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**Original Article**

# Effects of 2-hour nighttime nap on melatonin concentration and alertness during 12-hour simulated night work

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**Abstract: A nighttime nap is expected to mitigate melatonin suppression during night work by blocking light input to the retina, but it is unclear. In the present study, we investigated the effects of a nap break on melatonin level, subjective sleepiness, and vigilance performance during simulated night work. Eleven healthy young males (mean ± SD age: 22.2 ± 4.1 years) participated in counterbalanced crossover design experiments with two conditions (nap vs. no nap). The subjects performed 12-hour simulated night work from 21:00 to 09:00 h (illuminance: ~500 lx). Subjects with a nap condition took a nap for 2 hours in a dark room from 03:00, while subjects with a no nap condition continued the simulated night work. The results showed that immediately after the 2-h nap break, the melatonin level at 05:00 h temporarily recovered from light-induced melatonin suppression during the simulated night work but significantly suppressed again at 07:00 and 09:00 h. Subjective alertness and vigilance performance were impaired immediately after the nap break but subsequently enhanced. The results suggest that a single nap break for 2 hours could be a strategy to enhance alertness during the last part of night shift but inadequate for mitigating melatonin suppression.**

**Key words:** Night work, Nighttime nap, Melatonin suppression, Subjective sleepiness, Vigilance performance

## Introduction

Epidemiological studies conducted in the past few decades have provided evidence that night work is associated with risks of health problems including sleep disorder, cardiovascular disease, diabetes, obesity, depression, and cancer[1, 2]. Melatonin suppression caused by light exposure during night work together with chronic circadian misalignment has been thought to be partially responsible for those health problems[3, 4]. Melatonin is a hormone released from the pineal gland solely during the night; it contributes

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to internal biological rhythm regulation. Also, melatonin is known to have an antioxidant function, and it has been investigated as a promising hormone for cancer prevention and treatment[5].

Exposure to light at night can immediately inhibit pineal melatonin synthesis, and its inhibition continues as long as the light exposure continues. Melatonin suppression in response to light depends on the light intensity[6], spectral composition[7], and exposure duration[8]. Its suppression is strong in response to blue-enriched (more short-wavelength components) white light with a high intensity. The most effective way to prevent melatonin suppression during night work may therefore be to reduce the intensity of light and short-wavelength light as much as possible[9, 10]. However, bright and/or blue-enriched lighting is desirable for providing night workers with good visibility and alertness[11].

Many shift workers take an afternoon or evening nap, also known as a prophylactic nap, before starting night work to prevent occupational errors and accidents caused by severe sleepiness, reduced alertness, and fatigue. However, it is not easy to fall asleep in the early evening since the internal biological clock mediating physiological functions is not ready to sleep. On the other hand, taking a nighttime nap during night shift has also been suggested to relieve sleep pressure and recover alertness and performance losses[12].

The effects of nighttime naps ranging from a short duration (15 min) to a long duration (120 min) at different timings have been investigated in previous studies[13], but the optimal timing and duration of a nighttime nap are still controversial. In Japan, the Japanese Nursing Association recommends a nap of more than two hours if the night shift starts after 22:00 h and the working time exceeds eight hours. Considering that the first sleep cycle between non-REM sleep and REM sleep generally takes 1–2 hours, a nighttime nap for 120 min could be useful for sleep quality. Indeed, some previous studies have shown that a 120-min nap is superior to a 60-min nap in terms of sleep quality and performance[14, 15].

As an additional potential benefit of a scheduled nap for a relatively long duration, it is expected that a nap can mitigate melatonin suppression during night work[16]. It is hypothesized that napping in darkness could promote melatonin secretion by blocking light input to the retina. However, to our knowledge, this potential effect has not been investigated so far, and it is unclear how much pineal melatonin recovers during a nap break in night work. In the present study, we investigated the effects of a nap break on melatonin level, subjective sleepiness, and vigilance performance during simulated night work.

## Materials and Methods

*Subjects*

Eleven healthy university male students (mean ± SD age: 22.2 ± 4.1 years) participated in this study. None of the participants showed extreme morningness or extreme eveningness as assessed by a Japanese version of the Morningness-Eveningness Questionnaire. Subjects who had engaged in night work or who had experienced time zone travel in the previous one month or who had ever taken sleeping pills and tranquilizers were excluded from this study. All of the participants were non-smokers. Signed written informed consent, 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 conditions and procedures*

All of the subjects participated in two experimental conditions with a counterbalanced crossover design, including nap vs. no nap conditions. A wash-out period of 1 week was interposed between the conditions. Two chambers were prepared: chamber 1 for simulated night work and chamber 2 for a nap break or sleep. The temperature and relative humidity of both chambers were maintained at 25 degrees and 50%, respectively.

Each experimental condition was conducted for two consecutive nights. Prior to each experiment, the subjects were instructed to go to bed between 23:00 and 01:00 h and wake up between 07:00 and 09:00 h for one week. Wrist actigraphy with tri-axial accelerometers (MotionWatch 8, CamNtech Inc., UK) and a daily sleep diary were used to confirm sleep control implementation. Fig. 1 shows the details of the experimental protocols. The subjects arrived at our experimental facility at about 20:00 h and changed into comfortable clothes (their own home wear). After receiving brief instructions for the experiment, the subjects spent time in a dimly lit room (<15 lx, chamber 1) in a sitting position from 21:00 to 01:00 h. During this period, reading a book or using portable devices, including laptops, tablet pc, and smartphones, were allowed. The illuminance of the portable devices was always set to minimum brightness when the room light was adjusted to dim light (i.e., <5 lx as measured at a distance of 20 cm from the center of each screen). Subsequently, the subjects slept in darkness (chamber 2) from 01:00 to 09:00 h. Salivary samples were col-

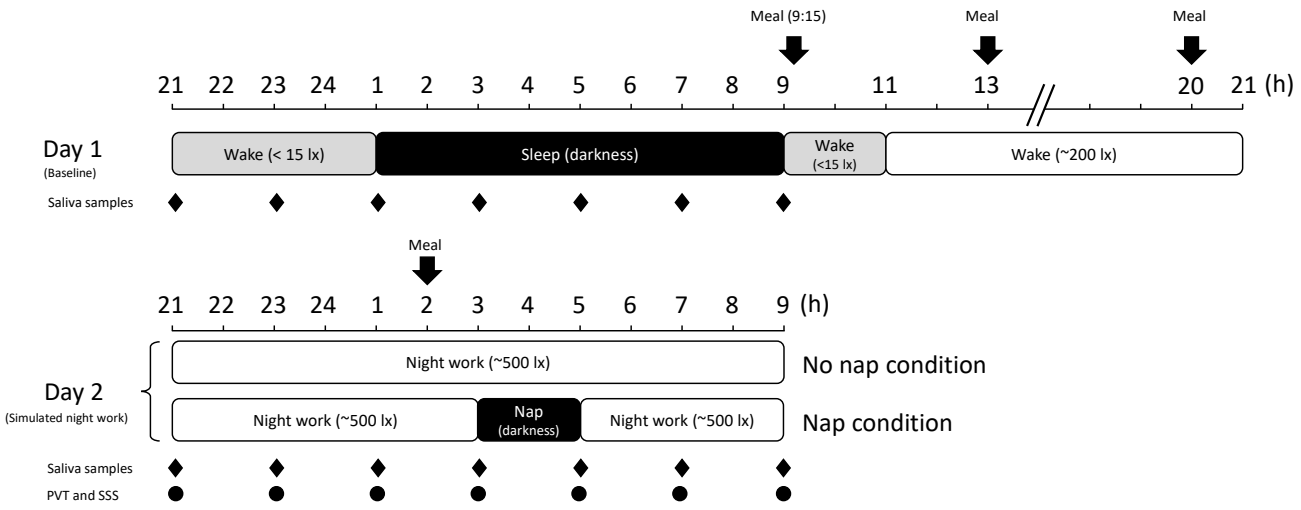


**Fig. 1. Experimental protocol with two conditions (nap vs. no nap) with a crossover design, showing light conditions, mealtime, and measurement timing. A one-week of wash-out period was interposed between the conditions.**

lected at 2-h intervals using a plain cotton plug (Salivette Sarstedt, Germany) from 21:00 to 09:00 h. When collecting salivary samples during the sleep period, the subjects were woken and instructed to raise their upper body.

On the next morning, the subjects moved into chamber 1 and had free time from 09:00 to 21:00 h. The illuminance was set to dim light (<15 lx) between 09:00 and 11:00 h and changed to light of medium intensity (~200 lx, 4,200 K, fluorescent light) between 11:00 and 21:00 h. The subjects were not allowed to leave the room (i.e., chamber 1) except for using the toilet. During the free time session, the subjects spent time reading a book or using portable devices. The subjects performed simulated night work under exposure to medium intensity light (~500 lx, 4,200 K, fluorescent light) from 21:00 to 09:00 h. During the simulated night work, participants were allowed to use a laptop to write documents and collect information via the internet except when conducting experimental tasks, including collecting salivary samples, PVT, and subjective sleepiness ratings. Also, the participants were instructed to stop all actions and rinse their mouths 15 minutes before saliva collection. Subjects with a nap condition took a nap for 2 hours in a dark room (i.e., chamber 2) from 03:00 h, while subjects with no nap condition continued the simulated night work. Salivary sample collection, sleepiness assessment (Stanford sleepiness scale: SSS), and a psychomotor vigilance test (PVT) were conducted at 2-h intervals from 21:00 to 09:00 h. Meals were provided four times at 09:15 (breakfast), 13:00 (lunch), 20:00 (supper), and 02:00 h (late night meal).

*Sample analysis*

Salivary melatonin concentration was determined using a radioimmunoassay (RK-DSM; Buhlmann Laboratories AG, Allschwil, Switzerland). Melatonin area under the curve (AUC) for the samples obtained between 21:00 and 09:00 h was calculated using the trapezoid method. In the PVT analysis, mean reciprocal reaction time (1/RT: reaction velocity) and 10% fastest reaction time (i.e., optimal alertness) were considered as dependent variables. Reaction time slower than 500 ms was counted as a lapse.

*Statistical analysis*

All of the results are presented as means ± standard error. SPSS 23.0 ((IBM© SPSS© Statistics) was used for all statistical analyses. In the statistical comparison of melatonin profiles between day 1 (baseline) and day 2 (simulated night work) or between nap and no nap conditions, repeated measures two-way ANOVA was performed separately in each experimental condition. Greenhouse-Geisser correction was performed when Mauchily's sphericity assumption was largely violated. Comparisons of melatonin concentrations at each time point and melatonin AUCs between day 1 (baseline) and day 2 (simulated night work) were performed using a two-sided, paired Student's *t-test*. Given that melatonin concentration could differ depending on the circadian rhythm, light exposure history, and food intake even in the same individual[17, 18], the amount of melatonin changes based on the melatonin concentration at 03:00 h (i.e., just before the nap intervention) were compared. Also, multiple comparisons with Holm correction were performed for the data obtained between 03:00 and 09:00 h.

In statistical comparisons between the nap conditions (i.e., nap vs. no nap) for subjective sleepiness (SSS), and performance (PVT reaction time), repeated-measures two-way ANOVA was conducted. A two-sided, paired Student's *t-test* was used for planned comparisons between the conditions (i.e., nap vs. no nap). The Wilcoxon matched-pairs signed-rank test was conducted to compare the numbers of lapses (i.e., nap vs. no nap) at each corresponding measurement time. A *p*-value of less than 0.05 was considered statistically significant. A *p*-value of less than 0.1 and greater than 0.05 was considered a significant tendency.

## Results

*Melatonin suppression*

Fig. 2 shows the average and individual melatonin profiles in each experimental condition. In the nap condition (Fig. 2A), repeated-measures two-way ANOVA with day (baseline vs. simulated night work) and time (03:00 ~ 09:00 h) showed main effects in day ($F_{1, 8}$=29.650, $p$=0.001) and time ($F_{3, 24}$=10.919, $p$<0.001). A significant interaction between day and time was found ($F_{3, 24}$=3.968, $p$=0.020). A paired *t-test* for melatonin concentrations between day 1 (baseline) and day 2 (simulated night work) at each time point showed that light exposure during simulated night work significantly suppressed melatonin compared to the baseline concentration at 03:00, 07:00, and 09:00 h ($p$<0.001, $p$=0.002, and $p$=0.006, respectively) but not at 05:00 h (i.e., shortly after the nap break) ($p$=0.197).

In the no nap condition (Fig. 2B), repeated-measures two-way ANOVA with day (baseline vs. simulated night work) and time (03:00 ~ 09:00 h) showed a main effect in day ($F_{1, 8}$=8.254, $p$=0.021) but not in time ($F_{1.327, 10.613}$=2.087, $p$=0.177; Greenhouse-Geisser corrected). There was no significant interaction between day and time ($F_{1.610, 12.876}$=0.065, $p$=0.904; Greenhouse-Geisser corrected). A paired *t-test* for melatonin concentrations at each time point showed that melatonin levels during simulated night work were significantly lower than those during baseline at 03:00 and 05:00 h ($p$=0.035 and $p$=0.042, respectively). Besides, there was a significant tendency for a lower melatonin concentration during the simulated night work than at baseline at 07:00 ($p$=0.062). There was no significant difference in melatonin concentration between baseline and simulated night work at 09:00 h ($p$=0.135).

Multiple comparisons with Holm correction among the time points (03:00 ~ 09:00 h) in each condition (i.e., nap and no nap conditions) were also performed. In the nap

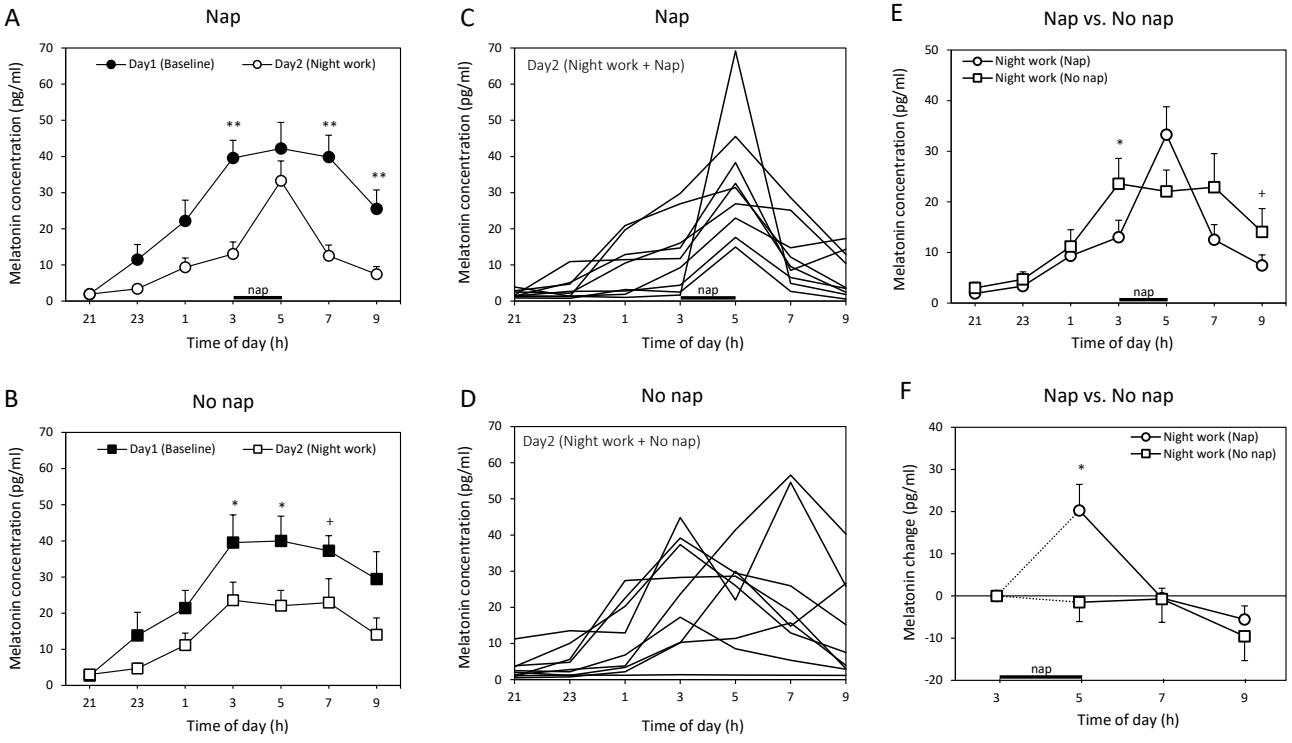


**Fig. 2. Comparison of salivary melatonin concentrations between the baseline day and the simulated night work (A and B) and individual melatonin profiles in each night work condition (C and D). Comparisons of melatonin concentrations (E) and melatonin changes (F) between nap and no nap conditions. **: $p$<0.01, *: $p$<0.05, +: $p$<0.1**

condition, melatonin concentration at 05:00 h was significantly greater than the concentrations at 03:00, 07:00, and 09:00 h ($p$=0.038, $p$=0.027, and $p$=0.006, respectively). In the no nap condition, however, there was no significant difference in melatonin concentrations among the time points.

In the direct comparison of melatonin profiles between nap and no nap conditions (Fig. 2E), repeated-measures ANOVA with condition (nap vs. no nap) and time (03:00 ~ 09:00 h) showed a main effect in time ($F_{3, 24}$=8.935, $p$<0.001) but not in condition ($F_{1, 8}$=1.577, $p$=0.245). A significant interaction between condition and time was found ($F_{3, 24}$=4.639, $p$=0.011). A paired *t-test* for melatonin concentrations at each time point showed that melatonin level at 03:00 h (i.e., before the nap intervention) was significantly higher in no nap condition than in nap condition ($p$=0.042). There was a significant tendency for a higher melatonin concentration in the no nap condition than in the nap condition at 09:00 h ($p$=0.055). However, there were no significant differences in melatonin levels between the nap and no nap conditions at 05:00 and 07:00 h ($p$=0.170 and $p$=0.091, respectively).

Comparison of relative melatonin changes, based on melatonin concentration at 03:00 h, between the nap and no nap conditions was also conducted (Fig. 2F). Repeated-measures ANOVA with condition (nap and no nap) and time (05:00 ~ 09:00 h) showed a main effect in time ($F_{2, 16}$=8.935, $p$<0.001) but not in condition ($F_{1, 8}$=2.678, $p$=0.140). A significant interaction between condition and time was found ($F_{2, 16}$=5.489, $p$=0.015). A paired *t-test* for melatonin concentrations at each time point showed that melatonin level at 05:00 h (i.e., soon after the nap intervention) was significantly greater in nap condition than in no nap condition ($p$=0.026). However, there were no significant differences in melatonin concentrations between nap and no nap conditions at 07:00 and 09:00 h ($p$=0.972 and $p$=0.507, respectively).

Fig. 3 shows the melatonin AUCs in each experimental condition. Repeated-measures ANOVA with condition (nap vs.no nap) and day (baseline vs. simulated night work) showed a main effect in day ($F_{1, 8}$=32.233, $p$<0.001). However, there were no main effect in condition ($F_{1, 8}$=0.173, $p$=0.688) and no significant interaction between condition and day ($F_{1, 8}$=0.537, $p$=0.485). A paired *t-test* was performed to compare melatonin AUCs between baseline and simulated night work in each experimental condition. In the nap condition, the melatonin AUC on day 2 (simulated night work) was significantly lower than that on day 1 (baseline) ($p$<0.001). In the no nap condition, the melatonin AUC on day 2 (simulated night work) was significantly lower than that on day 1 (baseline) ($p$=0.010). The percentage of melatonin AUC suppression in each condition was calculated based on the melatonin AUC on the baseline day and compared between the conditions (i.e., nap vs. no nap). There was no statistical difference ($p$=0.186, paired *t-test*).

*PVT*

The results for mean reciprocal response time (1/RT), 10% fastest response time, and lapses are shown in Fig. 4. In ANOVA for mean 1/RT, there were no significant main effects in condition (nap vs. no nap; $F_{1, 8}$=2.702, $p$=0.139) and time (03:00 ~ 09:00 h; $F_{1.392, 11.140}$=2.107, $p$=0.173; Greenhouse-Geisser corrected). However, an interaction between condition and time was found ($F_{1.404, 11.229}$=4.838, $p$=0.040; Greenhouse-Geisser corrected). Similar results were obtained in ANOVA for 10% fastest response time: no main effects in condition (nap vs. no nap; $F_{1, 8}$=1.202, $p$=0.305) and time (03:00 ~ 09:00 h; $F_{1.833, 14.664}$=2.269, $p$=0.141) but an interaction between condition and time ($F_{3, 24}$=7.636, $p$=0.001).

In the comparison of mean 1/RT (response speed) at each

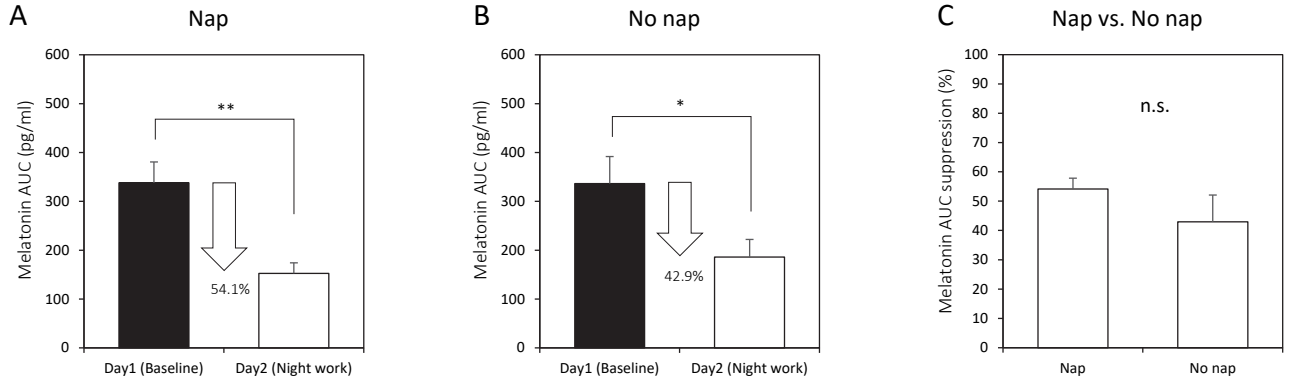


**Fig. 3. Comparison of melatonin AUC between the baseline day and the simulated night work day (A: nap condition, B: no nap condition). Comparison of melatonin AUC suppression (%) between nap and no nap conditions (C). **: $p$<0.01, *: $p$<0.05**

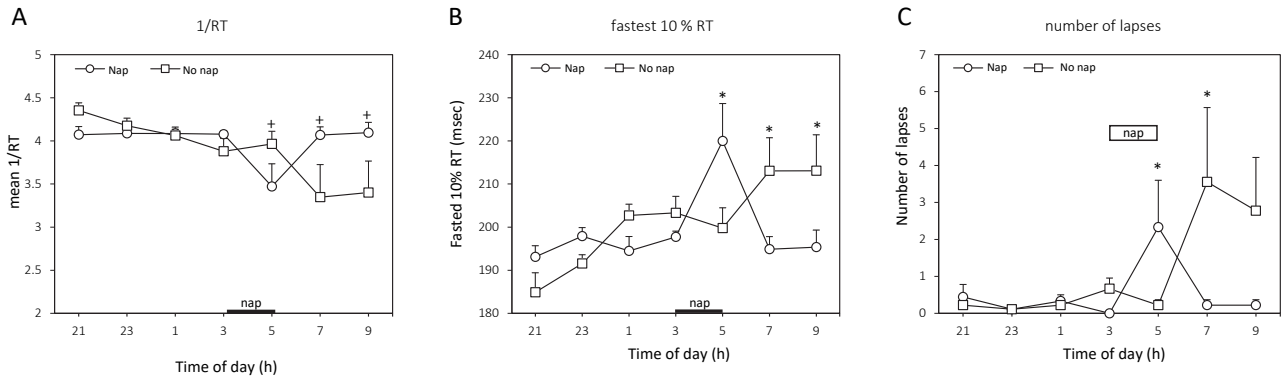


**Fig. 4. Comparison of PVT performance including 1/RT (A), fastest 10% RT (B), and number of lapses (C) between the nap and no nap conditions. *:$p$<0.05**

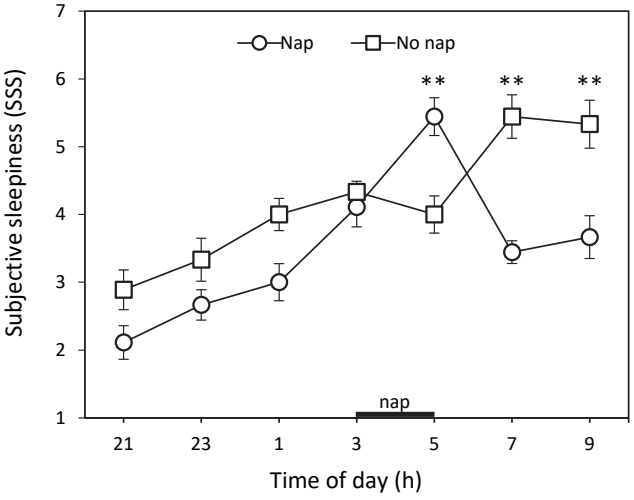


**Fig. 5. Comparison of subjective sleepiness (SSS) between the nap and no nap conditions. **:$p$<0.01**

time point (paired *t-test*), there were no significant differences between the conditions. However, the response speed in the nap break tended to be slower than that in the no nap condition at 05:00 h ($p$=0.085) and tended to be faster than that in the no nap condition at 07:00 and 09:00 h ($p$=0.058 and $p$=0.090, respectively).

The paired *t-test* for mean 10% fastest RT (optimal alertness) at each time point showed that optimal alertness was significantly worse in the nap condition than in the no nap condition at 05:00 h ($p$=0.047) but was significantly better at 07:00 h ($p$=0.020). There was also a significant tendency for better optimal alertness in the nap condition than in the no nap condition at 09:00 h ($p$=0.087).

A comparison of the numbers of lapses between the conditions at each time point (Wilcoxon signed-rank test) showed that the number of lapses was significantly greater in the nap condition than in the no nap condition at 05:00 h ($p$=0.041 and $p$=0.046, respectively). However, the number of lapses was significantly smaller in the nap condition than in the no nap condition at 07:00 h ($p$=0.046). There were no significant differences in the numbers of lapses between the conditions at 03:00 and 09:00 h.

*SSS*

The results for subjective sleepiness are shown in Fig. 5. There was a significant main effect of condition (nap vs. no nap; $F_{1, 8}$=9.729, $p$=0.014), but there was no main effect of time (03:00 ~ 09:00 h, $F_{3, 24}$=1.405, $p$=0.266). There was a significant interaction between condition and time ($F_{3, 24}$=18.600, $p$<0.001).

In the comparison of levels of sleepiness between the nap and no nap conditions at each time point (paired *t-test*), the level of sleepiness was significantly higher in the nap condition than in the no nap condition at 05:00 h ($p$=0.008). However, the levels of sleepiness were significantly lower in the nap condition than in the no nap condition at 07:00 and 09:00 h ($p$=0.001 and $p$=0.002, respectively).

## Discussion

The effects of nighttime naps on sleepiness and performance during night shift work have been established in many previous studies[15, 19–21]. In the present study, we also investigated the effect of blocking light during napping on melatonin recovery. Not surprisingly, the simulated night work without a nap break (i.e., constant light exposure condition) continuously inhibited pineal melatonin synthesis until the end of the experiment (Fig. 2B). In contrast, melatonin levels were recovered from light-induced suppression immediately after the 2-h nap break (Figs. 2A and 2F). However, in the result of direct comparison of melatonin between the nap and no nap conditions (Fig. 2E), there was no significant difference at 05:00 h, immediately after the

nap break. Contrary to our expectation, there was a significant difference in melatonin concentration between the nap and no nap conditions at 03:00 h, before the nap break. Hence, we calculated the percentage changes based on the melatonin concentration at 03:00 h in each condition and compared them. In the results, the melatonin change in the nap condition was found to be significantly greater than that in the nap condition at 05:00 h, immediately after the nap break (Fig. 2F). It is unclear why the differences in melatonin concentration emerged between the nap and no nap conditions even before the nap intervention. Unlike in the nap conditions, some participants might have relatively weak melatonin sensitivity to light in the no nap condition. As mentioned above, melatonin concentration could differ depending on the circadian rhythm, light exposure history, and food intake even in the same individual[17, 18]. Although we controlled the participants' sleep schedule for one week and meal, light exposure, and behavior the day before the simulated night work, melatonin secretions were not well controlled. In a future study, the secretion rhythm and/or amplitude of melatonin need to be tightly controlled to assess the effects of nighttime napping on melatonin more accurately.

In support of our hypothesis, blocking light during the nap break is thought to be responsible for melatonin recovery during the nap break. However, there might also be other factors influencing melatonin concentration. For instance, the results of a previous study suggested that melatonin concentration could be directly influenced by sleep[22]. According to that study, melatonin levels were increased (approximately 6.7%) during sleep compared to the levels during constant wakefulness in a dimly lit room (<10 lx). However, exposure to dim light might reduce melatonin levels, as the authors discussed in their paper. Also, there is a lack of further studies supporting the direct effect of sleep on melatonin. Moreover, a recent study has shown melatonin increment during sleep deprivation compared to that during sleep in female subjects, results that are inconsistent with previous results[23].

The increased melatonin concentration during the nap break was, however, rapidly diminished soon after the participants returned to the simulated night work, indicating that the beneficial effect of the nap break on melatonin is temporary. Furthermore, melatonin was excessively suppressed after the nap break more than we expected (Fig. 2). Consequently, the melatonin AUC changes (%) were almost identical in the nap and no nap conditions; instead, although there was no statistically significant difference, the AUC change in the nap condition was slightly greater than that in the no nap condition (Fig. 3). These results are thought to be partially related to the fact that the pineal gland does not store melatonin. Once melatonin is synthesized, it rapidly reaches all cellular tissues via capillaries and is degraded in the liver[24]. Another possible reason is that the nap break in a dark room resulted in recovery of retinal photosensitivity, which sensitized pineal melatonin to the light exposure during the simulated night work. Dark adaptation can result in complete recovery of bleached photopigments in the retina within an hour and increase the gain of the phototransduction cascade[25]. Furthermore, it has recently been reported that mRGC also has an ability of dark adaptation[26]. Jasser *et al.* demonstrated that 2-h dark adaptation can amplify light-induced melatonin suppression[27]. We also found that even a 10-min break in a nearly dark room (<1 lx) can promote melatonin suppression in response to a subsequent light stimulus[28].

Consistent with the results of previous studies[14, 21], the nighttime nap break was found to promote subjective and objective alertness. Overall, the simulated night shift work without a nap break increased subjective sleepiness and decreased PVT performance over time (Figs. 3 and 4). However, compared to those results, the subjective sleepiness ratings were decreased after the nap break. The values representing optimal alertness (i.e., fastest 10% RT) were improved; the response speeds (i.e., 1/RT) tended to be faster after the nap break. Also, the number of PVT lapses was decreased after the nap break.

Nonetheless, we also found some adverse effects of nap breaks on subjective sleepiness and PVT performance. The participants showed performance decrement shortly after the 2-h nap break: compared to the results at 05:00 h in the no nap condition, the level of subjective sleepiness was higher, the fastest 10% RT was slower, and the number of PVT lapses was larger in the nap condition. These results might be caused by early assessment timing in which the participant performed PVT and self-rating sleepiness approximately 10 min after waking from the nap break. However, it has been reported that awakening at the circadian nadir or in a deep sleep state can lead to severe sleep inertia, a state of impaired cognitive and vigilance performance, occurring immediately after awakening from a nighttime nap[29]. Sleep deprivation can also generate sleep inertia since it can increase the amount of slow-wave sleep in naps[30]. In the present study, the participants were awakened from the nap break at 05:00 h, generally near the circadian nadir; they did not take a diurnal nap before the start of the experiment. Considering the previous findings, these probably accelerated sleep inertia after the nap break.

In previous studies, it was shown that sleep inertia in the morning could be reduced by using a dawn simulation, a technique mimicking sunrise by gradually increasing illuminance from dim light before waking from sleep[31, 32]. From these findings, it is expected that the symptom of sleep inertia after a nap break could be improved using artificial dawn simulation during the nap break. Moreover, given that external photic stimuli can reach the retina via the eyelid[33], the increase in melatonin sensitivity could be restrained by conducting a dawn simulation during a nap break. If so, it might be possible to mitigate melatonin suppression following a nap break.

There are some limitations in the present study. It is unclear how much or how fast melatonin recovered during the 2-h nap break because we measured melatonin concentration at 2-hour intervals. Although melatonin levels can be altered by recent light exposure history[17] and food intake[18], we did not strictly control those factors. We did not assess the participants' sleep propensity during the 2-h nap break, including sleep latency, duration, and depth. The actual nap duration is usually shorter than the permitted nap duration, and it varies among individuals[34]. Nap duration might not directly influence melatonin recovery, but it could affect the results of subjective or objective alertness. The nap break was taken between 03:00 and 05:00 h regardless of individual differences in biological rhythm and chronotype. Therefore, the length and timing of the nap break might have been inappropriate for some participants.

It has been reported that the postural position can affect melatonin concentration. Some previous studies revealed that plasma and salivary melatonin concentrations are increased by changing the posture from the supine position to the standing position and are decreased by changing the posture reversely[35, 36]. This phenomenon probably occurred when the participants took a nap break or awakened from the nap in the present study. Considering those previous reports, the effect of the 2-h nap break on melatonin might have been underestimated in the present study.

The sample size might be small to generalize the results. All participants in this study were healthy young male adults. However, melatonin levels could decline with aging[37]. Several previous studies showed consistent results in which women exhibit significantly higher melatonin concentration than men[38, 39]. The magnitude of melatonin recovery during the nap break might be dependent on age-related and sex-related differences in melatonin levels. Moreover, it has also been known that the pineal melatonin sensitivity to light could differ depending on ages, crystalline lens transmittance, and genetic variations in the clock genes[40–43]. Therefore, it is necessary to verify the reproducibility of our findings considering the inter-individual differences mentioned above.

Interestingly, several previous studies have shown that even short sleep contributes to resetting circadian rhythms. One previous study showed that taking 4-h sleep regularly every day can maintain 24-h period rhythm of body temperature even with another irregular 4-h sleep[44]. Another previous study showed that taking 2-h sleep during nighttime sleep deprivation compensates the endogenous oscillation of core body temperature[45]. Additionally, in the same previous study, sleep for 2 hours contributed to an increase in slow-wave sleep and REM sleep. Given that nocturnal melatonin secretion or external melatonin administration is related to biological regulation[46, 47], the melatonin recovery during the 2-h nap break might somehow contribute to those previous findings. In a future study, the effect of a 2-h nap break on circadian phase shift after night shift work should be investigated.

Our findings indicate that taking a 2-h nighttime nap can temporarily recover melatonin concentration from severe suppression caused by light exposure during night work. A nap for 2 hours can induce sleep inertia but ultimately enhances subjective alertness and vigilance performance. The increase in melatonin during the nap break may imply the importance of melatonin secretion during the biological night again. However, the results in this study suggest a possibility that a nighttime nap in darkness might promote melatonin suppression on the following night work by increasing retinal sensitivity to light, which could lead to even worse results in the total amount of melatonin secretion. For now, it is difficult to conclude that nighttime napping for 2 hours can mitigate melatonin suppression during night shift work. However, it is expected that nighttime napping can provide beneficial effects on melatonin during night work if the increase in retinal photosensitivity by napping in darkness could be attenuated.

Although we examined the effects of a nap for 2 hours in a laboratory setting, most workplaces with night shift work do not allow their employees to take a nap during the night shifts. Even when a nap opportunity was allowed, the necessary number of staff has to be assigned to meet the task demand. Given recent increases in the night shift longer than 8 hours, such as 12 or 16 hours, it is recommended to take a planned nap for 2 hours during the night shifts to reduce health and safety risks[48].

## Fundings

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