Abstract  The present study evaluated the effects of exposure to light intensity in the morning on dim light melatonin onset (DLMO). The tested light intensities were 750 lux, 150 lux, 3000 lux, 6000 lux and 12,000 lux (horizontal illuminance at cornea), using commercial 5000 K fluorescent lamps. Eleven healthy males aged 21–31 participated in 2-day experiments for each light condition. On the first experimental day (day 1), subjects were exposed to dim light (<30 lux) for 3 h in the morning (09:00–12:00). On the same day, saliva samples were taken in dim light (<30 lux) every 30 min from 21:00 to 01:00 to determine the DLMO phase. The subjects were allowed to sleep from 01:00 to 08:00. On the second experimental day (day 2), the subjects were exposed to experimental light conditions for 3 h in the morning. The experimental schedule after light exposure was the same as on day 1. On comparing day 2 with day 1, significant phase advances of DLMO were obtained at 3000 lux, 6000 lux and 12,000 lux. These findings indicate that exposure to a necessary intensity from an ordinary light source, such as a fluorescent lamp, in the morning within one day affects melatonin secretion.

Keywords: light intensities, daytime exposure, circadian rhythm, dim light melatonin onset, human

Introduction

In order to maintain proper synchronization with the environment, the master circadian clock located in the suprachiasmatic nucleus of the anterior hypothalamus (Ralph et al., 1990) is very sensitive to the 24-h light/dark cycle (the earth’s rotation). It has been suggested that either nonvisual photoreceptors, consisting of intrinsically photosensitive retinal ganglion cells (Hattar et al., 2002; Lucas et al., 1999), or both nonvisual and classical visual photoreceptors consisting of cones (Gooley et al., in press) mediate the effects of light on the circadian rhythm. Modern humans are becoming unaccustomed to being exposed to the 24-h light/dark cycle in the modern environment, with 24-hour convenience stores and underground malls. The environment, thus, could contribute to desynchronization of the circadian rhythm with the 24 h life rhythm. The desynchronization of the circadian rhythm may be involved in health issues, such as circadian rhythm sleep disorders (Mundey et al., 2005).

The American Academy of Sleep Medicine recently recognized that timed exposure to bright light is an effective treatment for circadian rhythm sleep disorders, such as shift work disorder (SWD) and delayed sleep phase disorder (DSPD) (Morgenthaler et al., 2007; Sack et al., 2007). In the treatment of these disorders, it is preferable for daytime exposure to bright light to have a phase advance effect on the circadian clock. There is a need for research on the effect of light treatment, i.e., intensity and duration, in order to establish the optimal parameters, such as dosage. Some studies have reported that daytime exposure to bright light induces a phase advance of the circadian clock. Hashimoto et al. (Hashimoto et al., 1997) reported that daytime exposure (11:00–17:00) to bright light (5000 lux) for 3 days led to a phase advance in the onset of melatonin secretion in young adults. Exposure to bright light (6000 lux) after waking for 2 h between 03:00 and 08:00 for 4 days advanced the phase of the onset of melatonin secretion (Smith et al., 2009).

The previous findings, however, have been observed under the condition in which light exposure is strictly controlled for long periods. Given the practical application of light treatment to people such as shift workers, there is some question about the application of the previous findings. Phase dependence of a light-induced phase shift has been reported as a phase response curve (PRC) (Dawson et al., 1993; Honma and Honma, 1988; Khalsa et al., 2003). According to the PRC, phase advances are generated after the circadian phase 0 h, which is around the time when core body temperature is at its minimum, i.e., usually during the early hours of the morning, and phase delays are generated before the circadian phase 0 h. The bright light in the late afternoon and/or the evening could delay the
circadian rhythm, and may have a depression effect on the phase advances of the bright light in the morning. The present study, thus, investigated the effect of exposure to different light intensities on circadian rhythm in the condition in which the light exposure to the subject is minimally controlled. Dim light melatonin onset (DLMO) phase in saliva samples was evaluated to assess the phase shift of the circadian clock (Lewy et al., 1999). The concentration of urinary 6-sulphatoxymelatonin (aMT6s) in samples collected overnight was used to calculate the total volume of nocturnal melatonin secretion, because urinary aMT6s is a major urinary metabolite of melatonin and is very closely correlated with plasma and saliva melatonin levels (Nowak et al., 1987; Baskett et al., 1998; Cook et al., 2000).

Materials and Methods

Subjects
Fourteen male subjects aged 19–31 (mean ± S.D, 21.8 ± 3.3) years gave informed consent to participate in this study. All subjects were free from any medical condition at the time of the experiment. None of them had a history of psychiatric or sleep disorders. They were instructed to abstain from alcohol and caffeine for a day before the experiment. From 5 days prior to the experiment, they were asked to maintain a regular sleep–wake schedule (sleep onset between 01:00 and 02:00 and waking between 08:00 and 09:00).

Experimental procedure
The experiments were carried out between August and October 2008. The experiments began with a baseline day (Fig. 1). On the baseline day (day 1), the subjects arrived at the experimental chamber before 08:50 and were exposed to dim light (<30 lx) from 09:00 to 12:00. Then, they left the experimental chamber and were required to return to it before 20:45. The subjects provided saliva samples under a dim light condition (<30 lx) every 30 min from 21:00 to 01:00 for determination of the DLMO (Voutilios et al., 1997; Lewy et al., 1999). Saliva samples were collected directly in clear sterile plastic tubes using sterile plastic straws. The subjects were allowed to watch a movie and read a book in the experimental chamber. The illuminance level of the movie display was kept less than 2 lx because bright light from a movie display may suppress melatonin secretion (Higuchi et al., 2003). The subjects slept overnight in the experimental chamber (01:00–08:00). Lights were turned off during sleep time. The subjects provided a urine sample in a sterile light-resistant bottle at 08:00. Urine samples were collected again in the same bottle between 21:00 and 08:00. On the second day (day 2), the subjects had breakfast in the experimental chamber and were then exposed to different light conditions from 09:00 to 12:00. The subjects’ pupil sizes were measured with an electronic pupillometer (FP-10000II; T.M.I., Saitama, Japan) at 10:00. To control the direction of the subjects’ gaze during light exposure, each subject was required to watch a movie on a small 3.5-inch monitor placed in front of his face. The experimental schedule after light exposure was the same as that on day 1. Exposures to the five light conditions were conducted in five different experimental sets with one light intensity per set. Experiments were performed under different conditions in random order at intervals of more than 5 days. The ambient temperature in the experimental chamber was kept at 27°C.

Light conditions
The light conditions were the five intensities of 750, 1500, 3000, 6000, and 12,000 lux. A white fluorescent lamp (Panasonic Ltd. Co., Osaka, Japan), which acted as the light source, was placed in front of each subject. Figure 2 shows the relative spectral power distributions (SPD), correlated color temperature (CCT), color rendering index (CRI), and chromaticity (x, y) of the light source.
Hormone assays
Saliva samples were centrifuged at 1500 g for 5 min and then frozen at 330°C until assay. Melatonin levels in the samples were analysed in duplicate using a commercially available ELISA kit (Direct Saliva Melatonin ELISA; Buhlmann Laboratories, Allschwil, Switzerland) and mean values of the duplicates were calculated. The limit of detection of the kit was 0.5 pg/mL and the limit of quantification was 1.6–20.5 pg/mL. The intra- and interassay coefficients of variance were 12.6% and 22.9%, respectively.

Urine samples were pipetted into clear sterile plastic tubes, centrifuged at 2000 g for 5 min, and then frozen at −30°C until assay. To adjust for variation in the dilution of urine, creatinine-adjusted aMT6s concentrations were used (Klante et al., 1997; Graham et al., 1998). For the analysis of aMT6s and creatinine, we used the 6-sulphatoxymelatonin ELISA kit (Buhlmann Laboratories) and the Urinary Creatinine Detection Kit (Luminos, Michigan, US), respectively. The limit of detection of the aMT6s kit was 0.14 ng/mL and the limit of quantification was 1.5 ng/mL. The intra- and interassay coefficients of variation were 7.1% and 11.9%, respectively. The limit of detection of the creatinine kit was 0.019 ng/dL and the limit of quantification was 0.037 ng/dL. The intra- and interassay coefficients of variation were 2.4% and 3.2%, respectively. All urinary analyses were carried out in duplicate and the mean value of the duplicates was used.

Data analysis
Since there is substantial inter-individual variation in endogenous melatonin levels (Arendt, 1988; Burgess and Fogg, 2008), a salivary threshold was determined by calculating the mean of the five base melatonin values plus twice the standard deviation of these values (Voultsios et al., 1997). In this study, five base melatonin values were obtained from the saliva samples collected at 21:00 on day 1 of each light condition. The thresholds varied among subjects and ranged from 3.1 to 5.9 (mean±S.D, 4.0±0.8) pg/mL. The time of the DLMO phase was determined by linear interpolation between the time points before and after melatonin concentration increased and remained above the thresholds (Voultsios et al., 1997; Lewy et al., 1999). To directly compare the effect of different light conditions on the total volume of nocturnal melatonin secretion, the percentage increase in melatonin secretion was defined as [(aMT6s on day 2−aMT6s on day 1)/aMT6s on day 1]×100).

The mean aMT6s concentration and DLMO phase were compared between days 1 and 2 using a two-tailed paired t-test. Statistical analyses were performed using SPSS version 16.0 (SPSS, Chicago, IL, USA). Differences for which p < 0.05 were considered statistically significant.

Results
Data on three subjects were excluded from the analysis because DLMO times were not detected during the sampling period (21:00–01:00). Results for 11 subjects were therefore analyzed.

The mean illuminances of ambient light are shown in Table 1. According to the PRCs for light exposure (Lewy et al., 1992), ambient light may advance DLMO time in the early afternoon and may delay it in the evening. Therefore, the mean illuminances were separated into values for early afternoon (12:00–15:00), late afternoon (15:00–18:00), and evening (18:00–21:00). For each period, there were no significant differences in the mean illuminance between days 1 and 2 under any of the light conditions.

Table 2 shows DLMO phases for each day under each light condition. Comparison of data for days 1 and 2 shows that DLMO phases were significantly advanced by exposure to 3000, 6000, and 12000 lux. Significant advancement of the DLMO phase was not obtained at 750 and 1500 lux. For each period, there were no significant differences in the mean illuminance between days 1 and 2 under any of the light conditions.

Mean pupil size during light exposure and retinal irradiance are shown in Table 2. Table 3 shows DLMO phases for each day under each light condition. Comparison of data for days 1 and 2 shows that DLMO phases were significantly advanced by exposure to 3000, 6000, and 12000 lux. Significant advancement of the DLMO phase was not obtained at 750 and 1500 lux. Urinary aMT6s concentration on day 2 was significantly higher than that on day 1 in the 6000 lx and 12,000 lx conditions.

Table 1 Mean Illuminance levels of ambient light for each light condition (lx)

<table>
<thead>
<tr>
<th>Photopic illuminance at cornea (lx)</th>
<th>day 1</th>
<th>day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12:00–15:00</td>
<td>15:00–18:00</td>
</tr>
<tr>
<td>750</td>
<td>1589 (1359)</td>
<td>255 (381)</td>
</tr>
<tr>
<td>1500</td>
<td>1105 (1944)</td>
<td>146 (128)</td>
</tr>
<tr>
<td>3000</td>
<td>1591 (1719)</td>
<td>460 (409)</td>
</tr>
<tr>
<td>6000</td>
<td>2781 (2794)</td>
<td>518 (478)</td>
</tr>
<tr>
<td>12000</td>
<td>1495 (1176)</td>
<td>312 (501)</td>
</tr>
</tbody>
</table>

Mean(S.D.)
Table 3 Mean DLMO times (time of day) for each light condition. Standard deviations (S.D.) and phase advance (day 1–day 2) are in minutes.

<table>
<thead>
<tr>
<th>Photopic illuminance at cornea (lx)</th>
<th>DLMO day 1</th>
<th>DLMO day 2</th>
<th>Day 1–day 2 (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>750</td>
<td>22:58 (80)</td>
<td>22:50 (88)</td>
<td>8 (15)</td>
</tr>
<tr>
<td>1500</td>
<td>22:39 (76)</td>
<td>22:36 (66)</td>
<td>4 (20)</td>
</tr>
<tr>
<td>3000</td>
<td>22:02 (55)</td>
<td>21:47 (57)</td>
<td>15 (13)*</td>
</tr>
<tr>
<td>6000</td>
<td>22:23 (46)</td>
<td>21:57 (38)</td>
<td>26 (18)**</td>
</tr>
<tr>
<td>12000</td>
<td>22:40 (72)</td>
<td>22:13 (67)</td>
<td>27 (21)**</td>
</tr>
</tbody>
</table>

** p<0.01; * p<0.05 (day 1 vs. day 2)

Mean (S.D.)

![Graph](image)

**Fig. 3** aMT6s concentrations in each light condition.

Discussion

In earlier studies (Dijk et al., 1989; Hashimoto et al., 1997; Smith et al., 2009) daytime exposure to bright light advanced the phase of the circadian clock. For example, bright light exposure (3000 lux) in the early morning (03:00–09:00) within one day resulted in a phase advance of DLMO (Buresova et al., 1991). Our present findings also showed a significant advance of DLMO on exposure to bright light (>3000 lux). The present results were obtained without controlling light exposure during the afternoon (Table 1). The light of the afternoon, especially the late afternoon, may have a depression effect of the phase advance of bright light in the morning. The mean illuminances of the late afternoon (18:00–21:00) were less than 100 lux on each condition. In human studies (McIntyre et al., 1989; Aoki et al., 1998), light intensity less than 200 lx might not suppress melatonin secretion. Since melatonin could have a major physiological role in the circadian system (Brzezinski, 1997), the minimum light intensity delaying circadian phase might also be more than 200 lx. The ambient light of this study, thus, may not have an impact on the circadian phase.

While the DLMO was advanced by bright light of 3000 lux, the mean advancing amount of the DLMO at 3000 lux was lower than that at 6000 and 12000 lux. DLMO may be related to the amplitude of melatonin secretion as well as the circadian phase (Lewy et al., 1999). There was no difference in the urinary aMT6s concentration at 3000 lux. At 6000 and 12000 lux, however, the urinary aMT6s concentrations on day 2 were higher than those on day 1. These findings suggest that bright light of 6000 and 12000 lux advance the circadian phase and increase the amplitude of melatonin secretion. The increments of melatonin secretion, thus, might cause the higher advancing amount of the DLMO at 6000 and 12000 lux.

Smith et al. have reported significant difference of light-induced melatonin suppression after different prior photic history (Smith et al., 2004). This finding implies that a photic history may have an impact on photosensitivity to circadian rhythm. The present study was carried out in a season with long hours of daylight (between August and October). If conducted during a season with short hours of daylight, the circadian phase could be advanced by lower light intensity than 3000 lux. Following PRC by Khalsa et al., meanwhile, bright light acutely delayed the circadian phase approximately 6h before the circadian phase 0 h (Khalsa et al., 2003). The subjects of the present study were exposed to dim light at night (21:00–01:00) because of measuring DLMO. The light in this period acutely delayed the circadian phase. In modern society, there are many people who are exposed to bright light at home and/or in the workplace at night. For reliable data, therefore, bright light of more than 3000 lux may be appropriate.

One explanation for the advance of circadian rhythms after daytime exposure to bright light may be that in response to light in mammals, glutamate and pituitary adenylylate cyclase-activating peptide (PACAP) are released from the retinohypothalmic tract (RHT) and stimulate their receptors in the hypothalamic suprachiasmatic nucleus (SCN) neuron (Reppert and Weaver, 2002). The signalling induces acute expression of clock genes Per1 and Per2 (Albrecht et al., 1997; Shearman et al., 1997; Shigeyoshi et al., 1997). For animal studies, the antisense oligonucleotides against the Per1 gene inhibited both light-induced phase advance (Tischkau et al., 2003) and delay (Akiyama et al., 1999) of the circadian rhythms. The photic induction of the Per2 gene could be involved in the phase delay (Akiyama et al., 1999), but not in the phase advance (Tischkau et al., 2003). Furthermore, the light-induced phase advance or delay of the circadian clock is impaired in mice deficient in the Per1 or Per2 gene, respectively (Albrecht et al., 2001). The Per1 gene, thus, might play an important role in the advance of circadian rhythm. On the other hand, there is a possible reason for increased nocturnal melatonin secretion after daytime exposure to bright light: Bright light during the day may modify serotonergic function in the brain, resulting in an increase in melatonin secretion, which is a major metabolite of serotonin (5-HT) in the pineal gland. In animals, exposure to light during their subjective day attenuated the phase-shifting effect caused by a 5HT1a agonist (Penev et al., 1997) suggesting that the effect of bright light may involve a serotonergic mechanism. Despite a lack of systematic evidence, the phase and amplitude of the
melatonin rhythm may be affected by different parameters of daytime light exposure such as light intensity, duration, and timing, as well as the number of consecutive days.

Although our findings are limited, the present study provided applicational data that exposure to a minimum dose (3000 lx x 3 h) from an ordinary light source, such as a fluorescent lamp, in the morning has a phase-advancing effect within one day. These findings may be useful in adaptation to an artificial light environment by human beings as well as devising light treatments for circadian rhythm sleep disorders such as SWD and DSPD. The subjects in the present study, however, were exposed to bright light (mean 700–3000 lux) during daytime (12:00–15:00) and dim light (<30 lux) during nighttime (21:00–01:00). The light of these periods could have an impact on melatonin secretion and/or circadian rhythm. If the subjects were under less than 700 lux during the daytime, less amount of light exposure in the morning may have an impact; on the other hand, if they were under bright light during nighttime, more of the light could be appropriate as an application to light treatment. Furthermore, light-induced nocturnal melatonin suppression depends on duration of light exposure as well as on light intensity (Aoki et al., 1998). The wavelength composition of nocturnal light, also, affects light-induced melatonin suppression (Brainard et al., 2001; Lockley et al., 2003; Figueiro et al., 2006; Kozaki et al., 2008). In addition, photosensitivity may be affected by season (Higuchi et al., 2007b), eye color (Higuchi et al., 2007a), and aging (Herljevic et al., 2005). Further research is therefore needed to examine the parameters of light exposure and photosensitive variation in human beings.

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Correspondence to: Tomoaki Kozaki, Faculty of Design, Kyushu University, 4–9–1 Shiobaru, Minami-ku, Fukuoka 815–8540, Japan
Phone: 092−553−4531
Fax: 092−553−4531
e-mail: kozaki@design.kyushu-u.ac.jp