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Carryover effect on next-day sleepiness and psychomotor performance of nighttime administered antihistaminic drugs: a randomized controlled trial

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Background Antihistamines with strong sedative–hypnotic properties are frequently prescribed for insomnia secondary to allergy, but the potential risks of such administration have not been fully elucidated.
Subjects and methods This randomized, double-blind, placebo-controlled crossover study was conducted to evaluate next-day sleepiness and psychomotor performance following the administration of antihistamines. Twenty-two healthy male participants participated in four drug administration sessions with more than a 1-week interval between the sessions. Either zolpidem 10 mg, or diphenhydramine 50 mg, or ketotifen 1 mg, or a placebo was administered before sleep, and polysomnography was conducted to evaluate sleep. In the morning and afternoon of the day after administration, the participants were evaluated for subjective sleepiness, objective sleepiness, and psychomotor performance.
Results The antihistamines with high blood–brain barrier-crossing efficiency were significantly associated with sleepiness and psychomotor performance decline the next day. Ketotifen showed the strongest carryover effect, followed by diphenhydramine. Compared with the placebo, no significant carryover effect was observed with zolpidem.
Conclusion The results suggest that the risk–benefit balance should be considered in the ready use of antihistamines that easily cross the blood–brain barrier for alleviating secondary insomnia associated with allergies. Copyright © 2012 John Wiley & Sons, Ltd.

KEY WORDS—antihistaminic drugs; carryover effect; diphenhydramine; ketotifen; zolpidem

INTRODUCTION

The effectiveness of benzodiazepine and non-benzodiazepine hypnotics in treating insomnia secondary to physical and mental illness (comorbid insomnia) has been demonstrated by several randomized control trials (Asnis *et al.*, 1999; Fava *et al.*, 2006; Stewart *et al.*, 2006), and these drugs are now used as standard therapeutic drugs in clinical practice. However, many drugs with sedative–hypnotic properties are also used off-label to treat secondary insomnia, and a typical example of this is antihistamines commonly used to treat patients with atopic dermatitis and asthma. Antihistamines with strong sedative–hypnotic properties are often intentionally administered before sleep to ameliorate insomnia symptoms in addition to allergy symptoms.

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The strength of antihistamine sedative–hypnotic effects is determined by differences in the drug's ability to cross the blood–brain barrier (BBB) (Yanai and Tashiro, 2007). In general, first-generation antihistamines are highly effective in crossing the BBB but have low specificity toward histamine receptors. In contrast, second-generation antihistamines have a low tendency to cross the BBB and high specificity toward histamine receptors (Simons, 1994; Yanai and Tashiro, 2007). In fact, positron emission tomography showed that the first-generation antihistamine diphenhydramine (DPH) produced as high as 56% occupancy of histamine H1 receptors in the brain at 90 min postadministration (Zhang *et al.*, 2010). Because of the strong clinical sedative–hypnotic effects, DPH has been widely marketed as an over-the-counter hypnotic. Some of the second-generation antihistamines also have strong sedative–hypnotic properties. For example, the brain histamine H1 receptor occupancy of ketotifen (KTF) reaches 72% at 160 min postadministration (T_{max})

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(Tashiro *et al.*, 2006). A functional brain imaging study using near-infrared spectroscopy showed impaired psychomotor performance and significant decrease in cerebral blood flow response in the lateral prefrontal cortex 3 h after KTF administration, compared with the placebo group (Tsujii *et al.*, 2009). Several other studies have also shown that psychomotor performance is acutely and transiently suppressed after administration of an antihistamine with strong sedative–hypnotic effects (Weiler *et al.*, 2000; Tashiro *et al.*, 2005; Boyle *et al.*, 2006).

In this randomised, double-blind, placebo-controlled crossover study, we examined the risk–benefit balance of two types of antihistamines with effective BBB-crossing properties as a treatment for secondary insomnia, with the primary endpoints set as next-day objective and subjective sleepiness and psychomotor performance following administration before sleep.

MATERIALS AND METHODS

Participants

Healthy Japanese men were recruited for the study through newspaper advertisements and other media outlets. Before the study, the participants were interviewed by physicians, administered questionnaires including the Pittsburgh Sleep Quality Index and Epworth Sleepiness Scale, and underwent polysomnography (PSG) to screen out the presence of serious physical diseases, sleep disorders, and psychiatric diseases that might affect the study evaluation. Those taking hypnotics, tranquilizers, antihistamines, or other drugs with sedative–hypnotic properties were excluded. This yielded 22 healthy adult male participants (mean age, 22.2 ± 0.8 years; mean height, 171.5 ± 1.1 cm; mean body weight, 64.7 ± 2.3 kg; mean body mass index, 22.0 ± 0.8 kg/m^2). All the participants underwent and completed all the study sessions. Prior to screening, the participants received oral and written explanations of the purpose and methods of the study as well as expected drawbacks and possible occurrences. Written informed consent was obtained from all the participants. This study protocol was approved by the Ethics Committee of the National Center of Neurology and Psychiatry.

Study protocol and drug assignment

This random, double-blind, placebo-controlled crossover study involved four drug administration sessions (3 days, 2 nights per session) conducted with more than a 1-week interval between the sessions. Each session consisted of a night to adjust to the new sleep environment, a night to administer the drug and measure nocturnal PSG, and the day after to evaluate sleepiness and psychomotor performance (Figure 1). The sleep of the participants at home was continuously monitored for 1 week with an actigraph (AMI Inc.,

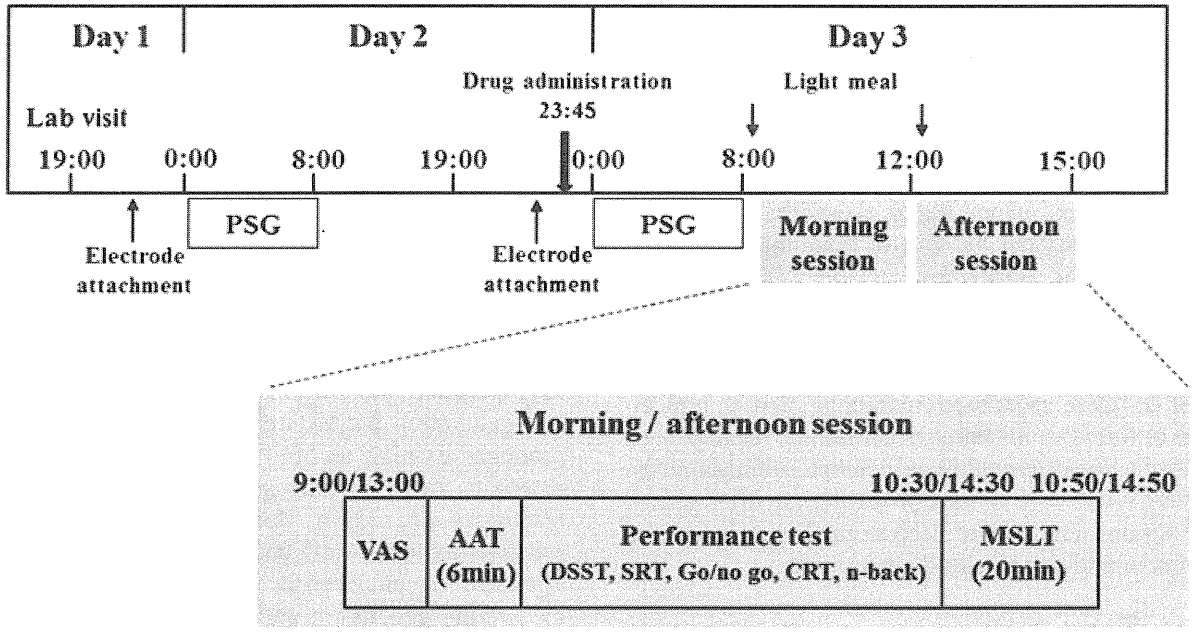

Figure 1. Experimental protocol. Order and time of tests for subjective and objective sleepiness and psychomotor performance conducted on the day after drug administration. PSG, polysomnography; VAS, Visual Analog Scale; AAT, Alpha Attenuation Test; DSST, Digit Symbol Substitution Test; SRT, simple reaction time; CRT, choice reaction time; MSLT, Multiple Sleep Latency Test

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Ardsley, NY, USA). The mean sleep onset time was set relative for each participant at midnight (00:00). Intake of caffeinated drinks, alcoholic drinks, and nicotine products was prohibited 24 h prior to each session. The participants arrived at the testing center at 19:00 on the evening of the adjustment night (Day 1), PSG sensors were attached, and lights were turned out at 00:00. Lights were turned on, and the participants were instructed to get out of bed at 08:00. At 23:45 on the night that the drug was administered (Day 2), the participants were orally administered either DPH 50 mg, or KTF 1 mg, or zolpidem (ZPD) 10 mg as the control standard drug, or placebo (antiflatulent with *Bifidobacterium* species) inserted into opaque capsules. Lights were turned off, and PSG measurements began. On the day after administration (Day 3), lights were turned on, and the participants were instructed to arise at 08:00. That morning, the participants consumed a breakfast of 500 kcal and were then evaluated for objective and subjective sleepiness (see the following text) and psychomotor performance during the morning (9–11 h after drug administration) and afternoon (13–15 h after drug administration). $T_{1/2}$ (T_{max}) for KTF, DPH, and ZPD in healthy Japanese adults are 6.72 h (2.8 h) (Novartis, 2002), 5–8 h (2–4 h) (Glazko *et al.*, 1974), and 2 h (1.7 h) (Buysse, 2011), respectively.

The study drugs were randomly assigned to the four administration sessions. The order of administration, which was based on a Latin square design, was determined by a controller unrelated to the study.

Measured parameters

Polysomnography. Polysomnography (Grass Technologies™, West Warwick, RI, USA) was conducted to measure sleep architecture on the evening that each drug was administered as well as sleepiness the next day. The sleep stages were recorded continuously throughout the night and determined using the Rechtschaffen and Kales criteria (Rechtschaffen and Kales, 1968), brain waves, eye movement, electromyogram of the mentalis muscle, and electrocardiogram. During the screening, thoracic–abdominal breathing, air flow determined with a thermistor, electromyography of a lower limb muscle (tibialis anterior), and arterial oxygen saturation were also measured to rule out disorders such as sleep respiratory disorders and periodic limb movement disorder.

Data analysis was conducted and double checked by a technician unfamiliar with the study and the participants. Sleep parameters were calculated as follows: total sleep time (TST), defined as the total period of sleep time while in bed from lights out to arising time, excluding any wakeful period; sleep efficiency, the ratio of TST during the total time in bed; sleep latency (SL), the period between going to bed and falling asleep; and rapid eye movement (REM) latency, the time from falling asleep to the first REM cycle. For each sleep stage (Stage 1, Stage 2, slow wave sleep, and REM), the emergence ratio to TST was computed. Regarding awakening while in bed, total awake time was composed of all wakeful periods during the total time in bed, whereas awake time after sleep onset excluded SL from the total awake time. Arousal was calculated by dividing the number of awakenings by TST shown by brain waves appearing over 3-s epochs during sleep.

Evaluation of sleepiness. A Visual Analog Scale (VAS) was used to evaluate subjective sleepiness on Day 3 (McCormack *et al.*, 1988). The participants were asked to mark their degree of sleepiness on a 0–100 mm straight line, where the left end represented the strongest state of sleepiness and the right end represented a state with no sleepiness. The distance from the left end was taken as the VAS score.

The Multiple Sleep Latency Test (MSLT) (Carskadon *et al.*, 1986) and Alpha Attenuation Test (Alloway *et al.*, 1997) were used to assess objective sleepiness. The MSLT measures SL during a 20-min session in which the participant has the chance to sleep, where SL is the time from lights out until the participant enters sleep. These sessions were conducted in the morning and afternoon of Day 3. The time the participant took to reach the state where he or she was in a sleep stage for over 50% of a 30-s epoch after lights off (SL) was measured every 30 s on the basis of the brainwave criteria of Rechtschaffen and Kales (1968). The mean SL for the morning + afternoon sessions was calculated. When participants did not enter sleep during the MSLT, SL was set at 20 min.

In the Alpha Attenuation Test, eyes were opened and closed alternatively three times over a 1-min period. The alpha attenuation coefficient (AAC) was obtained by dividing the 8–13 Hz α power value derived from electrodes 02-A1 at eyes closed by α power at eyes open (Alloway *et al.*, 1997). The closer AAC was to 0, the lower the level of wakefulness.

Tests of psychomotor performance. Psychomotor performance on Day 3 was assessed with the (i) *n*-back test, (ii) three different tasks to measure reaction time (RT) to a stimulus, and (iii) the Digit Symbol Substitution Test (DSST).

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(i) The *n*-back test is a working memory task that evaluates RT and error in recalling a stimulus *n* times back in a series of different stimuli (Kirchner, 1958). The participants are shown four consecutive circles at 1.8-s intervals on a computer monitor. The circles are arranged randomly, with one circle displayed red and the other three circles displayed blue. The participants are asked to push the numbered button that corresponds to the place where the red circle was *n* times before the currently displayed configuration. One-back tasks ask the placement directly before the current configuration. One-back, two-back, and three-back tasks were conducted in the present study. Each *n*-back task was conducted 60 times per session, and the mean RT and percent error were calculated. RTs less than 100 ms were considered too short to make a fair judgment and were thus excluded from the analysis.

(ii) Three different tasks were conducted as tests of RT: the simple reaction time (SRT) test, the go/no go test, and the choice reaction time (CRT) test. The SRT test measures the time required before detection of a given stimulus (Tiplady, 1988); the participants were asked to press a designated button as quickly as possible when a '+' symbol appeared on the computer monitor, and RT was measured. This was conducted 56 times over one session, and mean RT was calculated. The go/no go test involves participants immediately pressing a button when a go signal is displayed but remaining stationary when a no go signal is displayed. The go and no go signals are displayed in random order (Newman *et al.*, 1985). In this study, '○' was set as the go signal and '×' as the no go signal. Signals were displayed 50 times in one session, and the mean RT for the go signal was calculated. The CRT with multiple stimuli requires participants to respond to each stimulus appropriately (Sherwood and Kerr, 1993). In this study, nine squares (■) were shown in a 3 × 3 configuration, and one square (■) was randomly replaced with a triangle (▲). The participants were instructed to press the number key that corresponded to the placement of the different shape. The task was completed 60 times for one session. RTs less than 100 ms were considered too short to make a fair judgment and were excluded from the analysis.

(iii) The DSST assigns nine different shapes to the numbers one through nine. The participants were asked to enter the shape that corresponds to an indicated number (Wechsler, 1955). One session lasted 90 s, and the number of responses was determined by the number of correctly recorded shapes.

Statistical analysis

Because the Kolmogorov–Smirnov test showed normal distribution of PSG parameters in all drug sessions, Dunnett's test was conducted to compare the drug sessions. Morning and afternoon VAS normality, mean SL determined by the MSLT, AAC, and psychomotor test mean scores could not be substantiated. Therefore, Steel's test was conducted for between-drug session comparisons. SPSS for Windows 11.5.1 (SPSS Inc., Chicago, IL, USA) was used for Dunnett's test and Wilcoxon's signed rank sum test, with significance set at $p < 0.05$. The critical value of each session for the Steel test was set at 2.349. All data are shown as means standard error.

RESULTS

Influence of each drug on sleep architecture

The PSG parameters of each drug are shown in Table 1. Compared with the placebo, DPH had significantly

Table 1. Sleep parameters after administration of each drug

Sleep parameter	PCB	ZPD	DPH	KTF	*p*-value
Total sleep time (min)	441.9 ± 5.0	446.7 ± 5.3	444.1 ± 5.5	444.5 ± 3.5	n.s.
Sleep efficiency (%)	91.5 ± 1.2	92.4 ± 1.0	92.2 ± 1.2	92.0 ± 0.8	n.s.
Sleep latency (min)	13.2 ± 3.3	17.3 ± 4.5	17.6 ± 4.9	16.8 ± 3.5	n.s.
REM latency (min)	99.9 ± 10.8	96.0 ± 10.0	138.5 ± 13.5[a]	91.8 ± 10.2	<0.05
Time spent in sleep stage/total sleep time (%)					
Stage1	10.4 ± 1.0	8.2 ± 1.0	8.7 ± 0.8	8.7 ± 0.8	n.s.
Stage2	51.9 ± 1.2	51.6 ± 1.4	54.7 ± 1.1	55.1 ± 1.1	n.s.
Slow wave sleep	17.2 ± 1.4	20.8 ± 1.8	20.5 ± 1.6	17.3 ± 1.3	n.s.
REM sleep	20.5 ± 1.6	19.4 ± 0.9	16.2 ± 0.9[a]	18.9 ± 0.8	<0.05
Total awaking time (min)	41.2 ± 5.8	36.7 ± 5.0	37.8 ± 5.7	38.8 ± 4.0	n.s.
Wake after sleep onset (min)	28.3 ± 4.1	19.5 ± 2.0	21.1 ± 2.6	22.4 ± 2.7	n.s.
Arousal (no./h of sleep)	12.3 ± 0.9	10.4 ± 0.8	11.4 ± 0.9	11.7 ± 0.9	n.s.

REM, rapid eye movement; PCB, placebo; ZPD, zolpidem; DPH, diphenhydramine; KTF, ketotifen; n.s., not significant.
[a]Significant difference from placebo ($p < 0.05$) determined by Dunnett's test. Data are expressed as mean ± standard error of mean.

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longer REM latency (99.9 ± 10.8 vs. 138.5 ± 13.5 s) and reduced %REM (20.5 ± 1.6 vs. 16.2 ± 0.9%). No significant differences in other sleep parameters were observed between ZPD, antihistamines (DPH and KTF), and the placebo.

Assessment of subjective sleepiness postadministration

Table 2 shows subjective and objective sleepiness the day after administration of each drug and the results of the different psychomotor performance tasks. Among the different drug groups, significant differences were observed in mean next-day and next-morning sleepiness but not next-afternoon sleepiness.

Mean next-day sleepiness caused by ZPD was comparable with that caused by the placebo. It was significantly strong with KTF (test statistic = 2.723) and tended to be strong with DPH (test statistic = 2.019). Likewise, next-morning sleepiness caused by KTF was significantly strong (test statistic = 3.192) and that caused by DPH tended to be strong (test statistic = 2.223) compared with the placebo. Next-morning sleepiness was significantly stronger than next-afternoon sleepiness for all drug sessions. This difference was smallest with ZPD (VAS score 9.7 ± 5.3) and large for DPH and KTF (18.5 ± 5.9 and 21.4 ± 4.0, respectively).

Table 2. Subjective sleepiness, objective sleepiness, and psychomotor performance on the day after administration of each drug

		Treatment session			
Test	Time	PCB	ZPD	DPH	KTF
VAS	AM	59.5 ± 4.9[a]	57.6 ± 4.6[a]	74.0 ± 3.8[a]	80.2 ± 3.9[ab]
	PM	46.5 ± 5.6	47.9 ± 4.9	55.5 ± 4.7	58.9 ± 4.2
	Mean	53.0 ± 4.5	52.8 ± 4.0	64.8 ± 3.1	69.5 ± 3.5[b]
MSLT (min)	AM	4.2 ± 1.0	3.5 ± 0.8	2.3 ± 0.5	1.1 ± 0.1[ab]
	PM	5.0 ± 1.1	5.7 ± 1.3	3.4 ± 0.9	1.9 ± 0.3[b]
	Mean	4.6 ± 0.6	4.6 ± 0.9	2.9 ± 0.5	1.5 ± 0.2[b]
AAC	AM	2.8 ± 0.3[a]	2.8 ± 0.4	2.1 ± 0.2	1.6 ± 0.2[b]
	PM	2.1 ± 0.3	2.6 ± 0.4	2.3 ± 0.4	1.5 ± 0.2
	Mean	2.5 ± 0.2	2.7 ± 0.3	2.2 ± 0.2[b]	1.6 ± 0.1[b]
DSST	AM	77.8 ± 3.3	77.3 ± 1.6	73.5 ± 2.7	70.8 ± 2.5[a]
	PM	80.6 ± 3.1	79.6 ± 1.9	78.2 ± 3.8	77.8 ± 2.8
	Mean	79.2 ± 2.9	78.5 ± 1.6	75.9 ± 2.9	74.3 ± 2.3
SRT (ms)	AM	340.4 ± 32.6	328.1 ± 19.2	368.7 ± 30.0[a]	403.8 ± 30.8[ab]
	PM	320.6 ± 19.3	314.9 ± 14.0	336.5 ± 23.3	345.6 ± 20.4
	Mean	330.5 ± 24.1	321.5 ± 15.6	352.6 ± 22.8	374.7 ± 22.0
Go/no go (ms)	AM	507.3 ± 27.5	524.3 ± 32.9	677.3 ± 73.0	688.5 ± 51.5[ab]
	PM	502.1 ± 34.2	508.3 ± 36.7	583.5 ± 60.9	557.7 ± 38.6
	Mean	504.7 ± 21.7	516.5 ± 24.3	630.4 ± 47.5	623.1 ± 33.4
CRT (ms)	AM	685.0 ± 63.2	674.1 ± 44.3	747.8 ± 58.0[a]	867.5 ± 61.4[ab]
	PM	619.3 ± 40.0	597.9 ± 26.9	640.3 ± 39.6	722.8 ± 50.4
	Mean	652.2 ± 37.3	636.0 ± 26.3	694.1 ± 35.6	795.2 ± 40.8
One-back test %Error (%)	AM	6.7 ± 3.4	3.6 ± 1.3[b]	7.3 ± 2.4[b]	16.3 ± 4.5[b]
	PM	2.7 ± 1.1	5.7 ± 2.4[b]	5.4 ± 1.6[b]	8.3 ± 2.4[b]
	Mean	4.7 ± 1.7	4.7 ± 1.8	6.3 ± 1.5[b]	12.3 ± 2.6[b]
Two-back test %Error (%)	AM	9.1 ± 2.7	11.4 ± 3.5	15.1 ± 4.7	20.5 ± 3.6[b]
	PM	5.6 ± 1.9	9.2 ± 3.4	8.3 ± 2.6	13.9 ± 3.2[b]
	Mean	7.3 ± 1.6	10.3 ± 3.1	11.7 ± 2.7	17.2 ± 2.9[b]
Three-back test %Error (%)	AM	16.1 ± 3.5	15.4 ± 3.2	23.3 ± 4.6	30.2 ± 4.6[ab]
	PM	12.5 ± 2.7	10.4 ± 2.8	13.9 ± 3.6	19.8 ± 3.9
	Mean	14.3 ± 2.7	12.9 ± 2.4	18.6 ± 3.5	25.0 ± 3.8[b]
One-back test RT (ms)	AM	348.4 ± 29.4	338.9 ± 25.9	323.3 ± 26.3	359.0 ± 21.4
	PM	324.7 ± 30.8	327.7 ± 27.1	313.3 ± 19.2	328.0 ± 20.4
	Mean	336.6 ± 27.8	333.3 ± 25.8	318.3 ± 21.7	343.5 ± 19.5
Two-back test RT (ms)	AM	313.5 ± 26.0	299.0 ± 23.8	296.4 ± 29.0	338.5 ± 28.8[a]
	PM	276.5 ± 18.5	293.3 ± 23.2	266.2 ± 20.9	272.4 ± 18.4
	Mean	295.0 ± 20.2	296.2 ± 21.4	274.6 ± 23.2	305.5 ± 20.1
Three-back test RT (ms)	AM	287.3 ± 23.2	284.0 ± 22.1	317.4 ± 34.0	339.0 ± 27.6
	PM	254.3 ± 17.7	265.0 ± 17.6	267.3 ± 19.2	308.4 ± 35.8
	Mean	270.8 ± 17.9	274.5 ± 18.8	292.4 ± 24.4	323.7 ± 30.0

VAS, Visual Analog Scale; MSLT, Multiple Sleep Latency Test; AAC, alpha attenuation coefficient; DSST, Digit Symbol Substitution Test; SRT, simple reaction time; CRT, choice reaction time; RT, reaction time. PCB, placebo; ZPD, zolpidem; DPH, diphenhydramine; KTF, ketotifen.
[a]Significant difference from PM determined by Wilcoxon signed rank test ($p < 0.05$).
[b]Significant difference from placebo determined by Steel's test ($p < 0.05$). Data are expressed as mean ± standard error of mean.

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Assessment of objective sleepiness postadministration

Mean SL as determined by the MSLT on the day after administration of each drug is shown in Table 2 and Figure 2. Significant group differences in mean SL were observed for each drug for next-day SL as well as for next-morning SL and next-afternoon SL.

The mean next-day SL with ZPD was statistically equivalent to that for the placebo, whereas it tended to be reduced with DPH (test statistic = 1.760) and significantly reduced with KTF (test statistic = 3.357).

Similarly, the next-morning SL and next-afternoon SL with ZPD were statistically equivalent to those with the placebo. However, these values tended to be shorter with DPH compared with the placebo (test statistic = 2.066 and 2.159, respectively) and significantly shorter with KTF (test statistic = 4.202 and 3.474, respectively). For all drug sessions, next-morning SL was shorter than next-afternoon SL, and the difference was smallest with KTF.

Level of wakefulness based on AAC findings showed similar tendencies to that determined by the MSLT. For each session, significant differences in mean AAC were observed between the drug groups for next-day AAC and for next-morning AAC but not for next-afternoon AAC.

Mean next-day AAC with ZPD was statistically equivalent to that with the placebo. However, it was slightly lower with DPH and significantly lower with KTF (test statistic = 2.745). Similarly, compared with the placebo, next-morning AAC was somewhat higher with DPH (test statistic = 1.807) but was significantly lower with KTF (test statistic = 3.357).

Psychomotor performance scores postadministration

Results of the *n*-back test for each drug on the day after administration are shown in Table 2 and Figure 3. As the degree of difficulty increased from the one-back

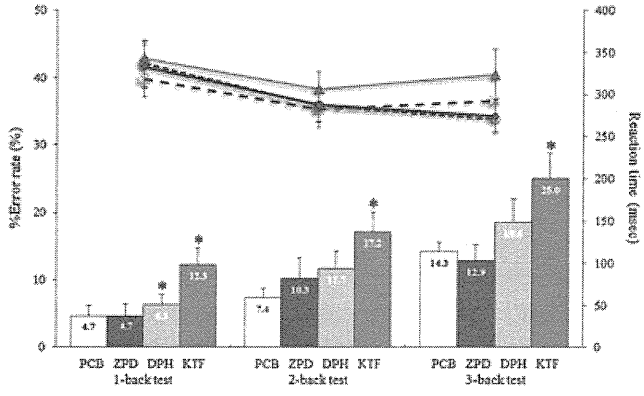

Figure 3. Psychomotor performance measured by the *n*-back test after administration of each drug. Percent error rate (left vertical axis, column) and reaction time (right vertical column, line) in the *n*-back test are shown by drug type. *Significant compared with placebo ($p < 0.05$; one-way analysis of variance followed by Dunnett's test). ZPD, zolpidem; DPH, diphenhydramine; KTF, ketotifen; PCB, placebo

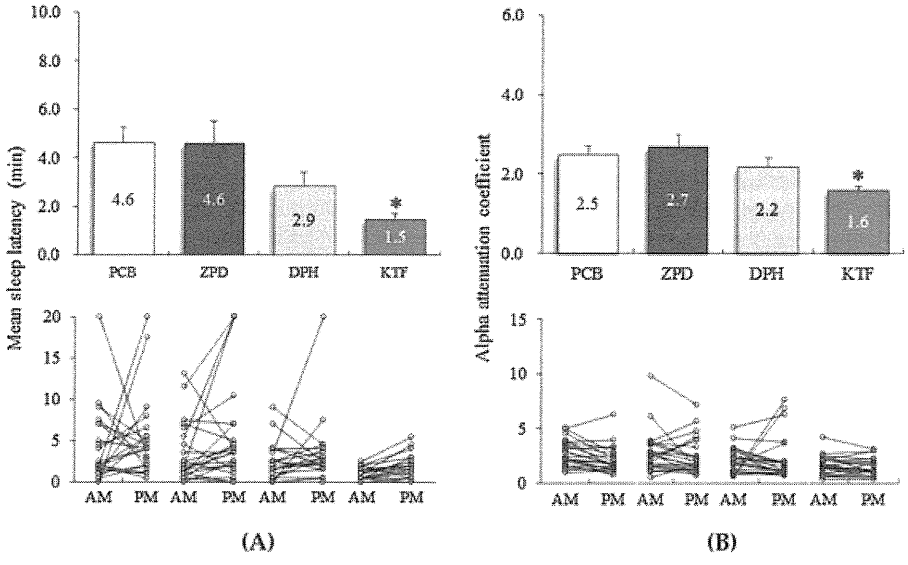

Figure 2. Objective sleepiness after administration of each drug measured by Multiple Sleep Latency Test (A) and alpha attenuation test (B). (A) Mean sleep latency (SL), by drug type, on the day after administration of each drug is shown in the upper panel. In the lower panel, SL of each participant during the morning and afternoon sessions is plotted. SL of 20 min indicates that the participants did not fall asleep during the sleep trial. (B) The upper panel shows the mean alpha attenuation coefficient, by drug type, on the day after administration of each drug. In the lower panel, the alpha attenuation coefficient of each participant during the morning and afternoon sessions are plotted. The smaller the value for SL and the alpha attenuation coefficient, the less wakeful the participant. *Significant compared with the placebo ($p < 0.05$; Steel's test). ZPD, zolpidem; DPH, diphenhydramine; KTF, ketotifen; PCB, placebo

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task to the three-back task, the percent error increased for all drug sessions. In terms of mean next-day percent error, the percent error with ZPD was similar to that with the placebo. However, it was significantly increased on the one-back task with DPH (test statistic = 2.441) and was increased on all back tests with KTF (one-back, two-back, and three-back tasks = 3.404, 2.981, and 2.370, respectively). In comparison with the placebo sessions, the next-morning percent error was significantly decreased on the one-back task with ZPD (test statistic = 3.521) and significantly increased on all back tasks with KTF (one-back, two-back, and three-back tasks = 4.953, 3.075, and 2.559, respectively). The next-afternoon percent error on the one-back and two-back tasks was significantly increased with KTF compared with the placebo (test statistic = 4.976 and 3.638, respectively). Although the next-morning percent error was higher than the next-afternoon one for all drug sessions, the largest difference was seen for the three-back task with KTF (10.4 ± 4.0, $p < 0.05$). In contrast, there were no significant differences in RT between the four sessions or between levels of difficulty ($n = 1$–3) (Table 2, Figure 3). However, next-morning RT on the two-back task was significantly longer than next-afternoon RT after KTF administration (Table 2).

On the three types of tasks for measuring RT in reaction to a stimulus, all drugs tended to show a prolonged time on the SRT, go/no go test, and CRT as task difficulty increased (Table 2). Mean next-day RT tended to be longer with DPH and KTF than with the placebo, but the differences were not significant. Next-morning RT was significantly longer for the SRT, go/no go test, and CRT following KTF administration compared with placebo administration (test statistics = 2.464, 2.927, and 2.441, respectively). In addition, next-morning RT was significantly longer than next-afternoon RT for the SRT and CRT after DPH administration and for the SRT, go/no go test, and CRT after KTF administration. No significant differences in the correct response rates for any of the tasks were observed between the four sessions or between different levels of difficulty (data not shown).

The number of correct responses on the DSST was in the order of placebo, ZPD, DPH, and KTF. However, no significant differences were seen between the drug groups for the mean number of correct responses for the next day, the next morning, or the next afternoon (Table 2). The number of responses tended to increase during the next-afternoon compared with the next-morning in all sessions. Whereas the differences in number of responses were insignificant following administration of the placebo, ZPD, and DPH, the number of responses the next morning after KTF administration was significantly lower than that the next afternoon.

DISCUSSION

To examine the carryover effects of DPH and KTF, which are first-generation and second-generation antihistamines with strong central sedative–hypnotic effects, we conducted a randomized, double-blind, placebo-controlled crossover study with the use of two controls, a placebo and the standard sleep aid ZPD. The sedative–hypnotic effects of these drugs were examined using various testing modalities such as subjective and objective sleepiness indicators, as well as suppression of psychomotor performance on multiple tasks (RT and accuracy). The results demonstrated that DPH and KTF are associated with a risk of significantly strong carryover effects, mainly hypnotic and sedative effects. Compared with the placebo, significantly strong subjective and objective sleepiness and suppression of psychomotor performance were observed the day after administration of KTF, followed by DPH. In general, first-generation antihistamines cross the BBB more effectively than second-generation antihistamines (Yanai and Tashiro, 2007), and the results of this study also suggest that first-generation antihistamines have a higher risk for sleepiness and poor psychomotor performance. However, DPH and KTF used in the present study are the first-generation and second-generation antihistamines, respectively, that readily cross the BBB, and therefore, it is necessary to keep in mind that, regardless of the generation, antihistamines with effective BBB-crossing properties develop strong carryover effects on the day after administration.

In contrast, ZPD, which is the standard medication for secondary insomnia, showed no carryover effects. In the psychomotor performance tests, sedative–hypnotic effects were more pronounced during more difficult tasks requiring working memory, as in the *n*-back task, than during relatively easy and simple tasks such as the SRT. Furthermore, decreases in accuracy, rather than in RT, were prominent after antihistamine administration.

No significant hypnotic and sedative carryover effects were observed after ZPD administration, the standard drug frequently used in clinical practice and used in this study as a control. ZPD is extremely fast acting, with short T_{max} at 1.7 h and $T_{1/2}$ at 2 h (Buysse, 2011), and hypnotic and sedative carryover effects are reported to be low (Rojas-Zamorano *et al.*, 2009). This held true for the participants in the present study as no significant differences were observed between ZPD and

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the placebo for any items, including mean SL on the MSLT, AAC, and psychomotor performance tests. This was in clear contrast to the results obtained with the antihistamines.

We also compared sleep architecture from the night before administration as it is a confounding factor in the assessment of sleepiness and psychomotor performance suppression on the day after drug administration. No significant differences were observed between the placebo, ZPD, DPH, and KTF for the majority of sleep parameters, including TST, sleep efficiency, and rates of deep sleep. Interestingly, after DPH administration, a prolonged REM latency (mean = 39 min) and decreased %REM (mean 4.3%) were observed in comparison with the placebo. The present findings are consistent with previous reports on the prolongation of REM latency and a reduction in the percentage of REM sleep with first-generation antihistamines such as chlorpheniramine (Adam and Oswald, 1986; AASM, 2005; Boyle *et al.*, 2006; Rojas-Zamorano *et al.*, 2009; Church *et al.*, 2010; Buysse, 2011). The mechanism behind this modulatory effect on REM sleep by DPH and chlorpheniramine is not yet understood. However, in the present study, KTF did not modulate REM sleep. Thus, changes in REM sleep must have had very limited influence over the hypnotic and sedative carryover effects that we observed on the day after administration of the antihistamines that readily cross the BBB. Future study may be needed to examine instead the risks of emergence of rebound REM sleep-related symptoms (insomnia, nightmares, and REM sleep behavior disorder) when DPH is suddenly terminated after long-term use. Overall, at least among the healthy participants in this study, we did not observe any apparent changes in sleep architecture that can explain the differences in sleepiness and psychomotor performance between ZPD and the antihistamines the day after administration.

The present findings suggest a risk of carryover effects far surpassing pharmacokinetic predictions for antihistamines that readily cross the BBB. The strength of the carryover effects of DPH and KTF was significantly high the next morning (9–11 h postadministration) and tended to decrease the next afternoon (13–15 h postadministration). At first glance, this appears to be a change associated with the blood kinetics of antihistamines. However, T_{max} is 2.8 h and $T_{1/2}$ is 6.72 h for KTF (in healthy Japanese adults) (Novartis, 2002). The corresponding values for DPH are 2–4 h and 5–8 h (Glazko *et al.*, 1974). Therefore, the concentration of KTF and DPH administered before sleep would have dropped to half of the peak concentration at the time of the next-morning assessment. Although serum drug concentrations were not measured in the present study, it appears that the sedative–hypnotic effects of KTF and DPH continued significantly longer than the period during which such carryover effects were expected from their blood kinetics. In a positron emission tomography study using LigandTracer for histamine receptors in the brain, Yanai and colleagues (2007) demonstrated a large discrepancy between blood kinetics and receptor occupancy of antihistamine in the brain (Zhang *et al.*, 2010). Among the participants in their two studies, the receptor occupancy was 56% at 1.5 h after DPH administration (Tashiro *et al.*, 2008) but remained as high as 45% even after 12 h (Zhang *et al.*, 2010). Receptor occupancy at 2.7 h after KTF administration has also shown to be as high as 70% (Tashiro *et al.*, 2006; Yanai and Tashiro, 2007). Although there is no data on the successive changes in receptor occupancy over the 2.7-h period after KTF administration, it is speculated that high levels of occupancy are maintained over many hours, given its clinical characteristics as a strong sedative, despite being a second-generation antihistamine. These findings indicate that some antihistamines do have carryover effects that constitute lasting sedative–hypnotic side effects even after blood drug levels have lowered (Yanai and Tashiro, 2007; Zhang *et al.*, 2010).

The following limitations of the present study should be considered in interpreting its clinical meaningfulness. Because this study tested healthy individuals, the usefulness of antihistamines that readily cross the BBB in the treatment of patients with allergies or insomnia cannot be determined from the results of this study. However, it is unlikely that the carryover effects of antihistamines would not occur in patients with physical illness or poor sleep. It is therefore necessary to clarify whether the benefits outweigh the risks of using such antihistamines to treat insomnia secondary to allergies and, more specifically, to confirm that antihistamines alleviate insomnia and that quality of life improvement cancels out any carryover effects these drugs bring about. At present, there have been no clinical trials to verify the risk–benefit balance of antihistamines that readily cross the BBB to treat insomnia secondary to allergies. We believe that prescriptions should be adjusted for each target symptom, with second-generation antihistamines with a lower BBB-crossing tendency being used to treat allergies and concomitant use of hypnotics to treat secondary insomnia. Important drug options would include the standard drugs such as ZPD, used as a control in this study whose treatment effect for secondary insomnia has been demonstrated (Asnis *et al.*, 1999), and hypnotics with low risks of sedative–hypnotic carryover effects and high ω1 receptor specificity (Depoortere *et al.*, 1986; Perrault *et al.*, 1990; Benavides *et al.*, 1992; Sanger *et al.*, 1994).

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CONCLUSION

This randomized, double-blind, placebo-controlled crossover study examined the carryover effects the day after administering antihistamines that readily cross the BBB before sleep. The results showed that antihistamines taken before sleep significantly increased subjective and objective sleepiness and significantly reduced psychomotor performance the next day, generating clinically identifiable sedative–hypnotic carryover effects. The carryover effects suggest the danger of their presence even following the lowering of blood drug levels. These carryover effects of antihistamines are adverse events that can greatly interfere with the quality of life of patients. Therefore, the present findings indicate that thorough consideration must be given to the risks of readily using antihistamines that easily cross the BBB for the treatment of insomnia secondary to allergies.

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CONFLICT OF INTEREST

No conflict of interest declared.

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Individual Traits and Environmental Factors Influencing Sleep Timing: A Study of 225 Japanese Couples

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Behavioral and physiological processes, such as sleep-wakefulness, thermoregulation, and hormone secretion, exhibit 24-h rhythms in most organisms. These biological rhythms are driven by the circadian clock system and are entrained by the external environment, which in the case of humans includes social time schedules. Couples might be ideal experimental subjects to discriminate between individual traits and environmental factors, as they share lifestyle habits but not genetic backgrounds. In this study, sleep timing was compared between married Japanese couples (n = 225) who had lived together for 1 yr or more (mean 17 yrs). Additionally, the authors evaluated the influence of individual traits and environmental factors on an individual's sleep timing per each couple. The results reveal that the sleep timings of a couple are mainly associated with the chronotypes of the husband and wife, whereas the sleep timings are significantly influenced by certain environmental factors. The findings suggest that chronotype remains one of the major determinants of an individual's sleep onset and wake times. Understanding an individual's chronotype may help improve the quality of life issues surrounding sleep. (Author correspondence: mishima@ncnp.go.jp)

Keywords: Chronotype, Circadian rhythms, Lifestyle habit, Society, Spouse

INTRODUCTION

The preferred timing of daily activities shows great variation among individuals. The type of individual difference known as morningness-eveningness preference (chronoytpe) has been well studied by questionnaires, including the 19-item self-report Horne-Östberg Morningness-Eveningness Questionnaire (MEQ) (Horne & Östberg, 1976). Morning types fall asleep and get up earlier than intermediate types, and still earlier than evening types (Horne & Östberg, 1976; Kerkhof, 1998; Tzischinsky & Shochat, 2011). Similarly, phases of physiological rhythms, such as core body temperature and the secretion of melatonin, cortisol, or thyroid-stimulating hormone, are advanced in morning types compared to intermediate types, and more so compared to evening types (Baehr et al., 2000; Duffy et al., 1999). Another questionnaire, the Munich ChronoType Questionnaire (MCTQ), developed by Roenneberg et al. (2003), collects data on actual sleep and wake times on work days and free days, and studies by Roenneberg et al. and others have shown age- and sex-related differences in chronotype (Adan & Natale, 2002; Carskadon et al., 1998; Randler, 2008, Roenneberg, 2004; Roenneberg et al., 2004). People have an early chronotype in childhood, which becomes later during puberty and adolescence, before returning to an earlier one, with an early sleep timing, in the late 20s. Generally, males prefer evening activities, whereas females prefer morning ones, but this sex-related difference disappears in the elderly (Foster & Roenneberg, 2008; Roenneberg et al., 2003). An individual's chronotype has also been shown to depend on dawn/dusk times, and the human circadian clock is predominantly entrained to sun time (Martinez-Nicolas et al., 2011; Roenneberg et al., 2007). These findings suggest that chronotype is associated with the circadian clock system.

The daily rhythms of behavior and physiology driven by the circadian clock system are entrained to environmental cues, such as light exposure, food intake, and work schedules, enabling us to adapt to changes in the external environment (Foster & Roenneberg, 2008; Gachon et al., 2004; Pittendrigh & Daan; 1974, Takahashi

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et al., 2008). Sleep-wake timing is one such rhythm that is influenced by both the circadian clock system and the external environment. Due to new social needs, most people in modern society are expected to adjust themselves to different time schedules (Åkerstedt et al., 2010; Wittmann et al., 2006). In this sense, environmental factors such as social time cues and lifestyle habits might strongly impact on an individual's sleep timing.

Couples might be ideal experimental subjects to discriminate between individual traits and environmental factors, as they share lifestyle habits, but not genetic backgrounds as in the case of twins (Barclay et al., 2010). Accordingly, in this study we evaluated the individual differences in sleep timing by comparing sleep onset time, wake time, and mid-sleep time between the spouses of 225 married couples who shared daily routines and housing for longer than or equal to a 1-yr period (average 17 yrs). Furthermore, we assessed the influence of individual traits (age, chronotype, and depressive mood) and environmental factors (work schedule, spouse's sleep timing, and lifestyle habits) on the determination of sleep onset and wake times.

METHODS

Subjects

The study population consisted of 225 married couples, part of a larger cohort of 1814 Japanese participants studied by Kitamura et al. (2010). The mean ± SD age of the husbands and wives were 44.39 ± 10.69 yrs and 42.12 ± 10.05 yrs, respectively (range 22–73 yrs and 21–72 yrs, respectively). The couples had lived together for between 1 and 48 yrs (mean ± SD: 17.04 ± 10.72 yrs), ate meals together 0–21 (mean ± SD: 9.51 ± 4.18) times/wk, and shared a bedroom 87.11% of the time. In relation to work patterns, 43 males (19.1% of husbands) and 81 females (36.0% of wives) were nightshift workers 1–2 times/wk, 12 males (5.3% of husbands) and 22 females (9.8% of wives) 3–4 times/wk, 9 males (4.0% of husband) and 5 female (2.2% of wives) ≥5 times/wk. The protocol was approved by the Institutional Ethics Committee of National Center of Neurology and Psychiatry. Written informed consent was obtained from each subject. The protocol and all the procedures were in agreement with the Declaration of Helsinki and met the ethical standards of the journal (Portaluppi et al., 2010).

Self-Assessment

The Japanese versions of all the listed questionnaires were used in this study. The Horne-Östberg MEQ (Horne & Östberg, 1976) was administered to assess subjects' diurnal preferences; its validity was previously confirmed in a Japanese population (Ishihara et al., 1984). Since chronotypes have been shown to change with age, age-adjusted MEQ scores were used to rate individuals' chronotypes: age-adjusted MEQ scores of 16–41 denote evening types, 42–58 denote intermediate types, and 59–86 denote morning types. Sleep quality and depressive mood were evaluated by the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989) and the Center for Epidemiological Studies Depression Scale (CES-D) (Radloff, 1977), respectively, to assess the presence/absence of depression and sleep disorders that could strongly modify the sleep state in each subject. A PSQI total score >5 was considered indicative of poor sleep quality, and a CES-D score ≥16 was considered indicative of depressive symptomatology.

Statistical Analysis

Sleep onset time (SOT) and wake time (WT) for the past month were determined by the values given in the PSQI. The sleep parameters were not obtained separately for work days or free days. Mid-sleep time (MT) was calculated as the midpoint between sleep onset time and wake time. Pearson's correlation analysis was performed to ascertain whether sleep onset time, wake time, or mid-sleep time was correlated between husband and wife, and to determine if the number of years each couple lived together correlated with absolute differences between husband and wife in terms of sleep onset time (ΔSO), wake time (ΔWT), or mid-sleep time (ΔMT). Statistical analyses were performed using SPSS version 11. Noncontinuous variables were analyzed as categorical data. Full model multiple regression analysis was performed to assess the influence of individual traits and external factors on a husband's or wife's sleep timing (sleep onset time or wake time) between spouses for the 225 couples. Variables tested in the analysis for individual traits were (i) age, (ii) chronotype, and (iii) presence of depressive mood (CES-D); and for environmental factors: (i) shiftwork schedule, (ii) spouse's shiftwork schedule, (iii) spouse's sleep onset time, (iv) spouse's wake time, (v) sharing a bedroom (not a sharing a bed but sleeping in the same room as is common in Japanese culture), (vi) number of years living together, and (vii) number of times/wk eating meals (breakfast, lunch, and supper) together. The variables of "chronotype" and "presence of depressive mood" were categorized according to the cutoff points described above in the section on Self-Assessment, and the variables of "shiftwork schedule," "spouse's shiftwork schedule," and "spouse's wake time" were categorized as described above in the section on Subjects. Variables with a p value <.05 were considered to be statistically significant.

RESULTS

Among the total of 450 participants, 79 males (35.1% of husbands) and 62 females (27.6% of wives) were defined as morning types (M), 125 males (55.6% of husbands) and 154 females (68.4% of wives) as intermediate types (I), and 21 males (9.3% of husbands) and 9 females (4.0% of wives) as evening types (E). Fifty-six males (24.9% of husbands) and 79 females (35.1% of wives) had poor sleep quality. In addition, 40 males (17.8% of husbands) and 63 females (28.0% of wives) had depressive mood. Out of the 225 couples, 120 (53.3%) had the same chronotypes (husband and wife: 26 M-M, 93 I-I, and 1

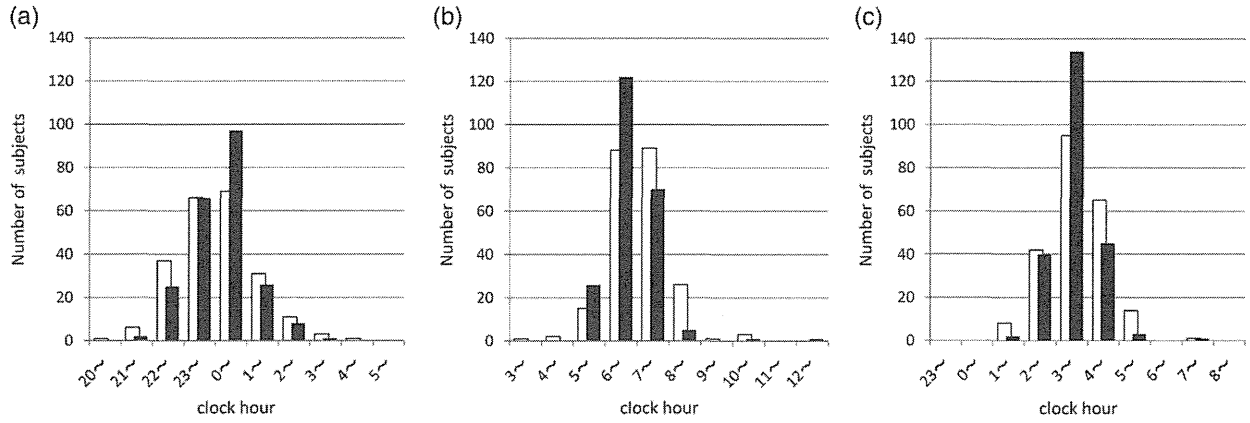

FIGURE 1. Frequency distribution of sleep onset time (A), wake time, (B), and mid-sleep time (C) for 225 husbands (open bar) and their wives (black bar) in our study sample. (A) Husband's sleep onset times ranged from 19:45 to 04:00 h, whereas wife's ranged from 20:35 to 02:30 h. (B) Husband's wake times ranged from 03:00 to 10:00 h, whereas wife's ranged from 04:30 to 11:30 h. (C) Husband's mid-sleep times ranged from 00:05 to 06:45 h, whereas wife's ranged from 00:43 to 07:00 h.

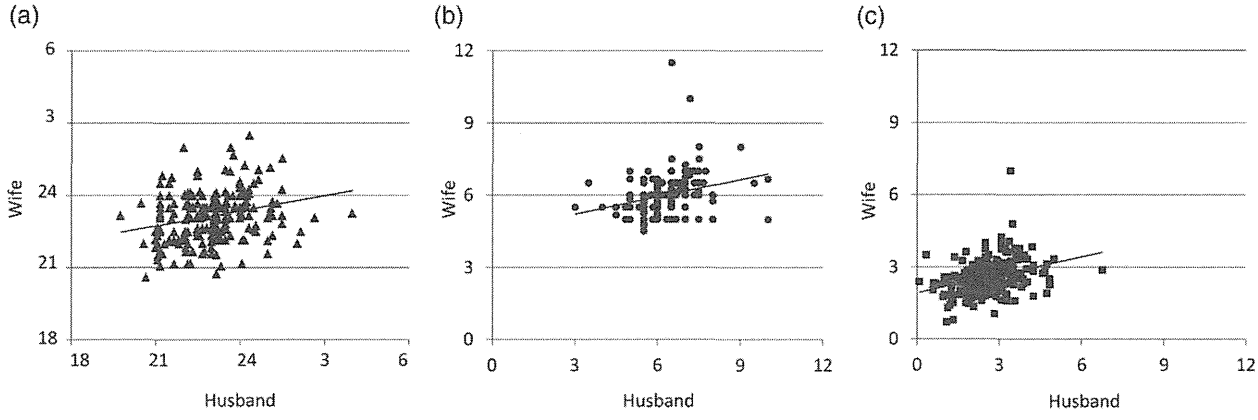

FIGURE 2. Comparison of (A) sleep onset time, (B) wake time, and (C) mid-sleep time between the husband (x-axis) and wife (y-axis) of 225 couples. Positive correlation was found between husband and wife's sleep onset times ($R = .259$, $p < .001$), wake times ($R = .285$, $p < .001$), and mid-sleep times ($R = .345$, $p < .001$).

E-E), whereas 105 (46.7%) had different chronotypes (husband and wife: 48 M-I, 5 M-E, 29 I-M, 3 I-E, 7 E-M, and 13 E-I). Couples did not differ by chronotype distribution ($\chi^2_4 = 5.30$, $p = .258$), and neither sleep quality distribution ($\chi^2_1 = .043$, $p = .837$) nor depressive mood distribution ($\chi^2_1 = .216$, $p = .642$) differed for the various combinations of chronotypes between husband and wife.

Sleep timing followed a normal distribution and varied widely among individuals (Figure 1). Husband's sleep onset time ranged from 19:45 to 04:00 h (mean ± SD: 23:00 ± 01:16 h), wake time from 03:00 to 10:00 h (mean ± SD: 06:21 ± 00:54 h), and mid-sleep time from 00:05 to 06:45 h (mean ± SD: 02:40 ± 00:57 h), whereas the corresponding values for the wife were 20:35 to 02:30 h (mean ± SD: 23:08 ± 01:01 h), 04:30 to 11:30 h (mean ± SD: 06:01 ± 00:46 h), and 00:43 to 07:00 h (mean ± SD: 02:35 ± 00:42 h). Sleep onset time, wake time, and mid-sleep time were compared between the husband and wife for each couple (Figure 2). A positive correlation was seen between spouses for sleep onset time ($R = .259$, $p < .001$), wake time ($R = .285$, $p < .001$), and mid-sleep time ($R = .345$, $p < .001$). On the contrary, there was no correlation between spouses for age-adjusted MEQ score ($R = .078$, $p = .244$). The absolute difference between spouses in terms of SO, WT, and MT (i.e., ΔSO, ΔMT, and ΔWT) ranged from .00 to 4.75 h (mean ± SD: 1.03 ± .95 h), from .00 to 5.00 h (mean ± SD: .72 ± .77 h), and from .00 to 3.88 h (mean ± SD: .72 ± .65 h), respectively. No significant correlation was seen between years living together and ΔSO ($R = .069$, $p = .303$), ΔWT ($R = -.064$, $p = .341$), or ΔMT ($R = -.006$, $p = .928$) (Figure 3).

Table 1 lists the influence of individual traits and environmental factors on husband's sleep timing. Husband's SOT was mainly influenced by his chronotype, followed by number of meals/wk eaten together. Husband's SOT was not related to his age, his depressive mood, his or his wife's shiftwork schedule, wife's SOT, wife's WT, sharing a bedroom, or years living together.

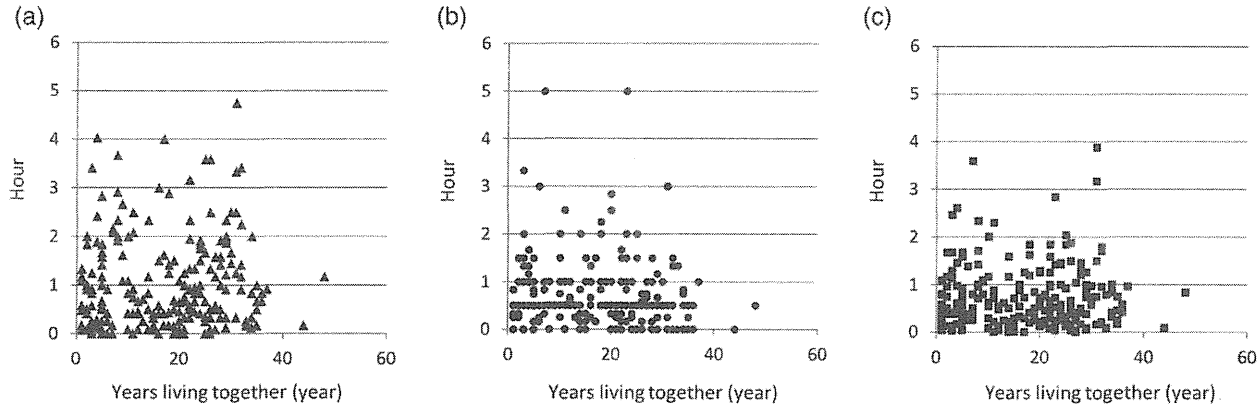

FIGURE 3. Comparison of absolute differences between husband and wife in (A) sleep onset time (ΔSO), (B) wake time (ΔWT), and (C) mid-sleep time (ΔMT) with number of years the couple had lived together. The years of living together did not correlate closely with either ΔSO ($R = .069$, $p = .303$), ΔWT ($R = -.064$, $p = .341$), or ΔMT ($R = -.006$, $p = .928$).

Similarly, husband's WT was mainly influenced by his own chronotype, followed by his wife's WT. No relationship was found between husband's WT and his age, his depressive mood, his or his wife's shiftwork schedule, wife's SOT, sharing a bedroom, years living together, or number of meals/wk eaten together. Overall, husband's SOT and WT were mainly and strongly associated with his chronotype.

Table 2lists the influence of individual traits and environmental factors on wife's sleep timing. Wife's SOT was mainly influenced by her chronotype, followed by husband's shiftwork schedule and husband's SOT. Her SOT showed no relationship with her age, her depressive mood, her shiftwork schedule, husband's WT, sharing a bedroom, years living together, or number of meal eaten times/wk together. Wife's WT was influenced mainly by her chronotype, followed by number of meals/wk eaten together and husband's WT. In contrast, wife's age, her depressive mood, her and her husband's shiftwork schedules, husband's SOT, sharing a bedroom, and years living together were not related to her WT. Overall, wife's sleep timing was

TABLE 1. Influence of individual traits and environmental factors on husband's sleep timing

Husband's sleep onset time	β	p
Age	0.199	0.195
Chronotype	0.537	**<.001**
Depressive mood	−0.028	0.602
Shiftwork schedule	−0.045	0.416
Wife's shiftwork schedule	−0.043	0.445
Wife's sleep onset time	0.106	0.061
Wife's wake time	0.095	0.095
Sharing bedroom	0.035	0.514
Years living together	−0.204	0.180
Meal times a week together	−0.157	**0.007**
$R = 0.649$; $F(10,214) = 15.601$; $p < 0.001$		

Husband's wake time	β	p
Age	−0.259	0.101
Chronotype	0.435	**<0.001**
Depressive mood	0.078	0.155
Shiftwork schedule	0.072	0.210
Wife's shiftwork schedule	0.016	0.781
Wife's sleep onset time	0.066	0.255
Wife's wake time	0.124	**0.034**
Sharing bedroom	0.086	0.121
Years living together	0.130	0.404
Meal times a week together	−0.068	0.253
$R = .623$; $F(10,214) = 13.589$; $p < .001$		

Note: Boldface = significance.

TABLE 2. Influence of individual traits and environmental factors on wife's sleep timing

Wife's sleep onset time	β	p
Age	0.112	0.511
Chronotype	0.209	**0.002**
Depressive mood	0.117	0.063
Shiftwork schedule	0.028	0.680
Husband's shiftwork schedule	−0.176	**0.008**
Husband's sleep onset time	0.179	**0.023**
Husband's wake time	0.111	0.160
Sharing bedroom	−0.029	0.647
Years living together	−0.015	0.929
Meal times a week together	0.020	0.777
$R = 0.413$; $F(10,214) = 4.404$; $p < 0.001$		

Wife's wake time	β	p
Age	−0.074	0.650
Chronotype	0.372	**<0.001**
Depressive mood	0.015	0.805
Shiftwork schedule	−0.001	0.991
Husband's shiftwork schedule	0.080	0.201
Husband's sleep onset time	0.076	0.305
Husband's wake time	0.173	**0.022**
Sharing bedroom	−0.001	0.990
Years living together	−0.023	0.887
Meal times a week together	0.181	**0.008**
$R = 0.503$; $F(10,214) = 7.248$; $p < 0.001$		

Note: Boldface = significance.

mainly associated with her chronotype and with her husband's sleep timing.

DISCUSSION

We found that sleep onset time, wake time, and midsleep time did correlate significantly, but not strongly, between husbands and wives who shared daily routines and housing. Furthermore, years living together showed no significant correlation with the differences in sleep timing between husband and wife. Multiple regression analysis showed that an individual's sleep timing was mainly influenced by chronotype. No significant correlation was found between the husband and wife's chronotype in this study. This is in contrast to other studies reporting a significant correlation, although the correlation is thought to be based on initial assortative mating rather than interaction during marriage (Hur et al., 1998; Randler & Kretz, 2011). Taken together, the results suggest that a couple's sleep timings do not synchronize the longer they live together, and thus the saying "Like husband, like wife" may not simply apply in the specific case of sleep habits.

Environmental factors (work schedule, spouse's sleep timing, lifestyle, etc.) have been reported to interfere with individual sleep-wake cycles (Leonhard & Randler, 2009; Meadows et al., 2009; Wittmann et al., 2006; Yamazaki et al., 2005). The data presented here also indicate that spouse's sleep timing, spouse's shiftwork schedule, and number of meals/wk eaten together influence the sleep timing. Notably, wife's SOT was associated with spouse's shiftwork schedule and SOT, although husband's SOT was not. The wife tended to go to bed earlier if her husband was a nightshift worker, and go to bed later if her husband went to bed later. Living together with a spouse appears to be a strong factor influencing women's sleep timing. Most couples sleep with a steady partner, and they report being less satisfied when sleeping alone (Troxel et al., 2007, 2010). It is likely that a husband and wife go to bed together (same timing) if they have a good marital relationship. Troxel et al. showed a bidirectional link between sleep and closeness of the couple's relationship (Hasler & Troxel, 2010; Troxel, 2010); couples with matched sleep-wake timing report a better relationship than those with unmatched sleep-wake timing. These findings imply that the association between sleep and relationship quality might explain the influence of "spouse" on an individual's sleep timing.

There are some limitations in this study. The survey was performed using self-rating questionnaires in a cross-sectional and retrospective design. The present results would have been strengthened if additional data had been collected using other tools, such as sleep logs or actigraphs, and if the study had been conducted in a prospective and longitudinal manner. Also, Leonhard and Randler (2009) have reported that children are a

strong factor influencing their mother's sleep timing; our data did not include information about children (number, age, sex, etc.). In addition, a major model for sleep regulation is a two-process model, where the two components of circadian drive and homeostatic drive interact with each other to regulate the sleep-wake cycle (Daan et al., 1984). As individual differences in nocturnal sleep pressure have some influence on the preferred timing of the sleep-wake cycles (Mongrain et al., 2006), data on homeostatic drive (slow-wave activity in non-rapid eye movement sleep, etc.) would have provided a better understanding of an individual's sleep timing.

CONCLUSION

The present findings demonstrate that chronotype is the major factor influencing an individual's sleep timing, followed by spouse's sleep timing, spouse's work schedule, or number of meals/wk eaten together. This study suggests that an individual's sleep timing is strongly associated with individual traits and chronotype, although environmental factors do significantly influence sleep onset and wake times. Our findings imply that recognizing an individual's chronotype may help promote better physical, emotional, and mental well-being by improving quality of life issues surrounding sleep.

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Sleep Debt Elicits Negative Emotional Reaction through Diminished Amygdala-Anterior Cingulate Functional Connectivity

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Abstract

Objectives: Sleep debt reportedly increases emotional instability, such as anxiety and confusion, in addition to sleepiness and psychomotor impairment. However, the neural basis of emotional instability due to sleep debt has yet to be elucidated. This study investigated changes in emotional responses that are elicited by the simulation of short-term sleep loss and the brain regions responsible for these changes.

Subjects and Methods: Fourteen healthy adult men aged 24.1±3.3 years (range, 20–32 years) participated in a within-subject crossover study consisting of 5-day sessions of both sleep debt (4 h for time in bed) and sleep control (8 h for time in bed). On the last day of each session, participants underwent polysomnography and completed the State-Trait Anxiety Inventory and Profile of Mood States questionnaires. In addition, functional magnetic resonance imaging was conducted while performing an emotional face viewing task.

Results: Restricted sleep over the 5-day period increased the activity of the left amygdala in response to the facial expression of fear, whereas a happy facial expression did not change the activity. Restricted sleep also resulted in a significant decrease in the functional connectivity between the amygdala and the ventral anterior cingulate cortex (vACC) in proportion to the degree of sleep debt (as indicated by the percentage of slow wave sleep and δ wave power). This decrease was significantly correlated with activation of the left amygdala and deterioration of subjective mood state.

Conclusion: The results of this study suggest that continuous and accumulating sleep debt that can be experienced in everyday life can downregulate the functional suppression of the amygdala by the vACC and consequently enhance the response of the amygdala to negative emotional stimuli. Such functional alteration in emotional control may, in part, be attributed to the neural basis of emotional instability during sleep debt.

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Introduction

Many people are now suffering from chronic sleep loss as a result of today's 24-h society, night-owl lifestyles, and prolonged work hours becoming a normal state of everyday life [1,2,3,4,5,6]. Sleep loss causes day-time sleepiness and psychomotor impairment, and can result in human errors and accidents [7,8,9].

Acute sleep deprivation has been shown to augment physiological and psychological reactions to emotional stimuli. For example, compared with normal sleep conditions, overnight total sleep deprivation enhances sympathetic reactions to unpleasant stimuli, such as dilation of the pupils and increased heart rate and blood pressure [10,11], declined task performance due to increased interference of working memory by unpleasant emotional stimuli [12], and increased changes in mood deterioration triggered even by weak emotional stressors [13]. According to functional brain imaging studies investigating the neural basis of emotional responses after acute sleep deprivation, unpleasant emotional stimuli increase the activity of the amygdala after overnight total sleep deprivation, suggesting a decline in functional connectivity between the amygdala and the medial prefrontal cortex (mPFC) which may reflect decreased inhibition by the frontal lobe [12,14].

In addition, Swann et al. have shown that response time to subliminal priming is shortened after having short hours of sleep over a 2-day period [15]. Because subliminal visual information is transmitted to the amygdala without going through the visual

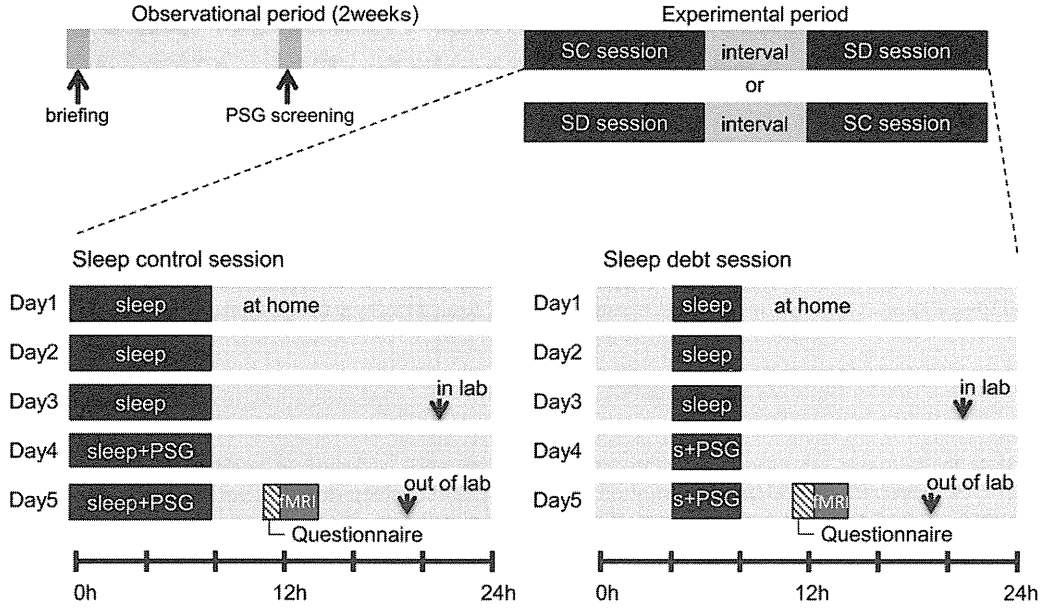

Figure 1. Experimental protocol. The study was conducted in a randomized crossover design, involving a sleep control (SC) and sleep debt (SD) session (for 5 days in each session) with a 2-week interval between the sessions. In the observational session before the experiment, participants visited the laboratory for a briefing session and gave their informed consent. One week later, participants came to the lab for PSG screening. One week after the PSG screening, the experimental sessions were started, with the order of the sessions counter-balanced across participants (i.e., SC-SD or SD-SC). Participants stayed at home on days 1–3 within each SC and SD session, according to the restrictive sleep-wake schedule that had been already instructed in the briefing (i.e., sleep time of 8 h for SC and 4 h for SD). Participants came to the lab on night 3 of the SC and SC sessions and spent the rest of the sessions (i.e., 2 days per session) in the sleep-lab with their sleep time controlled as instructed. On nights 3 and 4 in each session, participants underwent PSG measurement in the lab. On day 5, they completed questionnaires to check their mood state and sleepiness followed by fMRI scanning with an emotional task. SC, sleep control; SD, sleep debt; PSG, polysomnography; SSS, Stanford Sleepiness Scale; STAI, State-Trait Anxiety Inventory; POMS, Profile of Mood States.
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cortex [16,17,18,19,20] and subliminal and supraliminal stimulation induce different responses in the amygdala [21,22], it is possible that the transduction through subliminal signal pathways may also play an important role in changes in emotional responses to visual stimuli after sleep deprivation.

On the other hand, deteriorated mental and physical conditions due to sleep loss (partial sleep deprivation) are more likely to be caused by an accumulation of short sleep episodes over several days (sleep debt) than overnight total sleep deprivation [23]. Although sleep debt reportedly augments emotional instability (including anxiety and confusion), together with sleepiness, the feeling of fatigue, and deficits in psychomotor performance [24,25,26,27,28], the characteristics of emotional responses induced by sleep debt and the neural basis underlying such responses have not been studied extensively and therefore remain unclear.

In this study, we simulated continuous and accumulating sleep debt that can be experienced in everyday life (short hours of sleep over a 5-day period) to investigate changes in emotional responses caused by visual stimuli presented above and below the level of consciousness and the brain regions responsible for these changes.

Materials and Methods

Ethics Statement

This study was approved by the Ethics Committee of the National Center of Neurology and Psychiatry, Japan and was conducted in accordance with the Declaration of Helsinki.

Participants

This study involved 14 healthy, right-handed adult men (mean ± SD age, 24.1±3.32 years) who provided written informed consent to participate. All participants were Japanese and native Japanese speakers. A sleep log and actigraph (Ambulatory Monitoring Inc., Ardsley, NY) were used to monitor the sleep schedule of participants during the observational period (a 2-week period prior to the study) and the following experimental period. Using Cole's algorithm with optimal parameters [29], sleep-onset time, wake time, and the amount of time awake in bed were calculated from the actigraph data and were compared with the sleep log to confirm the absence of irregular life patterns, such as working in shifts or staying up all night. Overnight polysomnography (PSG) was also conducted during the observational period to examine for sleep disorders.

Exclusion criteria were as follows: a mean bedtime or wakeup time during the observational period outside of the hours 23:00–02:00 and 07:00–10:00, respectively(including shift worker); some form of sleep disorder; serious physical complication; psychiatric disorder; ocular disease, including achromatopsia; taking medication or substances inveterately that might affect the experimental data (e.g., steroids and drugs that induce drowsiness such as hypnotics and anti-histamines); caffeine intake of over 200 mg per day, heavy smoker (stressed by a 5-day smoking cessation) implanted metal object such as a pacemaker; working shifts (engaged in shift work in the 4 weeks preceding the study); or travelling to a country with a 6-h time difference in the 3 months preceding the study.

Sleep restriction protocol

Figure 1 shows the experimental protocol. All participants attended the briefing session concerning the experimental outline, underwent sleep electroencephalography (EEG) screening during the 2-week observational period, and participated in two 5-day experimental sessions. The number of hours in bed (i.e., after lights-out and during sleep) was 8 h/day in the sleep control (SC) session and 4 h in the sleep debt (SD) session. Both sessions were conducted as a crossover study with a 2-week interval between the sessions. During the interval, participants were asked to maintain a regular lifestyle without staying up all night or taking shift work.

In the SC session, based on the sleep log and actigram from the observation period, mean bedtime (23:00–02:00) for each individual was used as the start time for 8 h of sleep (wakeup time 07:00–10:00). In the SD session, bedtime started 4 h later (03:00–06:00) than that in the SC session and total hours in bed were 4 h (wakeup time 07:00–10:00).

In both sessions, participants stayed home for the first 3 days and then stayed in a laboratory room at the National Center of Neurology and Psychiatry for the next 2 days. To maintain the strict wakeup time at home, we sent a mail alert every 4 h, starting at the scheduled wakeup time until bedtime, and asked participants to answer the mail immediately. Participants were instructed by E-mail to refrain from caffeine and alcohol intake and smoking during the 5 days in which the sessions were held. In the laboratory, participants were under video camera surveillance, always assisted by a research attendant, and verbally awakened when in a drowsy state, such taking a nap or dozing off. During the wake period, participants were allowed to move freely around the laboratory, read and write, enjoy music and videos, play videogames, and engage in conversation with a researcher. Mineral water was always available, but the intake of caffeine and alcohol, smoking, and heavy exercise were restricted. Ambient temperature and humidity in the laboratory were maintained at 25±0.5°C and 50±5% RH, respectively.

MRI and emotional face viewing task

Magnetic resonance imaging (MRI) was performed on day 5 of the sessions. Participants were first served the same breakfast (~350-kcal sandwich) within 2 h of wakeup time before entering a room next to the MRI room 2–2.5 h after the wakeup time to answer a questionnaire about subjective sleepiness and mood. They underwent MRI 3–5 h after wakeup time.

During MRI, participants viewed faces with emotional expressions under two different conditions: (1) the conscious condition with a sufficient viewing time to allow supraliminal visual perception of an emotional facial expression and (2) the non-conscious condition with a brief viewing time to perform subliminal perception of an emotional facial expression followed by a neutral facial expression to mask the emotional facial image (Fig. 2).

We selected portraits of 16 individuals (4 Caucasian men, 4 Caucasian women, 4 Japanese men, and 4 Japanese women) from two standardized image sets ([30,31]www.atr-p.com/face-db.html) and created 3 facial images per individual representing the categories of fear, happy, and neutral facial expressions by masking the hair and background (48 images in total).

(1) Under the conscious condition, a fixation image was presented for 1000 ms, followed by one of the three types of facial expressions for 200 ms and then a blank image for 1000 ms. (2) Under the non-conscious condition, after presenting a fixation image for 1000 ms, either (i) a neutral facial image was presented for 26 ms, followed by a neutral facial image of another person of the same sex for 174 ms and then a blank image for 1000 ms, or (ii) a happy (fear) facial image was presented for 26 ms, followed by a neutral facial image of the same person for 174 ms and then a blank image for 1000 ms.

In both the conscious and non-conscious presentations, therefore, one trial consisted of the presentations of a fixation image, a facial image in the conscious condition or two images in the non-conscious condition, and a blank image. Nine trials composed one block. Among each block of 9 trials, a target stimulus was presented randomly, to which participants were to respond by pressing a button in order to keep themselves awake and focused on the images. After completing each block, a fixation image was shown on the screen for 15 s (baseline). A total of 12 image presentation blocks (i.e., one session) were conducted under the non-conscious and conscious conditions (6 blocks each) with a 15-s baseline period provided every 2 blocks, and a total of 2 sessions were performed with a 2-min break between the sessions. The order of block presentation was counter-balanced between participants and sessions.

Questionnaire

The Stanford Sleepiness Scale (SSS; [32]) was used to assess subjective sleepiness. Subjective mood was evaluated using state components of the State-Trait Anxiety Inventory (STAI-S; [33]), as well as the Profile of Mood States (POMS; [34]). During the SC and SD sessions, participants answered the questionnaire immediately prior to MRI.

Polysomnography (PSG) and delta wave power analysis

On nights 4 and 5 of each session, PSG was performed and analyzed using the Neurofax EEG-1200 (Nihon Kohden Corporation, Tokyo, Japan) with Ag/AgCl electrodes. The system recorded an electrocardiogram (ECG), electrooculogram (EOG), electromyogram of the chin (Chin-EMG), and electroencephalogram (EEG) at C3, C4, O1, and O2 sites in line with the International 10–20 system using the mastoid processes as reference points. The sampling rate was 200 Hz, and the hardware bandpass filter was set at 0.5–35 Hz. EEG recordings from C3-A2 were used to perform visual classification of sleep stages in 30-s epochs in accordance with international sleep scoring parameters [35].

The PSG data obtained on night 4 was excluded from the analysis to eliminate the first night effect [36]. The following sleep parameters were calculated from the PSG data taken on night 5: total sleep time (TST), duration of each sleep stage, duration of slow wave sleep (Stage 3+4; SWS) during the 2-h period after bedtime, sleep latency (SL), sleep efficiency (SE), percentage of SWS spent in TST (%SWS), and percentage of rapid eye movement (REM) sleep (%REM).

Because sleep debt increases the duration of SWS and delta power during the initial stage of sleep [27,37,38,39,40], during the first 2 h after bedtime the amounts of SWS ($SWS_{2\ h}$) and δ power ($\delta_{2\ h}$, 0.5–4 Hz) were used as objective indicators of sleep debt.

After visually excluding epochs containing body movement artifacts, all NREM sleep epochs (Stages 2–4) in the C3 EEG recording were analyzed using the fast Fourier transform (5.12-s hamming window, 2.5-s steps), and based on the power values obtained every 0.2 Hz, δ power (0.5–4 Hz) was calculated.

fMRI acquisition

The Siemens Magnetom Verio 3T MRI system was used in the analysis. To obtain reference images for analysis, structural images (T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE) images) were taken with the following sequence

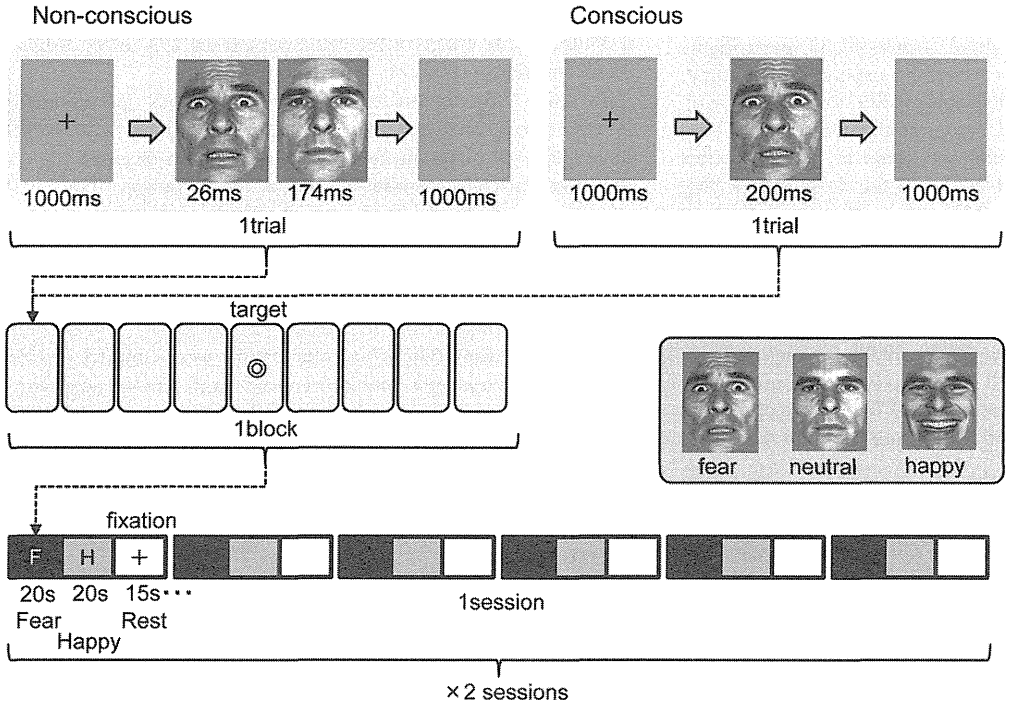

Figure 2. Design of emotional facial presentations. Facial pictures depicting fear or happy (i.e., emotional) or neutral expressions were used as the stimuli and were presented either non-consciously or consciously. In a non-conscious trial, an emotional image (either fear, happy, or neutral) was implicitly presented for 26 ms, followed by an explicit presentation for 174 ms of a neutral 'masking' face of the same identity as the preceding implicit emotional face (when the implicit face was neutral, the following explicit mask was of a different person of the same sex). Participants were required to press a button in response to each 'target' stimulus to keep themselves awake during the scanning.
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parameters: TR/TE = 1900/2.52 ms, voxel size = 1×1×1 mm, flip angle 9°, and field of view = 256×192 mm.

A single shot echo-planar imaging technique was used to obtain task-related functional MRI (fMRI) images. Settings were: TR/TE = 2500/25 ms, 30 axial slices, voxel size = 3×3×4 mm, 1-mm inter slice gap, flip angle 90°, matrix size = 64×64, and field of view = 192×192 mm. Of 137 scanning images obtained in each session, the first 5 images were excluded from analysis.

fMRI data analysis

SPM8 (Wellcome Department of Imaging Neuroscience, http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) was used in the analysis of functional brain imaging data. For each image, motion and slice timing correction as well as co-registration into an MPRAGE structural image was performed. MPRAGE imaging was carried out after PSG screening. The Montreal Neurological Institute (MNI) template was used for spatial normalization, and smoothing was performed using an 8-mm full width of half maximum Gaussian kernel. MRI time-series data that contained the three-dimensional blood oxygenation level dependent (BOLD) signals of each participant were analyzed using the first-level fixed effect model with general linear model (GLM) regression analysis. Using the canonical hemodynamic response model implemented in SPM8, a hypothetical hemodynamic time course corresponding to the stimulus presentations under each task condition was developed by convolving the canonical function. Thirteen hemodynamic models of time series corresponding to i) 6 conditions [3 categories of emotions (happy, fear, and neutral)×2 types of image presentation (conscious/non-conscious)], ii) target image presentation, and iii) 6 head motions as regressors were incorporated into the design matrix. Actual BOLD signals were analyzed voxel by voxel using the GLM, and the parameter estimate for each regressor was calculated. To subtract the low visual features, contrasts were created by subtracting the activity at the time of neutral facial image presentation from the activity at the time of emotional facial image presentation. Consequently, a total of 4 contrasts for a fear vs. neutral facial image and happy vs. neutral facial image under the conscious and non-conscious conditions were created.

To determine differences between the SC and SD sleep conditions, the value of the first-level contrast images in each SC and SD session were entered into a paired *t*-test implemented in SPM, with SC/SD as a within-subject factor.

Based on the hypothesis that sleep debt enhances the activity of the amygdala [14], we set the amygdala as the region of interest (ROI) and searched for the area where the activation was higher during the SD session than the SC session. Using the PickAtlas software (Wake Forest University (WFU), http://fmri.wfubmc.edu/downloads/WFU_PickAtlas_User_Manual.pdf) in the SPM Toolbox, masks for the amygdala on both sides were generated based on Anatomical Automatic Labeling (AAL), and each voxel in the mask was analyzed. Data were considered significant if p was less than 0.001 and the number of continuous voxels forming a cluster was greater than 5 within the amygdala ROIs. Furthermore, the significant cluster was corrected by family-wise error (FWE) correction within the amygdala ROIs ($p<0.05$, small volume correction [41]).

Functional connectivity analysis

To reveal the functional connectivity related to enhanced amygdala activation during sleep debt, a cluster in the left amygdala (peak MNI coordinates x = −14, y = 4, z = −18, 8