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Repeated exposures to daytime bright light increase nocturnal melatonin rise and maintain circadian phase in young subjects under fixed sleep schedule

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Takasu, Nana N, Satoko Hashimoto, Yujiro Yamanaka, Yusuke Tanahashi, Ayano Yamazaki, Sato Honma, and Ken-ichi Honma. Repeated exposures to daytime bright light increase nocturnal melatonin rise and maintain circadian phase in young subjects under fixed sleep schedule. *Am J Physiol Regul Integr Comp Physiol* 291: R1799–R1807, 2006. First published July 13, 2006; doi:10.1152/ajpregu.00211.2006.—Effects of two different light intensities during daytime were examined on human circadian rhythms in plasma melatonin, core body temperature, and wrist activity under a fixed sleep schedule. Sleep qualities as indicated by polysomnography and subjective sleepiness were also measured. In the first week, under dim light conditions (~10 lx), the onset and peak of nocturnal melatonin rise were significantly delayed, whereas the end of melatonin rise was not changed. The peak level of melatonin rise was not affected. As a result, the width of nocturnal melatonin rise was significantly shortened. In the second week, under bright light conditions (~5,000 lx), the phases of nocturnal melatonin rise were not changed further, but the peak level was significantly increased. Core body temperature at the initial sleep phase was progressively elevated during the course of dim light exposure and reached the maximum level at the first night of bright light conditions. Subjective sleepiness gradually declined in the course of dim light exposure and reached the minimum level at the first day of bright light. These findings indicate that repeated exposures to daytime bright light are effective in controlling the circadian phase and increasing the peak level of nocturnal melatonin rise in plasma and suggest a close correlation between phase-delay shifts of the onset of nocturnal melatonin rise or body temperature rhythm and daytime sleepiness.

light intensity; core temperature; sleepiness; polysomnography; entrainment

BRIGHT LIGHT is known to reset human circadian rhythms. Repeated exposures to bright light at 24-h intervals were shown to entrain free-running activity rhythms in subjects under temporal isolation (21), and a single pulse of bright light in the subjective morning produced phase-advance shifts and, in the subjective evening, yielded phase-delay shifts (19, 24, 31). The magnitude as well as the direction of phase shift was a function of the circadian phase in which a light pulse was given, which was expressed as a phase-response curve. The magnitude of a light-induced phase shift seems to be positively correlated with light intensity (5, 46). Generally, daytime bright light exerts small effects on the phase of circadian rhythms. In mammals, bright light affects the free-running period of circadian rhythm (1). However, in humans, the free-running period under temporal isolation was not much changed by changing light intensities from 0.5 to 1,000 lx (45). On the other hand, the circadian periods in blind subjects were significantly shorter than in sighted subjects (45). Besides the effects on the circadian rhythm, bright light at night was reported to suppress the nocturnal melatonin rise and decrease subjective sleepiness (4, 9, 29). Bright light also may influence thermoregulation (7, 13).

Bright light in daytime was reported to affect the nocturnal melatonin rise in humans. Hashimoto et al. (16) demonstrated significant phase-advance shifts of the onset as well as the peak of nocturnal melatonin rise in young subjects who were exposed to daytime bright light (~5,000 lx) for 3 days under temporal isolation. As a result, the area under the curve (AUC) of the nocturnal melatonin rise was increased. Park and Tokura (37) also reported significant increase in the amplitude of urinary melatonin rhythm in young subjects exposed to midday bright light (~5,000 lx) for 2 days. More recently, Mishima et al. (32) demonstrated a significant increase in the nocturnal melatonin rise and improvement of sleep quality in elderly residents at nursing homes after repeated exposures to midday bright light (~2,500 lx) for 4 wk. These findings suggested that daytime bright light increased plasma melatonin production. On the other hand, timed melatonin intake is known to facilitate nocturnal sleep in healthy subjects (3, 42, 47) and improves nocturnal sleep in elderly insomniacs (14, 15). Therefore, it is temping to postulate that enhanced nocturnal melatonin secretion by daytime bright light improves sleep quality. Bright light is known to stimulate the sympathetic nerves for a prolonged period (35, 36, 40), which may explain, at least in part, the elevation of nocturnal melatonin peak by daytime bright light. The purpose of the present study was to examine whether daytime bright light increases nocturnal melatonin rise and improves sleep quality in young healthy subjects under a strict sleep schedule.

METHODS

Subjects and Facility

Eight healthy male subjects (20–29 yr old) participated in this experiment as paid volunteers. General status of subjects was checked with a routine medical examination. None of the volunteers reported any sleep disorders. Subjects who had been abroad or had a job in the early morning or late night within 4 wk before the experiment were excluded. The subjects gave written informed consent. This study was approved by the ethical committee of Hokkaido University Graduate School of Medicine.

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The experiment was carried out in a living facility for temporal isolation. The isolation facility consists of four units shielded by sound- and light-proof materials and is air conditioned. Each unit was equipped with a bed, desk, comfortable chair, television, compact disc player, kitchen, and bathroom, as described elsewhere (21). Each subject stayed in a unit throughout the experiment. The room temperature and humidity were controlled within a narrow range (24 ± 2°C, 60 ± 10%). The main light source (fluorescent lighting) was fixed on the ceiling. The light was turned off during the sleeping period when the light intensity was under the limit of measurement (0.01 lx). The auxiliary dim light was equipped on the bed, bathroom, and desk. In the facility, the subjects were not allowed to use timing devices such as a watch, television, or radio.

Experimental Protocols

The protocol of the experiment is illustrated in Fig. 1. In the facility, the subjects were instructed to strictly follow the daily schedule consisting of an 8-h sleeping period and a 16-h waking period. During the waking period, they were exposed to light of two intensities, ~10 lx for the first 6 days (dim light conditions) and ~5,000 lx for the next 7 days (bright light conditions), except for a period of serial blood sampling (~10 lx). The light intensities were measured at the eye level of standing subjects. The subjects were instructed to keep a regular 8-h sleep schedule (0000 to 0800) for 2 wk before the experiment. The nocturnal melatonin levels in plasma were measured 1 wk before the experiment, and the peak phase was calculated. To make a phase relationship between sleep and circadian rhythms the same among the subjects, we determined the individual timings of lights off (bedtime) and lights on (waking up) in such a way that the peak of nocturnal melatonin rise should be located in the middle of the sleeping period. As a result, the bedtime was different from subject to subject (2300 in 1 subject, 0000 in 2 subjects, and 0100 in 5 subjects). Therefore, the results are illustrated in reference to zeitgeber time (ZT), where the bedtime was defined as ZT = 0. The melatonin peak was at ZT4.

The experiment started at 1700 in local time on the first day (defined as *day 1*). The previous night, a baseline measurement of sleep EEG was conducted at the facility. In the morning, the subjects returned home and came back to the facility again on the same day. The first serial blood sampling was performed from 1800 on *day 1* to 1800 on *days 2* (*days 1* and *2*). On the last day under the dim light conditions (*days 6* and *7*), the second serial blood sampling was conducted beginning at 1800 for 24 h. From the morning of the following day, the light condition was changed from dim to bright. On the last day under the bright light conditions (*days 14* and *15*), the third serial blood sampling was carried out from 1600 on *day 14* to 1800 on *day 15* (Fig. 1).

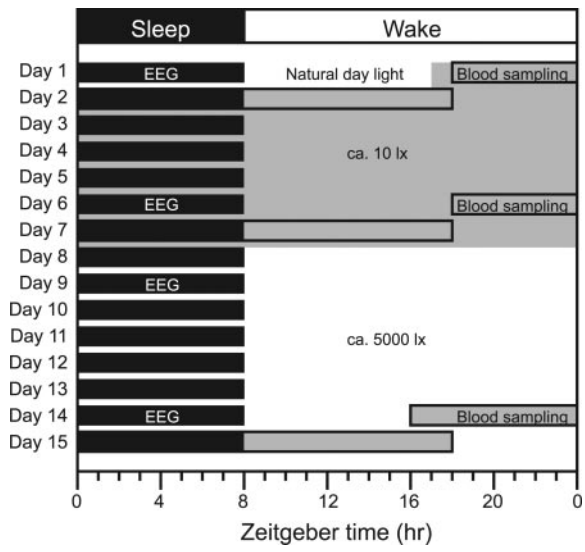

Fig. 1. Experimental protocol. Zeitgeber *time 0* (ZT0) represents the time that subjects went to bed. The shaded zone indicates dim light conditions (~10 lx), and the open zone indicates bright light conditions (~5,000 lx).

The subjects were awakened by a phone call. They were instructed to conduct performance tests and complete questionnaires every 2 h from lights on to 2 h before lights off. They took nutritionally balanced meals three times per day at 1, 7, and 13 h after waking up. The total calories were fixed to ~2,500 kcal/day. They took a shower at 12 h after waking up. In their free time, they were allowed to read books, listen to music, and watch videos. Physical exercise and napping were not permitted. The present experiment was performed from March to July.

Measurements

Plasma melatonin. Serial blood sampling for melatonin determination was performed at 1-h intervals for at least 24 h through an indwelling catheter with a heparin lock placed in the forearm (18). Blood sampling was performed under ~10 lx during the waking period and under <0.01 lx during the sleeping period with the aid of dim red light. Immediately after sampling, blood was centrifuged to separate plasma and frozen at −40°C until assay. Plasma melatonin was determined by radioimmunoassay (20). The sensitivity of the assay was 1.56 pg/ml, and the intra- and interassay variances were 6.6 and 7.0%, respectively.

Core body temperature. Rectal temperature was continuously measured by a thermistor probe with a line connected to a computer, except for short removals for defecation and bathing. The temperature data were fed into a computer at 10-min intervals.

Polysomnography. Sleep EEG were recorded continuously during the 8-h sleeping period on *day 1* (baseline), *day 6* (dim light conditions), *day 9* (bright light conditions), and *day 14* (bright light conditions). Sleep EEG on *day 1* were recorded just before the day of admission. Waking EEG were recorded during the waking period on *days 3*, *5*, *8*, and *13*.

Wrist activity. Wrist activity was measured continuously with a piezoelectric omnidirectional activity counterattached to the nondominant wrist (ACTIWATCH; Mini-Mitter, Bend, OR). The measurement was started from 2 wk before and ended 2 wk after the experiment, except for short removals for bathing. Wrist activity data were fed into a computer at 1-min intervals. The activity data and sleep diary were used to verify the subject's sleep-wake pattern.

Questionnaires for exhaustion and sleepiness. Subjective sleepiness and tiredness were evaluated with the Questionnaire for Subjective Symptoms in 2002 (QSS2002) (41) and the Visual Analog Scale (VAS) for sleepiness (33), which were conducted every day at 2-h intervals during the waking period. QSS2002 consists of five categories, and each category has five questions. The five categories are related to sleepiness, anxiety, drowsiness, tiredness, and visual symptoms. Each question is evaluated on a range from 1 (very alert) to 5 (very sleepy). Sleepiness is also evaluated from 0 (very alert) to 100 (very sleepy) with VAS (100 mm). In addition, computer-based performance tests (~10 min) were conducted on every occasion that questionnaires were given.

Data Analysis

The onset, peak, and end phases of nocturnal melatonin rise were determined geometrically as reported previously (18). The original melatonin rhythm was smoothed by a three-point moving average method. The amplitude of melatonin rhythm was defined as the difference between the peak value and the daytime baseline level at around 1600. The onset and end phases of nocturnal melatonin rise were defined as the points where the line of 20% amplitude crossed the ascending and descending limbs, respectively. The 20% amplitude

was determined using the melatonin rhythm on *days 1* and *2*. The peak phase was defined as the midpoint of the ascending and descending phases. The AUC of melatonin rhythm was defined as the area above the daytime baseline level. The melatonin data on *days 1* and *2* from one subject were missed because of technical failure and were excluded from the analyses.

Sleep EEG were scored according to Rechtschaffen and Kales's criteria (39). Time in bed (TIB) was defined as the time lying on the bed. Sleep latency (SL) was the time from lights off to the first appearance of stage 1 sleep (ST1), which is followed by stage 2 sleep (ST2) (sleep onset). Stage 3 sleep (ST3), stage 4 sleep (ST4), and rapid eye movement (REM) sleep were classified. REM latency (RL), the time from sleep onset to the first appearance of REM, wake after final waking (WAFA), the time from final awakening (sleep end) to the time of getting up, sleep period time (SPT), the time from sleep onset to sleep end, wake after sleep onset (WASO), the wake time during SPT, movement time (MT), total sleep time (TST), the WASO time subtracted from SPT, and sleep efficiency (SE), the ratio of TST to SPT, were all calculated. Power densities in the α (8.0–12.75 Hz) and θ (4.0–7.75 Hz) range of waking EEG were measured at 2-h intervals with eyes opened for 10 min and with eyes closed for 10 min.

Accumulated wrist activity was calculated separately in the sleeping and waking periods. They were averaged individually for 2 days immediately before the experiment and during the dim light (from *day 4* to *day 5*) and bright light conditions (from *day 11* to *day 12*).

Statistics

Friedman two-way analysis of variance and a post hoc Wilcoxon signed-rank test were used for the comparison between two conditions and during the course of experiment. Spearman's ranks correlation analysis was used to examine the correlation of two parameters.

RESULTS

Circadian Parameters of Plasma Melatonin Rhythm

Figure 2 illustrates the plasma melatonin rhythms in each of the subjects on *days 1* and *2* and the last days under the dim (*days 6* and *7*) and bright light conditions (*days 14* and *15*). The individual melatonin rhythms were smoothed. The averaged rhythms of seven subjects are depicted in Fig. 3. Friedman testing revealed significant differences among the times of day ($P < 0.001$) and between the light conditions ($P < 0.001$).

Table 1 demonstrates the circadian parameters of melatonin rhythm ($n = 7$). The amplitude of nocturnal melatonin rise was not changed under the dim light conditions, whereas it was significantly increased under the bright light conditions compared with the dim light conditions (Wilcoxon, $P < 0.05$). The onset phase was significantly delayed under the dim light (Wilcoxon, $P < 0.05$) and bright light conditions (Wilcoxon, $P < 0.05$) compared with *days 1* and *2*. There was no difference in the phases of melatonin rhythm between the dim and bright light conditions. On the other hand, the end phase was not significantly different among three rhythms. The width of nocturnal melatonin rise was significantly shortened under the dim light (Wilcoxon, $P < 0.05$), and bright light conditions (Wilcoxon, $P < 0.05$) compared with *days 1* and *2*. There was no significant difference in the AUC among three rhythms.

Circadian Rhythms in Core Body Temperature

Figure 4*A* illustrates the averaged circadian rhythms ($n = 8$) in core body temperature on *days 2* and *3*, *days 7* and *8*, *days 8* and *9*, and *days 13* and *14*. The days were selected to represent effects of each light condition. They were not actu-

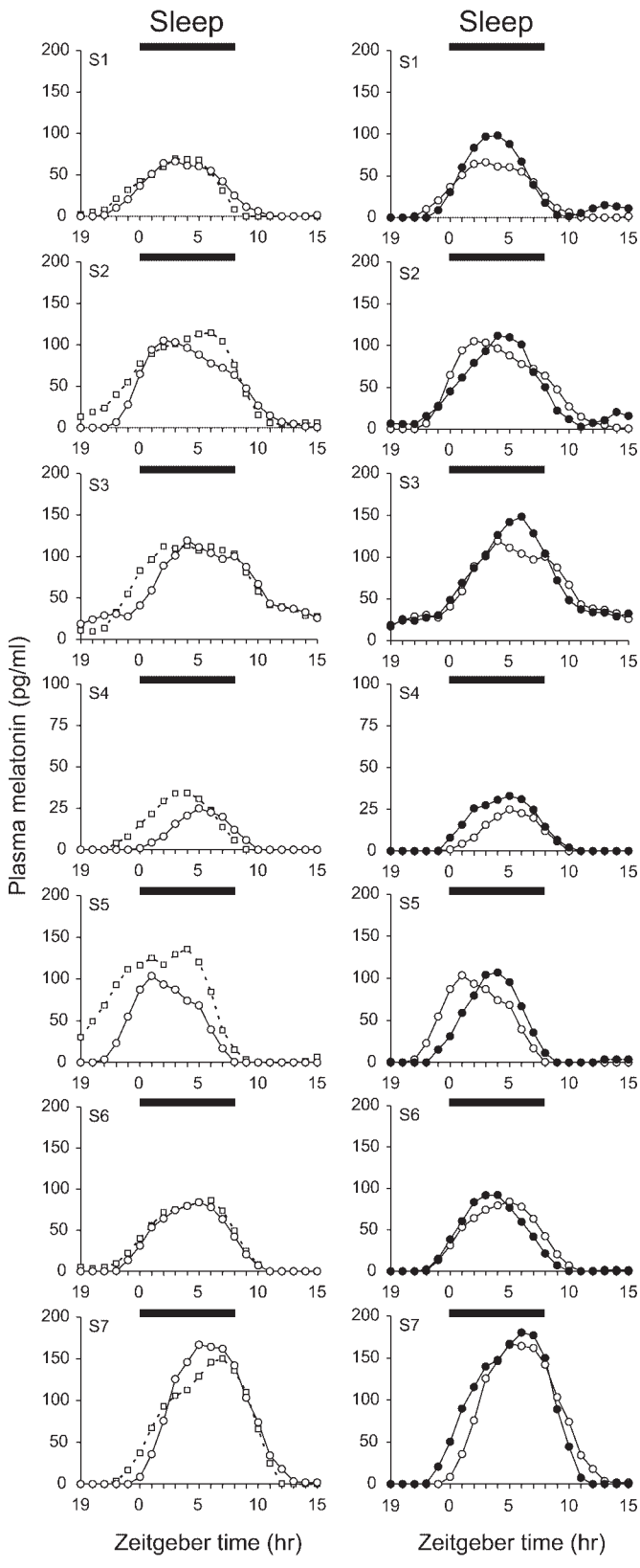

Fig. 2. Plasma melatonin rhythms in individual subjects ($n = 7$) on *days 1* and *2* (□, dashed line), *days 6* and *7* (○, solid line), and *days 14* and *15* (●, solid line). The abscissa indicates the time of day, where the time the subject went to bed is defined as ZT0. Solid horizontal bars indicate sleeping period.

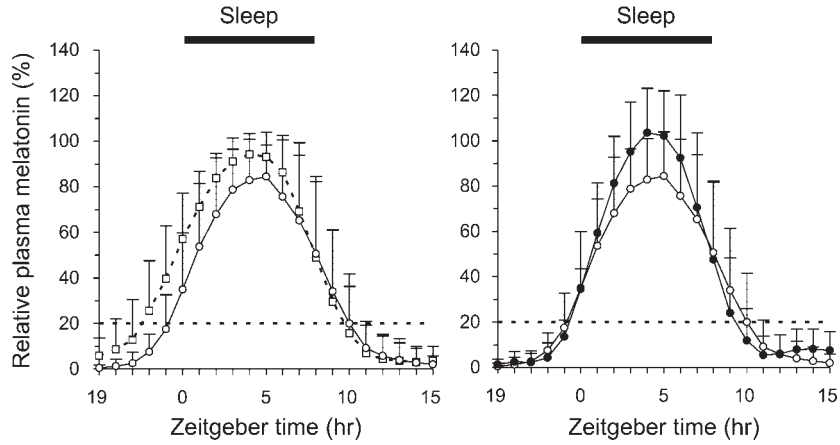

Fig. 3. Averaged melatonin rhythms on *days 1* and *2* (□, dashed line), *days 6* and *7* (○, solid line), and *days 14* and *15* (●, solid line). The abscissa indicates the time of day, where the time that subjects went to bed is defined as ZT0. Solid horizontal bars indicate sleeping periods. The melatonin values are standardized as mentioned in text. Dotted lines in each graph represent the 20% baseline. Values are expressed as means ± SD; $n = 7$.

ally the first and last days of each condition to exclude possible masking effects of blood sampling. Friedman testing revealed significant differences among the times of day ($P < 0.001$) and between the light conditions ($P < 0.001$). Core body temperature at a particular time of day was compared between *days 2* and *3* and *days 7* and *8*, between *days 7* and *8* and *days 8* and *9*, and between *days 8* and *9* and *days 13* and *14*. Core body temperature at ZT22, ZT0, and ZT1 was significantly higher on *days 7* and *8* than on *days 2* and *3* (Wilcoxon, $P < 0.05$). The body temperature at ZT10, ZT1, and ZT2 was significantly higher on *days 8* and *9* than on *days 7* and *8* (Wilcoxon, $P < 0.05$). Core body temperature at ZT10 was significantly lower on *days 13* and *14* than on *days 8* and *9* (Wilcoxon, $P < 0.05$). On the other hand, the amplitude of core body temperature in terms of a difference between the peak and trough was not significantly different throughout the experimental period. In the calculation of amplitude, the values at ZT21 were excluded because of possible effects of shower taking.

Figure 4*B* illustrates representative time courses of averaged core body temperature ($n = 8$) at particular times of day throughout the experiment. The body temperature at ZT16 ($r = 0.299$, $P < 0.01$), ZT17 ($r = 0.268$, $P < 0.01$), ZT18 ($r = 0.207$, $P < 0.05$), ZT19 ($r = 0.238$, $P < 0.05$), ZT22 ($r = 0.223$, $P < 0.05$), ZT23 ($r = 0.345$, $P < 0.01$), ZT0 ($r = 0.380$, $P < 0.01$), ZT1 ($r = 0.403$, $P < 0.01$), ZT2 ($r = 0.371$, $P < 0.01$), and ZT3 ($r = 0.227$, $P < 0.05$) was positively correlated with the experimental day (Spearman's rank correlation analysis). The correlation was further analyzed in two parts of the experiment separately, from *days 2* and *3* to *days 7* and *8* (dim light conditions) and from *days 8* and *9* to *days 14* and *15* (bright light conditions). A significant positive correlation was detected under the dim light conditions at ZT19 ($r = 0.404$, $P < 0.01$), ZT22 ($r = 0.306$, $P < 0.05$), ZT23 ($r = 0.356$, $P < 0.05$), and ZT0 ($r = 0.366$, $P < 0.05$). On the other hand, we detected no significant correlation under the bright light conditions at any time of day.

Sleep EEG and Sleepiness

The parameters of sleep EEG on *days 1*, *6*, *9*, and *14* are demonstrated in Table 2. ST2 was significantly shortened on *day 6* (Wilcoxon, $P < 0.05$), *day 9* (Wilcoxon, $P < 0.05$), and *day 14* (Wilcoxon, $P < 0.05$) compared with *day 1*. The sum of ST1 and ST2 was decreased on *day 6* (Wilcoxon, $P < 0.05$) and *day 9* (Wilcoxon, $P < 0.05$) compared with *day 1*. RL was significantly shortened on *day 9* (Wilcoxon, $P < 0.05$) and *day 14* (Wilcoxon, $P < 0.05$) compared with *day 1*. In addition, SPT was significantly decreased on *day 9* (Wilcoxon, $P < 0.05$) compared with either *day 1* or *day 6*. There were no significant differences in TST, SL, WAFA, SE, REM, WASO, ST1, ST3, ST4, SWS (ST3 + ST4), and MT among the days examined.

The power density of θ wave with eyes opened was significantly different at ZT18 (Friedman, $P < 0.01$) and was higher on *days 8* and *13* than on *day 3* (Wilcoxon, $P < 0.05$). The power density of α wave with eyes opened was significantly different at ZT14 (Friedman, $P < 0.01$) and was higher on *day 6* than on the other days (Wilcoxon, $P < 0.05$). No difference was observed in the power densities with eyes closed.

Figure 5, *A* and *C* illustrates subjective sleepiness throughout the waking period on *days 2*, *7*, *8*, and *13*. Friedman testing revealed significant differences among the times of day ($P < 0.001$) and between different days ($P < 0.001$). Sleepiness score at each ZT was compared between *day 2* and *day 7*, between *day 7* and *day 8*, and between *day 8* and *day 13*. The sleepiness scored by VAS was significantly lower at ZT14 and

Table 1. *Circadian phases, duration, amplitude, and AUC of nocturnal melatonin rise*

Day	Circadian Phase, h			Duration, h	Amplitude, pg/ml	AUC, pg/ml
	Onset	Peak	End			
1–2	22.2±1.6	4.1±1.8	10.0±2.5	11.7±2.2	98.6±39.5	885.3±412.6
6–7	24.0±1.8*	5.1±1.8*	10.2±2.4	10.2±2.1*	93.6±43.3	721.9±363.5
14–15	23.7±1.1*	4.7±1.4	9.6±2.0	9.9±1.7*	108.1±44.6†	782.4±379.5

Circadian phases (onset, peak, end), duration, amplitude, and area under the curve (AUC) of the nocturnal melatonin rise were measured on *days 1* and *2* (baseline), *days 6* and *7* (dim light), and *days 14* and *15* (bright light). Values are expressed as means ± SD, $n = 7$. *$P < 0.05$ vs. *days 1* and *2*. †$P < 0.05$ vs. *days 6* and *7*.

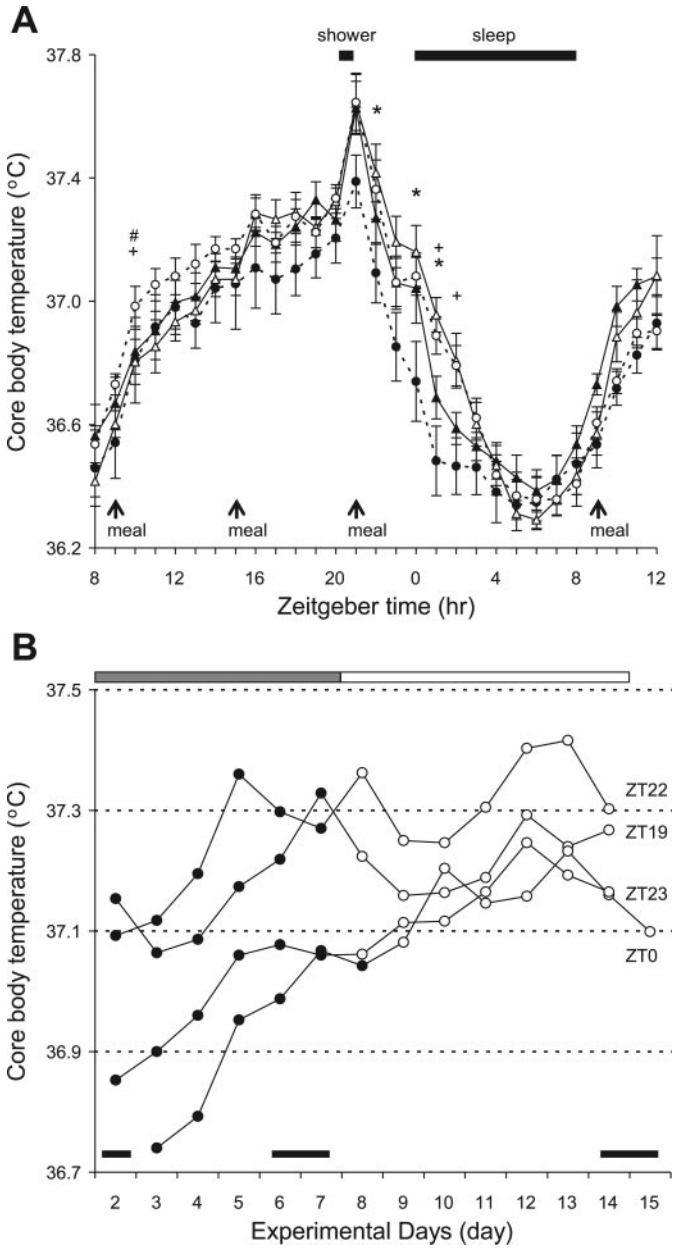

Fig. 4. *A*: circadian rhythms in core body temperature on *days 2* and *3* (●, dashed line), *days 7* and *8* (▲, solid line), *days 8* and *9* (○, dashed line), and *days 13* and *14* (△, solid line). The abscissa indicates the time of day, where the time that subjects went to bed is defined as ZT0. The solid horizontal bar indicates the sleeping period, and arrows indicate meal times. Values are expressed as means ± SE; $n = 8$. *$P < 0.05$, *days 2* and *3* vs. *days 7* and *8*. +$P < 0.05$, *days 7* and *8* vs. *days 8* and *9*. #$P < 0.05$, *days 8* and *9* vs. *days 13–14*. *B*: progressive changes in core body temperature at particular times of day (ZT19, ZT22, ZT23, and ZT0) in the course of the experiment. Closed and open circles indicate results under dim and bright light conditions, respectively. Shaded and open bars at *top* indicate dim and bright light conditions, respectively. Solid horizontal bars at *bottom* indicate periods of blood sampling. Values are expressed as means; $n = 8$.

ZT22 on *day 7* than on *day 2*. At ZT16, the score was significantly lower on *day 8* than on *day 7*. The sleepiness score by QSS2002 was significantly lower at ZT16 on *day 8* than on *day 7*.

Figure 5, *B* and *D* illustrates progressive changes of averaged sleepiness score at all ZT in the course of the experiment. The sleepiness evaluated by VAS was negatively correlated with the experimental day at ZT12, ZT14, ZT16, ZT18, and ZT22 and by QSS2002 at ZT12, ZT14, ZT16, and ZT18 [Spearman's rank correlation analysis: ZT12 ($r = -0.344$, $P < 0.01$), ZT14 ($r = -0.269$, $P < 0.01$), ZT16 ($r = -0.433$, $P < 0.01$), ZT18 ($r = -0.285$, $P < 0.01$), and ZT22 ($r = -0.198$, $P < 0.05$) by VAS; ZT12 ($r = -0.294$, $P < 0.01$), ZT14 ($r = -0.205$, $P < 0.05$), ZT16 ($r = -0.255$, $P < 0.01$), and ZT18 ($r = -0.217$, $P < 0.05$) by QSS2002]. The correlation was further analyzed in two parts of the experiment separately from *day 2* to *day 7* (dim light conditions) and from *day 8* to *day 14* (bright light conditions). A significant negative correlation was detected in the sleepiness score by VAS at ZT18 ($r = -0.456$, $P < 0.01$) and ZT22 ($r = -0.298$, $P < 0.05$) under the dim light conditions. There was no correlation at any ZT under the bright light conditions. In contrast, a significant correlation was not observed in the sleepiness score by QSS2002 under either the dim or bright light conditions.

Wrist Activity

Table 3 demonstrates the wrist activity before and during the experiment. The wrist activity during the waking period was significantly decreased in the dim (Wilcoxon, $P < 0.05$) and bright light conditions (Wilcoxon, $P < 0.05$) compared with that before the experiment. There was no difference in the wrist activity between the two light conditions. The wrist activity during the sleeping period was not different among the three conditions.

DISCUSSION

In the present study, we examined whether daytime bright light increased the amplitude of nocturnal melatonin rise in plasma and improved sleep quality in young healthy subjects. As demonstrated in Figs. 2 and 3, repeated exposures to bright light of ~5,000 lx during the waking period for 7 days significantly increased the amplitude of nocturnal melatonin rise compared with that observed after a 5-day stay under the dim light conditions (~10 lx). Previously, Hashimoto et al. (16) reported that bright light exposure (5,000 lx, from 1100 to 1700) for 3 consecutive days increased the AUC of nocturnal melatonin rise. An increase in the amplitude of urinary melatonin rhythm was also observed in young healthy subjects under bright light for 2 days (37). Mishima et al. (32) reported a robust effect of bright light on nocturnal melatonin in elderly insomniacs. In the latter subjects, the nocturnal melatonin level was significantly lower than in the young healthy subjects before bright light exposures. In the present study, the amplitude of nocturnal melatonin level under bright light was not different from that on *days 1* and *2*, suggesting that daytime bright light restored the nocturnal melatonin level that had been attenuated beforehand.

The mechanism of elevation of the nocturnal melatonin peak by daytime bright light is not well understood. The nocturnal melatonin rise is primarily regulated by the circadian pacemaker located in the suprachiasmatic nucleus (SCN) (34). The circadian signal from the SCN is conveyed through the paraventricular nucleus and the sympathetic pathways to the pineal gland (25), which releases the neurotransmitter norepinephrine from the postganglionic sympathetic nerve terminals. Norepinephrine activates the β-adrenergic receptors on the pineal

Table 2. *Sleep parameters*

Parameter	*Day 1*	*Day 6*	*Day 9*	*Day 14*
TIB, min	480.0±0.0	480.0±0.0	480.0±0.0	480.0±0.0
SPT, min	464.9±17.6	457.5±27.2	448.3±34.7*†	451.8±20.6
TST, min	451.3±21.9	438.0±27.6	432.5±31.2	434.5±21.7
SL, min	15.1±17.6	21.5±27.8	30.8±35.1	25.3±21.6
RL, min	88.6±39.2	67.2±35.3	63.1±11.1*	62.8±7.8*
WAFA, min	0.0±0.0	1.0±1.8	1.0±2.7	3.0±8.4
WASO, min	13.6±13.0	19.5±20.1	15.8±12.4	17.3±17.8
MT, min	3.1±2.7	4.7±3.5	3.3±2.8	3.4±2.1
ST1, min	19.1±17.3	14.5±10.7	18.3±13.1	17.2±14.7
ST2, min	246.3±26.5	215.0±36.0*	198.0±38.2*	210.8±29.4*
ST3, min	50.6±26.5	48.3±17.8	44.7±13.7	43.8±18.3
ST4, min	52.8±22.7	57.0±12.0	65.5±19.3	67.1±23.1
ST1+2, min	265.3±41.7	229.6±40.3*	216.3±45.6*	228.0±42.0
ST3+4, min	103.4±36.1	105.3±24.6	110.2±26.2	110.9±37.2
REM, min	79.5±27.2	98.4±28.7	102.6±30.3	92.1±27.8
WASO, %	2.9±2.8	4.2±4.3	3.4±2.7	3.8±3.8
MT, %	0.7±0.6	1.0±0.7	0.7±0.6	0.8±0.5
ST1, %	4.1±3.6	3.2±2.3	4.0±2.8	3.8±3.2
ST2, %	52.9±4.9	46.9±6.6*	43.9±6.5*	46.7±6.2*
ST3, %	10.9±5.6	10.6±3.9	10.0±2.9	9.8±4.2
ST4, %	11.5±5.2	12.6±3.2	15.0±5.8	14.8±4.9
ST1+2, %	57.0±8.2	50.0±7.5*	47.9±8.1*	50.4±8.7
ST3+4, %	22.3±7.9	23.2±6.0	24.9±7.3	24.6±8.2
REM, %	17.1±5.6	21.5±6.2	22.9±6.6	20.4±6.1
SE, %	97.1±2.8	95.8±4.3	96.6±2.7	96.2±3.8

Sleep parameters determined on *day 1* (baseline), *day 6* (dim light), *day 9* (bright light), and *day 14* (bright light) are shown. Values are expressed as means ± SD; $n = 8$. *$P < 0.05$ vs. *day 1*. †$P < 0.05$ vs. *day 6*.

cells and releases melatonin from the pineal gland (12). Light has dual effects on the nocturnal melatonin rise through the retinohypothalamic tract and SCN. Light regulates the phase of nocturnal melatonin rise by resetting the circadian pacemaker in the SCN (24, 46). In addition, light suppresses melatonin release from the pineal gland during the circadian phase, when the melatonin production is increased (29). The melatonin suppression seems to be mediated through the inhibition of the

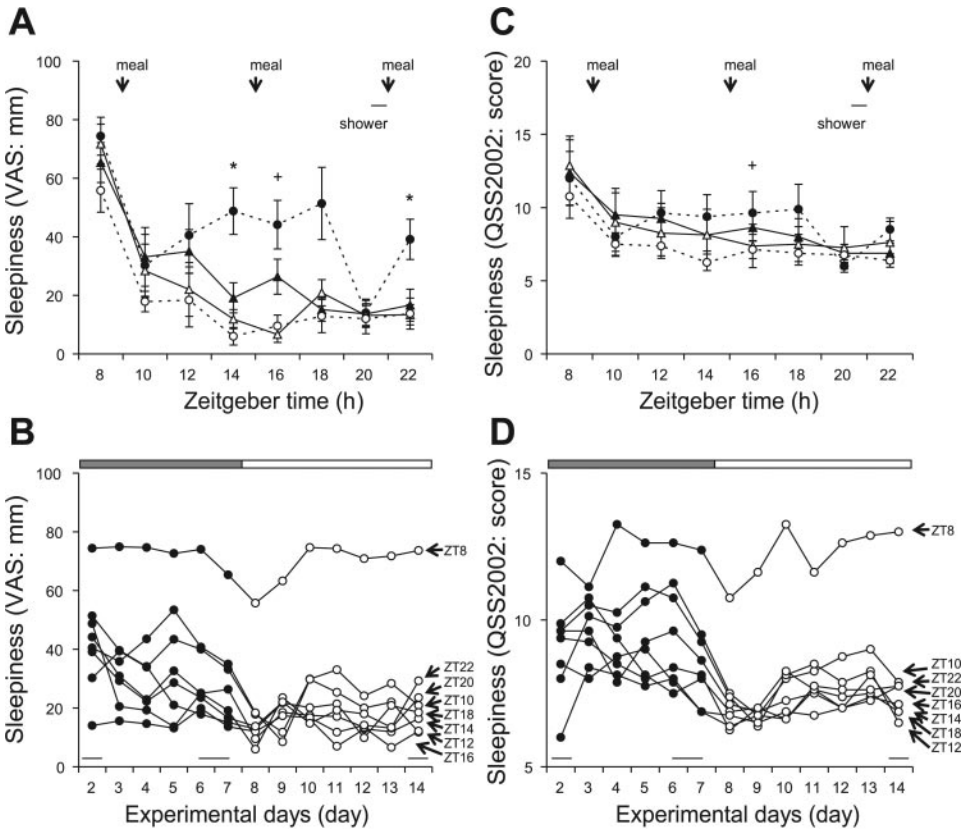

Fig. 5. *A* and *C*: changes in sleepiness in the course of the waking period. Sleepiness was evaluated using the Visual Analog Scale (VAS; *A*) and the Questionnaire for Subjective Symptoms in 2002 (QSS2002; *C*) on *day 2* (●, dashed line), *day 7* (▲, solid line), *day 8* (○, dashed line), and *day 13* (△, solid line). The abscissa indicates the time of day, where the wakeup time is defined as ZT8. Solid horizontal bars indicate times of shower taking, and arrows indicate meal times. Values are expressed as means ± SE; $n = 8$. *$P < 0.05$, *days 2* and *3* vs. *days 7* and *8*. +$P < 0.05$, *days 7* and *8* vs. *days 8* and *9*. *B* and *D*: progressive changes in sleepiness at all ZT throughout the experimental period. Sleepiness was evaluated using VAS (*B*) and QSS2002 (*D*). Closed and open circles indicate results under dim and bright light conditions, respectively. Shaded and open bars at *top* indicate the dim and bright light conditions, respectively. Solid horizontal bars indicate periods of blood sampling. Values are expressed as means; $n = 8$.

Table 3. *Wrist activity*

Day	Waking Period	Sleeping Period
Before experiment	11,547.8±5,635.6	902.7±484.8
Dim light	7,330.7±4,178.2*	1,088.4±868.3
Bright light	7,413.1±3,937.8*	1,668.1±2,170.8

Wrist activity was determined (counts/h) during the waking and sleeping periods before and during (dim light, *days 4* and *5*; bright light, *days 11* and *12*) the experiment. Values are expressed as means ± SD; $n = 8$. *$P < 0.05$ vs. before experiment.

activity of β-adrenergic receptors (6). Therefore, the nocturnal melatonin rise could be increased either by an increase in the amplitude of underlying circadian oscillation or an increase in the sensitivity of pineal gland to light signals such as upregulation of the β-adrenergic receptors. Previously, Masubuchi et al. (30) demonstrated in a human subject that the amplitude of nocturnal melatonin rise was reduced when the circadian melatonin and sleep wake rhythms were internally desynchronized, and the decreased amplitude was recovered when these rhythms were resynchronized. In the present study, the phase relationship between the circadian melatonin and sleep wake rhythms was essentially the same under the dim and bright light conditions (Fig. 3). Furthermore, the amplitude of rectal temperature rhythm was not changed throughout the experimental period. The changes in the state of underlying circadian oscillation were not likely the major cause of the increase in nocturnal melatonin rise.

Bright light is known to stimulate the sympathetic nerve system in animals, including humans. The effect persists for several hours after the termination of light exposure (35, 36, 40). The photic stimulation of the sympathetic nerves seems to mediate through the retinohypothalamic tract and SCN (36). Thus it is surmised that the exposure to daytime bright light exerts prolonged stimulation of the sympathetic nerves and activates β-adrenergic receptors of the pineal cells to increase melatonin secretion. A similar prolonged effect of bright light on the sympathetic nerve system has been proposed on the core body temperature around the initial sleep phase (7, 13). Bright light exposures immediately preceding nocturnal sleep prevented the initial decline of core body temperature that was otherwise observed (8, 27). The nocturnal decrease of deep body temperature is caused by increased heat loss due to nocturnal dilatation of peripheral vessels (2). Bright light was supposed to constrict the blood vessels by stimulating the sympathetic nerves so that the nocturnal heat loss was prevented to increase core body temperature (27). In the present study, the core body temperature at around the initial sleep phase was significantly higher on the first day under the bright light conditions than on the last day under the dim light conditions (Fig. 4*A*), which could also be interpreted as the result of sympathetic activation by bright light. On the other hand, nocturnal melatonin was reported to contribute to some extent to nocturnal decrease in core body temperature (26). Melatonin was suggested to dilate peripheral vessels and increase heat loss from the skin (26). In the present study, nocturnal melatonin rise was increased under the bright light conditions, but core body temperature at night was not changed. The vasodilation effect of melatonin, if any, might be canceled by persisting effects of bright light on the sympathetic nerves.

The onset and peak phases, but not the end phase of the nocturnal melatonin rise, were significantly delayed under the dim light conditions, resulting in shortening of the nocturnal melatonin rise. The phase shift was observed despite a strict sleep schedule. These findings suggest, first, that the strict sleep schedule under dim light of ~10 lx is not sufficient to keep the human circadian rhythm at the same phase as that in routine life and, second, that the onset and end of nocturnal melatonin rise are regulated by different mechanisms. A strict sleep schedule has been demonstrated insufficient to entrain the human circadian pacemaker (10, 17). However, further phase-delay shifts were prevented under the bright light conditions, although the onset of nocturnal melatonin rise was still significantly delayed compared with that on *days 1* and *2* (Fig. 3). Bright light in the morning phase advances the circadian melatonin rhythm, whereas that in the late evening phase delays the rhythm (24), which seemed to counterbalance under the present bright light conditions. The onset and end phases of nocturnal melatonin rise were not always shifted in parallel in response to light (11, 16, 28, 43). In rats, different circadian pacemakers have been proposed to drive the rising and falling phases of nocturnal melatonin, corresponding to the evening and morning oscillators for behavioral rhythms (22). A similar two-oscillator hypothesis was advanced in humans as well (44). Previously, Hashimoto et al. (16) demonstrated a phase-advance shift of the onset but not of the end of nocturnal melatonin rise in human subjects exposed to daytime bright light for 6 h, from 3 h after waking up to 7 h before bedtime. The finding supports an idea of differential effects of bright light on the onset and end of nocturnal melatonin rise. The lighting schedule might exclude the phase delay portion of a human phase-response curve from the perturbation by bright light (24).

The core body temperature at ZT22, ZT0, and ZT1 was significantly higher on the last night under dim light conditions than on the basal night. Progressive elevation of body temperature around the initial sleep phase was confirmed by a positive correlation with the experimental day during the dim light conditions (Fig. 4*B*). There was no correlation between body temperature and the experimental day under bright light conditions. Therefore, the elevation of core body temperature at the initial sleep phase is interpreted primarily as a result of phase-delay shifts of the circadian rhythm under dim light conditions, as demonstrated in the plasma melatonin rhythm (Fig. 3). Further phase-delay shifts of body temperature rhythm were not detected from the regression analyses, as in the case of plasma melatonin rhythm.

The EEG sleep parameters under the bright light conditions were not much different from those under the dim light conditions (Table 2). On the other hand, the amount of ST2 was significantly reduced in the dim and bright light conditions compared with the baseline. The reduction of ST2 may be related to the significant decline of daytime activities during the isolation experiment (Table 3). In contract, subjective sleepiness was changed in the course of the experiment and was highest on *day 2* and lowest on *day 8*, the first day of bright light (Fig. 5*A*). Bright light is known to decrease subjective sleepiness and increase alertness (4, 9, 38). However, in the present study, subjective sleepiness progressively declined under the dim light conditions and reached the minimum level on the first day of bright light (Fig. 5*B*). Subjective sleepiness

remained low under the bright light conditions. The time course throughout the experiment was similar to that of core body temperature at around the initial sleep phase (Figs. 4*B* and 5*B*). A close correlation between core body temperature and sleepiness has recently been suggested in humans (8, 23). The present finding suggests that the reduction of subjective sleepiness during the dim light conditions is due to phase-delay shifts of core body temperature at around the initial sleep phase, or the onset of nocturnal melatonin rise. The finding also suggests that a low level of subjective sleepiness under the bright light conditions is due to daytime bright light, or to elevated nocturnal melatonin peak. Clear effects of elevated melatonin peak were not detected on the parameters of sleep EEG, which was probably due to the age of subjects. Sleep of young subjects seems to be already good, and further improvements of sleep quality may be difficult to detect with ordinate sleep EEG.

In conclusion, exposures to daytime bright light for 7 days enhanced the nocturnal melatonin rise and prevented further phase-delay shifts of the circadian rhythm that was evident under the dim light conditions. Although clear effects of the elevated melatonin peak were not detected on the parameters of sleep EEG, bright light kept daytime sleepiness at low levels. These findings support the idea that daytime bright light is effective in controlling the circadian pacemaker in humans and reducing subjective sleepiness.

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