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*Am J Physiol Endocrinol Metab* 287:174-181, 2004. First published Mar 23, 2004;  
doi:10.1152/ajpendo.00385.2003

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

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Submitted 25 August 2003; accepted in final form 9 March 2004

**Gronfier, Claude, Kenneth P. Wright Jr., Richard E. Kronauer, Megan E. Jewett, and Charles A. Czeisler.** Efficacy of a single sequence of intermittent bright light pulses for delaying circadian phase in humans. *Am J Physiol Endocrinol Metab* 287: E174–E181, 2004. First published March 23, 2004; 10.1152/ajpendo.00385.2003.—It has been shown in animal studies that exposure to brief pulses of bright light can phase shift the circadian pacemaker and that the resetting action of light is most efficient during the first minutes of light exposure. In humans, multiple consecutive days of exposure to brief bright light pulses have been shown to phase shift the circadian pacemaker. The aim of the present study was to determine whether a single sequence of brief bright light pulses administered during the early biological night would phase delay the human circadian pacemaker. Twenty-one healthy young subjects underwent a 6.5-h light exposure session in one of three randomly assigned conditions: 1) continuous bright light of ~9,500 lux, 2) intermittent bright light (six 15-min bright light pulses of ~9,500 lux separated by 60 min of very dim light of <1 lux), and 3) continuous very dim light of <1 lux. Twenty subjects were included in the analysis. Core body temperature (CBT) and melatonin were used as phase markers of the circadian pacemaker. Phase delays of CBT and melatonin rhythms in response to intermittent bright light pulses were comparable to those measured after continuous bright light exposure, even though the total exposure to the intermittent bright light represented only 23% of the 6.5-h continuous exposure. These results demonstrate that a single sequence of intermittent bright light pulses can phase delay the human circadian pacemaker and show that intermittent pulses have a greater resetting efficacy on a per minute basis than does continuous exposure.

melatonin; circadian pacemaker; photoreception; phase shift

THE DAILY ALTERNATION OF LIGHT AND DARKNESS is the primary synchronizer of endogenous circadian oscillators to the 24-h day. The role of light as a phase-resetting agent of the human circadian pacemaker has been demonstrated in a variety of experiments using single or multiple sequences of continuous bright light exposure (for review see Ref. 17). The phase-shifting effect of bright light on the human pacemaker has been shown to be phase dependent (33). Exposure to several hours of bright light for up to 3 consecutive days has been found to reset the endogenous circadian rhythms of core body temperature, cortisol, melatonin, alertness, performance, and TSH by up to 12 h (1, 9, 12, 14–16, 18, 19, 21, 24, 25, 30, 48–50, 55). Moreover, it has been shown that even room light can significantly reset the phase of the human circadian clock (5, 6). In

fact, Zeitzer et al. (56) found that one-half of the maximum phase delay-resetting response achieved by a single exposure to bright light (~9,000 lux) during the early biological night can be obtained by ~1% of this light intensity, i.e., ordinary room light of ~100 lux.

It has been reported in studies of nocturnal animals that the mammalian circadian system is responsive to brief exposures of light. Nelson and Takahashi (42) demonstrated that pulses of bright light as short as 3 s produced measurable phase shifts in the golden hamster. They also showed that extending the duration of the stimulus beyond 5 min produced little additional phase shift. Furthermore, they found that the shift obtained by two 5-min light pulses separated in time by up to 1 h produced a phase-resetting response similar to that of a single 5-min pulse (43). Others have reported that the circadian system can be reset by a chain of very brief light pulses of 2 ms in mice (51) and 10  $\mu$ s in duration in rat (2). The structure of the physiological system responsible for such a dynamic resetting of the circadian pacemaker is not yet known. It has been hypothesized by Kronauer and colleagues (38, 39) to involve light-sensitive elements that are set to a “used” state after being exposed to light and slowly recover their initial “ready state” after a period of recycling after light removal.

To date there have been few systematic evaluations of the relative effectiveness of different durations of light exposure in the human circadian system (25), although the results of studies on the resetting response to intermittent bright light exposure suggest that humans are also responsive to brief pulses of light. Indeed, 3 consecutive days of exposure to six 40-min pulses of bright light (~5,000 lux) have been shown to phase delay circadian rhythms in humans (3). In other studies, 3–5 consecutive days of exposure to intermittent pulses of bright light (3,000–11,000 lux) as brief as 5 min in duration have been shown to induce phase advances of the endogenous circadian pacemaker in humans (10, 11, 45). These findings suggest that humans, like animals, are sensitive to brief bright light pulses. However, interpretation of the temporal dynamics of the resetting response was complicated by the use of multiple consecutive days of light exposure in those studies.

In the present study, therefore, we quantified the phase-resetting efficacy of a single sequence of intermittent bright light pulses given during the biological night compared with subjects' exposure to continuous bright light or to very dim light at the same circadian phase. On the basis of previous

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results, we anticipated a robust phase delay in the groups of subjects exposed to both continuous (33) and intermittent (45) bright light compared with small drifts in phase in the group of control subjects exposed to continuous very dim light due to the near-24-h period of the human circadian pacemaker (13, 33, 34, 54). In addition, on the basis of a mathematical model of the effect of light on the human circadian pacemaker developed by Kronauer and coworkers (32, 38, 39), we predicted that the resetting effect of the intermittent light exposure would be more efficient than continuous bright light exposure, as measured by the amount of shift induced per minute of bright light exposure.

## MATERIALS AND METHODS

### Subjects

Twenty-one healthy subjects participated in the study; 20 subjects [ $24.3 \pm 3.9$  (SD) yr, 15 males, 6 females, body mass index  $22.8 \pm 2.3$  (SD)  $\text{kg}/\text{m}^2$ ] were included in the analysis due to noncompliance to constant routine procedures by one subject. The subjects had no medical, psychiatric, or sleep disorders as determined by history, physical examination, electrocardiogram, blood and urine biochemical screening tests, and psychological screening questionnaires (Minnesota Multiphasic Personality Inventory and Beck Depression Inventory). A staff psychologist interviewed subjects and those with a history of or a current psychiatric pathology were excluded. Subjects reported that they were not taking any medication and were instructed to abstain from the use of alcohol, nicotine, recreational drugs, and foods or beverages containing caffeine for 3 wk before the study. All participants were drug free at the time of study as verified by a comprehensive toxic analysis conducted upon admission to the laboratory. All experimental procedures were carried out in accordance with the principles of the Declaration of Helsinki, and the protocol was approved by the Human Research Committee at the Brigham and Women's Hospital. Before beginning the protocol, all participants gave informed, written consent.

### Overall Study Design

Subjects were required to maintain a regular 8:16-h sleep-wake schedule at home for  $\geq 3$  wk before admission to the laboratory. To ensure compliance with this protocol, subjects were required to call into a date/time-stamped answering machine just before going to bed and immediately upon awakening, and the times were compared with sleep-wake logs on the day of admission. In addition, wrist activity and light exposure were monitored for 1 wk immediately before admission to the laboratory (Actiwatch-L; Mini Mitter, Sunriver, OR) and were used to verify the stability of their sleep-wake cycle during that last week and throughout the entire inpatient protocol.

Upon admission to the study on experimental *day 1*, subjects were maintained in an environment free from external time cues, including clocks, radios, television, visitors, and sunlight. Subjects maintained contact with staff members specifically trained to avoid communicating time of day or the nature of the experimental conditions to the subjects. Subjects were adapted to the laboratory with 3 baseline days (*days 1–3*), during which time they continued to sleep and wake at their habitual times (Fig. 1). To assess their endogenous circadian phase before the light stimulus and to appropriately center the light stimulus during the following scheduled episodes of wakefulness, subjects underwent a 26.2-h initial constant routine (CR1; procedure described below) from *day 4* to *day 5*. The duration of the constant routine, as well as the associated shift of the *sleep episodes 4* and *5* (see Fig. 1), was designed so that the 6.5-h light exposure session (centered in the middle of the wake period) would coincide with the delay portion of the human phase-response curve (33) and was started 9.05 h before habitual bedtime and ended 2.55 h before habitual wake

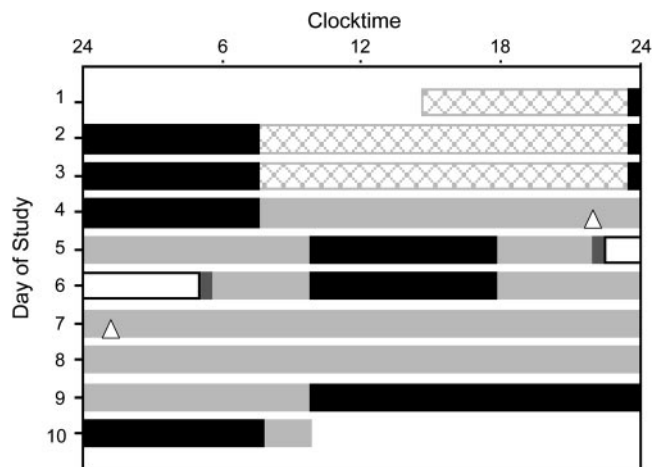


Fig. 1. Raster plot of the 10-day experimental protocol for *subject 19a1*. Scheduled sleep episodes (8 h in darkness) are illustrated as black bars. During baseline days (*days 1–3*), subjects were exposed to  $\sim 90$  lux during wakefulness (16 h). For the remainder of the study, except during the light exposure session, subjects were exposed to  $\sim 1.5$  lux during wakefulness (gray bars). An  $\sim 26.2$ -h constant routine (CR1) was scheduled on *days 4–5*. Light exposure session (white bar) was scheduled on *days 5–6* and consisted of 6.5 h of exposure centered 5.8 h before habitual wake time (i.e., started  $\sim 1.1$  h before habitual bedtime and ended  $\sim 2.6$  h before habitual waketime). After the light exposure day, subjects underwent a 64-h CR (CR2) and were discharged after an  $\sim 22$ -h recovery sleep episode.  $\Delta$ , Dim-light melatonin onsets (DLMO<sub>25%</sub>) in CR1 and CR2. For this subject, the light exposure [continuous bright light (BL) condition] generated a phase delay of 3.15 h in the DLMO<sub>25%</sub>.

time [corresponding to a center at  $\sim 3.5$  h before the subjects' core body temperature minimum (CBT<sub>min</sub>)].

On *day 5*, subjects were randomly assigned to one of the three light exposure conditions (described below). Then, they were scheduled to a final constant routine of 64-h duration (*days 6–9*) to reassess their endogenous circadian phase and thereby estimate the phase shift from the initial constant routine. Subjects were then discharged on *day 10*, after an  $\sim 22$ -h recovery sleep episode.

During wake episodes, subjects were free to move about the suite as desired, except that they were asked not to lie down, nap, or exercise. Subjects' compliance with the protocol was monitored by means of closed-circuit cameras and frequent interaction with technicians.

The experimental suites were equipped with ceiling-mounted cool-white (4,100 K) fluorescent lamps (T8 and T80; Phillips, Eindhoven, The Netherlands). A computer system automatically turned the lighting to the required preset intensity at the scheduled times. Maximum light intensities, as measured vertically at a height of  $\sim 1.83$  m with an IL1400 photometer (International Light, Newburyport, MA), were  $\sim 190$  lux during the waking hours of the 3 baseline days ( $\sim 90$  lux measured horizontally at a height of  $\sim 1.37$  m);  $< 8$  lux ( $\sim 1.5$  lux measured horizontally at a height of  $\sim 1.37$  m) during the constant routines and the day of light exposure; and, depending on the experimental condition (described below),  $< 1$  lux ( $\sim 0.5$  lux measured horizontally at a height of  $\sim 1.37$  m) or  $\sim 9,500$  lux (in the direction of gaze at a wall-mounted target) during the light exposure session. Sleep episodes were conducted in darkness. The experimental suites were equipped with hand-held terminals for online event recording, a porthole for 24-h blood sample collection with minimal sleep disturbance, a video camera, and a voice-activated audio system.

### Constant-Routine Procedure

The constant-routine (CR) procedure was used to assess the phase of two markers of the endogenous circadian pacemaker: the core body

temperature rhythm and the melatonin rhythm. The methodology that we used involved a refinement of a technique first proposed by Mills et al. (41), according to which the subjects are studied under constant environmental and behavioral conditions to eliminate or distribute across the circadian cycle the physiological responses evoked by environmental or behavioral stimuli, such as sleeping, eating, and changes in posture (22). The CR consisted of a regimen of enforced wakefulness in a semirecumbent posture in constant dim illumination of  $\sim 1.5$  lux. Subjects were required to maintain a very low level of physical activity and were not permitted to change their posture throughout the CR. This posture was also maintained for urine samples and bowel movements. Nutritional intake was divided into hourly isocaloric snacks to maintain an equal caloric intake across the circadian cycle. Caloric requirements were calculated with use of the Wilmore nomogram (53) to determine the basal metabolic rate and were adjusted upward by a 10% activity factor. Fluid intake was calculated for each subject to account for the sedentary nature of the CR. A staff member monitored the subject to help maintain wakefulness and to ensure compliance with the posture and activity level requirements.

#### Light Exposure Conditions

Subjects were randomly assigned to one of the three light exposure conditions (Fig. 2). The light exposure session of 6.5 h duration was centered 5.8 h before habitual wake time (i.e., it started  $\sim 1.1$  h before habitual bedtime and ended  $\sim 2.6$  h before habitual waketime) and therefore  $\sim 3.5$  h before  $CBT_{\min}$  (23). The three conditions were as follows: continuous bright light (BL) of  $\sim 9,500$  lux, defined as 100% bright light; intermittent bright light (IBL), consisting of six 15-min bright light pulses of  $\sim 9,500$  lux separated by 60 min of very dim light ( $<1$  lux), defined as 23% bright light; and continuous very dim light (VDL) of  $<1$  lux, defined as 0% bright light. In all three conditions, the 30 min before and the 30 min after the light exposure session were conducted in  $<1$  lux. Seven subjects were randomized to each condition. Subjects were seated in a chair from 2 h before until 2 h after the end of the light exposure session. A technician was present at all times during the light exposure session to ensure compliance with the protocol. During the light exposure sessions, subjects were instructed to fix their gaze on a target mounted on the wall of their suite for 5 of every 10 min and then allowed free gaze for 5 of every 10 min to ensure consistency of light exposure among subjects. This alternating fixed- and free-gaze episodes continued during the entire duration of the light exposure sessions. Light

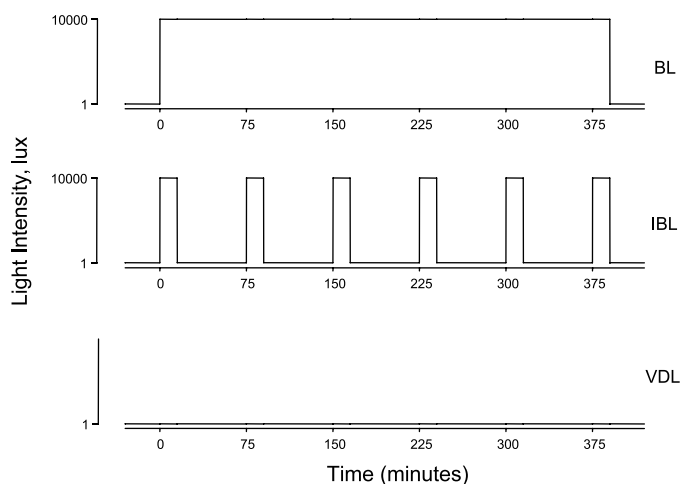


Fig. 2. Light exposure conditions. Subjects were exposed either to 6.5 h of BL ( $\sim 9,500$  lux), intermittent bright light [IBL; six 15-min bright light ( $\sim 9,500$  lux) pulses separated by 60 min of very dim light ( $<1$  lux)], or to very dim light (VDL;  $<1$  lux).

intensity measurements were taken in the direction of gaze every 5 min to ensure that subjects were exposed to the appropriate light intensity for the appropriate duration. Clear polycarbonate lenses filtered 99.9% of the light in the UV range from the light source. In addition, all subjects wore clear goggles (model no. S379, luminous transmittance 90%, UV absorption  $>99\%$ ; Uvex Safety, Smithfield, RI) during the exposure sessions to bright light for additional protection against UV exposure.

#### Data Collection

**Temperature data.** A real-time, online data acquisition system utilizing IBM PC-compatible computers was employed to monitor and collect temperature data. CBT was recorded continuously via a disposable rectal thermistor (Yellow Springs Instrument, Yellow Springs, OH), and room temperature was recorded by means of a permanent air temperature thermistor; both were recorded every minute.

**Hormonal data.** Blood samples were collected every 40 min during constant routines through an indwelling intravenous catheter that was inserted into a forearm vein on day 2 of the study, and every 5 to 10 min during the light exposure session (data will be presented elsewhere). A solution of heparinized saline (0.45% sodium chloride, 10 U heparin/ml) was infused at a slow rate (20 ml/h, 200 IU heparin/h) between blood samples. We provided participants with ferrous gluconate (324 mg) pills to be taken at breakfast and dinner 1) for a minimum of 1 wk before participation in the inpatient research protocol, 2) during the inpatient portion of the protocol, and 3) for 3 wk after completion of the protocol. Subjects' hemoglobin levels were tested every 1–2 days to ensure appropriate levels ( $>11.0$  for men,  $>10.3$  for women). Blood samples were collected in ethylenediaminetetraacetate- $K_2$  tubes and rapidly centrifuged at  $4^\circ\text{C}$ , and the plasma was stored at or below  $-25^\circ\text{C}$  until assayed. Samples were assayed for melatonin by use of radioimmunoassay techniques (Diagnostech/Pharmasan, Osceola, WI). The assay sensitivity was 2.5 pg/ml. The average intra-assay coefficients of variation were 6.4% below 50 pmol/l and 4.9% above.

#### Estimation of Circadian Phase

For temperature data collected during constant routines, a nonlinear least squares dual-harmonic regression model with correlated noise (8) was used. The time of the fitted  $CBT_{\min}$  was defined as the marker of the temperature phase. Data collected during the first 5 h of the CRs were excluded to minimize potential masking effects from the preceding sleep episode or from changes in posture from flat to the semirecumbent CR posture. To minimize uncertainty in the phase estimate, subjects for whom amplitude of the temperature rhythm was below  $0.14^\circ\text{C}$  were not included in the analysis (24) (Table 1). To minimize the effect of interindividual differences in circadian period on the phase shift assessments, only the first circadian oscillation of melatonin and CBT of the final CR was included in the analysis, and phase was estimated on the same time window length as for CR1.

For melatonin data, a three-harmonics least squares regression analysis was applied to data collected during CR1 (Fig. 3, thick line) to estimate the amplitude of each subject's melatonin rhythm (Fig. 3, dashed line). A threshold of 25% of the peak-to-trough amplitude of the fitted curve (54) was calculated (Fig. 3, dotted line) and applied to data of CR1 and CR2 (first circadian oscillation considered). Three markers of melatonin phase were computed for each CR data: 1) the midpoint ( $MP_{25\%}$ ) between 2) the upward dim-light melatonin onset ( $DLMOn_{25\%}$ ) and 3) the downward dim-light melatonin offset ( $DLMOff_{25\%}$ ) crossings of this 25% threshold.

Phase shifts of temperature and melatonin rhythms were calculated as the difference between the phase in CR1 and the phase in CR2.

Table 1. Timing of LES and individual phase shifts of endogenous circadian rhythms of temperature and melatonin in BL, IBL, and VDL exposure groups

Subject Code	Sex	Initial Phase (clocktime)		LES (clocktime)		Center LES Relative to		Phase Shift			
		CBT <sub>min</sub>	DLMOn <sub>25%</sub>	Beginning	End	CBT <sub>min</sub>	DLMOn <sub>25%</sub>	CBT <sub>min</sub>	DLMOn <sub>25%</sub>	MP <sub>25%</sub>	DLMOff <sub>25%</sub>
<b>Condition BL</b>											
19a1	F	4:17	22:57	23:32	6:02	-1.50	3.82	-2.70	-3.15	-2.65	-2.16
2006	M	3:27	20:55	21:57	4:27	-2.25	4.28	-2.45	-2.70	-2.05	-1.40
2012	F	4:51	23:16	23:04	5:34	-2.53	3.04	-3.72	-3.36	-3.31	-3.27
2036	M	6:50	23:26	23:01	5:31	-4.57	2.83	-2.03	-2.92	-2.76	-2.60
2041	F	1:57	20:32	21:32	4:02	-1.17	4.24	-2.48	-2.48	-2.25	-2.03
2043	M	5:23	22:50	23:04	5:34	-3.07	3.48	-4.02	-3.72	-3.54	-3.36
	Median	04:34	22:53	23:03	5:33	-2.39	3.65	-2.70	-3.03	-2.71	-2.38
	Mean±SD	4:28±1:40	22:19±1:16	22:42±0:46	5:12±0:46	-2.51±1.22	3.61±0.61	-2.98±0.85	-3.05±0.45	-2.76±0.58	-2.47±0.76
	SE	00:41	00:31	0:19	0:19	0.50	0.25	0.38	0.18	0.24	0.31
<b>Condition IBL</b>											
19b9	M	8:10	23:51	23:06	5:36	-5.82	2.49	-1.18	-2.12	-2.71	-3.28
19c4	F	4:50	22:22	23:06	5:36	-2.48	3.97	-1.43	-2.88	-2.43	-1.98
2004	M	5:10	0:40	23:01	5:31	-2.90	1.60	-3.55	-2.45	-1.91	-1.37
2024	M	5:31	22:48	22:58	5:28	-3.30	3.42	-1.73	-2.14	-1.64	-1.12
2035	M	5:03	22:12	22:01	4:31	-3.78	3.07	-3.07	-2.98	-2.30	-1.63
19c9	M	6:19	22:09	22:54	5:24	-4.17	4.00	-1.65	-2.28	-1.82	-1.38
2042	F	3:47	23:23	22:01	4:31	-2.52	1.87	-3.65	-2.34	-2.43	-2.52
	Median	5:10	22:48	22:58	5:28	-3.30	3.07	-1.73	-2.34	-2.30	-1.63
	Mean±SD	5:33±1:23	23:04±0:57	22:44±0:30	5:14±0:30	-3.57±1.17	2.92±0.96	-2.32±1.06	-2.46±0.34	-2.18±0.39	-1.90±0.77
	SE	0:31	0:22	0:11	0:11	0.44	0.36	0.40	0.13	0.15	0.29
<b>Condition VDL</b>											
19b1	M	6:17	0:10	23:32	6:02	-3.50	2.61	0.47	-0.33	-0.46	-0.58
19c3	M	6:08	23:39	23:43	6:13	-3.17	3.31	0.42	-0.52	-0.19	0.13
2008	F		21:49	21:58	4:28		3.39		-0.24	0.05	0.34
2018	M	6:06	23:23	23:11	5:41	-3.67	3.04	0.17	-0.29	-0.38	-0.47
2038	M	6:08	22:32	22:24	4:54	-4.48	3.11	1.32	0.06	0.19	0.33
2039	M	5:04	21:40	21:04	3:34	-4.75	2.65	0.98	-1.09	-0.82	-0.55
2056	M	8:10	0:26	0:02	6:32	-4.88	2.84	-0.25			-0.93
	Median	06:08	23:23	23:11	5:41	-4.07	3.04	0.44	-0.31	-0.28	-0.47
	Mean±SD	6:19±1:01	23:06±1:06	22:51±1:05	5:21±1:05	-4.08±0.72	2.99±0.30	0.52±0.56	-0.40±0.39	-0.27±0.37	-0.25±0.51
	SE	0:25	0:25	0:24	0:24	0.29	0.12	0.23	0.16	0.15	0.19

LES, light exposure sessions; CBT<sub>min</sub>, core body temperature minimum; DLMOn<sub>25%</sub>, dim-light melatonin onset; MP<sub>25%</sub>, midpoint; DLMOff<sub>25%</sub>, DLM offset; BL, continuous bright light; IBL, intermittent bright light; VDL very dim light. By convention, phase delays are negative (-) shifts; phase advances are positive shifts. Note that, because of the low amplitude of their CBT rhythms, phase shifts of CBT<sub>min</sub> are not given for subjects 2008 and 2041, and the center of the light exposure sessions relative to CBT<sub>min</sub> is not given for subject 2008. Because of missing blood samples, melatonin MP<sub>25%</sub> and DLMOn<sub>25%</sub> could not be calculated for subject 2056. Centers of the light exposure sessions and phase shifts are given in hours.

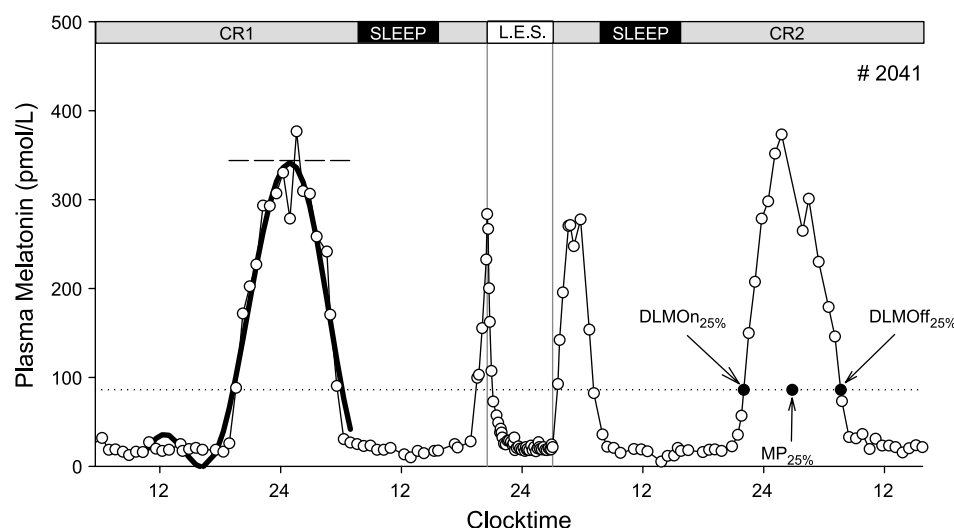


Fig. 3. Method used for melatonin phase assessment using DLMOn<sub>25%</sub>, DLM offset (DLMOff<sub>25%</sub>), and midpoint (MP<sub>25%</sub>) phase markers. Thick line, the 3-harmonics fitted curve; dashed line, the amplitude of the fitted curve; dotted line, 25% of the amplitude. The figure also illustrates the bright light exposure session (L.E.S.)-induced melatonin suppression. The study days corresponding to the profile are given at the top of the figure.

### Statistical Analysis

Repeated-measures ANOVA (condition  $\times$  phase marker) with Greenhouse-Geisser correction (only original degrees of freedom are reported), and Tukey's tests for post hoc multiple comparisons were used to compare phase shifts between phase markers, with  $H_0: \mu_{DLMO_{25\%}} = \mu_{MP_{25\%}} = \mu_{DLMO_{off25\%}} = \mu_{CBT_{min}}$ . Pearson correlations were calculated between phase shifts and initial phases of light exposure, between  $CBT_{min}$  and melatonin phase shifts, and between phase shifts of the melatonin phase markers ( $DLMO_{25\%}$  and  $DLMO_{off25\%}$ ).

### RESULTS

The light stimulus was, on average, centered  $3.15 \pm 0.72$  h after  $DLMO_{25\%}$  and  $3.39 \pm 1.28$  h before  $CBT_{min}$  [i.e., it started on average 5 min ( $0.09 \pm 0.72$  h) before  $DLMO_{25\%}$  and ended 5 min ( $0.09 \pm 1.56$  h) after  $CBT_{min}$ ; Table 1]. There were no significant differences among the three groups in the initial phases of the temperature and melatonin rhythms at which the light intervention occurred. There were minimal changes in phase observed in the control group of subjects exposed to VDL (median phase change of  $+0.44$  h for  $CBT_{min}$  and  $-0.31$  h for  $DLMO_{25\%}$ ; Fig. 4). A significantly greater phase delay was observed in subjects exposed either to BL [median phase delay =  $-2.70$  h for  $CBT_{min}$  ( $P < 0.0002$  vs. controls),  $3.03$  h for  $DLMO_{25\%}$  ( $P < 0.0002$  vs. controls),  $-2.71$  h for  $MP_{25\%}$  ( $P < 0.0002$  vs. controls), and  $-2.38$  h for  $DLMO_{off25\%}$  ( $P = 0.0003$  vs. controls)] or to IBL [median phase delay =  $-1.73$  h for  $CBT_{min}$  ( $P < 0.0003$  vs. controls),  $-2.34$  h for  $DLMO_{25\%}$  ( $P < 0.0002$  vs. controls),  $-2.30$  h for  $MP_{25\%}$  ( $P < 0.0002$  vs. controls), and  $-1.63$  h for  $DLMO_{off25\%}$  ( $P = 0.001$  vs. controls)]. As expected for the selected phase of light exposure, the BL and IBL groups demonstrated phase delays of their endogenous circadian rhythms of core body temperature and melatonin (Fig. 4).

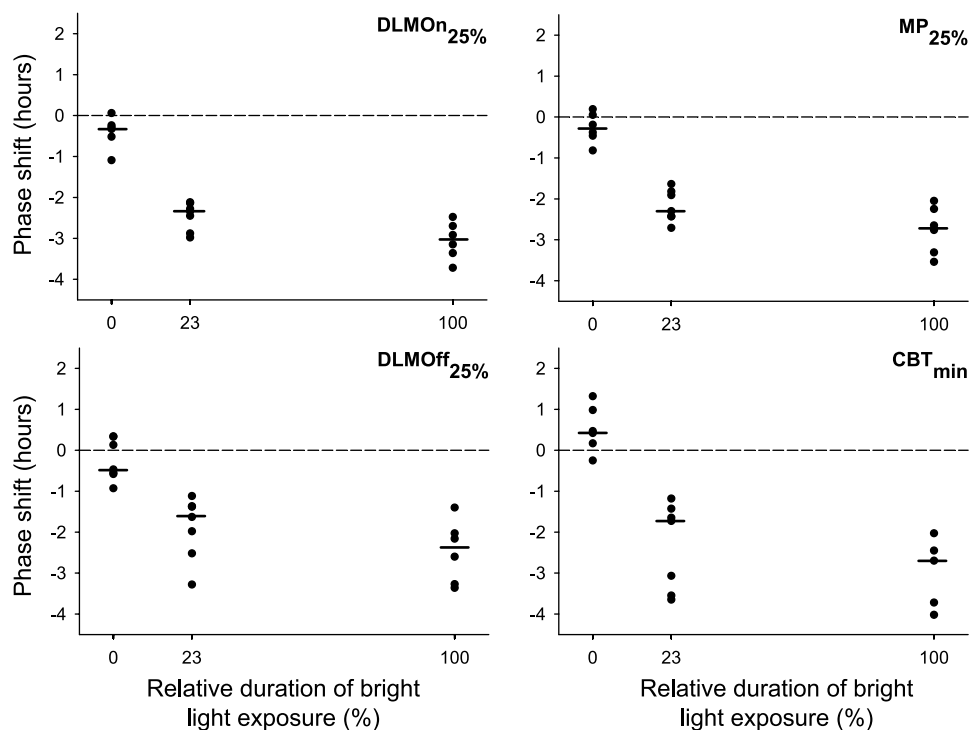
Although the phase shifts observed in the IBL group were slightly smaller than those observed in the BL group, no statistical differences were found for any marker (Fig. 4).

The resetting responses measured with  $CBT_{min}$  were more variable than those measured with melatonin (Table 1); however, we found highly significant and positive linear relationships between the markers of the two rhythms ( $r_{CBT_{min}/DLMO_{25\%}} = 0.896$ ,  $r_{CBT_{min}/MP_{25\%}} = 0.871$ , and  $r_{CBT_{min}/DLMO_{off25\%}} = 0.779$ ;  $P \leq 0.0001$  for all three correlation coefficients).

We found no significant linear correlation between observed melatonin phase shifts and the initial phases of light exposure ( $\phi_{init}$ ) within the limited range of initial phases to which subjects were exposed within each condition ( $r_{\phi_{init}/DLMO_{25\%}} = 0.182$ ,  $r_{\phi_{init}/MP_{25\%}} = 0.071$ ,  $r_{\phi_{init}/DLMO_{off25\%}} = 0.031$ ;  $P$  nonsignificant for all 3 correlation coefficients).

In appraising the variability of the phase shift data presented here (Table 1), we recognize three independent variability sources whose variances should sum: 1) noise in the individual phase assessments whose difference comprises the phases shift, 2) intersubject differences in the endogenous circadian period that lead to different phase drifts over the two cycles of shift assessments, and 3) intersubject differences in photic response arising either from sensitivity differences or from differences in the timing of the stimulus. Klerman et al. (36) found that the method of single-phase assessment yielding the lowest variance was a normalized level crossing on the rising front of the melatonin pulse. This variance could be as low as  $0.04 \text{ h}^2$  (50th %ile) so that, for phase shifts, the variance might be  $0.08 \text{ h}^2$ . For our results, the  $DLMO_{25\%}$  does indeed give consistently the lowest variance estimates. Czeisler et al. (13) estimated the standard deviation of circadian period for young adults to be  $0.13$  h so that a two-cycle phase drift would show a variance of  $0.07 \text{ h}^2$ . We recognize that variance estimates are extremely unreliable for data groups of six or seven members.

Fig. 4. Individual resetting responses to VDL (0%), IBL (23%) and BL (100%) exposure conditions, measured with core body temperature minimum ( $CBT_{min}$ ) and melatonin phase markers  $DLMO_{25\%}$ ,  $MP_{25\%}$ , and  $DLMO_{off25\%}$ . Phase-advance shifts are plotted as positive values, whereas phase-delay shifts are plotted as negative values. Horizontal bars, median phase shift observed in each condition.



However, the value of  $0.15 \text{ h}^2$  for the VDL condition is consistent with the sum of  $0.08$  and  $0.07 \text{ h}^2$  for sources 1 and 2 described above. The variance of  $0.20 \text{ h}^2$  for the BL condition suggests that perhaps as little as  $0.05 \text{ h}^2$  might be ascribed to source 3. However, by this reasoning, the variance of  $0.12 \text{ h}^2$  for the IBL condition is inexplicably small and may represent a remarkably homogeneous subject group with little difference in endogenous circadian period.

## DISCUSSION

The present results demonstrate that a single sequence of IBL exposure is effective in inducing phase delays of the human circadian timing system. The phase delays observed after IBL exposure were comparable to those measured after BL exposure (Fig. 4), even though the BL represented only 23% of the 6.5-h light exposure session. Given that the median phase delay measured with  $\text{DLMO}_{25\%}$  was  $-3.03 \text{ h}$  under BL condition (6.5 h of BL), and  $-2.34 \text{ h}$  in the IBL (1.5 h of bright light) condition, the phase-resetting ability of IBL exposure, when the reference  $-0.31\text{-h}$  phase shift measured in the control VDL condition was taken into account, was 3.2 times greater than that of BL exposure on a per minute basis of BL exposure.

The small change in phase observed in the control group may be attributed to the longer-than-24-h period of the circadian pacemaker (13) and the potentially weak entraining effect of nonphotic synchronizers (37). We conclude that the additional 2.06-h average change in phase ( $\text{DLMO}$ ) observed in the IBL group compared with the control group represents the direct effect of the exposure to IBL on the circadian pacemaker.

On the basis of previous studies (3, 10, 45), we expected a robust phase delay for both BL and IBL exposure. Indeed, the dynamic-resetting model developed by Kronauer et al. (32, 38, 39) predicted that the phase shift after IBL would be  $\sim 70\%$  of that after BL. In fact, we found that IBL was 69% as effective in shifting  $\text{CBT}_{\min}$ , 74% as effective in shifting  $\text{DLMO}_{25\%}$ , and 83 and 61% as effective in shifting  $\text{MP}_{25\%}$  and  $\text{DLMO}_{\text{off}25\%}$ , respectively, compared with BL. For all markers studied, the shifts after IBL exposure were not significantly different from those after BL exposure, and phase delay shifts after IBL and BL exposures were significantly greater than those after VDL exposure.

The mathematical model developed by Kronauer and colleagues (32, 38, 39) predicts that there is a greater activation rate (a measure of resetting effectiveness of light) at the very beginning of a light pulse. As activator elements are depleted, the activation rate becomes limited by the rate at which elements are recycled. This model is consequently characterized by two rate parameters: a forward rate,  $\alpha$ , that depends on light intensity,  $I$ , but is fixed at any given intensity, and a backward rate,  $\beta$ , that is independent of intensity. For any given  $I$ , at least two different temporal patterns are needed to estimate  $\alpha$  and  $\beta$  from the response data (38, 39). For  $I \cong 10,000 \text{ lux}$ ,  $\alpha$  and  $\beta$  were estimated to be  $\sim 15 \text{ min}^{-1}$  and  $90 \text{ min}^{-1}$ , respectively.

For a periodic stimulus pattern consisting of alternating light and dark episodes, we denote the fraction of cycle time that the light is "on" as the "duty cycle." If a window of time is available for stimulation, the maximum response will be generated by using light the entire time (duty cycle = 1). However,

most of this light is used inefficiently since after a time  $t \cong \alpha^{-1}$ , the incremental response is limited by the recycle rate and consequently is low. The maximally efficient use of a stimulus (i.e., maximum response per minute of stimulus) is found when the duty cycle is very low, but then the total response is also low. Intermediate duty cycles effect a compromise: less than maximum efficiency but total response not a great deal below maximum.

The present experiment was designed with several objectives. The first was to expose subjects to a single sequence of bright light pulses, as opposed to multiple sequences of exposure over several days. A second was to extend the range of the duration of near darkness from the former range of 20–45 min to 60 min. A third was to study a short duty cycle of 0.23. Both of these changes in intermittent light patterns, besides improving the accuracy of rate estimate, increase the practical usefulness of intermittent light intervention. Our fourth goal was to validate Kronauer's mathematical model of resetting efficacy of intermittent bright light (32, 38, 39); simulations based on existing rate estimates indicated that the pattern of light exposure used in this experiment would produce  $\sim 70\%$  of the resetting drive onto the circadian pacemaker achieved by continuous bright light.

These results are in agreement with animal studies that reported that the resetting action of light was most efficient at the beginning of the light exposure and that minimal additional phase shift was produced by further extension of the light stimulus (2, 42, 43, 51) due to a reduction in photic responsiveness of the circadian pacemaker (35).

It is now recognized that the visual photopic and scotopic systems are not the only photosensitive structures in the retina. Recent studies in mammals have identified a subset of retinal ganglion cells (RGCs) that are intrinsically photosensitive and are involved in conveying photic input to the circadian system (4, 26, 28, 44, 46). These cells contain a photopigment, melanopsin, that appears to have a broad role in nonvisual photoreception (27). Although the involvement of cryptochromes in phototransduction to the circadian system has been suggested (52), recent evidence suggests that these nonvisual (melanopsin-containing RGCs) along with the classical visual (rod and cones) photoreceptive systems account for all non-image-forming functions involved in pupillary light reflex and circadian photoentrainment (29, 40, 44).

Given the unusual light responses of the melanopsin-containing RGCs (4), it is possible that these cells embody the process L, proposed in the model of Kronauer et al. (38, 39), that intervenes between light and the pacemaker oscillator (process P). Process L can itself be parsed into two functions. One is a dynamic system in which activation elements are used and subsequently recycled. The other function is a simple power-law relation between the strength of light and the utilization rate parameter. The exponent for the power law inferred for human phase shift data is proportional to the 0.6 power of light intensity. In their report, Berson et al. (4) include data on the acute (1 s average) intracellular depolarization of a ganglion cell as a function of irradiance at several wavelengths of light. A plausible representation of the irradiance dependence is a power law with an exponent of 0.6 over a range of 1.5 to 2 log units (from threshold to saturation). It is possible that these ganglion cells might also embody the dynamic system portion of process L, but the data presented thus far do not cover the

temporal range required either to support or to reject this possibility. The response dynamics of rods and cones to light make them unlikely to embody process L. Further investigations, however, including exposures to intermittent light of different duty cycles and different wavelengths, are required to clarify this point.

These findings have important implications, as they provide a greater understanding of the effects of brief episodes of light on the human circadian timing system and suggest that the intermittent exposure to bright light that occurs in everyday life (47) may have a much greater impact on circadian entrainment than previously recognized. The present results also indicate that brief episodes of light exposure may be a cost- and time-effective way of resetting the circadian timing system in humans. Such strategies could be used not only to treat circadian misalignment related to the low light intensities (20, 54) associated with space missions, but also, as shown in the field setting, in which subjects are exposed to conflicting cues, to treat circadian misalignment associated with transmeridian travel, shiftwork (3, 7, 10, 31), and circadian sleep disorders such as advanced and delayed sleep phase syndrome.

#### ACKNOWLEDGMENTS

We thank the subject volunteers and subject recruiters, the laboratory technicians involved in data collection, and Dr. S. W. Lockley for comments on an early version of the manuscript.

#### GRANTS

The work described in this article was supported by Grant NAG 5-3952 from the National Aeronautics and Space Agency, and by General Clinical Research Center Grant GCRC-M01-RR-02635 from the National Center for Research Resources.

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