Human circadian pacemaker is sensitive to light throughout subjective day without evidence of transients

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Numerous reports have shown that, in human subjects, appropriately timed bright-light exposure induces phase shifts in a wide variety of circadian markers (for review, see Ref. 8). When light stimuli are timed to occur across the circadian cycle in a series of experiments, the phase shifts observed produce phase-response curves (PRCs) in humans similar to those observed in other organisms; phase delays occur during early and mid subjective night, and phase advances occur during late subjective night and subjective morning (11, 17, 20, 26). A great deal of attention has been focused on the "critical" region of human PRCs, which lies between the phase delay and phase advance portions of the PRC (1, 24, 25). This is because it is the critical region that determines whether a PRC is type 0 (with phase shifts as large as 12 h in the critical region) or type 1 (with phase shifts as small as 0 h in the critical region). However, it is also important to characterize the response of the human circadian system to light stimuli that occur outside the critical region, during the subjective day, when individuals are most likely to be exposed to bright light in naturalistic settings (7).

Thus, as described below, a study was conducted to investigate the following three questions in human subjects exposed to a three-cycle bright-light stimulus applied outside of the critical zone. 1) Does the PRC contain a "dead zone" of insensitivity to bright light during the subjective day? 2) Does the core body temperature reflect the true phase of the underlying circadian pacemaker when it is measured 1–2 days after the stimulus application? 3) Does the intensity and/or the timing of the background illumination, relative to the bright light, modulate the phase-shifting effects of the bright-light stimulus?

Sensitivity to Light Stimuli During Subjective Day

In rodents, Pohl (31) noted that PRCs differed greatly during the subjective day, depending on whether the animals were nocturnal or diurnal. Nocturnal rodents were insensitive to light for several hours during their subjective day, resulting in a dead zone (in which the circadian system does not shift in response to light stimuli) in their PRCs between the phase-advance and phase-delay regions (31). Those dead zones are characterized as regions of flat (0) slope for several hours, preceded and followed by regions of negative slope. Similar dead zones have been found in many species (21). However, Pohl (31) found that, in diurnal rodents, PRCs did not have dead zones during the subjective day but instead maintained constant negative slopes, indicating that the circadian pacemakers of those diurnal rodents were sensitive to light throughout their subjective day. A number of other organisms also have PRCs with no evidence of any dead zone (21). Although Pohl (31) found that the presence or absence of a dead zone was dependent on whether the animal was nocturnal or diurnal, some exceptions to this rule have since been reported for other organisms (21). This led us to question whether the human PRC to light contains a dead zone or whether it maintains a constant negative slope throughout the subjective day, as in most other diurnal mammals.

Although several PRCs to light have been reported in humans (11, 17, 20, 26), no studies have intensively investigated the response to light during the subjective day. For example, in 1989, we reported a type 0 PRC to three cycles of bright light, but of the 45 trials conducted in that study, only 17 occurred during the subjective day (i.e., from 3 to 19 h after the fitted temperature minimum) and only 26 occurred outside of the critical zone (i.e., between 1.5 and 22.5 h after the fitted temperature minimum) (11). In that study, the
PRC had a relatively constant negative slope during the subjective day. However, the smoothed fit to those data had a portion during the subjective day in which the slope appeared nearly flat (20), making it unclear whether humans might have a dead zone during which they are insensitive to the three-cycle stimulus used. To answer this question, a higher density of trials was required during the subjective day. Thus, to carefully assess the slope of the human PRC to three cycles of bright light, in the present study we have conducted 56 resetting trials in which the stimulus was centered outside of the critical zone, throughout the subjective day.

Time Course of Stable Phase Resetting of Core Body Temperature via Bright Light

It has been widely reported in many organisms that, after exposure to a phase-resetting stimulus, phase markers of the circadian system often display several cycles of non-steady-state behavior (referred to as “transients”) before reaching a stable phase position. The magnitude and direction of these transients are dependent on the particular organism and phase marker studied as well as the circadian phase of the stimulus administration (30). Pittendrigh et al. (30) proposed that such transients may not reflect the time course of the phase shift of the underlying pacemaker but instead may reflect the time it took for a given phase marker to realign with the shifted pacemaker. To test this hypothesis, Pittendrigh (28, 29) designed a double-pulse experiment in which the PRC to two stimuli applied several hours apart was compared with the steady-state PRC to a single light stimulus. Because the steady-state phase shifts observed in the double-pulse experiment could be predicted by simply iterating the single-pulse PRC, Pittendrigh concluded that the transients observed were a feature of the phase marker (referred to as a “slave oscillator”) rather than of the underlying pacemaker itself, which seemed to be reset rapidly (within 2 h of stimulus application). This result has since been observed in a number of other organisms as well (2, 3, 6, 16, 32; however, see also 13). Alternatively, the observation of transients could reflect an underlying dual-oscillator circadian system in which the coupled oscillators show differential responses to resetting stimuli (13, 14, 18).

Because the activity rhythm in rodents seemed to have more pronounced transients than the eclosion rhythm in Drosophila, which in turn showed more transients than the phototactic response rhythm in the single-celled organism Euglena, Pittendrigh et al. (30) proposed that the number of transient cycles required to reach a steady state may increase in more complex multicellular organisms, such as humans. Alternatively, these data could indicate that the number of transient cycles may depend on how tightly coupled the marker rhythm is to the pacemaker. However, although the phase-shifting response to bright-light stimuli has been widely explored in human subjects by use of a number of different circadian markers, potential transients in these markers have not been systematically studied. This is of particular concern in the interpretation of human PRCs that were constructed using subjects who participated in multiple consecutive trials (11, 20). In these consecutive trials, circadian phase and amplitude were measured during the 1–2 days immediately after the last stimulus cycle of the previous trial. This poststimulus phase assessment then became the initial phase reference point for the timing of the next stimulus application. Thus it was possible that either the circadian pacemaker or its phase markers may not have reached a steady-state phase position between the consecutive trials, thereby altering the shapes of the PRCs reported (11, 20).

To determine whether the phase marker (core body temperature) may have exhibited transients in the human PRCs reported previously (11, 20), one can compare the results of the steady-state trials (in which the initial circadian phase was known to be stable), to the results observed in the consecutive trials (in which the initial circadian phase was measured immediately after a prior stimulus application). If the phase marker were to exhibit transients, then for any given phase of stimulus application, the consecutive trials would induce different (usually larger) apparent phase shifts in the marker than the steady-state trials. In the 45 trials we reported in the three-cycle PRC (11), there did not appear to be any systematic difference between the results from the 35 consecutive trials compared with the results from the 10 steady-state trials, suggesting that, in each consecutive trial, the subject’s circadian system had reached a relatively stable phase position by the time of the poststimulus phase assessment. However, in that study, only five steady-state trials fell outside of the highly variable critical zone, so that it was not possible to conduct a thorough comparison with the consecutive trials. Thus, to investigate potential transients of core body temperature in the 56 trials in the study presented here, we have compared the results of 41 of these trials in which the circadian system had been given several weeks to stabilize before applying the three-cycle bright-light stimulus (steady-state trials) with the results of 13 of these trials in which the prestimulus phase assessment occurred immediately after a prior stimulus application (consecutive trials). In addition, because it is often difficult to distinguish between transients in a marker and masking in that marker (27), the phase and amplitude of the core body temperature were determined using a constant routine protocol that was specifically designed to minimize masking of this marker (10).

Effects of Intensity and Timing of Background Lighting Relative to Bright Light

Unlike PRCs reported in most other organisms, which are conducted against a background of darkness, in human PRCs, the bright-light stimulus is usually applied against a background of ordinary indoor room light. In addition, the bright-light stimuli often are not centered in the middle of the waking episode, so that the background room light is not distributed evenly.
around the bright-light episode. In our three-cycle PRC, we found that the timing of the background room light (~150 lx) relative to the 5-h bright-light stimulus (~10,000 lx) could have significant effects on the direction and magnitude of the resulting phase shifts observed (11). Thus, in that study, to construct a PRC that contained trials in which the bright light and the background room light were centered at different times, we proposed a weighted averaging technique that included both the room light (weighting = 0.27) and the bright light (weighting = 0.73) to estimate the circadian phase of the overall light stimulus applied (11). Recent findings have confirmed that ordinary indoor light is indeed capable of resetting the human circadian pacemaker (4). In fact, the relative weighting strength of room light (0.29) and bright light (0.71) observed in that study was very close to that used in the weighted averaging technique described above. However, the use of this averaging technique to construct the previously published PRC (11) assumed that the background lighting affected only the initial phase of the overall stimulus applied and did not modulate the strength of the bright-light stimulus.

To test this hypothesis, in the three-cycle trials presented here we have reduced the background lighting. Throughout all trials, with the exception of the bright-light applications, the subjects were exposed only to very dim light (10–15 lx) or darkness. The data from these dim-background trials were then compared with the data from the 150-1x-background trials reported earlier (11) to assess the contributions of the timing and intensity of the background lighting to the overall phase shifts observed.

MATERIALS AND METHODS

Subjects

Data from 43 young men (mean age ± SE, 22.9 ± 0.3 yr; range, 18–30 yr), who were free from medical, psychiatric, and self-reported sleep disorders, were analyzed. Subjects were instructed to abstain from caffeine, nicotine, alcohol, and drugs for 3 wk before their study, and they were drug free at the time of study, as verified by urinary toxicological analysis. All subjects denied a history of night work or shift work in the 3 yr before study, and none reported crossing time zones more than two times in the 3 mo before study. Subjects were instructed to keep a regular sleep-wake schedule (bedtimes and wake times within 1 h of self-selected target times) during the 3 wk before their admission to the laboratory. Adherence to a regular schedule during the week before admission was verified with wrist Actigraphy (Vitalog Monitoring, Redwood City, CA; Ambulatory Monitoring, Ardsley, NY).

Throughout each study, the subjects were maintained in an environment free of time cues. Each subject underwent an endogenous circadian phase and amplitude assessment after 3 baseline days in the laboratory (5, 10; see below). All 43 subjects completed the first segment of the protocol, involving assessment of their response to a three-cycle stimulus (41 steady-state dim-background trials, 2 off-center dim-background trials; see below); 13 of these subjects also completed a second segment of the protocol, measuring their response to an additional three cycles of stimulus (consecutive dim-background trials; see below) to assess possible transient behavior of the phase marker. An additional two subjects participated in the study, but their data were excluded because of poor core body temperature recordings (subject 1238) and insufficient light levels (subject 1013).

Steady-State Dim-Background Trial Protocol

Each study began with 3 baseline days and nights in the laboratory (Fig. 1A), with subjects scheduled to sleep for 8 h

![Fig. 1. Double raster plots of study protocols. Time of day is plotted on x-axis and successive days of experiment are plotted both beside and beneath each other. Filled bars, scheduled bed-rest episodes; stippled bars, constant-routine (CR) procedures; stippled area, when ambient lighting was 10–15 lx; open boxes, 5 h/day bright-light stimuli; circled X, estimated circadian phase of core body temperature minimum (CBTmin) during 1st constant routine (CR1). During 1st 3 days of all studies, 8-h bed-rest episodes were scheduled at habitual times and subjects were exposed to ~150 lx during their wake episodes. A: steady-state dim-background trial protocol for subjects in group 3. During 3-cycle stimulus application, center of both wake episode and 5-h bright-light stimulus was scheduled 7.5 h after CBTmin, as measured in CR1, and center of 8-h bed-rest episode was scheduled 12 h opposite center of wake episode. B: off-center dim-background trial protocol. During 3-cycle stimulus application, center of wake episode was scheduled 6.5 h after CBTmin, as measured in CR1, and center of 5 h bright-light stimulus was scheduled 1.5 h after CBTmin. Center of 8-h bed-rest episode was scheduled 12 h opposite center of wake episode. C: steady-state dim-background trial protocol for subjects in group 1 (experimental days 1–10) followed by consecutive dim-background trial protocol (experimental days 10–16). During both 3-cycle stimulus applications, center of wake episode and 5-h bright-light stimulus was scheduled 1.5 h after CBTmin, as measured in CR1, and center of 8-h bed-rest episode was scheduled 12 h opposite center of wake episode.]
at their habitual times. During the waking portion of the baseline days, subjects were exposed to ordinary indoor room light (~150 lx). The baseline days were included to ensure that each subject's circadian system had achieved a stable phase and amplitude before assessment. Each subject then began a prestimulus constant routine (CR1) to assess the endogenous circadian phase and amplitude of his core body temperature rhythm (see description below).

After CR1, each subject was assigned to one of the six stimulus groups described below. For each subject, the fitted core body temperature minimum (CBTmin, see below) of CR1 was used as a phase reference marker for determining the timing of the remainder of the protocol. The ending of CR1 was thus timed so that all subsequent protocol events within each stimulus group would fall at an identical phase relative to the prestimulus CBTmin. After an 8-h recovery sleep episode, subjects underwent 3 stimulus days, each consisting of 16 h of wakefulness in dim light (10–15 lx) and 8 h of scheduled bed rest in darkness. During the waking episode, subjects were exposed to a 5-h episode of bright light (7,000–13,000 lx; see description below), which was centered in the middle of the waking day. The protocols for the six stimulus groups were scheduled so that the center of the bright light would fall ~1.5 h after CBTmin (group 1, n = 7), ~3.5 h after CBTmin (group 2, n = 8), ~7.5 h after CBTmin (group 3, n = 6), ~12.0 h after CBTmin (group 4, n = 6), ~16.5 h after CBTmin (group 5, n = 6), or ~22.5 h after CBTmin (group 6, n = 7).

Also, one additional trial was conducted with the bright light centered ~20.5 h after CBTmin. Partial data from the subjects in groups 1 and 6 have previously been published (4, 12).

After three cycles on this schedule, a second constant routine (CR2) was carried out for 40 h. After another 8-h recovery sleep episode, subjects either were discharged from the laboratory (groups 2–5) or underwent the consecutive dim-background trial protocol described below (groups 1 and 6).

Two off-center dim-background trials were conducted using the same protocol and the same very dim background light as that described above for the steady-state dim-background trials, except that the center of the bright light occurred early in the waking day (3 h after wake time) rather than in the center of the waking day (8 h after wake time) (Fig. 1B). The bright light was centered ~1.5 h after CBTmin. The data from those two trials were analyzed separately from the other steady-state dim-background trials in case the timing of the activity or the timing of the dim-background light relative to the bright light affected the magnitude and direction of the phase shifts induced.

Consecutive Dim-Background Trial Protocol

After the steady-state dim-background trial protocol described above (Fig. 1C, experimental days 1–10), six of the seven subjects from group 6 and all seven subjects from group 1 were exposed to another three-cycle schedule at the same clock hours as in their steady-state dim-background trials (Fig. 1C, experimental days 10–16). A third constant routine (CR3) was then carried out for 40–50 h, and this was followed by a final recovery sleep episode of at least 8 h, after which the subjects were discharged from the laboratory.

Constant-Routine Procedure

The constant-routine (CR) procedure was designed to minimize, or distribute evenly across the circadian cycle, factors known to mask the endogenous rhythm of core body temperature (10). Core body temperature was collected throughout all studies at 1-min intervals from a rectal thermistor (Yellow Springs Instrument, Yellow Springs, OH). During the CRs, subjects were kept awake in a constant semirecumbent posture in dim light (10–15 lx) with minimal physical activity allowed, and food was distributed in hourly snacks across the 24-h day. Phase and amplitude of the core body temperature data were estimated by fitting to the data from the CRs a two-harmonic-regression model with first-order autoregressive noise (5). The period of the fundamental component of the model was constrained between 24.0 and 24.3 h (9). In the five cases in which the model estimated a time constant for autoregressive noise that was >2 SD above the mean (time constants >9.06 h), the same two-harmonic-regression model was used without autoregressive noise. Phase (referred to as CBTmin) was defined to be the average of the phases of the minima from the single-harmonic and composite waveforms of the fitted model, and amplitude was defined as half the distance between the maximum and minimum of the first harmonic component of the model.

Light Exposure

All the 5-h bright-light exposures were provided by ceiling-mounted cool-white fluorescent lamps (North American Philips Lighting, Bloomfield, NJ). Before the beginning of each bright-light exposure, ambient room illumination was increased from 10–15 to ~10,000 lx in six linearly graded steps lasting 5 min each. At the end of the 5-h episode, six 5-min steps were used to return the room light levels to 10–15 lx. Ocular light exposure was estimated three times per hour during the 5-h bright-light exposure from a sensor held at the level of each subject's forehead and pointed in the direction of gaze (International Light, Newburyport, MA). Subjects were instructed to gaze at a spot on the wall, at which point they were exposed to ~9,000 lx of light for 10 min of each 20 min during the 5-h bright-light episode. During the other 10 min of each 20-min segment, each subject's gaze was unrestricted. All subjects wore clear ultraviolet-excluding safety glasses (Uvex Winter Optical, Smithfield, RI) throughout each 5-h exposure to bright light.

Data Analysis

Constructing PRCs and phase transition curves. Initial phase was defined to be the time of the center of the bright-light stimulus relative to the time of the prestimulus CBTmin (assigned a value of 0). Phase shifts were defined to be the difference between the time of CBTmin in CR1 and CBTmin in CR2 (or between CR2 and CR3 in consecutive dim-background trials), with phase delays assigned negative values and phase advances assigned positive values, according to convention. Final phase was defined to be the initial phase plus the phase shift observed. Data from all trials were plotted in both PRCs (initial phase vs. phase shift) and phase transition curves (PTCs; initial phase vs. final phase) as described in J. ewett et al. (20). To determine the slope of the PRCs, linear regressions were fitted to the data. The slope of a PTC can be calculated by simply subtracting 1.0 from the fitted slope of the PRC to the same data; the intercept of a PTC is always the same as that of the PRC. Paired two-tailed Student's t-tests were performed to determine the significance of changes in circadian phase and amplitude between CR1 and CR2 and between CR2 and CR3. A one-way analysis of variance was used to compare the phase shifts observed in the six stimulus groups. All results are presented as means ± SD unless otherwise indicated.

Evaluation of transients. To evaluate transients, a linear regression fitted to the consecutive dim-background trial PRC
Fig. 2. Schematic illustration of anticipated results with (A) and without (B) lagging transients in phase marker (CBTmin). Hypothetical phase-response curves (PRCs) to steady-state trials are represented by solid lines, and hypothetical PRCs to consecutive trials are represented by dashed lines. Hypothetical results are shown for steady-state trials centered 5.5 h after CBTmin (●); steady-state trials centered 1.5 h after CBTmin (×); and consecutive trials centered 5.5 h after CBTmin (○).

(initial phases: 4.77–17.49 h) was compared with a linear regression fitted to a similar portion of the steady-state dim-background trial PRC (initial phases: 3.42–16.57 h). Student’s t-tests were used to compare the slopes and intercepts of the two regression lines. As can be seen in the schematic diagram (Fig. 2), if the circadian phase marker (CBTmin) were to show lagging transients in response to the three-cycle bright-light stimulus, we would expect to observe larger phase shifts in consecutive trials than in steady-state trials conducted at the same initial phase. This would result in the consecutive trial PRC (Fig. 2A, dashed line) having a steeper slope than the steady-state trial PRC (Fig. 2A, solid line). If, on the other hand, the marker were not to show transients, we would expect the consecutive PRC (Fig. 2B, dashed line) to be indistinguishable from the steady-state PRC (Fig. 2B, solid line). To illustrate this, consider the following two hypothetical cases in which we compare the phase shifts to a bright-light stimulus centered 5.5 h after CBTmin, in steady-state vs. consecutive trials.

**Case 1. If circadian phase marker were to exhibit transients.** We will look at the hypothetical results of two experiments (Fig. 1, A and C). Assume that a three-cycle stimulus is applied at an initial phase of 1.5 h after CBTmin in the first trial of a consecutive series (hence a steady-state trial) illustrated in Fig. 1C. Suppose that this induces a shift of +6 h in the pacemaker, but a phase shift of only +4 h is observed in the marker because of transients (Fig. 2A, ×). In the following consecutive trial, the stimulus is applied at the same clock hour as it was in the previous steady-state trial, which would appear from the marker to be at an initial phase of 5.5 h after CBTmin (due to prior transients in marker) but would actually occur at 7.5 h after CBTmin in the pacemaker. Suppose that this was to induce a +2 h phase shift in the pacemaker but only a +1.5-h phase shift in the marker. During this consecutive trial, the marker would also recover from the transients to the prior steady-state trial (which occurred ~7 days earlier), so that an additional +2-h phase shift would be observed in the marker, resulting in a total observed phase shift of +3.5 h in the consecutive trial (Fig. 2B, ×). In contrast, if the stimulus were centered 5.5 h after CBTmin in a steady-state trial (Fig. 1A), the pacemaker would be shifted +3 h, with a phase shift of only +2 h observed in the marker because of transients (Fig. 2A, filled circle). Thus the +3.5-h phase shift observed in the consecutive trial at an initial phase of 5.5 h after CBTmin would be 1.5 h larger than the +2-h phase shift observed in a steady-state trial at the same initial phase.

**Case 2. If circadian phase marker were not to exhibit transients.** We will consider the same two experiments discussed above in case 1. In the first experiment, in the steady-state trial that occurs before the consecutive trial, the stimulus (applied at an initial phase of 1.5 h after CBTmin) induces a shift of +4 h in both the pacemaker and the marker (Fig. 2B, ×). In the following consecutive trial, the stimulus is applied at an initial phase of 5.5 h after CBTmin, and induces a +2-h phase shift in both the marker and the pacemaker (Fig. 2B, open circle). In the second experiment, the stimulus is centered 5.5 h after CBTmin, in a steady-state trial, and both the pacemaker and the marker shift +2 h (Fig. 2B, filled circle). This is the same magnitude as the phase shift observed in the consecutive trial conducted at the same initial phase.

Photic sensitivity throughout subjective day. A PRC was constructed using all 56 trials (both consecutive and steady-state trials), and a linear regression was fitted to the entire PRC. To determine whether a dead zone might exist near the crossover (10.6 h after CBTmin) of the PRC to all 56 trials, a linear regression fitted to the data before the crossover (initial phases <10.6 h) was compared with a linear regression fitted to the data after the crossover (initial phases >10.6 h). The Student’s t-test was then used to determine whether the two regression lines projected to different crossover points, which would indicate the presence of a dead zone.

Evaluation of effects of background lighting. To investigate the effects of dim (10–15 lx) vs. moderate (~150 lx) back- ground lighting on the phase shifts achieved with a three-cycle bright-light stimulus, the PRC of the 56 studies reported here (referred to as dim-background trials, including both steady-state and consecutive trials) was compared with an equivalent portion (initial phases between 1.47 h and 22.55 h after CBTmin) of the PRC conducted under ~150-lx background first reported by Czeisler et al. (11) and further analyzed by J ewett et al. (20). The initial phases for the 150-lx-background PRC were determined using the weighted averaging technique described by Czeisler et al. (11). To ensure that the findings reported here were not the result of using this weighted averaging technique to calculate initial phase, PRCs to the 150-lx-background trials were also constructed using the center of the bright-light stimulus for initial phase. Linear regressions were fitted to the dim-background and 150-lx-background PRCs, and the slopes and intercepts of the regression lines were compared using the Student’s t-test.

To determine the effects of the timing of the 150-lx-background light relative to the bright light, the 150-lx-background trials were divided into those in which the 5-h bright-light stimulus was centered near (within ±1.0 h) the middle of the waking day (n = 8, referred to as centered 150-lx trials) and those in which the stimulus was centered 4.74 ± 1.29 h away from the middle of the waking day (n = 14, referred to as off-center 150-lx trials). Linear regressions were then fitted to each data set and compared with the linear regression to the PRC of the dim-background trials using the Student’s t-test.
RESULTS

Steady-State Dim-Background Trials

All stimulus groups except group 4 (light centered ~12.0 h after CBTmin) showed significant phase shifts in CBTmin between CR1 and CR2 (Table 1). No stimulus group showed any significant change in circadian amplitude between CR1 and CR2. The timing of the bright-light stimulus relative to the CBTmin of CR1 had a significant effect (F5,34 = 182.91, P < 0.0001) on the resulting phase shift achieved (Fig. 3, filled circles). The steady-state dim-background trial PRC showed a highly linear relationship between initial and final phase (r² = 0.96, P < 0.0001), with a fitted slope of −0.56 ± 0.02 (equivalent to a PTC slope of 0.44) and an ordinate intercept of 5.87 ± 0.25 h.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Initial Phase, h</th>
<th>Phase Shift, h</th>
<th>Initial Amplitude, °C</th>
<th>Amplitude Change, °C</th>
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</thead>
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<tr>
<td>1</td>
<td>7</td>
<td>1.55 ± 0.12</td>
<td>+4.45 ± 0.94***</td>
<td>0.27 ± 0.03</td>
<td>−0.03 ± 0.04 (NS)</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>3.49 ± 0.05</td>
<td>+4.13 ± 0.53***</td>
<td>0.29 ± 0.08</td>
<td>+0.05 ± 0.07 (NS)</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>7.59 ± 0.38</td>
<td>+1.61 ± 0.74</td>
<td>0.31 ± 0.03</td>
<td>+0.01 ± 0.08 (NS)</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>11.99 ± 0.11</td>
<td>−0.15 ± 0.03 (NS)</td>
<td>0.29 ± 0.11</td>
<td>+0.03 ± 0.00 (NS)</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>16.45 ± 0.07</td>
<td>−3.2 ± 0.71**</td>
<td>0.31 ± 0.06</td>
<td>−0.02 ± 0.06 (NS)</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>22.51 ± 0.03</td>
<td>−7.06 ± 1.29***</td>
<td>0.28 ± 0.12</td>
<td>+0.01 ± 0.07 (NS)</td>
</tr>
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</table>

NS, P > 0.05; *P < 0.004; ***P < 0.0002; ***P < 0.0001.

The phase and amplitude data from the two off-center dim-background trials (Fig. 3, open triangles) were indistinguishable from those of group 1, which had the bright light centered at the same circadian phase (~1.5 h after CBTmin). Data from the off-center dim-background trials were thus pooled with the data from the steady-state dim-background trials for all further analyses.

Consecutive Trials

The consecutive dim-background trials showed no significant change in circadian amplitude and a significant change in circadian phase (P < 0.0007) between CR2 and CR3 (Fig. 3, open circles). The initial amplitudes (0.26 ± 0.06°C) for the consecutive dim-background trials (measured in CR2) were statistically indistinguishable from the initial amplitudes (0.29 ± 0.08°C) for the steady-state dim-background trials (measured in CR1). When plotted in a PRC, the data from the consecutive dim-background trials were also highly linear (r² = 0.90, P < 0.0001), with a fitted slope of −0.53 ± 0.05 (equivalent to a PTC slope of 0.47) and an intercept of 5.79 ± 0.64 h (Fig. 3A, dashed line). The slope and intercept of the regression line fitted to the consecutive dim-background trial PRC showed no significant difference from the slope (~0.55 ± 0.03) and intercept (6.03 ± 0.30) of the regression line fitted to an equivalent portion of the steady-state dim-background trial PRC (r² = 0.94, P < 0.0001; Fig. 3A, solid line). The consecutive dim-background trial data were thus pooled with the steady-state dim-background trial data for all further analyses.

Pooled Data From All 56 Trials

The data from the steady-state, off-center, and consecutive dim-background trials were combined, and a linear regression (r² = 0.95, P < 0.0001) was fitted to the PRC of all 56 trials (referred to below as dim-background trials). The PRC had a fitted slope of −0.56 ± 0.02 (equivalent to a PTC slope of 0.44) and an intercept of 5.89 ± 0.21 h (Figs. 3B and 3A, solid lines). The PRC was highly linear, with a constant negative slope that was significantly different from zero (P < 0.0001). There was no evidence of a dead zone (defined as a portion of PRC with a slope of 0) near the crossover, which occurred 10.6 h after CBTmin. A linear regression (r² = 0.72, P < 0.0001) fitted to the data with initial phases
Dim-Background Lighting vs. 150-lx-Background Lighting

The slope and intercept of the line fitted to the PRC of the dim-background trials were significantly different (P < 0.0001) from the slope (−0.50 ± 0.04) and intercept (4.50 ± 0.55 h) of the linear regression (r² = 0.86, P < 0.0001) fitted to the data with initial phases after 10.6 h (n = 29). Thus the pooled PRC did not have even a small portion of flat slope between the phase advances and the phase delays.

DISCUSSION

Photic Sensitivity Throughout Subjective Day

The data from the steady-state dim-background trials indicate that when a three-cycle bright-light stimulus is applied outside of the critical region (~1.5 to ~22.5 h after CBTmin), highly reproducible phase shifts are achieved without substantial changes in circadian amplitude. As can be seen in Table 1, the intersubject variability in the magnitude of the phase shifts observed in each stimulus group is quite small, relative to the overall estimated error of 1.3 h for the two phase assessment procedures conducted in each trial (23). Because the assessments of phase and amplitude occurred before and after the three-cycle stimulus application, the current study cannot determine whether circadian amplitude was modified during stimulus application outside the critical zone. However, the findings presented here do indicate that if the circadian amplitude was reset by the bright-light stimulus, it had fully recovered by the time it was measured in the poststimulus CR.

In a reanalysis of our 1989 PRC, we found that in those 150-lx-background trials (in which subjects participated in multiple consecutive trials), stimuli applied during hours 0–12 before CBTmin resulted in significantly larger final amplitudes than stimuli applied during hours 12–24 after CBTmin (20). However, in the steady-state dim-background three-cycle trials presented here in which the initial conditions were carefully controlled, this was not the case. In fact, in the present study, no stimulus group showed any significant difference between the prestimulus circadian amplitude measured in CR1 and the poststimulus amplitude measured in CR2. This is most likely due to the fact that, unlike the current study, our 1989 PRC included trials inside the critical region (1.5 h before to 1.5 h after CBTmin) in which circadian amplitude is more sensitive to bright-light stimuli (20). This hypothesis is supported by a repetition of the analysis of final amplitudes from the 1989 PRC using only the trials in which the light was centered 1.48 to 22.55 h after CBTmin (as in the current study). Such analysis (one-tailed Student's t-test) reveals no significant difference in final amplitude between the trials in which the light was centered during the 12 h before the CBTmin and
no evidence of transients in CBT min measured after stimuli applied during the 12 h after CBT min still exists (P < 0.07).

The PRC presented in Fig. 3B indicates that human subjects do not have a dead zone (defined to be a portion of the PRC with a slope of 0) in response to the three-cycle stimulus and instead are sensitive to bright light throughout their subjective day. This finding is consistent with the predictions of a mathematical model of the effects of bright light on the human circadian pacemaker (19, 22). Even near the crossover region in which the PRC changes from phase advances to phase delays, there is no evidence of a dead zone. Instead, the data have a constant negative slope throughout the PRC. Furthermore, a linear regression through the advance portion of the PRC projects to the same crossover point as a linear regression through the delay portion, suggesting that there is no detectable region of flat slope at the crossover. Thus the human circadian system clearly does not have the typical 5- to 10-h dead zone observed in some other organisms (21). However, it remains possible that because the bright-light stimulus was 5 h long and was presented across three circadian cycles, we may have blurred the edges of a very small dead zone of 1 or 2 h, which might exist near the crossover region in which the density of data points is fairly low. Further trials would need to be performed on either side of the crossover region to determine with certainty whether such a very small dead zone exists.

Despite the absence of an observed dead zone, it remains important to identify the circadian phase at which light stimuli do not shift the human circadian pacemaker (i.e., crossover between phase advances and phase delays). Although the three-cycle PRC shown in Fig. 3B crosses the zero-phase-shift line when the bright light is centered 10.6 h after CBT min, this does not accurately reflect the circadian phase of minimum responsiveness, since the three-cycle PRC does not take into account the drift due to a non-24-h circadian period that occurs during the 5 days between the initial and final phase assessments in each trial (12). To correct for this, we calculate that the ~24.2-h period of the human circadian pacemaker (9), together with the ~0.56 slope of the PRC in Fig. 3B, would result in an overall delay in the observed final phases of ~0.7 h. Thus, to offset the effects of circadian period during this study, the entire PRC in Fig. 3 must be shifted up by ~0.7 h. This operation results in a derived crossover of 11.9 h, indicating that when the average drift due to circadian period is taken into account, the circadian phase at which the bright-light stimulus does not induce any phase shift is ~11.9 h after CBT min.

No Evidence of Transients in CBT min Measured After Three-Cycle Bright-Light Stimulus

In all the trials conducted in this study, the fitted minimum of the endogenous core body temperature (CBT min) was used as the phase marker of the underlying circadian pacemaker. Studies in other organisms have indicated that even though the pacemaker itself may shift rapidly in response to a stimulus, the phase markers used may not reflect this phase shift until several circadian cycles later (3, 6, 16, 28–30, 32). Therefore, if the poststimulus CBT min measured in CR2 in our experiments were to display this type of lagging transients, the magnitude of the phase shifts measured in the steady-state dim-background trials (using CBT min as a marker) would be smaller than the actual phase shifts achieved in the pacemaker. During the following consecutive dim-background trials, however, the phase marker would recover from the transients to the previous steady-state dim-background trials, resulting in larger apparent phase shifts in the consecutive dim-background trials than would have been expected from the PRC derived from the steady-state dim-background trials. Thus, if transients existed in our phase marker, CBT min, we would have expected the slope of the line fitted to the consecutive dim-background PRC to be steeper (due to larger phase shifts) than the line fitted to the steady-state dim-background PRC. However, as can be seen in Fig. 3A, the consecutive dim-background PRC and the steady-state dim-background PRC are nearly identical (slopes = 0.~0.53 ± 0.05 and ~0.55 ± 0.03, respectively). This suggests that any transients in CBT min are rapidly dissipated, so that within one to two circadian cycles after the completion of the three-cycle stimulus application, the CBT min reflects the true phase of the underlying pacemaker. This finding suggests that the shape of the PRC we reported in 1989, which was constructed using a mixture of steady-state 150-lx and consecutive 150-lx trials, was not altered by transients in the phase marker during the subjective day (11). However, further studies aimed at this specific question must be conducted to determine whether light stimuli centered in the highly sensitive critical zone also induce minimal transients in CBT min or whether more substantial transients are produced in that region. In addition, potential transients in other markers need to be investigated in human subjects. For example, a study by Hashimoto et al. (15) suggests that bright light applied during the subjective day may induce transients in the offset of plasma melatonin secretion.

Timing of Background Room Light Modulates Resetting Response to Bright Light

When bright light was centered in the middle of the waking day, the level of the background lighting before and after the 5-h bright-light stimulus did not significantly change the magnitude of the phase shifts achieved. As can be seen in Fig. 4, the PRC to the centered 150-lx trials (dashed line) and the PRC to the pooled dim-background trials (solid line) are nearly identical. This indicates that, in the centered 150-lx trials, neither the ~150-lx lighting before and after the 5-h bright-light stimulus nor the ~150-lx lighting during the CRs altered the phase shifts observed. This is in contrast to the results of the off-center 150-lx trials, in which we found that the relative placement of the bright light within the waking day affected the magnitude of the phase shifts induced. As can be seen in Fig.
4, in trials with a background of ~150 lx, the PRC has a much more shallow slope when the bright light was not centered in the waking day (off-center 150-1x trials), with smaller phase advances and a much earlier crossover than when the bright light was centered in the waking day (centered 150-1x trials). This is surprising, given that the initial phases of the 150-1x background trials were calculated using a weighted average of the midpoint of the bright light (weighting = 73%) and the midpoint of the room light (weighting = 27%) to take into account the timing of the background light (11). Recent findings suggest that the relative strength of bright light (~9,500 lx) to room light (~180 lx) is 71 to 29% (4), which is in close agreement with the estimate used. This suggests that the off-center timing of the room light relative to the bright light interacted with the resetting response in a more complicated manner than by simply changing the initial phase of the overall light exposure. The off-center ~150-1x background light seems to have blunted the magnitude of the phase shifts observed, especially in the phase advance region of the PRC. This finding was the same for all the off-center 150-1x trials, regardless of whether the bright light was scheduled early or late in the waking day or whether initial phase was defined to be the center of the bright-light stimulus or a weighted average of both the room light and the bright light. The absence of such an observed effect in the results of the two off-center dim-background trials suggests that this is a photic rather than a behavioral effect (i.e., related to timing of scheduled light exposure rather than timing of sleep-wake cycle), although further studies are required to substantiate this conclusion.

Perspectives

The results of the current study demonstrate that human subjects are sensitive to bright light throughout their subjective day. Furthermore, our findings indicate that these phase shifts occur rapidly, within 2–3 days, without any evidence of transients in core body temperature. For diurnal species, such as humans, the absence of a dead zone may allow the circadian system to rapidly achieve stable entrainment in response to exposure to environmental light stimuli, which typically occur during the subjective day. If the human circadian system had the prolonged period of insensitivity to light during the subjective day that has been observed in many nocturnal species, stable entrainment could take many more cycles to be achieved. Humans are exposed to many different levels of light during the day (7); our data indicate that all of this light exposure contributes to entrainment.

Our findings have important implications for patients who need to receive phototherapy to treat circadian misalignment without interfering with their sleep-wake schedule. Our data suggest that small-to-moderate phase advances (2–3 h in 3 days) can be achieved by exposing patients to bright light during the first 8 h of their habitual waking day. For patients sleeping during the nighttime hours, this can be achieved by using exposure to sunlight as a simple and inexpensive method of phototherapy. In addition, small-to-moderate phase delays can be achieved using exposure to artificial sources of bright light (or sunlight, when available) during the last 8 h of the habitual waking day. Further studies are needed to understand how the timing and level of background lighting might affect these phase shifts induced by bright-light stimuli during the subjective day. However, our data indicate that there are a broad range of circadian phases available in the waking day during which light can be used as an effective method for adjusting circadian phase in humans.

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