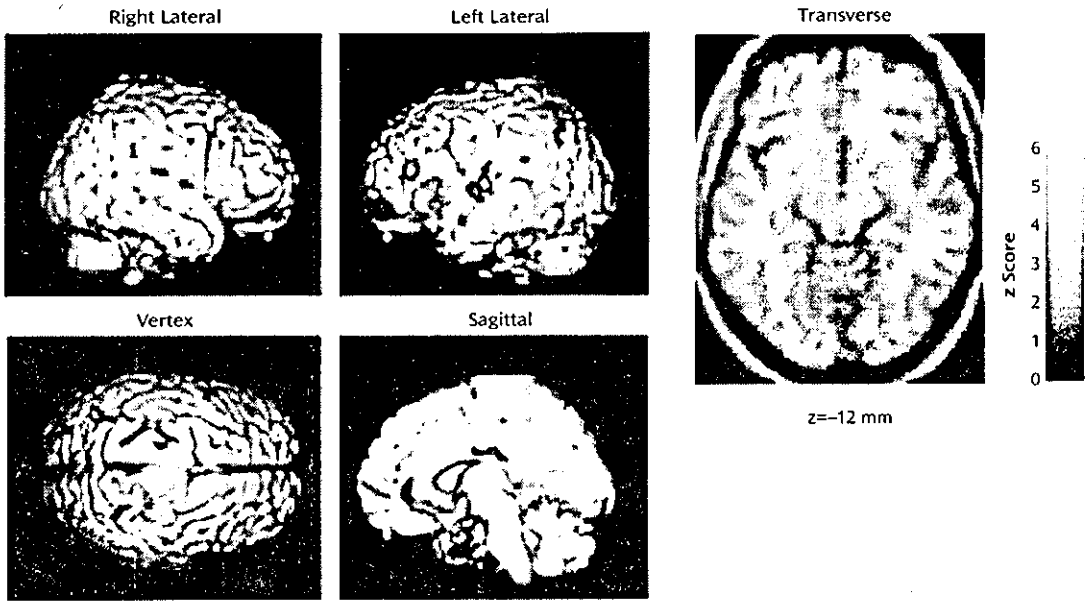
FIGURE 1. Surface Projections and a Transverse Section of Brain Regions Showing Significantly Lower Levels of Blood Flow in Nine Normal Volunteers During Non-REM Sleep After Triazolam Administration Than After Placebo Administration



brain rather than on the other wake-promoting structures. Therefore, the hypnotic effect of the benzodiazepines may result mainly from inhibition of the forebrain control system for wakefulness. This is supported by the finding in our previous study (8) that the basal forebrain is deactivated during deep non-REM sleep in normal humans, suggesting that deactivation of the basal forebrain is involved in the non-REM sleep networks. Additionally, our present finding that triazolam deactivated the amygdaloid complexes, which are involved in emotional response, including anxiety and fear, during non-REM sleep, suggests that the anxiolytic effect of the benzodiazepines is also associated with their hypnotic effect.

Most benzodiazepines, including triazolam, induce particular changes in EEG activity and sleep stages. They decrease sleep latency and night awakenings, but, in terms of sleep architecture, they reduce slow wave sleep and REM sleep. Thus, the benzodiazepines have been mysterious agents with a paradoxical effect, and the mechanism explaining this effect has remained unclear. The present finding that triazolam deactivates the basal forebrain may account for the paradoxical effect. The basal forebrain is structurally and functionally heterogeneous. Since the basal forebrain contains the type of tonic neurons that are active specifically in wakefulness and the type that are active specifically in sleep (10), and since the latter type of neurons are particularly related to the induction of slow wave sleep (11), relatively diffuse deactivation of this region might facilitate initiation of sleep but inhibit slow wave sleep.

The left neocortical regions, including the superior temporal gyrus, precentral gyrus, superior frontal gyrus (prefrontal cortex), and superior parietal gyrus, were deactivated after triazolam administration. Asymmetric changes in rCBF during the sedative state induced by midazolam, a short-acting benzodiazepine, have been reported (12): midazolam was shown to decrease rCBF in the left prefrontal cortex in a dose-dependent fashion. The deactivation of the left neocortical regions by triazolam in the present study is basically consistent with the finding of the midazolam study, suggesting that left-hand cortical areas are more sensitive to the modulation of benzodiazepines.

Received Feb. 25, 2003; revision received Sept. 2, 2003; accepted Sept. 5, 2003. From the National Center Hospital for Mental, Nervous and Muscular Disorders. Address reprint requests to Dr. Kajimura, Musashi Clinic for Mental and Sleep Disorders, SF Bldg. 2F, 1-7-17 Misono-cho, Kodaira, Tokyo 187-0041, Japan; kajimura@sa2.so-net.ne.jp (e-mail).

Supported by a grant from the Ministry of Health, Labor and Welfare of Japan.

The authors thank Dr. Pierre Maquet for his critical comments and suggestions.

References

1. Müller WE: Pharmacology of the GABAergic/benzodiazepine system, in Pharmacology of Sleep. Edited by Kales A. Berlin, Springer-Verlag, 1995, pp 211–242
2. Maquet P: Functional neuroimaging of normal human sleep by positron emission tomography. J Sleep Res 2000; 9:207–231
3. Finelli LA, Landolt HP, Buck A, Roth C, Berthold T, Borbely AA, Achermann P: Functional neuroanatomy of human sleep

states after zolpidem and placebo: a $H_2{}^{15}O$-PET study. J Sleep Res 2000; 9:161–173
4. Kajimura N, Kato M, Okuma T, Sekimoto M, Watanabe T, Takahashi K: A quantitative sleep EEG study on the effects of benzodiazepine and zopiclone in schizophrenic patients. Schizophr Res 1995; 15:303–312
5. Rechtschaffen A, Kales A (eds): A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects: NIH Publication 204. Bethesda, Md, Public Health Service, National Institutes of Health, 1968
6. Herscovitch P, Markham J, Raichle ME: Brain blood flow measured with intravenous $H_2{}^{15}O$, I: theory and error analysis. J Nucl Med 1983; 24:782–789
7. Raichle ME, Martin WRW, Herscovitch P, Mintum MA, Makkam J: Brain blood flow measured with intravenous $H_2{}^{15}O$, II: implementation and validation. J Nucl Med 1983; 24:790–798
8. Kajimura N, Uchiyama M, Takayama Y, Uchida S, Uema T, Kato M, Sekimoto M, Watanabe T, Nakajima T, Horikoshi S, Ogawa K, Nishikawa M, Hiroki M, Kudo Y, Matsuda H, Okawa M, Takahashi K: Activity of midbrain reticular formation and neocortex during the progression of human non-rapid eye movement sleep. J Neurosci 1999; 19:10065–10073
9. Talairach J, Tournoux P: Co-Planar Stereotaxic Atlas of the Human Brain: Three-Dimensional Proportional System. Stuttgart, Germany, Georg Thieme, 1988
10. Sakai F, Mansari M, Jin JS, Zhang JG, Vanni-Mercier G: Posterior hypothalamus in the regulation of wakefulness and paradoxical sleep, in The Diencephalon and Sleep. Edited by Mancia M, Marini G. New York, Raven Press, 1990, pp 171–198
11. Koyama Y, Hayaishi O: Firing of neurons in the preoptic/anterior hypothalamic areas in rat: its possible involvement in slow wave sleep and paradoxical sleep. Neurosci Res 1994; 19:31–38
12. Veselis RA, Reinsel RA, Beattie BJ, Mawlawi OR, Feshchenko VA, DiResta GR, Larson SM, Blasberg RG: Midazolam changes cerebral blood flow in discrete brain regions: an $H_2{}^{15}O$ positron emission tomography study. Anesthesiology 1997; 87:1106–1117

Available online at www.sciencedirect.com
SCIENCE DIRECT
Neuroscience Research 49 (2004) 387–393
Neuroscience Research
www.elsevier.com/locate/neures

Time estimation during nocturnal sleep in human subjects

Sayaka Aritake [a,b], Makoto Uchiyama [b,*], Hirokuni Tagaya [b], Hiroyuki Suzuki [b], Kenichi Kuriyama [b], Akiko Ozaki [b], Xin Tan [b], Kayo Shibui [b], Yuichi Kamei [c], Yoshiro Okubo [d], Kiyohisa Takahashi [e]

[a] *Department of Life Sciences and Bio-informatics, Graduate School of Allied Health Sciences, Tokyo Medical and Dental University, Yushima, Bunkyo-ku, Tokyo, 113-0034, Japan*
[b] *Department of Psychophysiology, National Institute of Mental Health, National Center of Neurology and Psychiatry, Kohnodai, Ichikawa, Chiba, 272-0827, Japan*
[c] *Department of Psychiatry, Kohnodai Hospital, National Center of Neurology and Psychiatry, Kohnodai, Ichikawa, Chiba, 272-8516, Japan*
[d] *Department of Neuropsychiatry, Nippon Medical School, Sendagi, Bunkyo-ku, Tokyo, 113-8602, Japan*
[e] *Japan Foundation for Neuroscience and Mental Health, Kodaira, Tokyo, 187-8551, Japan*

Received 29 January 2004; accepted 30 April 2004

Available online 20 June 2004

Abstract

It has been postulated that time estimation during nocturnal sleep in humans can be explained by an interval timing clock inside the brain. However, no systematic investigations have been carried out with respect to how the human brain perceives the passage of time during sleep. The brain mechanisms of over- or underestimation of time spent in sleep have not yet been clarified. Here, we carried out an experimental study in which 11 healthy volunteers participated in time estimation trials scheduled six times during 9 h nocturnal sleep periods, under carefully controlled conditions. The time estimation ratio (TER: a ratio of subjective passage of time to actual time interval) decreased significantly from the first to the sixth trial. Individual TER was positively correlated with slow wave sleep prior to the trial, while it was negatively correlated with REM sleep. Our results indicate that the human brain has an ability to estimate the passage of time during nocturnal sleep without referring to time cues, and that the accuracy of this function fluctuates from overestimation in the early hours of sleep to underestimation in the last hours of sleep.

Keywords: Time estimation; Interval timing clock; Cognitive science; Sleep; Insomnia; Circadian rhythm

1. Introduction

Animals are likely to have two endogenous time-keeping systems: a circadian clock that provides the time of day, and an interval timing clock that measures the passage of time, like a stopwatch (Morell, 1996). The circadian clock is driven by a self-sustaining oscillator with a period of about 24 h, which is located in the suprachiasmatic nuclei of the hypothalamus and is synchronized to the light–dark cycle (Moore-Ede et al., 1983). In contrast, the interval timing clock counts the number of signals that are generated in certain intervals (Gibbon and Church, 1981; Roberts and Holder, 1984). These clocks help animals to anticipate risks and opportunities in the environment and to survive.

Previous studies suggest that humans have an ability to perceive the passage of time without referring to an external clock or stopwatch. In experimental settings, this ability has been studied using a time production protocol (Kuriyama et al., 2003; Miro et al., 2003), in which subjects indicate an instructed interval; or by using a time estimation protocol, in which subjects estimate a given interval (Webb and Ross, 1972; Lavie and Webb, 1975; Aschoff, 1998b; Kuriyama et al., 2003). Functional imaging studies in humans have suggested that a certain brain network is responsible for the ability to perceive the passage of time (Maquet et al., 1996; Harrington et al., 1998; Pouthas et al., 1999; Schubotz et al., 2000).

* Corresponding author. Tel.: +81 47 375 4756; fax: +81 47 375 4771.
E-mail address: macoto@ncnp-k.go.jp (M. Uchiyama).

0168-0102/$ – see front matter
doi:10.1016/j.neures.2004.04.006

Several human sleep studies have suggested that the function that enables humans to perceive the passage of time is also available during sleep. Some individuals seem to have the ability to time the end of their nocturnal sleep without using an alarm clock (Omwake and Loranz, 1933; Tart, 1970; Lavie et al., 1979; Bell, 1980; Hawkins, 1989; Carskadon et al., 1990; Hawkins and Shaw, 1990; Moorcroft et al., 1997; Moorcroft and Breitenstein, 2000). A more recent study suggested that healthy humans' ACTH level peaks earlier in the morning hours, when instructed to rise earlier, without reference to a clock (Born et al., 1999). These observations may be explained by assuming the existence of an interval timing clock that is persistently activated during sleep. However, other possible mechanisms may enable individuals to perceive the passage of time that do not require the existence of an interval timing clock, the degree of sleep satiation might act as a signal to inform the individual that it is morning; or a strong will of the individual to rise at certain times in the morning might affect his/her sleep structure and results in signals being sent during shallow sleep in the morning (Watanabe, 1969).

To investigate whether humans have the ability to estimate the passage of time during sleep, it is necessary to wake subjects at various time intervals from the onset of sleep and ask them for a subjective estimation of the passage of time, without paying attention to the time that has passed when they were sleeping.

For the investigation reported here, we studied the changes of subjective time estimation as a function of sleep progression and investigated the relationship between subjective time estimation and sleep structure in healthy human subjects.

2. Materials and methods

2.1. Participants

Eleven healthy males aged from 18 to 23 years (mean age 20.5 years), who had regular sleep habits, participated in the study. They gave their written informed consent after the possible risks and details of the study were explained to them. A physician and a psychiatrist examined them and found that no participant suffered from neurological or psychiatric disorders, or had a history of using any psychoactive drugs. Subjects were instructed to keep to a regular sleep–wake schedule; record their sleeping patterns in a sleep log; and to abstain from caffeine, nicotine, and alcohol for a week prior to the experiment. All the participants wore a wrist activity recorder (Actiwatch-L, Mini-Mitter co., Inc. Bend, OR, USA) for a week prior to the experiment. Sleep onset and offset times were determined using Actiware Sleep software (V3. 2j Mini-Mitter co., Inc. Bend, OR, USA). The details recorded in participants' sleep logs, together with their sleep onset and offset times, confirmed that they had regular sleep–wake schedules. Since participants' attention to the time could potentially affect their results, we did not disclose the study objectives until the end of the study. The study protocol was approved by the Intramural Research Board of National Center of Neurology and Psychiatry (NCNP).

2.2. Experimental procedures

All the experiments were performed in the time isolation facility of NCNP. The participants entered a three-day laboratory experimental protocol (Fig. 1). During the

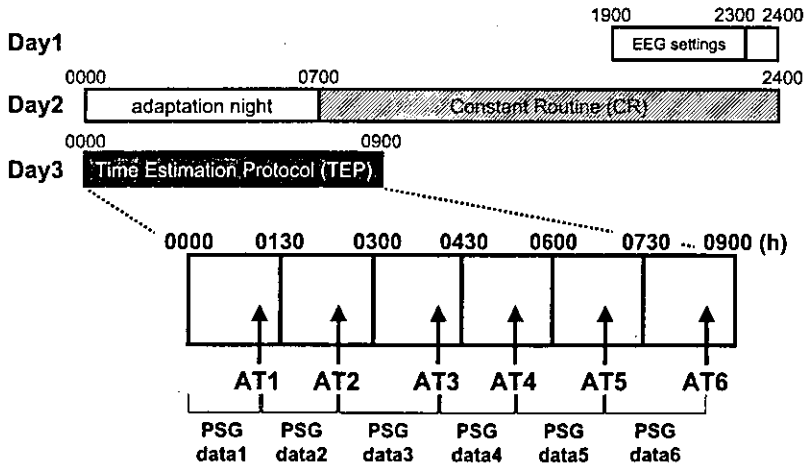


Fig. 1. On day 1, upon arriving at the laboratory at 19:00 h, participants were instructed to retire at 23:00 h and stay in bed until 07:00 h (adaptation night). On day 2, a constant routine (CR) for 17 h was conducted between 07:00 and 24:00 h. During this period, the participants were kept awake in a semi-recumbent position under room light conditions (180 lux). On day 3, a time estimation protocol (TEP) was conducted between 00:00 and 09:00 h. The 9-h polysomnography (PSG) recording periods were divided into six 90-min periods. We woke the participants and conducted a structured interview once every 90-min period (awakening trial: AT). Participants were woken for an AT when (1) they had slept for longer than 45 min since the time of lights out and the end of the prior AT; and (2) sleep stage 2 had continued for more than 3 min.

experiments, participants were not given any time cues, except for planned notification of the beginning of the time estimation protocol (TEP).

On day 1, they arrived at the laboratory at 19:00 h. During the entire experimental protocol, the participants were not allowed to have any instruments that provided information on time. The setting of electroencephalogram (EEG), electrooculogram (EOG), chin surface electromyogram (EMG), and electrocardiogram (ECG) electrodes was completed between 20:00 and 22:00 h. The participants were instructed to retire at 23:00 h (adaptation night). Polysomnography (PSG) recordings were undertaken between 23:00 and 07:00 h under complete dark condition (<0.1 lux).

On day 2, a constant routine (CR) for 17 h was conducted between 07:00 and 24:00 h. After being woken at 07:00 h, the participants were enforced to keep awake in a semi-recumbent position under room light condition (180 lux). Polysomnographic recordings were carried out throughout the CR. The investigators monitored the participants' behavioral status via a video monitoring system. The participants took isocaloric meals (470 kcal) [Calorie mate: Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan] every 3.5–4.5 h and 200–300 ml of mineral water per hour.

On day 3, TEP was conducted between 00:00 and 09:00 h. At 00:00 h, the participants were informed that it was 00:00 h and entered the PSG study. Two PSG experts monitored PSG recordings online via a CRT monitoring system (Neurofax, Nihon Kohden, Tokyo, Japan).

2.3. Time estimation protocol (TEP)

The 9-h PSG recording period was divided into six 90-min periods. The period from lights out to the sixth trial was 436.8 ± 44.5 min (mean ± S.D.). We awoke the participants and conducted a structured interview once per 90-min period (awakening trial: AT). Participants were woken for each AT when (1) they had slept for longer than 45 min since the time of lights out and the end of the prior AT; and (2) sleep stage 2 had continued for more than 3 min. When these criteria were not satisfied, the participants were woken at the end of each 90-min periods and the data was excluded.

In the structured interview we asked the following questions to determine participants' natural expression of time estimation, without encouraging them to focus their attention on time.

1. Subjective amount of dreaming (1, none; 2, a small amount; 3, a moderate amount; or 4, a lot);
2. Subjective amount of sleep (1, none; 2, a small amount; 3, a moderate amount; or 4, a lot);
3. Subjective sleep quality (1, very poor; 2, rather poor; 3, good; or 4, very good);
4. Subjective time of day;
5. Subjective sleepiness (1, very sleepy; 2, sleepy; 3, alert; or 4, very alert);
6. Subjective mood (1, bad; 2, rather bad; 3, good; or 4, very good);
7. Subjective level of energy (1, very exhausted; 2, exhausted; 3, vigor; or 4, very vigor);
8. Subjective level of tension (1, very strained; 2, strained; 3, relaxed; or 4, very relaxed);
9. Subjective level of refreshment (1, not at all refreshed; 2, a little refreshed; 3, somewhat refreshed; or 4, refreshed).

The structured interview was performed within 2 min of waking under dim light conditions (<8 lux). While the participants remained in a lying position on the bed, the interviewer was instructed to take care that participants did not guess the real purpose of the study. We told the participants that the aim of the study was to investigate the correlation between PSG parameters and subjective feelings of sleep. We confirmed that none of the participants had determined the real purpose of the investigation until the end of the study. Throughout the study, participants were given no information on the exact number and timing of meals during the CR or on the number of ATs in the TEP. PSG measurements were carried out continuously during the study and stored in a digital EEG system (Neurofax, Nihon Kohden, Tokyo, Japan). PSG measurements comprised EEG recordings (C3–A2, C4–A1, and O1–A2) in conformity with the 10–20 electrode system, and EOG (left-A2 and right-A1), EMG (chin) and ECG recordings.

2.4. Measurements

Subjective time interval was defined as the time difference between subjective times of day, which were obtained at successive ATs. Time estimation ratio (TER), as an indicator of subjective time estimation, was calculated by dividing a subjective time interval by an actual time interval (Campbell et al., 2001) (Fig. 2). PSG data obtained between successive ATs were scored in epochs of 30 s according to the standard criteria (Rechtschaffen and Kales, 1968). Time percentages for stages W, 1, 2, 3 + 4 and REM (%W, %S1, %S2, %S3 + 4 and %SREM) were calculated for all PSG recordings.

2.5. Statistical analyses

Randomized block ANOVAs (trial × subject) were carried out to analyze the time course of TER and time percentages of sleep stages. The relationship between TER and time percentages of sleep stages was examined using Pearson's correlation coefficient. Statview version 5.0 (SAS Institute, Cary, NC, USA) was used for all statistical analyses. The level of significance was set at $P < 0.05$.

3. Results

The difference between estimated and actual times of day is plotted against the actual time of AT in Fig. 3. The difference was greater in the early half of the night,

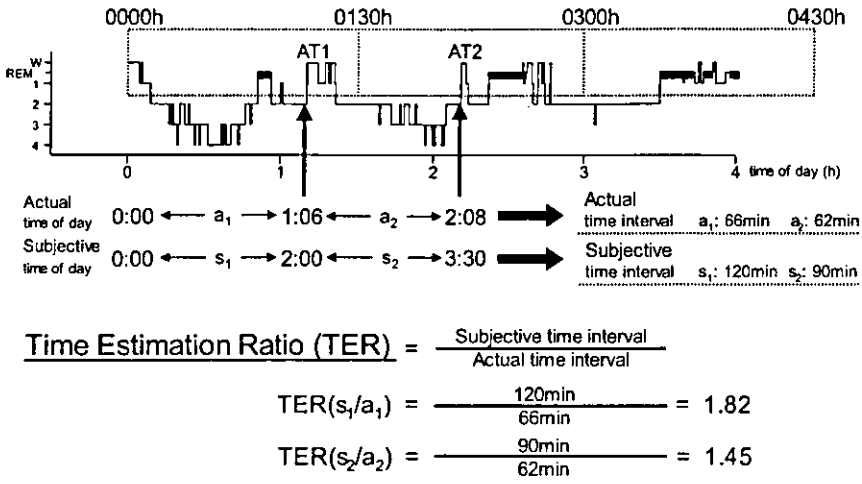


Fig. 2. Subjective time interval was defined as the time difference between subjective times of the day, which were obtained at successive ATs. Actual time interval was defined as the time difference between successive ATs. Time estimation ratio (TER), as an indicator of subjective time estimation, was calculated by dividing a subjective time interval (s_1 or s_2) by an actual time interval (a_1 or a_2) (Campbell et al., 2001).

whereas it lessened in the latter half of the night i.e. the passage of subjective time changed with the progression of sleep.

The profiles of mean TER are shown in Fig. 4. Randomized block ANOVA revealed a significant effect of AT number [F (5,10) = 11.93, $P < 0.0001$] on the TER. The value of TER was nearly doubled in AT1 and decreased toward 0.6 in accordance with the progression of sleep.

The time percentages of sleep stages are shown in Fig. 5. A significant effect of AT number was found in %S3 + 4 [F (5,10) = 7.44, $P < 0.0001$] and %SREM [F (5,10) = 4.67, $P = 0.0018$]. The percentage of S3 + 4 decreased throughout the night, but %SREM showed an increase towards morning. Trial number had no significant effect in %W, %S1, and %S2.

A significant positive correlation was found between TER and %S3 + 4 [$r = 0.29$, $P = 0.0259$]. A significant negative correlation was found between TER and %SREM [$r = -0.38$, $P = 0.0028$]. TER was not significantly correlated with %W, %S1 or %S2. To reduce inter-individual variations in sleep structure, we averaged the TER, %S3 + 4 and %SREM/AT data across all the participants. We found a significant positive correlation between the average TER and the average %S3 + 4 [$r = 0.94$, $P = 0.0027$], and a significant negative correlation between the average TER and the average %SREM [$r = -0.87$, $P = 0.0205$]. These were more robust compared to the individually calculated correlations.

To investigate whether subjective sleepiness during an AT may affect the TER, we examined the relationship between TER and subjective sleepiness during the ATs. Randomized block ANOVA analysis revealed no significant effect of subjective sleepiness on time estimation.

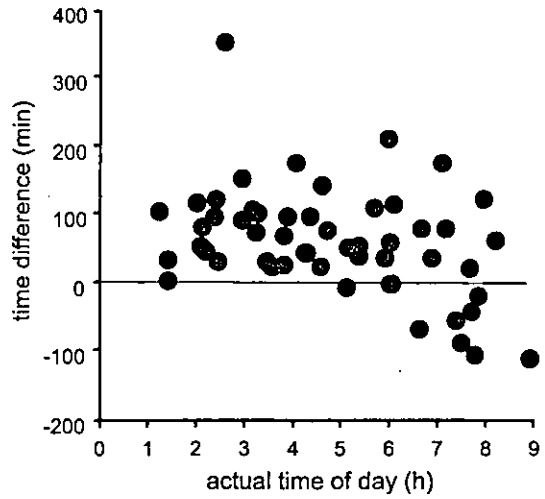


Fig. 3. The vertical axis represents the deviation between the subjective time of day and actual time of the ATs. The horizontal axis represents the actual time of the ATs. The difference was greater in the early half of the night, and lessened in the latter half of the night.

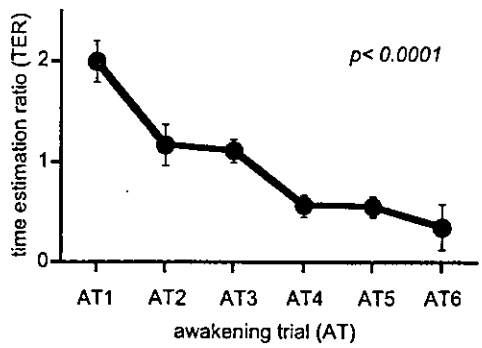


Fig. 4. The vertical axis represents the time estimation ratio (TER). The horizontal axis represents the AT number. Randomized block ANOVA analysis revealed a significant effect of AT number on TER. The value of TER was nearly doubled at AT1, and decreased toward 0.6 in accordance with the progression of sleep.

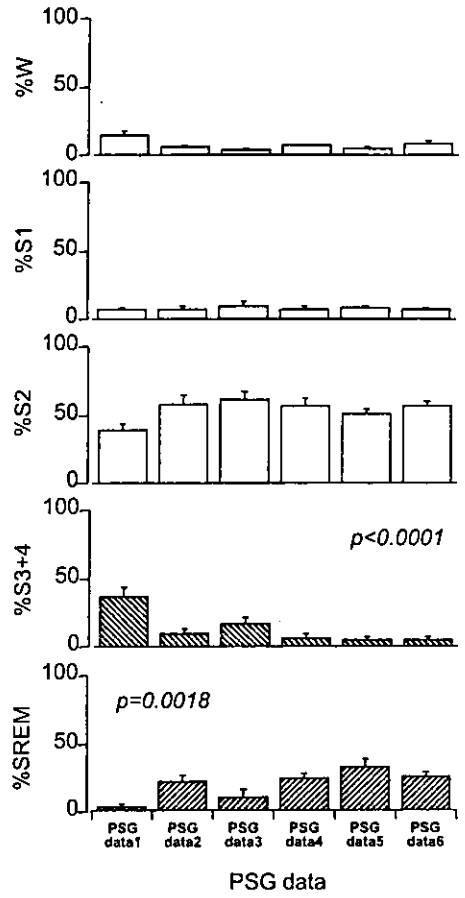


Fig. 5. Trial number had a significant effect in %S3 + 4 and %SREM. The percentage of S3 + 4 decreased through the night and the %SREM showed an increase towards morning. There was no significant effect of AT number in %W, %S1 or %S2.

4. Discussion

In this study, we investigated changes in subjective time estimation during nocturnal sleep as a function of sleep progression, and found that TER decreased with the progression of sleep, with the largest value occurring in the early hours and the smallest value in the last hours of nocturnal sleep. This is the first documentation of individual estimations of the passage of time during nocturnal sleep changes with the progression of sleep.

Several studies have demonstrated evidence that humans have an ability to estimate the passage of time during sleep (Brush, 1930; Lewis, 1969; Zung and Wilson, 1971; Moiseeva, 1975; Hartocollis, 1980; Vanable et al., 2000). Prior studies in human experimental psychology have revealed that some individuals are able to wake accurately at a predetermined time without using a watch or clock (Moorcroft et al., 1997; Born et al., 1999; Moorcroft and Breitenstein, 2000). Born et al. (1999) found that instructing healthy subjects to wake at an earlier predetermined time was associated with earlier ACTH peaks in the morning, suggesting a biological mechanism for the ability to keep track of the passage of time during sleep.

However, there is only a limited understanding regarding the physiological mechanisms of human time estimation during sleep, possibly because of methodological limitations to investigations. Fujisawa (1966), in an attempt to investigate human time estimation during sleep, conducted a study in which participants were woken randomly during all-night PSG recordings, and found that the progression of sleep and stages of sleep upon waking influenced the accuracy of human time estimation. Latash and Danilin (1972) conducted a similar experiment and observed the effects of preceding sleep stages on time estimation. Lavie et al. (1979) conducted a study in which participants were required to wake at predetermined times in the morning, and have reported that the ability to wake accurately at specific times may depend on an individual's motivational level and sleep stage upon waking. Campbell (1986) conducted a study in which human subjects, under isolated conditions, were instructed to estimate the passage of time without any external time cues, and found that subjects' state (sleep or wakefulness), during the time estimation trial, strongly influenced the results. These previous studies suggest that the brain has a mechanism for estimating the passage of time during sleep, and that this function is likely to be influenced by sleep state when a time estimation trial is carried out. This assumption may be supported by a finding by Miro et al. (2003) that human time estimation during a wakeful state is strongly influenced by sleepiness. Therefore, the findings of these prior studies on human time estimation during sleep seem more an indication of sleep state upon waking or consequent fluctuations in alertness of the individual at the time of questioning, rather than a brain process.

Alternatively, to study the process of time estimation in the brain, experiments should be carefully controlled so that subjects are woken in a consistent sleep state and are questioned at a consistent level of alertness. Since awakening trials in the present study were undertaken when subjects were 3-min into sleep stage 2, our finding that TER decreased with the progression of sleep was unlikely to be due to variations of sleep stages upon AT. The finding that subjective sleepiness at an AT did not differ with respect to sleep progression suggests that there were no marked effects of subjective sleepiness on the characteristic temporal pattern of TER in the present study. Thus, the changes in subjective time estimation, during sleep in this study, were likely to reflect a process in the brain that provides an accurate estimation of elapsed time during sleep.

Further analysis of sleep structure, during the period in which the participants estimated the passage of time, revealed that slow wave sleep decreased and REM sleep increased with the progression of sleep, but no other sleep stages significantly changed. A correlation analysis showed that the percentage of slow wave sleep in the estimated period was positively correlated with TER, and the percentage of REM sleep was negatively correlated. This suggests that

sleep structure in a given estimated period may be a determinant factor in the accuracy of time estimation. Latash and Danilin (1972) postulated that differences in time estimation during sleep may be attributed to sleep structure in the estimated period, although they did not control for sleep stages upon waking. Campbell (1986) demonstrated that the percentage of a given sleep stage in the estimated period had a marked influence on the accuracy of time estimation. From these reports, it may be concluded that the level of cortical activation has an effect on time estimation. In this regard, it may be interpreted from our correlation analysis that greater cortical suppression (longer period of slow wave sleep) is associated with overestimation of time, and its relative activation (REM sleep) is associated with underestimation.

Another possible explanation might be drawn. It is well documented that the temporal distributions of slow wave sleep and REM sleep are reversal in regard to the progress of sleep. Therefore, the phenomena having time effects as a function of sleep progression show the negative or positive correlation with slow wave sleep and REM sleep. Thus, all of these parameters could be overt manifestations of processes under control of the circadian rhythm (Aschoff, 1994, 1998a; Campbell et al., 2001; Kuriyama et al., 2003). The previous findings that time estimation during wakefulness is under the influence of circadian rhythm may be applicable to time estimation during sleep, given that time estimation mechanisms during sleep and wakefulness share common brain systems.

Further study of time estimation during sleep in humans would contribute to the field of sleep medicine in furthering knowledge regarding sleep state misperception (Thorpy, 1997) or psychophysiological insomnia (Salin-Pascual et al., 1992; Vanable et al., 2000).

Acknowledgements

This study was supported in part by a Research Grant for Nervous and Mental Disorders (11–3) and a Health Science Grant (15130301) from the Ministry of Health, Labor and Welfare, and a Grant-in-aid for Scientific Research (13470200) by the Ministry of Education, Science and Culture.

References

Aschoff, J., 1994. The timing of defecation within the sleep–wake cycle of humans during temporal isolation. J. Biol. Rhythms 9 (1), 43–50.

Aschoff, J., 1998a. Circadian parameters as individual characteristics. J. Biol. Rhythms 13 (2), 123–131.

Aschoff, J., 1998b. Human perception of short and long time intervals: its correlation with body temperature and the duration of wake time. J. Biol. Rhythms 13 (5), 437–442.

Bell, C.R., 1980. Awaking from sleep at a pre-set time. Percept. Mot. Skills 50, 503–508.

Born, J., Hansen, K., Marshall, L., Molle, M., Fehm, H.L., 1999. Timing the end of nocturnal sleep. Nature 397, 29–30.

Brush, E.N., 1930. Observations on temporal judgement during sleep. Am. J. Psycho. 42, 408–411.

Campbell, S.S., 1986. Estimation of empty time. Human neurobiol. 5, 205–207.

Campbell, S.S., Murphy, P.J., Boothroyd, C.E., 2001. Long-term time estimation is influenced by circadian phase. Physiol. Behav. 72, 589–593.

Carskadon, M.A., Herman, K.B., Davis, S.S., 1990. Arousals in college students: can they "Beat the clock"? Sleep Res. 19, 115.

Fujisawa, K., 1966. Subjective experiences during sleep. Psychiatria et Neurologica Japonica 68 (12), 1477–1491 (in Japanese).

Gibbon, J., Church, R.M., 1981. Time left: linear vs. logarithmic subjective time. J. Exp. Psychol. Anim. Behav. Process. 7 (2), 87–107.

Harrington, D.L., Haaland, K.Y., Knight, R.T., 1998. Cortical networks underlying mechanisms of time perception. J. Neurosci. 18 (3), 1085–1095.

Hartocollis, P., 1980. Time and the dream. J. Am. Psychoanal. Assoc. 28 (4), 861–877.

Hawkins, J., 1989. Sleep disturbance in intentional self-awakenings: behavior-genetic and transient factors. Percept. Mot. Skills 69, 507–510.

Hawkins, J., Shaw, P., 1990. Sleep satisfaction and intentional self-awakening: an alternative protocol for self-report data. Percept. Mot. Skills 70, 447–450.

Kuriyama, K., Uchiyama, M., Suzuki, H., Tagaya, H., Ozaki, A., Aritake, S., Kamei, Y., Nishikawa, T., Takahashi, K., 2003. Circadian fluctuation of time perception in human subjects. Neurosci. Res. 46, 23–31.

Latash, L.P., Danilin, V.P., 1972. Subjective estimation of the duration of time periods in night sleep. Nat. New Biol. 236 (64), 94–95.

Lavie, P., Webb, W.B., 1975. Time estimates in a long-term time-free environment. Am. J. Psychol. 88 (2), 177–186.

Lavie, P., Oksenberg, A., Zomer, J., 1979. It's time, you must wake up now. Percept. Mot. Skills 49, 447–450.

Lewis, S.A., 1969. Subjective estimates of sleep: an EEG evaluation. Br. J. Psychol. 60 (2), 203–208.

Maquet, P., Lejeune, H., Pouthas, V., Bonnet, M., Casini, L., Macar, F., Timsit-Berthier, M., Vidal, F., Ferrara, A., Degueldre, C., Quaglia, L., Delfiore, G., Luxen, A., Wood, R., Mazziota, J.C., Comar, D., 1996. Brain activation induced by estimation of duration: a PET study. Neuroimage 3 (2), 119–126.

Miro, E., Cano, M.C., Espinosa-Fernandez, L., Buela-Casal, G., 2003. Time estimation during prolonged sleep deprivation and its relation to activation measures. Human Factors 45 (1), 148–159.

Moiseeva, N.I., 1975. The characteristics of EEG activity and the subjective estimation of time during dreams of different structure. Electroencephalogr. Clin. Neurophysiol. 38, 569–577.

Moorcroft, W.H., Kayser, K.H., Griggs, A.J., 1997. Subjective and objective confirmation of the ability to self-awaken at a self-predetermined time without using external means. Sleep 20 (1), 40–45.

Moorcroft, W.H., Breitenstein, J.L., 2000. Awareness of time during sleep. Ann. Med. 32, 236–238.

Moore-Ede, M.C., Czeisler, C.A., Richardson, G.S., 1983. Circadian timekeeping in health and disease. I. Basic properties of circadian pacemakers. N. Engl. J. Med. 309, 469–476.

Morell, V., 1996. Setting a biological stopwatch. Science 271, 905–906.

Omwake, K.T., Loranz, M., 1933. Study of ability to wake at a specified time. J. Appl. Psychol. 17, 468–474.

Pouthas, V., Maquet, P., Garnero, L., Ferrandez, A.M., Renault, B., 1999. Neural bases of time estimation: a PET and ERP study. Electroencephalogr. Clin. Neurophysiol. 50 (Suppl.), 598–603.

Rechtschaffen, A., Kales, A., 1968. A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Washington, DC, United States Public Health Service, US Government Printing Office.

Roberts, S., Holder, M.D., 1984. What starts an internal clock? J. Exp. Psychol. Anim. Behav. Process. 10 (3), 273–296.

Salin-Pascual, R.J., Roehrs, T.A., Merlotti, L.A., Zorick, F., Roth, T., 1992. Long-term study of the sleep of insomnia patients with sleep state misperception and other insomnia patients. Am. J. Psychiatry 149, 904–908.

Schubotz, R.I., Friederici, A.D., von Cramon, D.Y., 2000. Time perception and motor timing: a common cortical and subcortical basis revealed by fMRI. Neuroimage 11 (1), 1–12.

Tart, C.T., 1970. Waking from sleep at a pre-selected time. J. Am. Soc. Psychosom. Dent. Med. 17, 3–16.

Thorpy, M.J., 1997. International classification of sleep disorders, Diagnostic and Coding Manual (revised), American Sleep Disorders Association, Rochester, Minnesota, 32-35.

Vanable, P.A., Aikens, J.E., Tadimeti, L., Caruana-Montaldo, B., Mendelson, W.B., 2000. Sleep latency and duration estimates among sleep disorder patients: variability as a function of sleep disorder diagnosis, sleep history, and psychological characteristics. Sleep 23 (1), 71–79.

Watanabe, H., 1969. A clinical study on the phenomenon of awaking at a pre-determined time (sommeil attentif). Psychiatria et Neurologica Japonica 71 (7), 631–652 (in Japanese).

Webb, W.B., Ross, W., 1972. Estimation of the passing of four consecutive hours. Percept. Mot. Skills 35, 768–770.

Zung, W.W., Wilson, W.P., 1971. Time estimation during sleep. Biol. Psychiat. 3, 159–164.

Sleep and Biological Rhythms 2004; 2: 57–64

ORIGINAL ARTICLE

Sleep habits and factors associated with short sleep duration among Japanese high-school students: A community study

Hirokuni TAGAYA,[1] Makoto UCHIYAMA,[1] Takashi OHIDA,[2] Yuichi KAMEI,[3] Kayo SHIBUI,[1] Akiko OZAKI,[1] Xin TAN,[1] Hiroyuki SUZUKI,[1] Sayaka Aritake,[1] Lan LI[1] and Kiyohisa TAKAHASHI[4]

[1]Department of Psychophysiology, National Institute of Mental Health, National Center of Neurology and Psychiatry, Ichikawa, [2]Department of Public Health, Nihon University School of Medicine, Tokyo, [3]Department of Psychiatry, Kohnodai Hospital, NCNP, Ichikawa and [4]Japan Foundation for Neuroscience and Mental Health, Kodaira, Japan

Abstract

To clarify the sleep habits, and predictors thereof, in Japanese high-school students, a community study was conducted. A total of 3833 students were selected randomly from the 34 full-time high schools in two suburban cities in Japan. The response rate was 90.7% (n=3478). The students completed the Japanese version of the Pittsburgh Sleep Questionnaire Index (PSQI-J), the Japanese version of the 12-item General Health Questionnaire (GHQ12-J), and a questionnaire on sociodemographic characteristics, daily life, domestic situations, and perceived physical health. Mean bed and rise times were found to be 00:03 and 06:33 h, respectively, resulting in a sleep duration of 380.0 min. A multivariate logistic regression analysis revealed that a short sleep duration (i.e. less than 6 h; prevalence: 25.6%) was significantly associated with a later bedtime, a higher global PSQI-J score, an earlier rise time, being female, a longer study duration outside school hours, and a longer commuting duration, but not with a higher GHQ12-J score. Short sleep duration in Japanese high-school students is associated with their lifestyle as well as sleep problems, but not with psychosomatic problems.

Key words: adolescents, community survey, high-school students, insufficient sleep, lifestyle.

INTRODUCTION

During adolescence, the sleep-wake pattern changes dramatically. Previous epidemiological studies on the sleep habits of adolescents have reported that older teenagers sleep less than younger teenagers, the timing of sleep is delayed in older versus younger teenagers, and there is an increasingly large discrepancy between school night and weekend sleep schedules with age in teenagers.[1] Biologically, it is believed that the human sleep-wake pattern is determined by an interplay between sleep debt and circadian sleep propensity.[2] It has been reported that the required sleep duration of adolescents for optimal daytime alertness is between 8.25 and 9.2 h,[3] and that the intrinsic circadian timing system shows a phase delay that is associated with puberty.[4–6] Socially, the sleep duration of adolescents seems to be shortened by various social constraints, interactions with peers, academic obligations, extracurricular activities, employment, and school start time.[1] Consequently, shortened sleep in adolescents leads to insufficient sleep,[7,8] daytime sleepiness,[7,8] and habitual naps.[9–12]

Correspondence: Dr H Tagaya, Department of Psychophysiology, National Institute of Mental Health, NCNP, 1-7-3, Kohnodai, Ichikawa J272-0827, Japan. Email: tagaya@ncnp-k.go.jp

Accepted for publication 5 December 2003.

Some cross-sectional surveys on sleep habits have demonstrated that in Japan, changes in the sleep pattern during adolescence are consistent with the results of surveys conducted in other countries.[13–16] However, three of them[13,14,16] have not investigated the relationship between sleep habits and lifestyles, and the rest[15] have not selected their sample properly.

To determine the sleep patterns of Japanese high-school students in detail, a community survey was carried out. The sleep habits and factors associated with short sleep duration (i.e. less than 6 h per night) were studied among a cohort of Japanese high-school students.

METHODS

Participants and procedure

This epidemiological study was carried out in the cities of Chiba and Yotsukaido, which lie in typical suburban areas of Japan, and are located approximately 40 km east of the center of Tokyo. The area has a population of approximately 950 000. We explained the aims of the study to the educational committee of the appropriate local governments and obtained their permission to carry out the survey.

The survey was performed between 26 June and 1 July 2000. One class in each grade from all of the 34 full-time high schools in these cities was randomly sampled (Fig. 1). Well-trained school nurses administered the anonymous questionnaire to the students during a health education class. Students were informed about the aim of the study, and were then asked whether they were willing to participate. All of the students who attended the class gave their consent. Before rating the questionnaire, the students were told to read the instructions carefully, and were given the opportunity to ask questions about the study and the questionnaire. The school nurses monitored the session and answered questions as needed to ensure that all questionnaires would be completed correctly. Approximately 30 min were required to complete the questionnaire.

Measures

A self-administered anonymous questionnaire, which is described below, was developed to investigate sleep habits, sleep problems, physical and mental status, and lifestyle. The questionnaire consisted of three sections: the modified Japanese version of Pittsburgh Sleep Questionnaire Index (PSQI-J),[17,18] the Japanese version of the 12-item General Health Questionnaire (GHQ12-J),[19,20] and a questionnaire on sociodemographic characteristics, lifestyle, domestic situations, and perceived physical health. The PSQI-J assesses perceived sleep duration, perceived sleep latency, and the frequency and severity of specific sleep-related problems during the previous month.[17,18] To separate the sleep habits during weekdays and those on weekends, we added questions for sleep habits on weekends. The global PSQI-J score was calculated according to a standard method.[17]

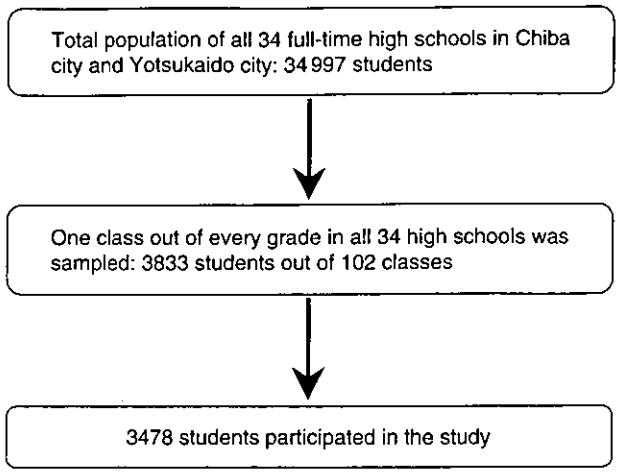


Figure 1 Sampling procedures. The 34 high schools in Chiba and Yotsukaido had a total student population of 34 997. Of the 3833 students sampled from that population (102 classes), 3478 (90.7%) participated in the survey and returned the questionnaires.

The GHQ12-J is a widely used, self-administered questionnaire that was designed as a screening tool for mental illness. It assesses 12 symptoms of psychiatric disorders during the previous month. The responses are scored in a binominal fashion (symptom present: 'not at all' = 0, 'same as usual' = 0, 'more than usual' = 1, and 'much more than usual' = 1). The presence of psychiatric symptoms is indicated when the sum of the 12 responses exceeds six (i.e. when the GHQ12-J score exceeds 0.5).[20]

The personal characteristics comprised age, grade, gender, type of school attended (public/private and co-education/single-gender school), type of school course (general/vocational), and planning future course after graduation (college/junior college/vocational school/employment/'not decided'). The following items were embedded in the questionnaire on lifestyle; participation in extracurricular activities (yes/no), type of extracurricular activities (sports/cultural), duration of

extracurricular activities (hours per day), duration of study outside school hours (hours per day), duration of watching television and/or playing PC games (hours per day), duration of commuting between home and school (hours per day), duration of part-time work (hours per day), family size, existence of a night worker in the family (yes/no), perceived bedroom situation (good/fair/poor), and number of people sharing the subject's bedroom. Perceived physical health was assessed by using a Likert scale (good/fair/bad).

Statistical analysis

In order to assess the effects of grade and gender on: (i) bedtime; (ii) rise time; (iii) perceived sleep duration; (iv) duration of extracurricular activity; (v) duration of study outside school hours; (vi) duration of watching television and/or playing PC games; (vii) duration of commuting between home and school; and (viii) duration of part-time work, we performed analyses of variance (ANOVAs), with gender and grade as independent variables.

The factors associated with short sleep duration were examined with the aid of a series of logistic regression analyses. Independent variables were converted into binominal factors: bedroom situation and perceived physical health ('good' or 'fair'=0 and 'bad'=1); parametric values were dichotomized at 25 or 75 percentile values. All variables were initially examined in univariate models. To control confounding factors and to determine the main correlates, we then performed multivariate logistic regression analyses for all variables that showed a significant correlation in univariate models. Statistical tests of the regression estimates or odds ratios (ORs) were based on Wald statistics. Odds ratios and their 95% confidence intervals (CIs) are presented to show the association.

All of the analyses were performed by using StatView, Version 5.0.1 for Macintosh (SAS Institute, Cary, NC, USA). The level of statistical significance was set at $P<0.05$.

RESULTS

Sample characteristics

The 34 high schools in Chiba and Yotsukaibo had a student population of 34 997. Of the 3833 students sampled from this population, 3478 (90.7%) participated in the survey and returned valid questionnaires (Fig. 1). The sociodemographic characteristics of the sample are shown in Table 1. The sample consisted of 1639 male students (47.1%) and 1839 female students (52.8%) whose mean (SD) age was 16.5 (1.0) years (range 15–18 years). The number of students who attended a private school was 795 (22.5%), 283 (8.1%) attended a girls' school,

Table 1 Sociodemographic characteristics of the study sample

	n	%
Grade		
First	1219	35.0
Second	1185	34.1
Third	1074	30.9
Sex		
Male	1639	47.1
Female	1839	52.9
Public or private school		
Public school	2693	77.4
Private school	785	22.6
Course		
General course	3075	88.4
Vocational course	403	11.6
Type of school		
Co-education	3195	91.9
Girls' school	283	8.1
Extracurricular activity		
Yes	1816	52.2
Athletic club	1162	33.4
Cultural club	552	15.9
Missing data	102	4.1
No	1637	47.1
Missing data	25	0.7
Future course		
College/junior college	2142	61.6
Other	1319	37.9
Missing data	17	0.5
Part-time work		
Yes	383	11.0
No	3072	88.3
Missing data	23	0.7
Size of family		
<5	2912	83.7
≥5	542	15.6
Missing data	24	0.7
Night workers in family		
Yes	468	13.5
No	2983	85.8
Missing data	27	0.8
Bedroom sharing		
Not sharing	2784	80.0
Sharing	670	19.3
Missing data	24	0.7

and 403 (11.6%) majored in a vocational course. Eighteen hundred and sixteen students (52.2%) participated in an extracurricular activity; 1162 students (33.4%) participated in athletic activities and 552 (15.9%) participated in cultural activities. Among all of the participants, 2142 students (61.6%) planned to proceed to college or junior college, and 383 students (11.0%) were working part-time. The mean family size in this cohort was 4.6 (1.1), 468 students (13.5%) had family members who were night workers, and 670 students (19.3%) shared a bedroom with other family members.

Habitual sleep and daily life

Table 2 shows the sleep habits and lifestyle of the participants. The mean bedtime (SD) was 00:03 (01:06) hours, and it was later for the male students than for the female students ($F_{1,2}=10.4$). The mean bedtime was later as the grade went up ($F_{1,2}=109.5$). The mean rise time was 06:33 (00:47) hours, and it was later for the male students than for the female students ($F_{1,2}=285.6$). The mean rise time was also later as the grade went up ($F_{1,2}=30.5$). The mean perceived sleep duration was 380.0 (66.0) min, and it was shorter for the female students than for the male students ($F_{1}=59.4$). The mean perceived sleep duration reduced as the grade went up ($F_{1,2}=73.8$). The mean perceived sleep latency was 16.8 (19.4) min. In 1665 students (47.8%), the global PSQI score exceeded the cut-off value[15] and was larger for female students than for male students ($F_{1,2}=10.8$), and increased as the grade went up ($F_{1,2}=21.9$).

The students spent 68.9 (77.8) min studying outside school hours, 133.5 (94.0) min watching television and/or playing PC games, and 75.5 (47.2) min commuting every day. There was a significant main effect of gender on the duration of study outside school hours and commuting, and a significant main effect of grade on the duration of study outside school hours and watching TV and/or playing PC games.

Health problems

The GHQ12-J score was higher for the female than for the male students, and increased as the grade went up. Analysis of variance revealed a significant main effect of gender ($F_{1,2}=55.6$) and grade ($F_{1,2}=7.3$). Psychiatric problems, as defined by the GHQ12-J questionnaire, were recognized in 802 students (23.1%), and 728 students (20.9%) reported that their perceived physical health was bad.

Table 2 Sleep habits and lifestyle of the sample

		Male			Female			Significant effects		
	Overall	First	Second	Third	First	Second	Third	Grade	Gender	Interaction
Bedtime (hours)	00:03 ± 01:06	23:46 ± 01:04	00:07 ± 01:08	00:24 ± 01:09	23:50 ± 01:05	00:03 ± 01:05	00:11 ± 01:01	**		**
Rise time (hours)	06:33 ± 00:47	06:36 ± 00:48	06:43 ± 01:06	06:49 ± 00:42	06:20 ± 00:36	06:28 ± 00:38	06:25 ± 00:39	**	**	*
Sleep duration (min)	380.0 ± 66.0	401.5 ± 62.0	383.3 ± 68.3	372.6 ± 67.1	381.7 ± 64.9	375.9 ± 67.0	363.8 ± 60.5	**	**	*
Sleep latency (min)	16.8 ± 19.4	15.8 ± 14.5	17.4 ± 18.4	18.4 ± 19.6	17.9 ± 24.4	14.6 ± 14.4	16.9 ± 22.4			**
Global PSQI score	5.7 ± 2.5	5.1 ± 2.3	5.7 ± 2.5	5.9 ± 2.5	5.5 ± 2.4	5.9 ± 2.4	6.1 ± 2.5	**	**	
Sum of GHQ-12 score	4.2 ± 3.1	3.6 ± 2.9	3.9 ± 3.1	4.1 ± 3.2	4.3 ± 3.2	4.8 ± 3.0	4.7 ± 3.1	**	**	
Study outside school hours (min/day)	68.9 ± 77.8	51.2 ± 60.5	40.6 ± 50.0	95.7 ± 97.2	70.1 ± 78.2	50.9 ± 55.1	109.9 ± 92.2	**	**	
TV and/or PC games (min/day)	133.5 ± 94.0	129.3 ± 93.8	141.3 ± 106.3	132.5 ± 95.3	137.2 ± 86.2	140.8 ± 93.1	118.3 ± 88.1	**		*
Commuting (min/day)	75.5 ± 47.2	68.5 ± 45.2	73.9 ± 49.3	69.7 ± 46.0	81.9 ± 47.9	78.4 ± 48.7	78.8 ± 44.3		**	

Results are presented as mean ± SD. *$P<0.05$; **$P<0.01$.

Table 3 Prevalence of short sleepers (perceived sleep duration of less than 6 h)

	Total	Short sleeper		
	n	*n*	%	95% CI
Males				
First grade	573	85	14.8	(13.7–16.0)
Second grade	537	119	22.2	(20.8–23.5)
Third grade	529	155	29.3	(27.8–30.8)
Females				
First grade	646	159	24.6	(23.2–26.0)
Second grade	648	189	29.2	(27.7–30.7)
Third grade	545	184	33.8	(32.2–35.3)
Overall	3478	891	25.6	(24.2–27.1)

Short sleeper

Eight hundred and ninety-one students (25.6%) were categorized as short sleepers (i.e. their perceived sleep duration was less than 6 h; Table 3). The prevalence of short sleepers was 14.8–29.3% for the male students and 24.6–33.8% for the females.

Factors associated with short sleep duration

Univariate logistic regression analyses were performed for 37 independent variables, 15 of which exhibited significant correlations with short sleep duration (Table 4). To control for confounding factors and to determine the main correlates of short sleep duration, all significant variables in the univariate models were submitted to a multivariate model. Adjusted ORs and their 95% CIs in the final model are shown in Table 4.

Multivariate logistic regression analysis revealed that short sleep duration was significantly associated with being female (OR=1.7, 95%CI: 1.4–2.2) and having a later bedtime (OR=22.5, 95%CI: 16.2–31.2), an earlier rise time (OR=3.8, 95%CI: 2.8–5.1), a high global PSQI-J score (OR=12.7, 95%CI: 9.6–16.7), a long study duration outside school hours (OR=2.0, 95%CI: 1.5–2.8), and a long commuting duration (OR=1.6, 95%CI: 1.2–2.2).

DISCUSSION

In this community survey, it was found that the average sleep duration of Japanese high-school students was 380 min, that older students slept less than younger students, and that female students slept less than male students. By using a multivariate logistic regression analysis, it was found that a short sleep duration was associated with sleep habits and lifestyle as well as a high global PSQI-J score, but not with a high GHQ12-J score or physical problems.

The sleep habits of adolescents in Japan have not been investigated until recently. There are some studies in which short sleep duration in Japanese adolescents has been examined,[15,16] however, in those studies, subjects were not sampled properly, their questionnaires were not standardized, or they did not take into account confounding factors in their analyses. In the first instance, the community study presented here documented the sleep habits among Japanese high-school students by using a random sampling procedure, standardized questionnaires, and multivariate statistical analyses.

It has been well acknowledged that a reduction in sleep duration, a delay in the sleep phase, and an increase in the discrepancy between sleep habits on school nights and on weekend nights are observed during adolescence.[1] These changes were reported to be caused by intrinsic biological changes[3–6] and environmental social factors.[1] The circadian system is likely to be susceptible to a phase delay during puberty, and social factors such as academic obligation, extracurricular activities, employment, and entertainment are known to suspend bedtime in adolescents, while the socially enforced school start time determines their rise time.[1] We suspect that the sleep duration among adolescents, which is under the influence of the pubertal circadian system as well as social constrains, may be shorter than what is required biologically, leading to insufficient sleep, daytime sleepiness, and habitual naps in these individuals.[21]

The mean sleep duration of the high-school students in the present study was 380 min. Previous surveys conducted in other countries have demonstrated that sleep duration is shorter in East Asia[9,22,23] (380–440 min in late teens) compared with Europe,[7,24–27] North America,[28] South America,[4] and Africa[11] (460–490 min in late teens). Te cultural difference between East Asia and other areas and different instruments may at least partially explain this difference. Another explanation may be drawn from the effect of naps on night sleep. Recent studies have suggested that taking a nap in the evening is a common occurrence among Japanese adolescents.[10,14] It has been shown that in an experimental setting, having an evening nap decreases the sleep debt,[2] so the late bedtime experienced by Japanese high-school

Table 4 Significant correlations of short sleep duration, as assessed by logistic regression analysis

	Total	Short sleep duration		Crude		Adjusted	
	n	*n*	%	OR	95% CI	OR	95% CI
Grade							
First	1219	244	20.2				
Second	1185	308	26.0	1.4	1.2–1.7*		
Third	1074	339	31.6	1.8	1.5–2.2*		
Gender							
Male	1639	359	21.9				
Female	1839	532	28.9	1.5	1.2–1.7*	1.7	1.4–2.2*
School type							
Co-educated	3195	799	25.0				
Girls' school	283	92	32.5	1.4	1.1–1.9*		
Bedtime							
Before 01:00 hours	3037	535	17.6				
After 01:00 hours	428	350	81.8	21.0	16.1–27.3*	22.5	16.2–31.2*
Perceived sleep latency							
≤20 min	2892	737	25.5				
>20min	502	138	27.5	1.3	1.1–1.5*		
Rise time							
After 06:00 hours	3021	697	23.1				
Before 06.00 hours	424	182	42.9	2.5	2.0–3.1*	3.8	2.8–5.1*
Global PSQI score							
≤7	2544	371	14.6				
>7	764	479	62.7	9.8	8.2–11.8*	12.7	9.6–16.7*
Sum of GHQ12-J score							
≤6	2656	637	24.0				
>6	802	249	31.1	1.4	1.2–1.7*		
Extracurricular activity							
Yes	1816	409	22.5				
No	1637	477	29.1	1.4	1.2–1.7*		
Duration of extracurricular activity							
≥1 h	1638	355	21.7				
<1 h	1690	492	29.1	1.5	1.3–1.7*		
Duration of study outside school hours							
≤2 h	2868	667	23.3				
>2 h	467	190	40.7	2.3	1.9–2.8*	2.0	1.5–2.8*
Commuting duration							
≤2 h	3039	753	24.8				
>2 h	383	127	33.2	1.5	1.2–1.9*	1.6	1.2–2.2*
Part-time work							
No	3072	763	24.8				
Yes	383	119	31.1	1.4	1.1–1.7*		
Bedroom environment							
Good	2589	631	24.4				
Bad	847	249	29.4	1.3	1.1–1.5*		
Perceived physical status							
Not bad	2727	627	23.0				
Bad	728	257	35.3	1.8	1.5–2.2*		

*$P<0.01$.

students might be influenced by the habit of taking naps in the evening.

Short sleep duration was associated with female students in the present study. A similar gender difference in perceived sleep duration has been reported in other community surveys in the USA,[29] Taiwan,[9] and in four of the European countries,[25] but not in Korea,[23] China[22] or Italy.[27] Further study is required to clarify the differences in the effect of gender on sleep duration among these countries.

In the present study, multivariate logistic regression analyses have revealed that short sleep duration in Japanese high-school students is associated with late sleep onset and poor sleep quality rather than an early rise time. We have also demonstrated that short sleep duration is not associated with psychiatric or physical problems. Insufficient sleep was reported to result in excessive daytime sleepiness and deteriorated daytime performance.[30,31] However, it remains unclear whether short sleep duration causes poor academic performance in adolescents. Eliasson *et al.* reported that academic performance in adolescents was not correlated with perceived sleep duration.[32]

Among environmental factors, the long commuting duration showed strongest association with short sleep duration. It has been reported that school start time is the strongest factor determining rise time in adolescents.[1] As this survey has been conducted in a homogenous community, the difference of school start times was minimal (less than 15 min) between schools. Our result indicates that commuting duration influences the rise time and sleep duration of adolescents, under equivalent school start times.

In conclusion, the sleep duration of Japanese high-school students was shorter compared with students from other countries in North and South America, Europe and Africa, but was comparable to that in East Asian countries. Short sleep duration was associated with multiple factors, and a late bedtime was the most significant predictor. Psychiatric and physical problems were not associated with short sleep duration.

ACKNOWLEDGMENTS

The authors would like to thank Ms Michiyo Hayashi of Chiba Prefectural Makuhari General High School and Ms Izumi Takahashi of Chiba Prefectural Kotehashi High School for conducting the survey. This study was supported, in part, by Health Science Grants from the Ministry of Health, Welfare and Labor, a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, and a Research Grant for Nervous and Mental Disorders.

REFERENCES

1 Carskadon MA. Factors influencing sleep patterns of adolescents. In: Carskadon MA, ed. *Adolescent Sleep Patterns*. Cambridge: Cambridge University Press, 2002; 4–26.
2 Borbely AA. A two process model of sleep regulation. *Hum. Neurobiol.* 1982; **1**: 195–204.
3 Carskadon MA, Harvey K, Duke P, Anders TF, Litt IF, Dement WC. Pubertal changes in daytime sleepiness. *Sleep* 1980; **2**: 453–60.
4 Andrade MM, Benedito-Silva AA, Menna-Barreto L. Correlations between morningness-eveningness character, sleep habits and temperature rhythm in adolescents. *Braz. J. Med. Biol. Res.* 1992; **25**: 835–9.
5 Carskadon MA, Vieira C, Acebo C. Association between puberty and delayed phase preference. *Sleep* 1993; **16**: 258–62.
6 Laberge L, Petit D, Simard C, Vitaro F, Tremblay RE, Montplaisir J. Development of sleep patterns in early adolescence. *J. Sleep Res.* 2001; **10**: 59–67.
7 Rugg-Gunn AJ, Hackett AF, Appleton DR, Eastoe JE. Bedtimes of 11–14-year-old children in north-east England. *J. Biosoc. Sci.* 1984; **16**: 291–7.
8 Strauch I, Meier B. Sleep need in adolescents: a longitudinal approach. *Sleep* 1988; **11**: 378–86.
9 Gau SF, Soong WT. Sleep problems of junior high school students in Taipei. *Sleep* 1995; **18**: 667–73.
10 Fukuda K, Ishihara K. Routine evening naps and nighttime sleep patterns in junior high and high school students. *Psychiatry Clin. Neurosci.* 2002; **56**: 229–30.
11 Reid A, Maldonado CC, Baker FC. Sleep behavior of South African adolescents. *Sleep* 2002; **25**: 423–7.
12 Thorleifsdottir B, Bjornsson JK, Benediktsdottir B, Gislason T, Kristbjarnarson H. Sleep and sleep habits from childhood to young adulthood over a 10-year period. *J. Psychosom. Res.* 2002; **53**: 529–37.
13 NHK Housou-Bunka-Kenkyujo, NHK Kokumin-Seikatsujikan-Chousa 2000. The national time use survey 2000. Tokyo: Nihon Housou Shuppan Kyokai, 2001 (In Japanese).
14 Park YM, Matsumoto K, Seo YJ, Kang MJ, Nagashima H. Changes of sleep or waking habits by age and sex in Japanese. *Percept. Mot. Skills* 2002; **94**: 1199–213.
15 Arakawa M, Taira K, Tanaka H *et al.* A survey of junior high school students' sleep habit and lifestyle in Okinawa. *Psychiatry Clin. Neurosci.* 2001; **55**: 211–12.
16 Takemura T, Funaki K, Kanbayashi T *et al.* Sleep habits of students attending elementary schools, and junior and senior high schools in Akita prefecture. *Psychiatry Clin. Neurosci.* 2002; **56**: 241–2.

17 Buysse DJ, Reynolds 3rd CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. *Psychiatry Res.* 1989; **28**: 193–213.
18 Doi Y, Minowa M, Uchiyama M *et al.* Psychometric assessment of subjective sleep quality using the Japanese version of the Pittsburgh Sleep Quality Index (PSQI-J) in psychiatric disordered and control subjects. *Psychiatry Res.* 2000; **97**: 165–72.
19 Goldberg DP, Rickels K, Downing R, Hesbacher P. A comparison of two psychiatric screening tests. *Br. J. Psychiatry* 1976; **129**: 61–7.
20 Doi Y, Minowa M. Factor structure of the 12-item General Health Questionnaire in the Japanese general adult population. *Psychiatry Clin. Neurosci.* 2003; **57**: 379–83.
21 Carskadon MA, Wolfson AR, Acebo C, Tzischinsky O, Seifer R. Adolescent sleep patterns, circadian timing, and sleepiness at a transition to early school days. *Sleep* 1998; **21**: 871–81.
22 Liu X, Uchiyama M, Okawa M, Kurita H. Prevalence and correlates of self-reported sleep problems among Chinese adolescents. *Sleep* 2000; **23**: 27–34.
23 Shin C, Kim J, Lee S, Ahn Y, Joo S. Sleep habits, excessive daytime sleepiness and school performance in high school students. *Psychiatry Clin. Neurosci.* 2003; **57**: 451–3.
24 Ferreira AM, Clemente V, Gozal D *et al.* Snoring in Portuguese primary school children. *Pediatrics* 2000; **106**: E64.
25 Ohayon MM, Roberts RE, Zulley J, Smirne S, Priest RG. Prevalence and patterns of problematic sleep among older adolescents. *J. Am. Acad. Child. Adolesc. Psychiatry* 2000; **39**: 1549–56.
26 Neveus T, Cnattingius S, Olsson U, Hetta J. Sleep habits and sleep problems among a community sample of schoolchildren. *Acta Paediatr.* 2001; **90**: 1450–5.
27 Giannotti F, Cortesi F, Sebastiani T, Ottaviano S. Circadian preference, sleep and daytime behaviour in adolescence. *J. Sleep Res.* 2002; **11**: 191–9.
28 Pollak CP, Bright D. Caffeine consumption and weekly sleep patterns in US seventh-, eighth-, and ninth-graders. *Pediatrics* 2003; **111**: 42–6.
29 Yarcheski A, Mahon NE. A study of sleep during adolescence. *J. Pediatr. Nurs.* 1994; **9**: 357–67.
30 Wolfson AR, Carskadon MA. Sleep schedules and daytime functioning in adolescents. *Child Dev.* 1998; **69**: 875–87.
31 Drake C, Nickel C, Burduvali E, Roth T, Jefferson C, Pietro B. The pediatric daytime sleepiness scale (PDSS): Sleep habits and school outcomes in middle-school children. *Sleep* 2003; **26**: 455–8.
32 Eliasson A, King J, Gould B. Association of sleep and academic performance. *Sleep Breath* 2002; **6**: 45–8.

Clock Genes in Cell Clocks: Roles, Actions, and Mysteries

Hitoshi Okamura[1]
Department of Brain Sciences,
Kobe University Graduate School of Medicine, Kobe, Japan

Abstract Cellular events must be organized in the time dimension as well as in the space dimension for many proteins to perform their cellular functions effectively. The intracellular molecular oscillating loops that compose the cell's circadian clock coordinate the timing of the expression of a variety of genes with basic or specific cellular functions. In mammals, the temporal pattern of clock gene expression generated in each SCN neuron is coupled to those of other cells and, amplified, spreads its signals through the brain and then, via feeding behavior, glucocorticoids, and sympathetic nerves, to peripheral organs. These peripheral organs have their own circadian clocks. In some tissues, such as liver, there is also a clock-regulating cell cycle, which interacts strongly with the components and temporal organization of the circadian clock. Some tissues, however, such as testis, express clock genes whose function, if any, remains unclear. Furthermore, circadian clock function may be suspended in differentiating tissue. Thus, the prominence of circadian organization may not apply equally to all tissues under all conditions.

Key words cell clock, circadian rhythms, integration mechanisms, cell division, differentiation

Virtually all organisms display biological rhythms that are controlled by the circadian clock. In mammals, in the early 1970s, brain-lesion, metabolic, and electrophysiological studies indicated that the central circadian oscillator is located in the hypothalamic SCN of the brain (see Klein et al., 1991). From these efforts, it is now clear that the overt rhythms of sleep-wake cycles, hormone levels (e.g., melatonin and corticosterone), and peripheral organ function are closely linked to the SCN rhythms of neuronal firing. However, the day-night rhythms of physiological parameters in peripheral organs such as cell division (Scheving et al., 1974) and enzymatic activity (Reinberg and Halberg, 1971) remained descriptive and poorly understood. The recent discovery of clock genes and their rhythmic expression in many peripheral organs helps explain many previous results (Schibler and Sassone-Corsi, 2002). Moreover, it has been shown that both central and peripheral organs contain an essentially identical circadian molecular core oscilla-

1. To whom all correspondence should be addressed: Hitoshi Okamura, MD, PhD, Division of Molecular Brain Science, Department of Brain Sciences, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan; e-mail: okamurah@kobe-u.ac.jp.

JOURNAL OF BIOLOGICAL RHYTHMS, Vol. 19 No. 5, October 2004 388-399
DOI: 10.1177/0748730404269169

tor (composed of an autoregulatory transcription/posttranscription/translation–based feedback loop involving a set of clock genes; Balsalobre at al., 1998; Yagita et al., 2001). It is not entirely clear why so many highly differentiated tissues and cells in mammals express a common core clock oscillating system. Is the cell clock physiologically important in all cell types? Is there a general function for the core clock oscillator in the cell? Is the cell clock involved in pathophysiology and disease? Does cellular time inside one cell affect the cellular time in neighboring or nearby cells?

These and other questions remain to be fully answered or understood. Here, I will consider the timing system at the cellular and molecular level (called the cell clock) and their effects on some cellular functions (e.g., cell cycles and basal metabolism). I begin the story with a brief summary of the molecular clock in mammals; then describe how the cell clock regulates the cell cycle; then consider the role, if any, of the cell clock in cellular differentiation; and, finally, address the nature of interactions between clocks in different tissues.

THE CELLULAR CORE OSCILLATORY LOOP

We can consider the cellular circadian oscillation in mammals to start with the transcription of 2 genes: *mPer1* and *mPer2*. The observation that *mPer1* and *mPer2* double-knockout mice became immediately arrhythmic in constant darkness, whereas mice with *mPer2* alone knocked out remained rhythmic for days, suggests that *mPer1* and *mPer2* execute primarily redundant functions in the generation of circadian rhythms (Bae et al., 2001; Zheng et al., 2001). Expression of these genes (see Hardin, 2004 [this issue]) is stimulated by heterodimers formed by the bHLH-PAS proteins CLOCK and BMAL1 (Fig. 1). The mPERs are made in the cytoplasm, translocate into the nucleus, and form a negative complex composed of mCRY1, mCRY2, mPER1, mPER2, mPER3, and mTIM, in various combinations, that suppresses the transcription of the *mPer1* and *mPer2* genes by binding to the positive factors CLOCK and BMAL1. Phosphorylation of mPER1 and mPER2 by casein kinase Iε is crucial for determining the circadian period length. Numerous results point strongly to the importance of this and other posttranscriptional and posttranslational regulatory mechanisms in the cell clock (see Harms et al., 2004 [this issue]). Furthermore, there is growing evidence that clock proteins are regulated dynamically in both spatial (nuclear and cytoplasmic) and temporal (production and degradation) dimensions. The clock proteins mPER1 and mPER2 usually shuttle between the cytoplasm and the nucleus and are easily degraded by ubiquitination and the proteasome pathway (Yagita et al., 2002). Ubiquitination of mPER proteins is inhibited by the presence of mCRY proteins. Since mCRY protein can also be ubiquitinated when mPER proteins are absent (Yagita et al., 2002), the mPER/mCRY dimer is stabilized against degradation, suppresses *mPer1* and *mPer2* transcription, and shuts off mPER synthesis (Fig. 1). Since it is speculated that the transcription level of *mPer* genes is determined essentially by the concentration of mPER/mCRY dimer in the nucleus, restarting *mPer* transcription depends on the export of the mPER proteins out of the nucleus by the CRM1/Exportin1 nuclear export machinery. The consequent decrease of mPER destabilizes mCRY, and the decrease of mCRY then allows *mPer1* and *mPer2* gene transcription to restart.

CELL CLOCKS IN MAMMALS

mPer genes are strongly and rhythmically expressed in SCN cells (Shigeyoshi et al., 1997; Zylka et al., 1998; Takumi et al., 1998), with their expression peaks in phase with SCN electrical activity (Inouye and Kawamura, 1979). Curiously, however, the pioneering papers also reported that non-SCN cells—indeed, most mammalian tissues—also express clock genes. Later, it was reported that in mammals, in vivo, clock genes actually oscillate in various organs, including liver, lung, and blood vessels (Schibler and Sassone-Corsi, 2002). Surprisingly, Balsalobre et al. (1998) reported that even fibroblast cell lines, which do not show rhythms of clock gene expression under basal conditions, do show clear rhythmic expression of clock genes for several cycles after brief treatment with high concentrations of serum. Since TPA, forskolin, and endothelin can also induce rhythmic expression of clock genes, it appears that all tissues are capable of rhythmic expression of clock genes after stimulation (and synchronization) by the appropriate external stimuli (Yagita et al., 2001).

The existence of autonomously rhythmic clock genes in peripheral cells and tissues raises the fundamental question, Are there any differences in the components of the core oscillatory loop in central

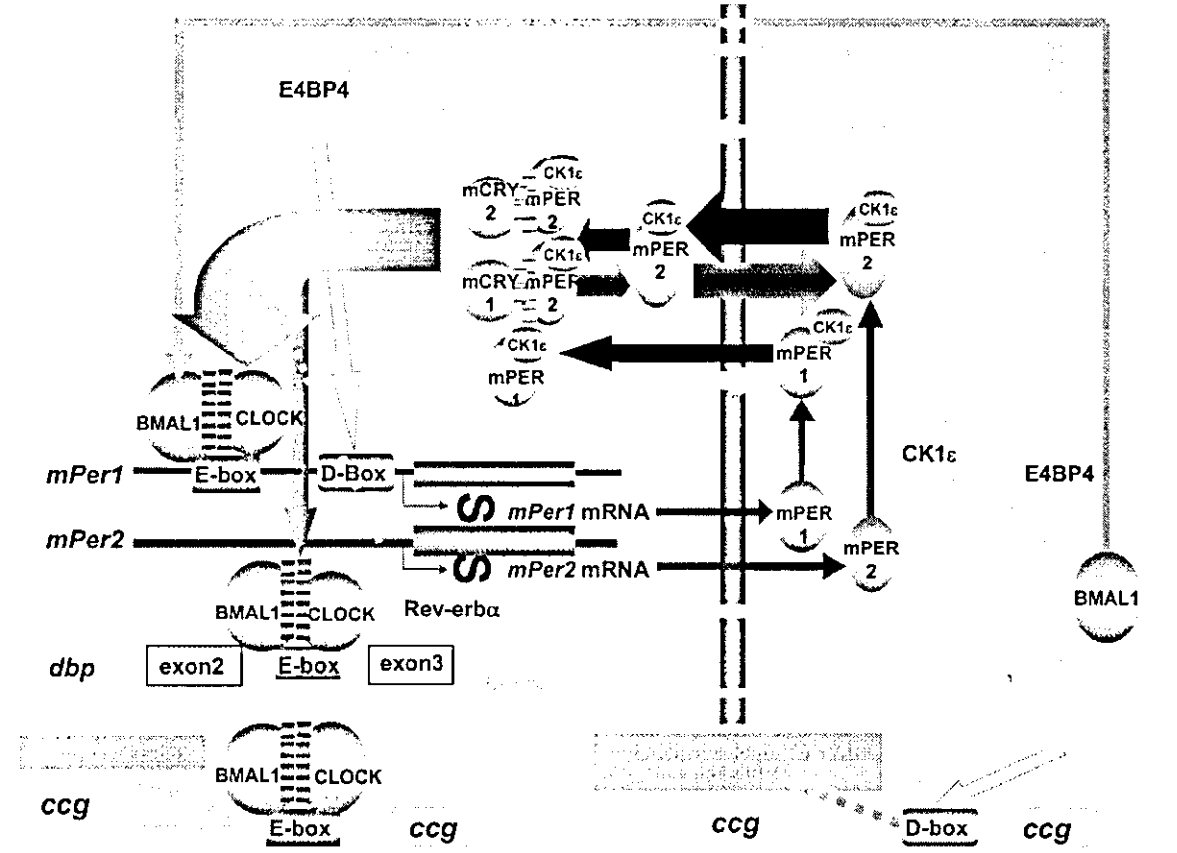


Figure 1. Molecular mechanisms of the mammalian circadian clock. BMAL1/CLOCK heterodimer binds to E-box of *mPer1* and *mPer2* genes to accelerate their transcription. mPER2 protein is produced in the cytoplasm, then phosphorylated by casein kinase 1ε (CK1ε). mPER2 protein shuttles between nucleus and cytoplasm via the CRM1/Exportin1 nuclear export system and is stabilized by the binding of mCRY1 or mCRY2. BMAL1/CLOCK also binds to E-box in clock-controlled genes (*ccgs*) and accelerates their transcription. The core negative autoregulatory feedback loop provided by *mPer1* and *mPer2* runs on, accompanying the move of an accessory PAR proteins loop. PAR proteins and E4BP4 competitively bind to D-box with opposite effects (Mitsui et al., 2001). E-box regulates Rev-erbα, which in turn regulates nighttime expression of *Bmal1* and *e4bp4*.

versus peripheral cells? Yagita et al. (2001) addressed this question by using spontaneously immortalized mouse embryonic fibroblasts from wild-type and *Cry1*$^{-/-}$*Cry2*$^{-/-}$ mice. Both wild-type and *Cry1*$^{-/-}$*Cry2*$^{-/-}$ cell lines showed clock properties similar to those found in the SCN of wild-type and *Cry1*$^{-/-}$*Cry2*$^{-/-}$ mice, respectively. These included (1) temporal expression profiles of all known clock genes, (2) the phases of the various mRNA rhythms (i.e., antiphase oscillation of *Bmal1* and *mPer* genes), (3) the delay between maximum mRNA levels and appearance of nuclear mPER1 and mPER2 protein, (4) the inability to produce oscillations in the absence of functional *mCry* genes, and (5) the control of period length by mCRY proteins. These, and similar results from other studies and tissues (e.g., using *mPer1*-promoter-luciferase transgenic or *mPer2*-luciferase knock-in animals; Yamazaki et al., 2000; Asai et al., 2001; Yoo et al., 2004), strongly support the conclusion that the components and oscillatory mechanism of central clocks (in the SCN) and of peripheral clocks are essentially identical.

CORE CLOCK REGULATES CLOCK-CONTROLLED GENES

The cell clock coordinates the timing of the expression of a variety of genes with specific cellular functions. Gene array studies have demonstrated that