value between 0.05 and 0.10 (54).

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FIGURE LEGENDS:

Figure 1: Double raster plot of experimental protocol. After three baseline days, during which time subjects continued to sleep (black bars) and wake at their habitual times, subjects are scheduled to a constant routine (CR1). The forced-desynchrony procedure (FD) was used to estimate the intrinsic circadian period (τ). The red dashed line illustrates the drift in phase corresponding to a 24.2 h period. A second CR (CR2) was used to re-assess circadian phase and subjects were then scheduled to sleep at their habitual phase angle of entrainment (assessed from CR1) to five 24-h days. Re-entrainment was verified by measurement of phase during CR3. Participants were then scheduled to a "30-day" entrainment segment (assigned to one of three light conditions described in Fig. 2), a subject-dependent T-cycle of τ + 1 h, calculated by adding one hour to each subject's τ. The two blue bars displayed at the end of the waking day show the relative timing of bright light pulses received by subjects assigned to the modulated light exposure (MLE) condition. CR4 was used to assess the circadian phase after the entrainment segment, and participants were discharged after three 24-h recovery days.

Figure 2: Daily melatonin phase estimates (MEL_{on}) throughout the forced-desynchrony protocol.

Figure 3: Relationship between intrinsic circadian period and phase angle of entrainment measured on CR1 as the difference in time between habitual bedtime (lights off) and MEL_{on}. Subjects with a shorter circadian period showed a larger phase angle. The high correlation coefficient ($r=0.946$, $p<0.001$) indicates that a single phase estimate may provide a tool for accurately estimating intrinsic circadian period.

Figure 4: Timing between lights off and melatonin onset (MEL_{on}) and between lights on and melatonin offset (MEL_{off}) at different times during the protocol. Open symbols are used on D2 and D3 for masked MEL_{on} and MEL_{off} (occurring under ~90 lux of light). The successful re-entrainment following forced-desynchrony is clear (as measured by a phase angle in CR3 comparable to that in D2, D3 and CR1) despite the wide range of phase angles achieved immediately at the end of forced desynchrony (CR2).

Figure 5: Change in phase angle of entrainment between MEL_{off} and the T-cycle in the three light conditions. Phase angles are plotted relative to the phase angle measured on day 3. Three of the four subjects in the 25 lux condition did not maintain entrainment, as melatonin offset gradually drifted to an earlier time. After a transitory drift, subjects in the 100 lux and the MLE conditions entrained to the τ + 1 h T-cycle (Table 1), although at a different phase angle.

TABLES

Table: Circadian period estimates

		Forced desynchrony			Entrainment trial			Entrainment
Subject	**Group**	**τ_t (SD)**	**τ_m(SD)**	**τ**	**T-cycle**	**τ_{obs}**	**τ_{obs} CI-95%**	**status**
2195	25 lux	23.47±0.03	23.47±0.01	23.47	24.47	24.46	24.43-24.50	**+**
2209	25 lux	23.57±0.04	23.59±0.01	23.58	24.58	24.31	24.25-24.36	**-**
22T1	25 lux	23.73±0.07	23.76±0.04	23.75	24.75	23.75	23.70-23.79	**-**
2313	25 lux	24.02±0.04	24.09±0.02	24.05	25.05	24.74	24.71-24.77	**-**
2123	100 lux	24.24±0.04	24.24±0.01	24.24	25.24	25.25	25.23-25.27	**+**
22F2	100 lux	24.24±0.07	24.24±0.01	24.24	25.24	25.26	25.22-25.30	**+**
2109	100 lux	24.24±0.05	24.36±0.02	24.30	25.30	25.27	25.24-25.31	**+**
2072	100 lux	24.23±0.06	24.42±0.05	24.33	25.33	25.33	25.28-25.39	**+**
2196	MLE	23.82±0.07	23.91±0.01	23.87	24.87	24.88	24.85-24.91	**+**
2210	MLE	24.22±0.04	24.28±0.02	24.25	25.25	25.25	25.20-25.30	**+**
2082	MLE	24.33±0.03	24.37±0.03	24.35	25.49	25.49	25.47-25.51	**+**
2111	MLE	24.44±0.03	24.52±0.01	24.48	25.48	25.48	25.46-25.51	**+**

Circadian periods measured during forced-desynchrony on core body temperature rhythms (τ_t) and plasma melatonin rhythms (τ_m). Subjects were considered as entrained (+) when the 95% confidence interval (CI) of their observed period (τ_{obs}) measured during the entrainment trial included the period of the imposed T-cycle. They were considered non-entrained (-) otherwise. Experimental conditions: 25 lux, 100 lux, modulated light exposure (MLE).

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FIGURES

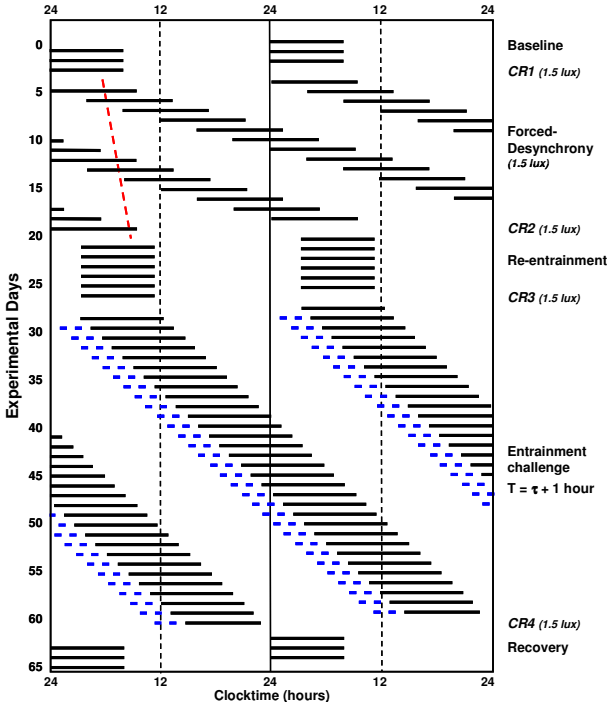

Figure 1

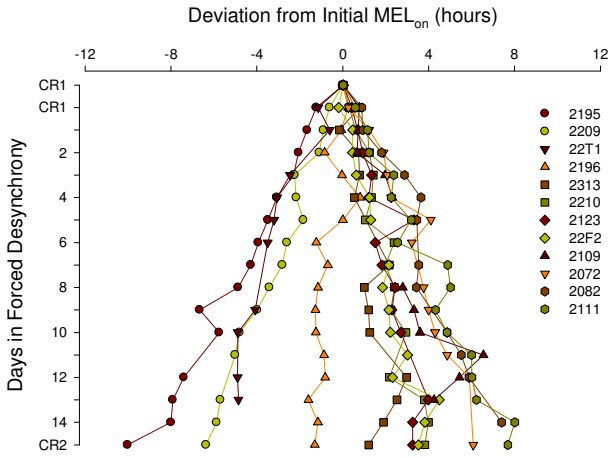

Figure 2

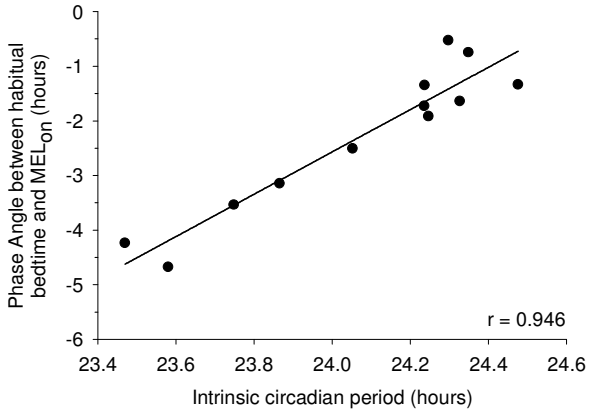

Figure 3

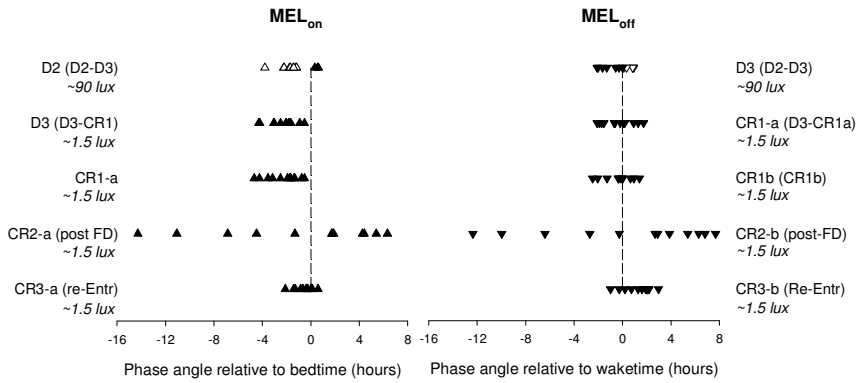

Figure 4

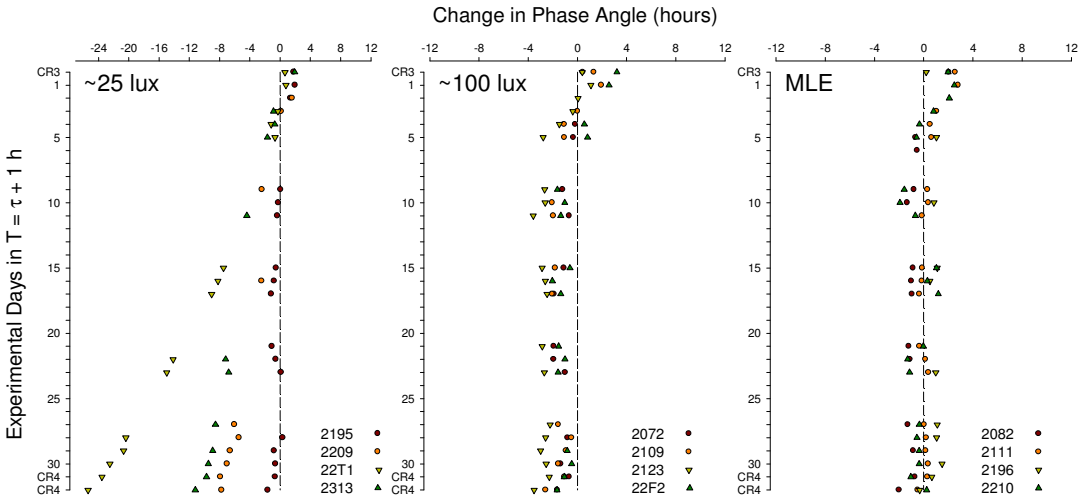

Figure 5