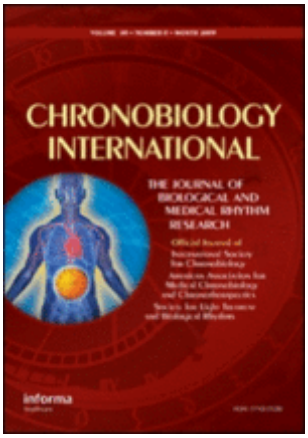

Melatonin suppression during a simulated night shift in medium intensity light is increased by 10-minute breaks in dim light and decreased by 10-minute breaks in bright light

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Complete List of Authors:	LEE, SANG-IL; Kyushu University Graduate School of Design Faculty of Design, Department of Human Science; Hokkaido University Faculty of Engineering, Division on Human Environmental Systems Kinoshita, Saki; Kyushu University, Graduate School of Integrated Frontier Science, Department of Kansei Science Noguchi, Anna; Kyushu University, Graduate School of Integrated Frontier Science, Department of Kansei Science Eto, Taisuke; Kyushu University, Graduate School of Integrated Frontier Science, Department of Kansei Science Ohashi, Michihiro; Kyushu University, Graduate School of Integrated Frontier Science, Department of Kansei Science Email address Nishimura, Yuki; Kyushu University, Graduate School of Integrated Frontier Science, Department of Kansei Science Email address; National Institute of Occupational Safety and Health Japan Noborito District Maeda, Kaho; Japan Aerospace Exploration Agency Institute of Aerospace Technology Tsukuba Space Center, Ground Facilities Department Motomura, Yuki; Kyushu University Graduate School of Design Faculty of Design, Department of Human Science Awata, Yasuhiro; Japan Aerospace Exploration Agency Institute of Aerospace Technology Tsukuba Space Center, Ground Facilities Department Higuchi, Shigekazu; Kyushu University Graduate School of Design Faculty of Design, Department of Human Science
Keywords:	Humans, Night shift work, Intermittent light at night exposure, Light adaptation, Short duration, Melatonin suppression, Subjective sleepiness, Performance

Melatonin suppression during a simulated night shift in medium intensity light is increased by 10-minute breaks in dim light and decreased by 10-minute breaks in bright light

Sang-il Lee[a, b], Saki Kinoshita[c], Anna Noguchi[c], Taisuke Eto[c], Michihiro Ohashi[c], Yuki Nishimura[c, e], Kaho Maeda[d], Yuki Motomura[a], Yasuhiro Awata[d], and Shigekazu Higuchi[a]*

[a]*Department of Human Science, Faculty of Design, Kyushu University, Fukuoka, Japan;* [b]*Division of Human Environmental Systems, Faculty of Engineering, Hokkaido University, Sapporo, Japan;* [c]*Department of Kansei Science, Graduate School of Integrated Frontier Science, Kyushu University, Fukuoka, Japan;* [d]*Ground Facilities Department, Japan Aerospace Exploration Agency, Tsukuba, Japan;* [e]*National Institute of Occupational Safety and Health, Kawasaki, Japan*

*corresponding author:
Shigekazu Higuchi, Ph.D
Department of Human Science
Faculty of design, Kyushu University
4-9-1 Shiobaru, Minami-ku, Fukuoka, 815-8540, Japan

Abstract

Exposure to light at night results in disruption of endogenous circadian rhythmicity and/or suppression of pineal melatonin, which can consequently lead to acute or chronic adverse health problems. In the present study, we investigated whether exposure to very dim light or very bright light for a short duration influences melatonin suppression, subjective sleepiness, and performance during exposure to constant moderately bright light. Twenty-four healthy male university students were divided into two experimental groups: Half of them (mean age: 20.0 ± 0.9 years) participated in an experiment for short-duration (10 min) light conditions of medium intensity light (430 lx, medium breaks) vs. very dim light (< 1 lx, dim breaks) and the other half (mean age: 21.3 ± 2.5 years) participated in an experiment for short-duration light conditions of medium intensity light (430 lx, medium breaks) vs. very bright light (4700 lx, bright breaks). Each simulated night shift consisting of 5 sets (each including 50-minute night work and 10-minute break) was performed from 01:00 to 06:00h. The subjects were exposed to medium intensity light (550 lx) during the night work. Each 10-minute break was conducted every hour from 02:00 to 06:00h. Salivary melatonin concentrations were measured, subjective sleepiness was assessed, the psychomotor vigilance task was performed at hourly intervals from 21:00h until the end of the experiment. Compared to melatonin suppression between 04:00 and 06:00h in the condition of medium breaks, the condition of dim breaks significantly promoted melatonin suppression and the condition of bright breaks significantly diminished melatonin suppression. However, there was no remarkable effect of either dim breaks or bright breaks on subjective sleepiness and performance of the psychomotor vigilance task. Our findings suggest that periodic exposure to light for short durations during exposure to a constant light environment affects the sensitivity of pineal melatonin to constant light depending on the difference between light intensities in the two light conditions (i.e., short light exposure vs. constant light exposure). Also, our findings indicate that exposure to light of various intensities at night could be a factor influencing the light-induced melatonin suppression in real night work settings.

Keywords: Humans; Night shift work; Intermittent light at night exposure; Light adaptation; Short duration; Melatonin suppression; Subjective sleepiness; Performance

Introduction

The 24-h light-dark cycle in nature is known as the strongest zeitgeber (i.e., time giver) for almost all mammalians. In humans, the suprachiasmatic nuclei (SCN), i.e., the circadian pacemaker, regulates circadian rhythmicity throughout the body via phototransduction input from ganglion cells in the retina (Weaver 1998). For instance, the SCN restrains pineal melatonin production during the daytime and allows melatonin secretion during the night. It has been believed that the release timing and the amount of melatonin secretion are related to the regulation of physiological and behavioral circadian rhythm (Macchi & Bruce 2004). However, the extension of daylight duration in modern life by using artificial lighting at night is likely to lead to acute melatonin suppression and circadian disturbances, which are partially responsible for some health problems (Smolensky et al. 2015; Smolensky et al. 2016; Lunn et al. 2017; Touitou et al. 2017).

The magnitude of melatonin suppression varies depending on light intensity (Zeitzer et al. 2000), exposure duration (Aoki et al. 1998), and wavelength composition (Brainard et al. 2001). Recently, however, there is growing evidence that prior light history has an impact on the magnitude of melatonin suppression in response to a subsequent light stimulus at night. Several studies have suggested that less daylight can increase melatonin sensitivity to light at night (Hebert et al. 2002; Smith et al. 2004). A field study with human subjects showed that melatonin suppression in response to 500 lx light at night was greater following exposure to dim light (wearing dark goggles with 2% transmission lenses) for one week than following exposure to bright light for one week (Hebert et al. 2002). Similar results were obtained in laboratory studies on melatonin suppression (Smith et al. 2004) and circadian phase shift (Chang et al. 2011). The results of those studies, however, were likely to have been affected by great differences in the intensities of prior light conditions. One study, however, showed significantly dampened melatonin suppression in response to blue light (460 nm monochromatic light) following 2-h exposure to dim white light (18 lx) compared to that following 2-h dark adaptation (Jasser et al. 2006). Taken together, the results suggest that melatonin sensitivity to a light stimulus can be increased or decreased depending on the relative intensity of a prior light stimulus to the target light stimulus.

Although the mechanism involved in the effect of prior light history is not clear, it seems that a long period of photic adaptation alters the absolute response threshold of photoreceptors and/or photosensory inputs from the photoreceptors to the

SCN. In humans, the visual photoreceptors (i.e., rods and cones) and especially a small subset of retinal ganglion cells expressing melanopsin (mRGC) contribute to non-visual effects on the circadian system (Gooley et al. 2012); those are designed to adapt to various changes in ambient light in a short time. For instance, exposure of the eyes to bright light desensitizes the visual photoreceptors to facilitate response to intensity increment (i.e., light adaptation) (Fain et al. 2001). Conversely, darkness fully recovers the photoreceptors from the desensitized state (i.e., dark adaptation) (Lamb & Pugh 2004). It should be noted, however, that the time required for photo-regeneration becomes longer as the photopigment bleaches more. Recently, evidence of the adaptation capacity of mRGC to light has been provided (Wong et al. 2005). One study with young human subjects, for example, showed that blocking short-wavelength light, which dramatically activates the mRGC, by using orange-colored contact lenses (i.e., blue light-filtering lenses) immediately reduced melatonin suppression but that the reduction in melatonin suppression disappeared 16 days after wearing the blue light-filtering lenses (Gimenez et al. 2014).

If two lights of different intensities are emitted alternately for long or short durations, can the effects of the short duration exposure be ignored? Some researchers have demonstrated that using intermittent light (e.g., alternate exposure to bright light and darkness) during the night for multiple days is effective for shifting the phase of human circadian rhythm (Baehr et al. 1999; Rimmer et al. 2000; Crowley et al. 2003; Gronfier et al. 2004; Smith et al. 2009). A mathematical model of the effects of brief light on the human circadian pacemaker has been proposed for explaining the results of previous investigations (Kronauer et al. 1999). Recent studies, however, showed that even brief (12 minutes or shorter) exposure to bright light was able to elicit circadian phase delay and melatonin suppression after previous adaptation to dim light during a constant routine (Chang et al. 2012; Rahman et al. 2017).

Although there is a possibility that exposure to darkness or dimmer light or brighter light can alter (at least temporarily) the sensitivity of non-visual responses to a subsequent light stimulus, the intermittent light conditions used in most previous studies are far from real working conditions. Furthermore, the effects of short exposure to very dim light during night work under light of constant medium intensity are unknown. In the present study, we therefore investigated whether short-duration exposure to very dim light or very bright light influences melatonin suppression, subjective sleepiness, and performance during exposure to constant light of medium intensity.

Materials and Methods

Subjects

Twenty-four healthy male university students participated in this study. Twelve subjects (mean ± SD age: 20.0 ± 0.9 years) participated in Experiment 1 and the remaining 12 subjects (mean ± SD age: 21.3 ± 2.5 years) participated in Experiment 2. None of the participants showed extreme morningness or extreme eveningness as assessed by a Japanese version of the Morningness-Eveningness Questionnaire (Ishihara et al. 1984). Subjects who had engaged in night shift work or who had experienced time zone travel (i.e., at least > 1 time zone) in the previous three months were excluded from the study. Signed written informed consent to take part in the research study, which was approved by the Ethical Committee of Kyushu University, was obtained from all participants. The experiments were conducted in accordance with the Declaration of Helsinki.

Experimental light conditions

The vertical illuminance and irradiance of each light condition were measured at eye level in a sitting position using an illuminance spectroradiometer (CL-500A, KONICA MINOLTA INC., Japan). Two experimental chambers were used for the experiment (chamber 1) and for 10 min breaks (chamber 2). In chamber 1, light-emitting diode (LED) ceiling lights (HH-LC569A, Panasonic Inc., Japan) were set up for exposure to constant medium intensity light (~550 lx). Fluorescent ceiling lights (FPL36CW, Panasonic Inc., Japan) were used for medium breaks (~430 lx) or bright breaks (~4700 lx). For dim breaks (<1 lx), incandescent bulbs were installed on the floor as indirect lighting. Detailed information on each light condition is given in Table 1. Melanopic lux was calculated using an excel-based toolbox provided by the Lucas Group at the University of Manchester (Enezi et al. 2011). (http://lucasgroup.lab.manchester.ac.uk/measuringmelanopicilluminance/).

Procedure

Two experiments with a crossover design, including dim breaks vs. medium breaks (Experiment 1) and bright breaks vs. medium breaks (Experiment 2), were conducted to investigate the effects of each short-duration (10 min) light exposure condition on melatonin suppression, subjective sleepiness, and performance during exposure to constant bright light. Each participant was therefore required to visit our laboratory

twice with an interval of 2 weeks. Six participants simultaneously participated in each experiment. Prior to the experiment, participants were instructed to sleep for more than 7 hours between 00:00 and 08:00h for one week. An accelerometry-based activity monitor (Lifecorder plus, Suzuken Co Ltd, Japan) and daily sleep diary were used to confirm the implementation of sleep intervention during the control period. Furthermore, each participant sent us a message via a mobile phone shortly before bedtime and shortly after waking up. If there was no message from a participant, we called the participant to confirm his situation. The participants were instructed not to drink alcohol from three days before the experiment. Also, excessive exercise, napping, and caffeine consumption were not allowed commencing the day before the experiment.

The procedures used for Experiment 1 and Experiment 2 were same (Figure 1). The participants arrived at the experimental facility at about 12:00h and dressed into the experimental clothes (short-sleeved T-shirt, short pants, and no socks) after receiving brief instructions for the experiment. The participants stayed in chamber 1 in a sitting position from 13:00h until the end of the experiment. The room illuminance was 275 lx (vertical illuminance at eye level) from 13:00 to 19:00h. The light illuminance was then changed into a dim light (< 10 lx) from 19:00 to 01:00h. The participants were allowed to use portable devices (e.g., smartphone, tablet pc, laptop, set to minimum brightness) and read books between 19:00 and 00:00h. For reference, the illuminances of the self-illuminating portable devices were measured under 2 lx at a distance of 20 cm from the center of each screen. The participants started simulated night work in a sitting position from 00:00 to 06:00h (practice session between 00:00 and 01:00h) in chamber 1. The light illuminance was changed to medium intensity light (550 lx) from 01:00 until 06:00h. Each break condition was conducted every hour from 02:00 to 06:00h in chamber 2 and lasted 10 min. During each break, all of the participants performed light stretching (~2 min in a standing position) and a word chain game (Shiritori, ~5 min in a sitting position) and answered questionnaires (~3 min in a sitting position) that addressed subjective sleepiness (Karolinska sleepiness scale, KSS) and ‘dummy variables’ (e.g., physical fatigue and mood state). Each break was followed by 50-min of simulated night work in which the participants conducted PVT and answered the KSS and dummy questionnaires for 10 min followed by card games for 20 min. After a 5-min rest period, the participants conducted PVT, collected saliva, and answered the KSS for 15 min (Figure 1b). The order of the break conditions in each experiment (i.e., Experiment 1 or Experiment 2) was random for each participant. For example, some participants

performed the medium breaks on the first visit and the bright breaks (or dim breaks) on the next visit, and vice versa for other participants. Participants had dinner at 19:00h (typical Japanese food, the same dishes for all participants in every experiment) and a late-night snack at 23:30h (rice, miso soup). No drinks except for water were provided throughout each experiment.

Salivary samples were collected hourly using a plain cotton plug (Salivette Sarstedt, Germany) from 21:00h until the end of the experiment. Participants did not drink any water for 15 min prior to each salivary sample collection. Subjective sleepiness was evaluated using the KSS at 1-hour intervals from 21:00 to 06:00h, 5 min before collecting the salivary sample. Subjective sleepiness during each break was also evaluated to confirm the acute effects of the break on sleepiness. Each participant performed the Psychomotor Vigilance Task (PVT) twice with a 1-hour interval, soon after the break and shortly before the next break. The PVT was performed for 5 min using Presentation (Neurobehavioral Systems Inc., Albany, CA). A visual stimulus was displayed randomly on the PC display at intervals of 2 to 10 sec. Participants were instructed to press the space key on the keyboard as soon as possible after the appearance of the visual stimulus. A beep sound was emitted from each earphone if the participants did not react within 3000 msec.

Sample analysis

Salivary melatonin concentrations were measured by radioimmunoassay kit (RK-DSM; Buhlmann Laboratories AG, Allschwil, Switzerland). Melatonin area under the curve (AUC; trapezoidal approximation) between 21:00 and 06:00h was calculated to evaluate the overall effect of each break condition on melatonin suppression. Data of three participants were excluded from analysis of the results for bright breaks, since each participant showed a gap longer than 1 hour in the time of dim light melatonin onset (DLMO) between the conditions (medium breaks vs. bright breaks). DLMO was determined by linear interpolation between two time points at which melatonin concentration crossed the 3.0 pg/ml threshold (Benloucif et al. 2008).

Statistical analysis

In statistical comparisons between the conditions (medium break vs. bright break or medium break vs. dim break) for the melatonin profile, subjective sleepiness (KSS), performance (PVT), repeated-measures two-way ANOVA (SPSS 23.0, IBM© SPSS©

Statistics) with light conditions and time (during the simulated night work) as independent factors was conducted. Greenhouse-Geisser correction was performed when Mauchily's sphericity assumption was largely violated. A two-sided, paired Student's t-test was used for planned comparisons between the light conditions during the night work when a significant interaction between the independent factors was found. For the comparison of numbers of PVT lapses between the conditions, the Wilcoxon signed-rank test was conducted. A P-value of less than 0.05 was considered statistically significant.

Results

Melatonin suppression

Figure 2 shows the melatonin profiles obtained from each experiment. In both experiments, melatonin gradually increased under the dim light condition (21:00-01:00h) but was immediately attenuated by light exposure (550 lx) from 01:00h in both experiments. However, the aspects of melatonin suppression were different with the light conditions during breaks.

In Experiment 1 (Figure 2a), repeated-measures two-way ANOVA with light condition (medium break vs. dim break) and time (01:00~06:00h) showed a main effect in light condition ($F_{1, 11} = 6.966$, $P = 0.027$) but not in time ($F_{1.951, 21.463} = 0.511$, $P = 0.603$, ns). There was a significant interaction between condition and time ($F_{2.827, 31.095} = 3.826$, $P = 0.021$). A paired t-test for melatonin concentrations at each time point showed that the dim break resulted in greater melatonin suppression than did the medium break at 04:00, 05:00, and 06:00h ($P = 0.011$, $P = 0.007$, and $P = 0.001$, respectively). A comparison of the melatonin AUCs between the conditions showed that there was a significant tendency for lower melatonin concentration in the dim break condition compared to that in the medium break condition.

Similarly, in Experiment 2 (Figure 2b), repeated-measures two-way ANOVA with light condition (medium break vs. bright break) and time (01:00~06:00h) showed a main effect in light condition ($F_{1, 8} = 9.837$, $P = 0.014$) but not in time ($F_{5, 40} = 0.981$, $P = 0.441$, ns). A significant interaction between condition and time was found ($F_{5, 40} = 4.484$, $P = 0.002$). A paired t-test for melatonin concentrations at each time point showed that the bright break resulted in lower melatonin suppression than did the medium break at 04:00, 05:00, and 06:00h ($P = 0.009$, $P = 0.038$, and P = 0.001, respectively). There was also a significant tendency for higher melatonin concentration

in the bright breaks than in the medium breaks at 05:00h (paired t-test, $P = 0.050$). The melatonin AUC in the bright breaks was significantly greater than that in the medium breaks.

Subjective sleepiness (KSS)

The results for subjective sleepiness are shown in Figure 3. In Experiment 1 (dim breaks), subjective sleepiness gradually increased over time during the simulated night work span ($F_{8, 34.031} = 9.066$; $p < 0.001$), but it showed almost the same pattern in the conditions (medium vs. dim) ($F_{1, 11} = 0.723$; $p = 0.413$). In addition, no significant interaction was found between conditions and time ($F_{8, 42.824} = 1.709$; $p = 0.167$). Similarly, in Experiment 2 (bright breaks), there was a significant main effect of time ($F_{8, 64} = 14.030$; $p < 0.001$), but no main effect of condition ($F_{1, 8} = 0.018$; $p = 0.897$), and no interaction between condition and time ($F_{8, 64} = 1.195$; $p = 0.316$) were found.

PVT

Figure 4 shows the results for reaction speed (mean reciprocal reaction time: mean 1/RT) (Basner & Dinges 2011) in the two experiments. In ANOVA analysis for mean 1/RT, there were significant main effects of time in Experiment 1 (dim breaks) ($F_{8, 27.005} = 8.729$; $p = 0.001$) and in Experiment 2 (bright breaks) ($F_{8, 16.421} = 11.593$; $p = 0.001$). However, mean 1/RT was not significantly different between conditions in both experiments ($F_{1, 11} = 0.0004$; $p = 0.984$ in Experiment 1, $F_{1, 8} = 0.018$; $p = 0.897$ in Experiment 2). Also, no interactions between conditions and time were found ($F_{8, 88} = 0.927$; $p = 0.499$ in Experiment 1, $F_{8, 22.129} = 1.073$; $p = 0.377$ in Experiment 2).

A comparison of the numbers of lapses at each time point (Wilcoxon signed-rank test) showed that there was no significant difference at any time points between medium breaks and dim breaks and between medium breaks and bright breaks (Figure 4).

Discussion

In the present study, we investigated whether periodic short-duration exposures (for 10 min at hourly intervals) to very bright (bright breaks) or very dim light (dim breaks) affect physiological responses including melatonin suppression, subjective sleepiness, and performance during exposure to constant medium intensity light. We found that both the dim breaks and bright breaks indirectly, rather than directly, affected melatonin suppression

during the experiments: the dim breaks promoted melatonin suppression, whereas the bright breaks diminished melatonin suppression. A possible reason for these results is that the dim breaks or the bright breaks sensitized or desensitized pineal melatonin to the subsequent constant light exposure during the experiment.

The effect of brief light exposure during each break on melatonin synthesis was first observed at the 04:00h time point, just before the 3rd break in both experiments, and the sensitized or desensitized states lasted until the end of the experiment (i.e., 06:00h). However, this does not mean that the first break had no effect at all, but rather it seems that adaptation to dim light for 4 hours before the start of the experiment caused strong melatonin suppression by sensitizing melatonin responsiveness to light (see melatonin suppressions at 02:00h in Figure 2). This may indicate that melatonin sensitivity can be changed shortly after short-duration exposure to light. Our results might suggest that using brief photo-adaptation probably enables real-time adjustment of melatonin sensitivity against a current light stimulus depending on relative photic intensity.

Although the results of the present study are similar to previous findings of prior light history having an effect on melatonin (see Introduction section), the underlying mechanisms responsible for the results appear to be different. In previous studies, it is more likely to be a compensatory adaptation by long-term adaptation of photoreceptors to a given photo-environment. On the other hand, although the mechanisms by which short adaptations during breaks contribute to melatonin sensitivity are not known, they seem to be associated with bleaching and recovery mechanism of photoreceptors in the retina. For instance, the bleached photopigments might be partially recovered by the dim breaks, leading to an increase in gain of the phototransduction cascade. Likewise, profound bleaching of a substantial fraction of the photopigments due to exposure to very bright light during the bright break might lead to attenuation of melatonin sensitivity to the subsequent light (Fain et al. 2001). On the other hand, visual photoreceptors, including rods and cones, saturate at a relatively low-intensity level of light (Lucas et al. 2003). Given that mRGC compensate the functional limitations of the visual photoreceptors for higher light intensities (Gooley et al. 2012), it might be more important to understand whether the mRGC has such capacity of light adaptation. The photopigment melanopsin has been shown to be homologous to invertebrate opsin (rhabdomeric opsin) (Shichida & Matsuyama 2009), and it has therefore been hypothesized that melanopsin uses the rhabdomeric phototransduction cascade (Hillman

et al. 1983). Although controversial, several previous studies have provided evidence of a bi- or tri-stable signaling state in mammalian melanopsin including, for example, red-light enhancement for pupil response to blue light (Graham et al. 2008; Mure et al. 2009; Emanuel & Do 2015).

Another in vitro study demonstrated that prior light stimulus alters the sensitivity of rat mRGC to subsequent light exposure in a way similar to that of photoreceptor adaptation, rather than neural network adaptation: a brief flash desensitized the cells whereas darkness re-sensitized the cells without synaptic inputs from rods and cones (Wong et al. 2005). According to the study, mRGC completed light adaptation (i.e., desensitization) within 5 min. On the other hand, the kinetics of dark adaptation appeared to be even slower for mRGC than for rods, as the cell showed a striking increase in sensitivity after 30~40 min of dark adaptation and kept increasing for at least 2 h and 40 min. More recent studies, however, have shown that synaptic inputs from the classical photoreceptors (i.e., rods and cones) via the inner plexiform layer to mRGC increase the sensitivity of mRGC to light (Wong et al. 2007). Based on these results, exposure to very bright light for 10 min (i.e., bright breaks) might be sufficient to cause a massive decrease in the photosensitivity of mRGC, and this phenomenon likely lead to the attenuation of melatonin suppression in Experiment 1. However, recovery in near darkness for 10 min (i.e., dim breaks) was probably not sufficient to elicit a significant increase in the photosensitivity of mRGC; rather, synaptically mediated signals from partially dark-adapted classical photoreceptors might be more responsible for the promotion of melatonin suppression in Experiment 2.

There were no remarkable effects of dim breaks or bright breaks on alertness, i.e., subjective sleepiness and performance of the psychomotor vigilance task. As an indirect alerting effect of light via retinal projection to the SCN, the magnitude of melatonin suppression was thought to be involved in subjective sleepiness (Cajochen 2007). Nonetheless, subjective sleepiness or reaction speed (i.e., mean 1/RT) was consistently increased or decreased over time in a similar pattern regardless of the break conditions. Similarly, unlike medium breaks, bright breaks and dim breaks did not have an additional effect on the number of lapses. On the other hand, as a direct alerting effect of light (Souman et al. 2018), the bright breaks were expected to be able to delay the decrease in alertness. However, we could not find such a beneficial effect even when we compared the reaction velocities or numbers of lapses before and after the bright breaks (Figure 4b and Figure 5b). Moreover, although acute reduction of

subjective sleepiness tended to emerge during each break session, it seems to be a temporal effect associated with moving to a break room or a light stretch during each break session, rather than than a direct alerting effect of light during each break. Nevertheless, it should be noted that the participants were continuously exposed to medium intensity light during the simulated night work, and this might have diluted the additional effects of bright breaks or dim breaks on alertness considering the dose-response relationship between light intensity and alertness (Cajochen et al. 2000). Also, high sleep pressure due to prior wakefulness might be partially responsible for the results, since the participants did not take a nap before the start of the experiments.

Epidemiological studies conducted over the past few decades have suggested adverse relationships of night shift work with acute and chronic adverse health problems (Kantermann et al. 2010; Parent et al. 2012; Evans & Davidson 2013; Kamdar et al. 2013). Direct effects of exposure to bright white light, especially blue-enriched light, on the circadian system, such as melatonin suppression and circadian misalignment, for example, between the biological clock and the social-behavioral cycle have been suspected as factors involved in the risks (Wittmann et al. 2006; Touitou et al. 2017). Nonetheless, in some ways, exposure to bright white light (i.e., blue-enriched light) is also helpful for keeping night workers awake and providing better visibility, leading to better performance and fewer accidents due to human errors (Cajochen 2007; Chellappa et al. 2011; Kraneburg et al. 2017). For attenuation of melatonin suppression without a negative effect on performance, the use of lighting with less short-wavelength components (Kozaki et al. 2008) and wearing blue light-filtering goggles (Kayumov et al. 2005), or a red-visor cap (Higuchi et al. 2011), have been proposed. In addition to these proposals, a countermeasure for night shift workers is also suggested by our findings that bright breaks can reduce melatonin suppression by light without having adverse effects on sleepiness or performance.

However, a field study in which the effect of bright light exposure during a short break (~20 min) in night work on melatonin was investigated showed no such desensitization in melatonin suppression. In that field study, the subjects showed greater melatonin suppression in night work when they took a break with exposure to bright light (2500 lx) than when they took a break with exposure to normal light (300 lx) (Lowden et al. 2004). However, a limitation of that field study is that the timing and duration of the breaks were not strictly controlled. Another limitation is that the subjects were allowed to leave the workplace for a short period. These limitations, however,

rather remind us about a question if similar results could be obtained by conducting bright breaks in a real night workplace. Additionally, it is uncertain in the present study whether the hourly repetitive execution of breaks was essential to achieve persistent effects on melatonin suppression. Although taking rest breaks is known to be effective for decreasing accident risks, recovering from physical fatigue and maintaining arousal level, taking breaks more than once per hour tends to disturb work (Tucker 2003). In this regard, it is essential to clarify the minimum number of breaks that is necessary to obtain the same results as those in the present study.

In the present study, although the pace of melatonin synthesis was remarkably diminished after light exposure (~550 lx), we did not observe dramatic melatonin suppression as found in some previous studies using a protocol and illuminance level similar to the present study. For example, McIntyre et al. (1989) reported that 1-h light exposure (500 lx) from midnight caused about 40% suppression of melatonin compared to the melatonin concentration just before light exposure (Mcintyre et al. 1989). Laakso et al. (1993) reported that melatonin suppression following 1-h light exposure from 23:00h amounted to as much as 53% (Laakso et al. 1993). Ethnicity might be partially responsible for the inconsistency in melatonin suppression induced by nocturnal light exposure. Higuchi et al. (2007) reported that melatonin suppression following 2-h light exposure (1000 lx) was greater in Caucasian than Asian subjects (Higuchi et al. 2007a). Although Aoki et al. (1998) also found that melatonin suppression amounted to as much as 40.1% following 2-h light exposure (500 lx) in Asian subjects (Aoki et al. 1998), dark adaptation by 5-h sleep before the light exposure possibly influenced the result (Jasser et al. 2006). Subjects in the previous study were directly exposed to a specially designed light source in a fixed position. However, in the present study, we used ceiling light, and the gaze of each participant was not strictly fixed; hence, the light intensity reaching the retina might have been less than 550 lx in the present study.

There is a question that remains unanswered: Can bright breaks or dim breaks modify the circadian phase shift caused by light exposure? There have been practical interventions using intermittent light to entrain the circadian clock of shift workers to long-term night shift duty (Baehr et al. 1999; Crowley et al. 2003; Smith et al. 2009; Smith & Eastman 2012). Intermittent light was used for multiple days in those previous studies, mainly to delay the phase of the circadian pacemaker. However, the results for melatonin in the present study indicate the possibility that circadian phase delay during night work can not only be promoted by conducting dim breaks, but it can also be

attenuated by conducting bright breaks. Given the greater melatonin suppression in the condition of dim breaks than in the condition of medium breaks, conducting dim breaks during night work can probably cause a larger phase delay than can continuous exposure to medium intensity light. Modulation of circadian phase to both advance and delay might be easier by conducting bright or dim breaks based on the human phase response curve (St Hilaire et al. 2012).

This study has several limitations. We measured salivary melatonin levels at hourly intervals; hence, acute effects of breaks on melatonin could not be determined in the present study. All of the participants in this study were healthy young male adults. However, inter-individual differences in the sensitivity of pineal melatonin have been shown in previous studies. Although there is still lack of agreement, there has been an accumulation of evidence indicating an age-dependent difference in pineal melatonin sensitivity (Charman 2003; Higuchi et al. 2014; Lee et al. 2018). Also, one study has suggested greater sensitivity in females than in males (Monteleone et al. 1995). The experiments in this study were conducted in different seasons: The experiment for dim breaks was conducted in summer (July), while the experiment for bright breaks was conducted in winter (from January to February). It has been reported that melatonin suppression by light at night is greater in winter than in summer (Higuchi et al. 2007b). Therefore, it is necessary to verify the reproducibility of our findings for different seasons.

In addition, inter-individual differences in the photo-sensitivity of pineal melatonin have been shown in previous studies (Higuchi et al. 2008; Santhi et al. 2012; Phillips et al. 2019). Indeed, in the present study, some participants showed strong melatonin suppression during light exposure, while others, especially participants who had a relatively low melatonin level at 01:00h (e.g., below 10 pg/ml), showed weak melatonin suppression. Furthermore, some participants showed quick recovery from the melatonin suppression and an increase in melatonin concentration over time. It remains unclear what causes the individual differences, but several recent studies have suggested that genetic variations in the clock genes are associated with inter-individual differences in melatonin suppression (Chellappa et al. 2012; Akiyama et al. 2017). Further investigation should be carried out to identify the individual differences in non-visual photo-sensitivity.

Our findings suggest that periodic exposure to light for a short duration during exposure to constant light affects melatonin sensitivity to the constant light depending

on the difference between light intensities in the light conditions (i.e., exposure to short light vs. exposure to constant light). In most previous studies, the effects of light with fixed intensity and/or spectral composition on the circadian system were investigated. However, humans generally do not stay at the same place for long duration; the light environment surrounding us frequently changes in real life. In this regard, the findings in the present study suggest that exposure to light of various intensities at night could be a factor influencing the light-induced melatonin suppression in real life.

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Declaration of interest statement

The authors report no conflict of interest.

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646 Table 1. Light conditions used in this study

	Night Shift Light (01:00-06:00h)		10 Min Breaks (4 per night, 1/h)	
	Medium Intensity Light	Medium Intensity Light	Bright Light	Dim Light
Illuminance (lx)	550	430	4700	1
Color temperature (K)	4500	3850	5000	-
Photon flux ($log_{10}1/cm^2/sec$)	14.70	14.54	15.62	12.63
Melanopic lux	83.37	52.86	787.84	0.09

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Figure 1. Experimental protocol (a) and details of the experimental tasks during the break and during the simulated night work (b). Participants always stayed in chamber 1 from 13:00h to the end of the experiment (i.e., 06:00h the next morning) except for when they took breaks in chamber 2.

Figure 2. Melatonin profiles (means ± standard error) and AUCs (means + standard error) in Experiment 1 (a: medium breaks vs. dim breaks) and Experiment 2 (b: medium breaks vs. bright breaks). The black arrows indicate the times when breaks were conducted. **: $p < 0.01$, *: $p < 0.05$

Figure 3. Subjective sleepiness (means ± standard error) in Experiment 1 (a: medium breaks vs. dim breaks) and Experiment 2 (b: medium breaks vs. bright breaks). The black arrows indicate the times when breaks were conducted.

Figure 4. Cognitive performance (i.e., reaction speed [mean 1/RT]; a, b) and number of lapses (c, d) in Experiment 1 (medium breaks vs. dim breaks; left columns) and Experiment 2 (medium breaks vs. bright breaks; right columns). The black arrows indicate the times when breaks were conducted.

Figure 1

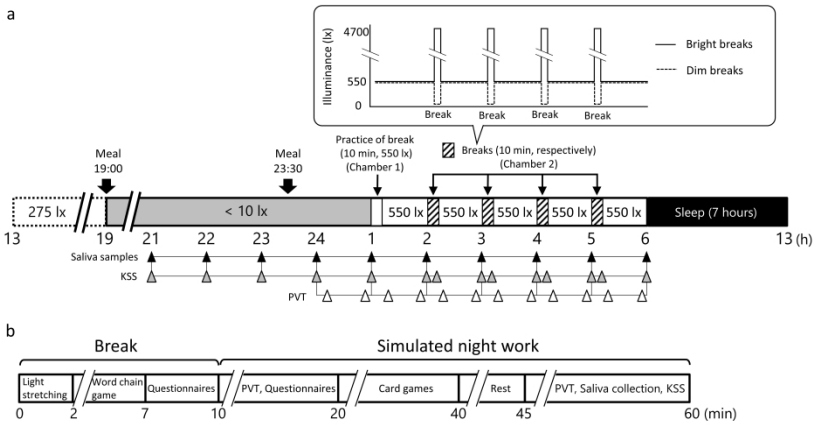
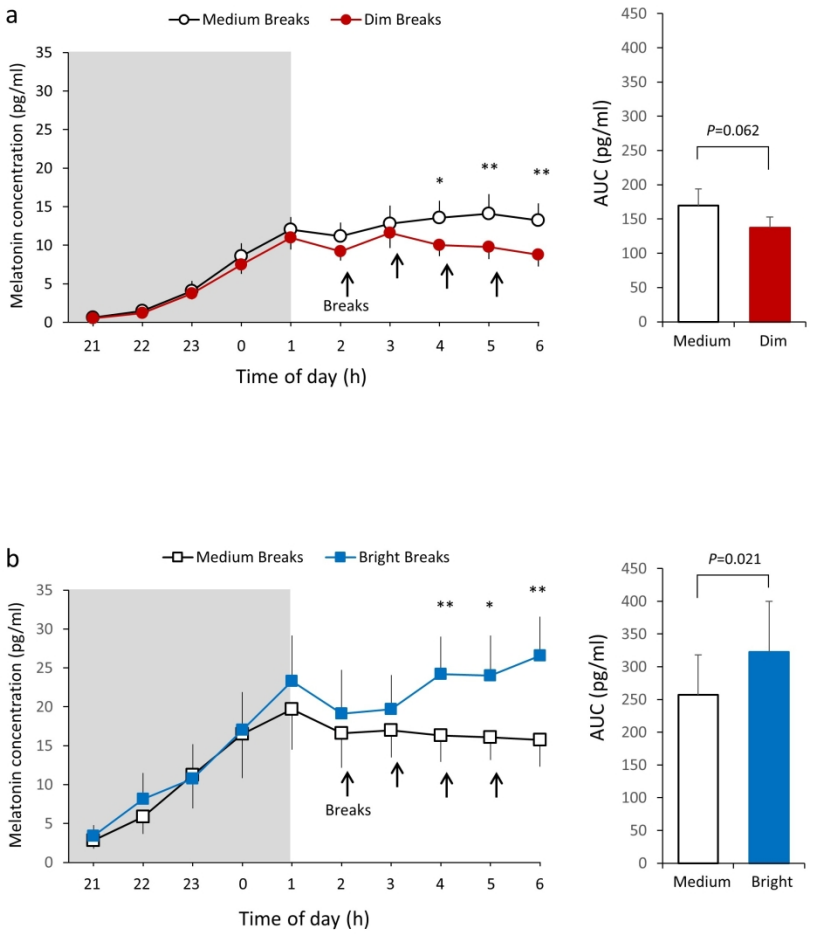


Figure 1. Experimental protocol (a) and details of the experimental tasks during the break and during the simulated night work (b). Participants always stayed in chamber 1 from 13:00h to the end of the experiment (i.e., 06:00h the next morning) except for when they took breaks in chamber 2.

338x190mm (600 x 600 DPI)

Figure 2



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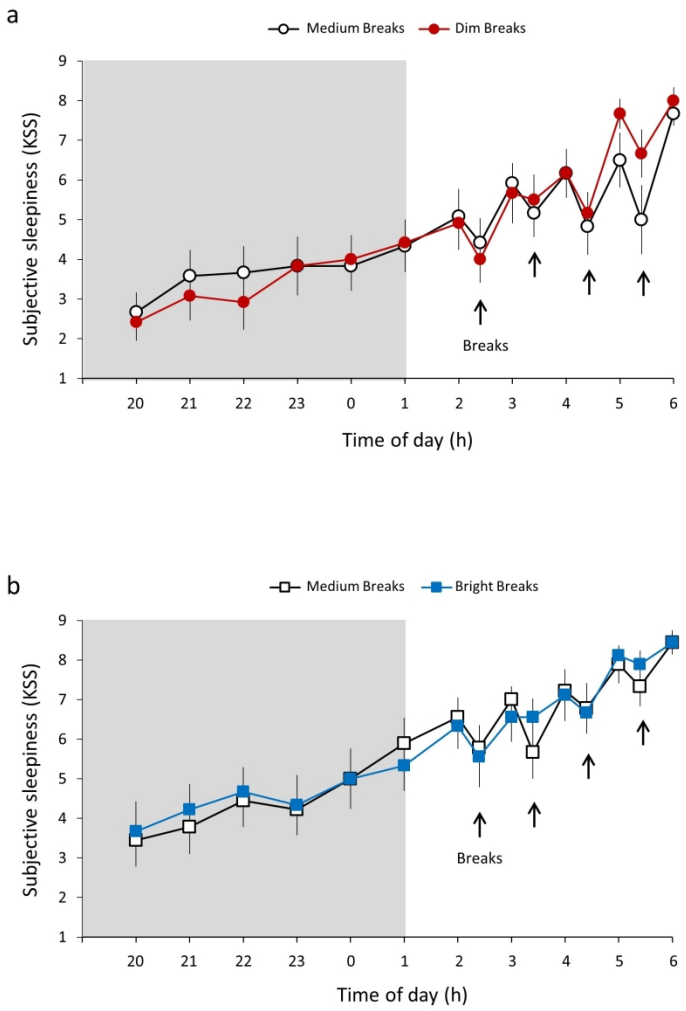
190x275mm (300 x 300 DPI)

Figure 3



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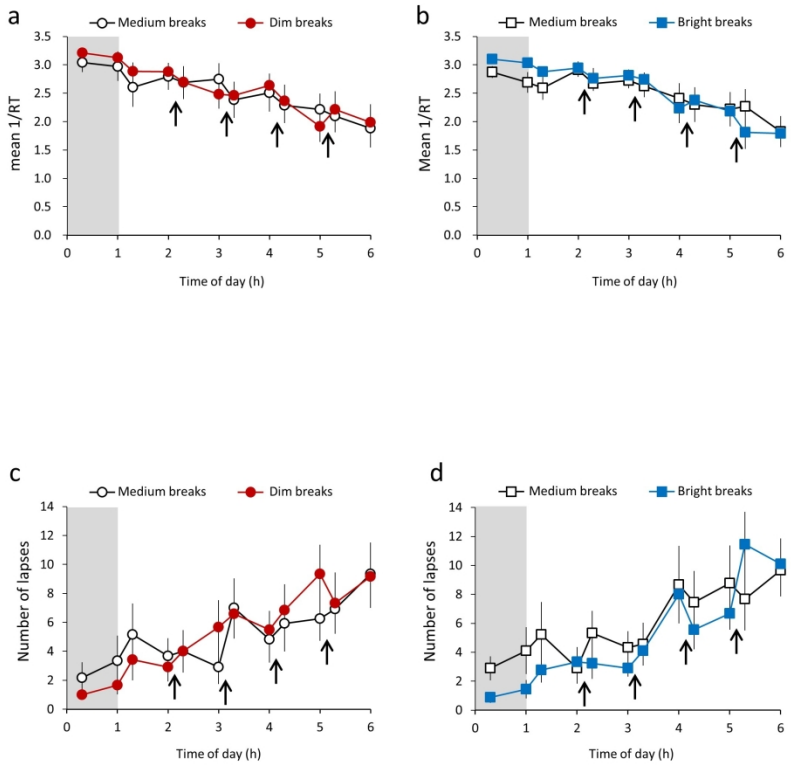

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