別紙5

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Suzuki K, Ohida T, Kaneita Y, Yokoyama E, Uchiyama M	Daytime sleepiness, sleep habits and occupational accidents among hospital nurses	Journal of Advanced Nursing	52	445-453	2005
Doi Y, Minowa M, Uchiyama M, Okawa M	Sleep-medication for symptomatic insomnia in the general population of Japan	Sleep and Biological Rhythms	vol. 3	149-157	2005
Hayakawa T, Uchiyama M, Kamei Y, Shibui K, Tagaya H, Asada T, Okawa M, Urata J, Takahashi K	Clinical Analyses of Sighted Patients with Non-24-Hour Sleep-Wake Syndrome: A Study of 57 Consecutively Diagnosed Cases	Sleep	28	945-952	2005
Kuriyama K, Uchiyama M, Suzuki H, Tagaya H, Ozaki A, Aritake S, Shibui K, Xin T, Lan L, Kamei Y, Takahashi K	Diurnal fluctuation of time perception under 30-h sustained wakefulness	Neurosci Res	53	123-128	2005
Kaneita Y, Ohida T, Uchiyama M, Takemura S, Kawahara K, Yokoyama E, Miyake T, Harano S, Suzuki K, Yagi Y, Kaneko A, Tsutsui T, Akashiba T	Excessive daytime sleepiness among Japanese General population	J Epidemiol	15	1-8	2005
Hiroki M, Uema T, Kajimura N, Ogawa K, Nishikawa M, Kato M, Watanabe T, Nakajima T, Takano H, Imabayashi E, Ohnishi T, Takayama Y, Matsda H, Uchiyama M, Okawa M, Takahashi K, Fukuyama H	Cerebral White Matter Blood Flow Is Constant During Human Non-Rapid Eye Movement Sleep : A Positron Emission Tomographic Study	J Appl Physiol	98	1846-1854	2005
Kaneita Y, Ohida T, Uchiyama M, Takemura S, Kawahara K, Yokoyama E, Miyake T, Harano S, Suzuki K, Fujita T	The Relationship Between Depression and Sleep Disturbances	J Clin Psychiatry	67	196-203	2005
Asai T, Kaneita Y, Uchiyama M, S Takemura, Asai, S Yokoyama E, Miyake T, Harano S, Suzuki, K Ibuka E, Kaneko A, Tsutsui, T Ohida T	Epidemiological study of the relationship between sleep disturbances and somatic and psychological complaints among the Japanese general population	Sleep and Biological Rhythms	4	55-62	2005
Saifur Rohman M, Emoto N, Nonaka H, Okura R, Nishimura M, Yagita K, van der Horst GT, Matsuo M, Okamura H, Yokoyama M	Circadian clock genes directly regulate expression of the Na^+/H^+ exchanger NHE3 in the kidney	*Kidney Int*	67	1410-1419	2005
Yamamoto Y, Yagita Y, Okamura H	Role of cyclic *mPer2* expression in mammalian cellular clock	*Mol. Cell Biol.*	25	1912-1921	2005
Iijima M, Yamaguchi S, van der Horst GT, Bonnefont X, Okamura H, Shibata S	Altered food-anticipatory activity rhythm in Cryptochrome- deficient mice	*Neurosci Res*	52	166-173	2005
Dong X, Yagita Y, Zhang J, Okamura H	Expression of ubiquitin-related enzymes in the suprachiasmatic nucleus with special reference to ubiquitin carboxy-terminal hydrolase UchL1	*Biomed. Res*	26	43-49	2005

Tamanini F, Yagita K, Okamura H, van der Horst GT	Nucleocytoplasmic shuttling of clock proteins	*Methods Enzymol*	393	418-435	2005
Masubuchi S, Kataoka N, Sassone-Corsi P, Okamura H	Mouse Period1 (mPER1) acts as a circadian adapter to entrain the oscillator to environmental light/dark cycles by regulating mPER2 protein	*J. Neurosci.*	25	4719-4724	2005
Kitahama K., Araneda S, Geffard M, Sei H, Okamura H	Tyramine-immunoreactive neuronal structures in the rat brain: abundance in the median eminence of the mediobasal hypothalamus	*Neurosci. Lett.*	383	215-219	2005
Masuki S., Todo T, Nakano Y, Okamura H, Nose H	Reduced alpha-adrenoceptor responsiveness and enhanced baroreflex sensitivity in Cry-deficient mice lacking biological clock	*J. Physiology (London)*	566	213-224	2005
Ishida A, Mutoh T, Ueyama T, Bando H, Masubuchi S, Nakahara D, Tsujimoto G, Okamura H	Light activates the adrenal gland: Timing of gene expression and glucocorticoid release	*Cell Metabolism*	2	297-307	2005
Masamizu Y, Ohtsuka T, Takashima Y, Nagahara H, Takenaka Y, Yoshikawa K, Okamura H, Kageyama R	Real-time imaging of the somite segmentation clock: reveration of unstable oscillators in the individual presomitic mesoderm cells	*Proc. Natl. Acad. Sci. USA.*	103	1313-1318	2006
Hashimoto N, Kido Y, Uchida T, Asahara S, Shigeyama Y, Matsuda T, Takeda A, Tsuchihashi D, Nishizawa A, Ogawa W, Fujimoto Y, Okamura H, Karen C. Arden KC, Herrera P, Tetsuo Noda T, and Kasuga M	Ablation of PDK1 in pancreatic β cells induces diabetes as a result of loss of β-cell mass	*Nature Genetics,*	in press		2006
Nishiyama T, Ikeda M, Iwata N, Suzuki T, Kitajima T, Yamanouchi Y, Sekine Y, Iyo M, Harano M, Komiyama T, Yamada M, Sora I, Ujike H, Inada T, Furukawa T, Ozaki N	Haplotype association between GABAA receptor gamma2 subunit gene (GABRG2) and methamphetamine use disorder	Pharmacogenomics J	5	89-95	2005
Saito S, Ikeda M, Iwata N, Suzuki T, Kitajima T, Yamanouchi Y, Kinoshita Y, Takahashi N, Inada T, Ozaki N	No association was found between a functional SNP in ZDHHC8 and schizophrenia in a Japanese case-control population	Neurosci Lett	374	21-24	2005
Hakamata Y, Takahashi N, Ishihara R, Saito S, Ozaki N, Honjo S, Ono Y, Inada T:.	No association between monoamine oxidase A promoter polymorphism and personality traits in Japanese females	Neurosci Lett,	389	121-3	2005
Iidaka T, Ozaki N, Matsumoto A, Nogawa J, Kinoshita Y, Suzuki T, Iwata N, Yamamoto Y, Okada T, Sadato N	A variant C178T in the regulatory region of the serotonin receptor gene HTR3A modulates neural activation in the human amygdala	J Neurosci	25	6460-6	2005
Mishima K, Tozawa T, Satoh K, Saitoh H, Mishima Y.	The 3111T/C polymorphism of hClock is associated with evening preference and delayed sleep timing in a Japanese population sample	Am J Med Genet B Neuropsychiatr Genet	133	101-4	2005

Mishima Y, Hozumi S, Shimizu T, Hishikawa Y, Mishima K	Passive body heating ameliorates sleep disturbances in patients with vascular dementia without circadian phase-shifting	Am J Geriatr Psychiatry	13	369-76	2005
Mizuno K, Inoue Y, Tanaka H, Komada Y, Saito H, Mishima K, Shirakawa S	Heart rate variability under acute simulated microgravity during daytime waking state and nocturnal sleep: Comparison of horizontal and 6 degrees head-down bed rest	Neurosci Lett	383	115-20	
Nakamura W, Yamazaki S, Takasu NN, Mishima K, Block GD	Differential response of Period1 expression within the suprachiasmatic nucleus	J Neurosci	25	5481-7	2005

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
Okamura H	Biological clock in the liver	J.-F. Dufour and P.-A. Clavien	Signaling Pathways in Liver Dieseases	Springer-Verlag	Berlin	2005	391-403
Okamura H, Ueyama T, Masubuchi S, Kitahama K	Morphology of DOPAergic neurons in mammals	Y. Misu and Y. Goshima	Neurobiology of DOPA as a Neurotransmitter	CRC Press in Taylor & Francis	Boca Raton	2006	47-62
内山真	高齢者の睡眠障害	内山真	ローテーターのための睡眠医学－特に各種身体疾患に伴う睡眠障害について－	アステラス製薬㈱	大阪	2005	1-8
内山真	睡眠障害の疫学的調査	内山真	ローテーターのための睡眠医学－特に各種身体疾患に伴う睡眠障害について－	アステラス製薬㈱	大阪	2005	190-194
内山真	睡眠障害の分類(2)：年齢別特徴	内山真	ローテーターのための睡眠医学－特に各種身体疾患に伴う睡眠障害について－	アステラス製薬㈱	大阪	2005	204-208
金圭子，内山真	ストレスと睡眠		ストレスの事典	朝倉書店	東京	2005	55-59
三島和夫	不眠症：内科疾患および精神疾患に伴う不眠	上島国利，市橋秀夫，保坂隆，朝田隆	精神科臨床ニューアプローチ8：睡眠障害・物質関連障害	メディカルビュー社	東京	2006	84-95
田ヶ谷浩邦	睡眠障害の分類(1)：睡眠障害の主要分類法	内山真，土井永史，清水徹男	ローテーターのための睡眠医学-特に各種身体疾患に伴う睡眠障害について-	アステラス製薬株式会社	大阪	2005	195-203
田ヶ谷浩邦，内山真	不眠症	野村総一郎，高橋祥友，川上健一	健康管理室で役立つこころの医学	南江堂	東京	2005	45-50
田ヶ谷浩邦，内山真	睡眠薬の半減期の違いは臨床に反映されるか？	上島国利，三村将，中込和幸，平島奈津子	EBM 精神疾患の治療 2006-2007	中外医薬社	東京	2006	290-299

IV.　研究成果の刊行物・別刷

Clinical Analyses of Sighted Patients with Non-24-Hour Sleep-Wake Syndrome: A Study of 57 Consecutively Diagnosed Cases

Tatsuro Hayakawa, MD[1]; Makoto Uchiyama, MD, PhD[2]; Yuichi Kamei, MD, PhD[1]; Kayo Shibui, MD, PhD[2]; Hirokuni Tagaya, MD, PhD[2]; Takashi Asada, MD, PhD[3]; Masako Okawa, MD, PhD[4]; Jujiro Urata, MD[1]; Kiyohisa Takahashi MD, PhD[5]

[1]Department of Psychiatry, Kohnodai Hospital, National Center of Neurology and Psychiatry, Kohnodai, Ichikawa, Japan; [2]Department of Psychophysiology, National Institute of Mental Health, National Center of Neurology and Psychiatry, Ogawa-Higashi, Kodaira, Japan; [3]Department of Psychiatry, Institute of Clinical Medicine, University of Tsukuba, Tennodai, Tsukuba, Japan; [4]Department of Psychiatry, Shiga University of Medical Science, Seta, Tsukinowacho, Otsu, Japan; [5]National Center of Neurology and Psychiatry. Ogawa-higashi, Kodaira, Japan

Study Objectives: The objective of this study was to clarify the clinical features of sighted patients with non–24-hour sleep-wake syndrome.

Design: Clinical analyses of consecutive patients suffering from non–24-hour sleep-wake syndrome.

Setting: The sleep disorders clinic at Kohnodai Hospital, National Center of Neurology and Psychiatry, Japan.

Patients: Fifty-seven patients who were diagnosed consecutively as having non–24-hour sleep-wake syndrome between 1991 and 2001 were included in the study.

Measurements and Results: The clinical features and sleep characteristics of the patients were analyzed. A semistructured psychiatric interview that included the criteria for Axis I or II disorders of Diagnostic and Statistical Manual of Mental Disorders, Third Edition-Revised was conducted, and relationships between psychiatric problems and non–24-hour sleep-wake syndrome were analyzed. The patient cohort included 41 (72%) men and 16 (28%) women. The onset of non–24-hour sleep-wake syndrome had occurred during the teenage years in 63% of the cohort, and the mean (± SD) period of the sleep–wake cycle was 24.9 ± 0.4 hours (range 24.4-26.5 hours). The mean sleep length of the patients was 9.3 ± 1.3 hours, and 44% of them had a sleep length of between 9 and 10 hours. Psychiatric disorders had preceded the onset of non–24-hour sleep-wake syndrome in 16 patients (28%); of the remaining 41 patients, 14 (34%) developed major depression after the onset of non–24-hour sleep-wake syndrome.

Conclusions: These results represent the first detailed clinical review of a relatively large number of sighted patients with non–24-hour sleep-wake syndrome.

Keywords: Non-24-hour sleep-wake syndrome, circadian rhythm, evening type, sleep disorder, delayed sleep-phase syndrome, psychiatric disorder, mood disorder, depression

Citation: Hayakawa T; Uchiyama M; Kamei Y et al. Clinical analyses of sighted patients with non–24-hour sleep-wake syndrome: a study of 57 consecutively diagnosed cases. *SLEEP* 2005;28(8):945-952.

INTRODUCTION

THE ENVIRONMENTAL LIGHT-DARK CYCLE IS THE STRONGEST SYNCHRONIZER OF THE CIRCADIAN RHYTHM IN HUMANS AND OTHER ANIMALS.[1] Individuals who live isolated from a normal 24-hour light-dark cycle exhibit a sleep-wake cycle that is longer than 24 hours.[1,2] This long cycle leads to progressively later spontaneous bedtimes and wake times.

Non-24-hour sleep-wake syndrome (non-24-hour sleep-wake syndrome) is a rare condition that is characterized by a chronic steady pattern of about 1-hour delays in spontaneous sleep-onset and wake times in individuals living under normal environmental conditions.[3] The *International Classification of Sleep Disorders* (ICSD)[3] provides the following criteria for diagnosing non-24-hour sleep-wake syndrome in a clinical setting: (1) primary complaint of either difficulty initiating sleep or difficulty in awakening, (2) progressive delays of sleep onset and offset with the inability to maintain stable entrainment to a 24-hour sleep-wake pattern, and (3) presence of the sleep pattern for at least 6 weeks.

Some previous clinical reports[4-36] have described individual or a few patients with non-24-hour sleep-wake syndrome. One study documented 322 patients having circadian rhythm sleep disorders, including 39 patients with non-24-hour sleep-wake syndrome, and reported some clinical characteristics of circadian rhythm sleep disorders as a whole.[37] However, so far there has been no published study analyzing a large number of patients suffering from non-24-hour sleep-wake syndrome. According to the ICSD,[3] the clinical characteristics of non-24-hour sleep-wake syndrome, such as age at onset, sex ratio, or familial pattern, have not been clarified. In addition, the etiologic factors that affect the development of non-24-hour sleep-wake syndrome remain to be elucidated. Although there is little question that blindness is a strong predisposing factor for non-24-hour sleep-wake syndrome,[3] the pathogenetic mechanisms underlying the development of the disorder in sighted humans have not been established.[3,38,39] Psychiatric disorders are thought to be associated with non-24-hour sleep-wake syndrome in sighted patients,[12,20,23,26,35] but no detailed clinical investigation has been carried out to confirm this causality. In the study presented here, we recruited patients with circadian rhythm sleep disorders to obtain a large cohort of sighted people with non-24-hour sleep-wake syndrome and studied their clinical features.

Disclosure Statement

This was not an industry supported study. Drs. Uchiyama, Hayakawa, Kamei, Shibui, Tagaya, Asada, Okawa, and Takahashi have indicated no financial conflicts of interest.

Submitted for publication July 2004
Accepted for publication April 2005

Address correspondence to: Makoto Uchiyama, MD, PhD, Department of Psychophysiology, National Institute of Mental Health, NCNP. Ogawa-Higashi, Kodaira, 187-8502 Japan. Tel: 81 42 346 2071, Fax: 81 42 346 2072

SUBJECTS AND METHODS

Recruitment of Patients

Subjects were recruited for the present study by advertising in the media for people who had sleep problems due to a delay or an advance of the major sleep phase, an irregular sleep phase, or an incapacity to synchronize to a 24-hour day, as well as by giving information on circadian rhythm sleep disorders to general physicians and psychiatrists in the neighboring cities. We then sent a 6-week sleep-log form to be completed by the responders, together with a questionnaire comprising questions on sleep habits and psychosocial status. Thereafter, those who returned the sleep log and questionnaire were referred to our sleep disorders clinic at Kohnodai Hospital, National Center of Neurology and Psychiatry. Among the participants who visited the sleep disorders clinic of the institute for treatment between 1991 and 2001, 392 were diagnosed according to the ICSD criteria as having circadian rhythm sleep disorders by sleep disorder specialists (TH, YK, MU, and MO). After an observation period (4-6 weeks) when no therapeutic interventions were conducted, treatments of circadian rhythm sleep disorders were initiated. The study protocol was approved by the Institutional Review Board of the National Center of Neurology and Psychiatry, and each subject gave his or her informed consent after the procedures and the possible risks of the experiment had been explained in detail.

Diagnostic Procedures

Diagnoses were made by applying the ICSD criteria for non-24-hour sleep-wake syndrome. On the first visit, we asked the patients to keep a detailed sleep log for at least 4 to 6 weeks, together with actigraphic assessment for a consecutive 2-week period (Mini-Motionlogger, Ambulatory Monitoring Inc., Ardsley, NY). Of the 392 patients who were diagnosed as having circadian rhythm sleep disorders, 41 men and 16 women (26.2 ± 8.5 years, mean ± SD) were diagnosed as having non-24-hour sleep-wake syndrome. The onset of non-24-hour sleep-wake syndrome was defined as the time when free running of the sleep-wake cycle began. An ophthalmologic specialist (KS) found no ophthalmologic abnormalities, except for myopia. Three psychiatrists (TH, YK, and MU) conducted a semistructured psychiatric interview,[38] which included the criteria for Axis I or II disorders of the *Diagnostic and Statistical Manual of Mental Disorders, Third Edition-Revised*[40] and an assessment of social functioning (descriptive form). Histories of shift work, psychiatric problems, physical problems, and preceding sleep disorders, as well as family history of circadian rhythm sleep disorders and psychiatric and neurologic disorders, were also investigated and reviewed by the psychiatrists. The clinical backgrounds of the patients are shown in Table 1.

All of the patients underwent examinations performed by the general physicians at the hospital, together with blood counts, urine examinations, serum biochemistry, electrocardiography, routine electroencephalography, and brain computed tomography or magnetic resonance imaging.

Evaluation of Sleep Log

Inspecting the sleep logs during the 4 to 6 weeks of the observation period from the first visit, 2 raters independently determined sleep onset and offset times in 30-minute bins. All the patients visited us when they were able to sleep during the nighttime and stay awake during the daytime. A delay of sleep onset longer than 4 hours per day was defined as a delayed phase jump. The patient was considered as a delayed-phase jumper when delayed-phase jumps were recognized at least 4 times per month during the observation period. Regression lines were fitted through the sleep-onset times for the 4 weeks from the first visit. The periods of the sleep-wake cycles were computed by adding the slope of the regression line of sleep-onset times to 24 hours.[38] A rhythm was considered not to be entrained to a 24-hour day when the 95% confidence intervals of the period did not cross 24 hours.

Table 1— Characteristics of 57 Consecutive Patients Diagnosed With Non-24 Hour Sleep-Wake Syndrome*

Characteristic	No. (%)
Sex	
Men	41 (72)
Women	16 (28)
Age at onset, y	
mean ± SD	20.2 ± 7.0
< 10	0 (0)
10-19	36 (63)
20-29	13 (23)
30-39	6 (11)
40-49	2 (3)
Marital status	
Married	6 (11)
Unmarried	51 (89)
Presence of family or roommate	
Yes	45 (79)
No	12 (21)
Social status at first visit	
Student	20 (35)
Employed	12 (21)
Part-time worker	3 (5)
Unemployed	22 (39)
Premorbid status	
Psychiatric problems	16 (28)
Physical problems	1 (2)
Delayed sleep-phase syndrome	15 (26)
Family history of mental, sleep, or neurologic disorder	
Yes	5 (9)
No	52 (91)

*Data are presented as number (%) unless otherwise indicated.

Evaluation of Actigraphic Data

Excluding missing data, we obtained at least 10 consecutive days of actigraphic data during the 2-week actigraphic assessment that started from the first visit (the first 2 weeks of the observation period). Based on automatically generated data (Action3 software, Ambulatory Monitoring, Inc.), sleep onset and offset times and sleep length were calculated in 5-minute bins. To calculate the period of the sleep-wake cycle based on the 10 days of actigraphic assessment, regression lines were fitted through the sleep-onset times in a manner similar to that described above (Figure 1).

Statistical Analyses

Statistical analyses were performed with Statview 5 for a Ma-

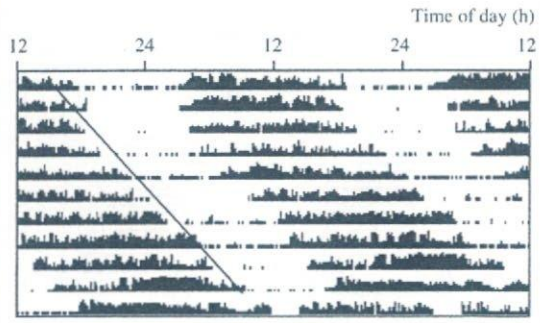

Figure 1—Wrist-activity measurements obtained from a representative patient (30-year-old man). To determine the period of the sleep-wake cycle, regression lines were fitted through the sleep-onset time obtained during 10 consecutive days. Linear-regression functions in this subject were computed with respect to sleep onset (Y = 20.99 - 1.73 * X; R = 0.99; 95% confidence interval, 1.54-1.92).

cintosh computer. The χ^2 test was used to compare categorical data from 2-dimensional tables, and the Mann-Whitney U test was used to compare group differences. The Spearman rank order correlation test was used to investigate correlation between 2 items. All numeric data are presented as the mean ± SD. We accepted a P value less than 5% as statistically significant.

RESULTS

Clinical Characteristics

The patient cohort was male-dominated, comprising 41 (72%) men and 16 (28%) women (the ratio of men to women was 2.6:1). There was no significant difference between the mean age of the male and female patients (26.3 ± 9.2 years and 25.8 ± 6.9 years, respectively). The mean age at onset of non-24-hour sleep-wake syndrome was 20.2 ± 7.0 years, with sex having no effect (age at onset of non-24-hour sleep-wake syndrome: 20.8 ± 7.6 years and 18.8 ± 5.1 years for men and women, respectively). As seen in Table 1, all of the patients were over the age of 10 years at the onset of the non-24-hour sleep-wake syndrome. Indeed, most of the patients (63%) were in their teens at the time of onset. The mean interval between the onset of non-24-hour sleep-wake syndrome and the first visit to the sleep disorders clinic was 5.9 ± 6.2 years. With regard to premorbid status, 16 (28%) of the patients had psychiatric problems, 1 (2%) had physical problems, and 15 (26%) had suffered from persistent sleep phase delay diagnosed as delayed sleep phase syndrome prior to the onset of non-24-hour sleep-wake syndrome. Fifty-six patients (98%) had a history of severely disrupted social functioning, such as temporary absence from school, leaving school, temporary retirement from office work, or retirement. Six patients (11%) had been married, and 45 (79%) had lived with their family or a roommate. Regarding social status, at the first visit to the sleep disorders clinic, 35% were students, 21% were employed, 5% worked part time, and 39% were unemployed. None of the patients had a history of head injuries or meningitis, developmental abnormality in childhood, or significant sleep problems before the age of 10 years. A family history of sleep, psychiatric, or neurologic disorders was noted in 5 patients (Table 1); these included major depression (n = 1, 1.8%), delayed sleep-phase syndrome (n = 2, 3.5%), and schizophrenia (n = 2, 3.5%). The physical examinations performed at referral, including blood counts, urine examinations, serum biochemistry, electrocardiography, routine electroencephalogram, and brain computed tomography or magnetic resonance imaging, did not reveal any marked somatic disorders or neurologic conditions in any of the subjects.

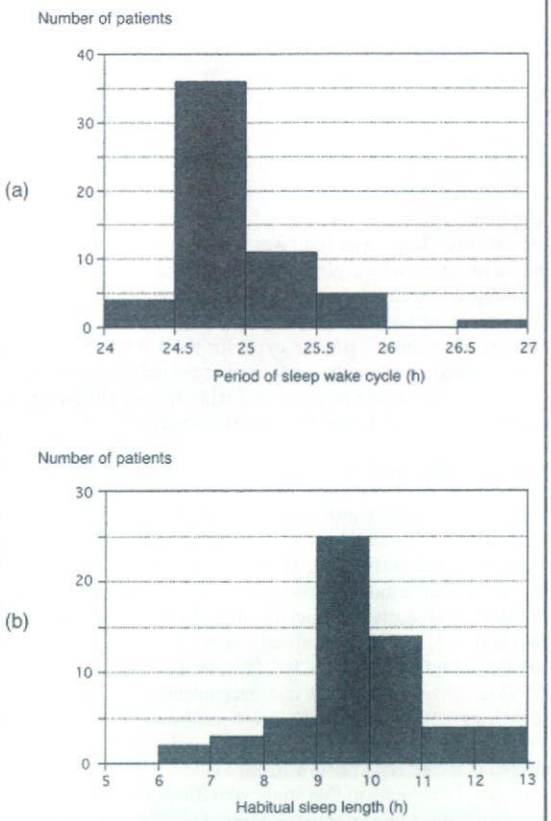

Figure 2—a. Distribution of periods of the sleep-wake cycles. The periods of the sleep-wake cycles for 57 patients are plotted with a 0.5-hour bin. The highest frequency was found at between 24.5 and 25 hours. Mean, median, and SD of the period were 24.9, 24.8, and 0.4 hours, respectively.
b. Distribution of periods of the sleep length. The sleep lengths for the patients are plotted with 1-hour bins. The highest frequency was observed between 9 hours and 10 hours. Mean, median, and SD of the sleep length were 9.3, 9.0, and 1.3 hours, respectively.

Sleep Characteristics

The period of the sleep-wake cycle obtained from 10-day actigraphic assessment was longer than 24 hours in all of the patients, a finding that was confirmed by the fact that in any of the patients the 95% confidence intervals for the period did not cross 24 hours. In the 10-day actigraphic assessment, a single delayed-phase jump was found in 8 of the delayed-phase jumpers, but there were none in any of the non–delayed-phase jumpers. The mean period of the sleep-wake cycle for the patients was 24.9 ± 0.4 hours (range 24.4-26.5 hours; Figure 2a). The mean sleep

length of the patients was 9.3 ± 1.3 hours, the most prevalent range being 9 to 10 hours (experienced by 44% of the patients; Figure 2b). There was no correlation between the period of the sleep-wake cycle and sleep length. Neither the period of the sleep-wake cycle nor sleep length was affected by sex (U-test). Differences in social status (employed, unemployed, and student) did not have significant effects on the period of the sleep-wake cycle nor on the sleep length (Kruskal Wallis nonparametric analysis of variance). Thirty-one patients (54%) were considered as delayed-phase jumpers. The delayed-phase jumps were found when the patients' sleep-onset time was delayed into the daytime. The period of sleep-wake cycle for 10-day actigraphic assessment did not differ between the delayed-phase jumpers (24.9 ± 0.5 hours) and the non–delayed-phase jumpers (24.9 ± 0.4 hours) (U-test). The period of the sleep-wake cycle for the 4-week sleep-log assessment was significantly prolonged in the delayed-phase jumpers (26.1 ± 0.8 hours) as compared with the non–delayed-phase jumpers (24.9 ± 0.5 hours) ($P < .0001$, U-test).

Psychiatric Problems

A semistructured psychiatric interview revealed that psychiatric disorders preceded the onset of non-24-hour sleep-wake syndrome in 16 patients (28%). These included adjustment disorders (n = 6, 10.5%), major depression (n = 3, 5.3%), schizophrenia (n = 2, 3.5%), obsessive-compulsive disorder (n = 2, 3.5%), social phobia (n = 1, 1.8%), generalized anxiety disorder (n = 1, 1.8%), and mental retardation (n = 1, 1.8%). In 15 of these 16 patients (93.7%), social withdrawal had accompanied the development of their psychiatric disorders. One typical case of a patient with a psychiatric disorder and associated social withdrawal is documented in a case report below (Case 1).

Of the 41 patients in this study who did not have a history of psychiatric disorders prior to the onset of non-24-hour sleep-wake syndrome, 14 (34%) developed major depression. No other psychiatric disorders developed after the onset of non-24-hour sleep-wake syndrome. The mean age of onset of the non-24-hour sleep-wake syndrome in the patients who developed major depression (23.6 ± 2.4 years) was significantly higher than that of the group who did not develop depression (19.2 ± 1.0 years; U test, $P < .05$).

In 5 of the patients who developed major depression after the onset of non-24-hour sleep-wake syndrome, their depressive complaints were aggravated when their sleep episodes occurred out of phase (ie, when they slept during the daytime) and slightly ameliorated when their sleep episode occurred in phase (ie, when they slept during the nighttime), as described for Case 2 below.

Case 1

This patient was a 26-year-old woman who had left school at the age of 16 years because of adjustment disorder. After leaving school, her sleep pattern was such that she fell asleep at 2:00 AM and awoke at 11:00 AM. At the age of 17 years, she enrolled at a night school but did not attend regularly because of difficulty coping with school life. At the age of 19 years, she became unable to fall asleep until the morning and unable to wake up until the late afternoon and stayed at home almost all day. After a couple of months, there began a gradual and daily delay in her sleep-onset time. At the age of 21 years, this patient consulted a clinic and was treated with benzodiazepine hypnotics, but these were ineffective.

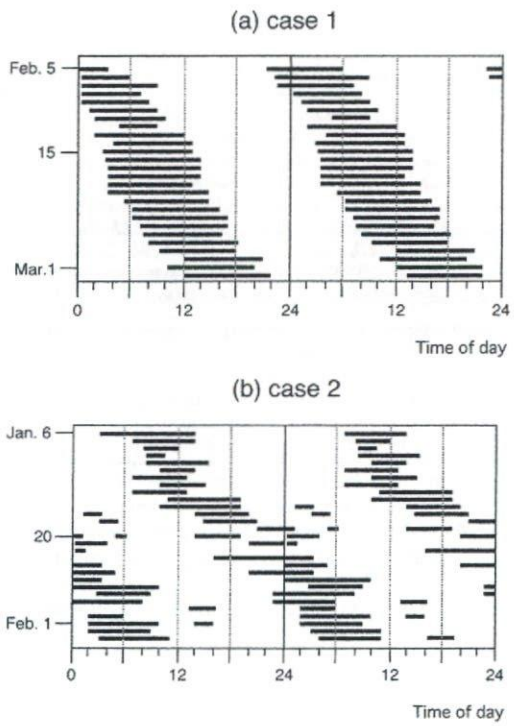

Figure 3—a. Self-reported sleep-wake records of a 26-year-old woman who was diagnosed as having non-24 sleep-wake syndrome before therapeutic intervention, double-plotted in raster format. Sleep episodes are shown as black bars.
b. Self-reported sleep-wake records of a 22-year-old male student who was diagnosed with non-24 sleep-wake syndrome before therapeutic intervention, double-plotted in raster format. Sleep episodes are shown as black bars.

On referral to our clinic, no abnormal findings were detected in routine electroencephalogram and magnetic resonance imaging investigations, blood count, biochemistry, or thyroid function test. A semistructured psychiatric interview revealed that she had adjustment disorder (according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*). Her sleep-wake record before the therapeutic intervention is documented in Figure 3a. Her sleep-wake rhythm exhibited a free-running sleep-wake cycle with a 24.8-hour period. She was diagnosed as having non-24-hour sleep-wake syndrome on the basis of the ICSD. There was no family history of circadian rhythm sleep disorders or psychiatric disorders, and her medical history was also unremarkable.

Case 2

This patient was a 22-year-old male student who, at the age of 15 years, began to have difficulty falling asleep until 3:00 AM and difficulty rising in the morning, resulting in frequent absences from morning classes at school. When he entered college at the age of 18 years, he noticed that his sleep onset was gradually delayed each day. After several months, he started complaining

of dysphoric mood and loss of interest. His depressive symptoms tended to be worse while his sleep phase was in the daytime. At the age of 19 years, he failed to attend college because of his sleep problems and depression and finally left college at the age of 21 years.

On referral to our clinic, no abnormal findings were detected in routine electroencephalogram and magnetic resonance imaging investigations, blood count, biochemistry, or thyroid function test. A semistructured psychiatric interview revealed that he had major depression (according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*). His sleep-wake rhythm before any therapeutic intervention is documented in Figure 3b; it exhibited a free-running sleep-wake cycle with a 24.7-hour period. He was diagnosed as having non-24-hour sleep-wake syndrome on the basis of the ICSD. There was no family history of circadian rhythm sleep disorders or psychiatric disorders, and his medical history was also unremarkable.

DISCUSSION

This is the first study of a large cohort of sighted patients suffering from non-24-hour sleep-wake syndrome. The prevalence of non-24-hour sleep-wake syndrome in the general population has not been established, but it is presumed to be rare.[3] Although the exact prevalence of non-24-hour sleep-wake syndrome in the blind has not been clarified, it does seem to occur relatively frequently in totally blind individuals.[4-10] Furthermore, Lockley et al[41] have reported that 57% of subjects without any light perception exhibited a free-running melatonin rhythm. Sack et al[42] have also reported that 50% of totally blind subjects had free-running plasma melatonin rhythms. These reports indicate that development of non-24-hour sleep-wake syndrome may be attributable to a free-running circadian pacemaker.

Non-24-hour sleep-wake syndrome has also been reported to occur in sighted individuals,[11-36] but the basic characteristics of such patients, such as sex, age, or age at onset, remain unknown.[3] In the study described here, of the 57 consecutively diagnosed patients with non-24-hour sleep-wake syndrome, 72% were men, making it a male-dominant cohort. This is comparable with previous studies: of the 39 sighted patients reported in previous studies[11-36] (Table 2), 33 (85%) were male.

The detailed clinical histories taken in the present study revealed that the commencement of a free-running sleep-wake cycle occurred mostly when patients were in their teens (63%) or 20s (23%). In none of the patients did onset of non-24-hour sleep-wake syndrome occur before the age of 10 years. In previous studies of sighted people with non-24-hour sleep-wake syndrome, the age of onset was also reported to be predominantly the teens (38%) and 20s (43%), as shown in Table 2. Indeed, of the 39 cases described in all of the previous studies, only 1 patient (2.6%), who also suffered from mental retardation, experienced onset of non-24-hour sleep-wake syndrome before the age of 10 years[33] (Table 2). Thus, the results of our study, together with those of the previous studies of sighted patients with non-24-hour sleep-wake syndrome, indicate that the onset of non-24-hour sleep-wake syndrome in sighted patients occurs predominantly in

Table 2—Published Reports of Sighted Patients With Non–24-Hour Sleep-Wake Syndrome

Author	Year	No.	Sex	Age, y		Social status at first visit	Period of sleep-wake cycle, h	Premorbid Psychiatric Problems	Premorbid Sleep Disorder
				First visit	Onset				
Eliott[11]	1970	1	M	NA	NA	NA	26		
Kokkoris[12]	1978	1	M	34	26	Unemployed	24.8	Schizoid personality	
Weber[13]	1980	1	M	28	24	College student	25.6		
Kamgar-Parsi[14]	1983	1	M	32	22	Unemployed	25.1		DSPS
Wollman[15]	1986	1	M	26	22	Student	27.4		
Sugita[16]	1987	1	M	25	23	Student	25		
Eastman[17]	1988	1	M	26	High school	NA	25		
Hoban[18]	1989	1	F	40	NA	Artist	25.1		
Moriya[19]	1990	1	M	16	15	Student	24.2-25		
Sasaki[20]	1990	1	F	22	21	NA	NA	Bipolar affective disorder	
Ohta[21]	1991	1	M	17	15	Student	24.6		
Oren[22]	1992	2	M	22, 28	NA	NA	NA		DSPS
Tagaya[23]	1993	1	M	20	18	Unemployed	25.3	Schizophrenia	
Emens[24]	1994	1	M	31	22	NA	25.2		
Tomoda[25]	1994	1	M	18	17	Student	25		
McArthur[26]	1996	1	M	41	41	Computer programmer	25.1	Depression, social phobia	DSPS
Uchiyama[27]	1996	1	M	30	18	Designer	27.2		
Yamadera[28]	1996	13	10 M	23.2*	19.8*	7 students, 3 unemployed, 2 employed, 1 part time worker	NA		
Nakamura[29]	1997	1	M	26	NA	Student	NA		DSPS
Shibui[30]	1998	1	M	43	41	Local government officer	25.8		
Hashimoto[31]	1998	1	M	26	26	Student	NA		
Hayakawa[32]	1998	1	M	20	16	Student	25		
Akaboshi[33]	2000	1	M	5	4	Kindergarten	24.9	Mental retardation	
Watanabe[34]	2000	1	M	17	17	NA	NA		
Morinobu[35]	2002	1	M	22	20	NA	NA	OCD	
Boivin[36]	2003	1	F	39	32	NA	NA		

*Mean, NA: refers to not available, OCD: obsessive-compulsive disorders, DSPS: delayed sleep-phase syndrome.

the teens and 20s.

In the study presented here, 56 of the 57 patients (98%) had a history of disturbed social functioning due to inability to regularly attend school or work. Only 1 patient (2%), who worked as a designer, had no consequential problems in his work schedule; this was probably due to his flexible work schedule—he went to work when he rose and worked for about 8 hours each day.

We found that 26% of our patients had suffered from persistent sleep-phase delay, which was diagnosed as delayed sleep-phase syndrome, prior to the onset of non-24-hour sleep-wake syndrome. A review of the previous relevant studies (Table 2) showed that persistent sleep-phase delay preceded the symptoms of non-24-hour sleep-wake syndrome in 5 out of 39 sighted patients (13%).[14,22,26,29] Oren et al[22] reported that 2 patients with delayed sleep-phase syndrome had developed non-24-hour sleep-wake syndrome after chronotherapy in which their sleep phase was scheduled to be delayed by 3 to 4 hours in an attempt to obtain the desired sleep phase, suggesting that, in patients with delayed sleep-phase syndrome, an enforced sleep-phase delay is a trigger for non-24-hour sleep-wake syndrome and that these 2 circadian rhythm sleep disorders share a common pathology.

The mean habitual sleep length in the patients in our study was 9.3 ± 1.3 hours. The habitual sleep length reported in the 6 previously published relevant articles ranged between 7.6 and 10 hours (mean 8.8 hours) in sighted people with non-24-hour sleep-wake syndrome.[12-15,26,34] Previous controlled studies have reported that sleep length is longer in patients with non-24-hour sleep-wake syndrome (9.2 hours) than in age-matched controls (7.3 hours).[43] The present study and most previous studies strongly suggest that habitual sleep length appears to be longer in patients with non-24-hour sleep-wake syndrome.

In the current study, the patient's sleep-wake cycle was objectively evaluated by using wrist-worn actigraphy for 2 weeks when the patient's sleep started during the nighttime and when delayed-phase jumps or irregular sleep patterns due to out-of-phase sleep episodes were least likely to occur.[27] We found that the mean period of the sleep-wake cycle for this 10-day actigraphic observation was 24.9 ± 0.4 hours. This sleep-wake period is comparable to that observed in classic human free-running experiments (around 25 hours), in which the subjects are allowed to select when they rise and retire, thus timings of light on and light-off.[44] The sleep-wake period is slightly longer than that of the circadian temperature or melatonin rhythm in totally blind patients (24.3-24.5 hours)[41-44] or normal subjects living under a forced desynchrony protocol (24.2 hours),[47] in which the sleep-wake cycle is forced to be outside the range of entrainment of the human circadian pacemaker and zeitgebers are either eliminated, minimized, or evenly distributed across the circadian cycle. Emens et al[24] reported that a sighted man with non-24-hour sleep-wake syndrome and a sleep-wake cycle of 25.17 hours under a normal 24-hour day-night condition displayed a shorter core body temperature rhythm of 24.50 hours under the forced desynchrony protocol for 3 weeks. It seems that his sleep-wake period was prolonged under a self-selected light-dark cycle relative to his endogenous circadian period and that an illumination on the delay portion of the human phase-response curve was likely to be responsible for this phenomenon.

In the present study, 54% of the patients were classified as delayed-phase jumpers, who displayed more than 3 delayed-phase jumps per month. An analysis of self-written sleep logs for 4 weeks showed that the period of the sleep-wake cycle was longer in delayed-phase jumpers (26.1 hours) than in non–delayed-phase jumpers (24.9h), suggesting that delayed-phase jumps that occur primarily when the patient's sleep started during the daytime prolonged the period of the sleep-wake cycle in the delayed-phase jumpers. We previously observed sleep-wake cycle and core body temperature rhythm in a patient with non-24-hour sleep-wake syndrome and found that an extremely long period of core body temperature rhythm (31.2 hours) with delayed-phase jumps of sleep onset appeared when his sleep onset was delayed into the daytime, whereas core body temperature rhythm and sleep-wake cycle displayed a period of 24.6 hours when his sleep onset occurred during the nighttime.[27] Similar variation of the period of the sleep-wake cycle has been observed in other early case reports of sighted patients with non-24-hour sleep-wake syndrome[12-15] but has not been recognized in totally blind patients with the syndrome.

Our previous case control studies have demonstrated that sleep in patients with non-24-hour sleep-wake syndrome is delayed relative to core body temperature rhythm[43] and melatonin rhythm under dim light condition[38] in comparison with age-matched controls. This altered phase relation between sleep and endogenous circadian phase, which has also been reported in other single case reports,[12,18] is likely to provide an opportunity that the patient's delay portion of the phase-response curve to light is more strongly illuminated. Thus, we postulate that the longer circadian period in sighted patients with non-24-hour sleep-wake syndrome in the present study, as compared with that in blind patients with non-24-hour sleep-wake syndrome, may be a consequence of light-provoked prolongation of the circadian period; we also propose that the extreme manifestation of such provoked prolongation might be a delayed-phase jump. This also provides an explanation for the observation by Emens et al[24] and a possible similarity between individuals in temporal isolation under a self-selected light-dark schedule and sighted individuals with non-24-hour sleep-wake syndrome.

Of the total cohort in the present study, 28% had developed psychiatric problems before the onset of non-24-hour sleep-wake syndrome. This suggests that withdrawal due to psychiatric problems is one of the etiologic factors of non-24-hour sleep-wake syndrome. There have been several reports of sighted patients with non-24-hour sleep-wake syndrome preceded by schizophrenia, bipolar disorder, depression, obsessive-compulsive disorder, or schizoid personality.[12,20,23,26,35] Kokkoris et al[12] have reported on a patient in whom onset of non-24-hour sleep-wake syndrome was preceded by development of a schizoid personality and postulated that this patient's non-24-hour sleep-wake syndrome sleep-wake cycles were the result of either a primary defect in the mechanism underlying entrainment or weakened social zeitgebers due to a personality disorder. Tagaya et al[23] have described a patient in whom the onset of non-24-hour sleep-wake syndrome was preceded by schizophrenia and noted that the sleep-wake cycle, which was related to the severity of the psychiatric symptoms, might be prolonged by social isolation.

Among the present patients who had no psychiatric problems before the onset of non-24-hour sleep-wake syndrome, 14 (34%) developed major depression thereafter. In 5 of these, the symptoms of depression were exacerbated when their sleep episodes occurred out of phase (ie, when they slept during the daytime) and slightly ameliorated when their sleep episode occurred in phase (ie, when they slept during the night). This suggests that a reduc-

tion in exposure to sunlight is a possible cause of depression, as described with respect to seasonal affective disorder.[48] The mean age at onset of the non-24-hour sleep-wake syndrome in those patients who developed major depression was 23.6 ± 2.4 years. It is possible that these patients had tried to adapt themselves to their social life and failed, leading to psychological stresses that could have precipitated their depression.

Some reports have indicated that depression is the most common psychopathology associated with delayed sleep-phase syndrome,[49,50] but no reports have described the relationship between non-24-hour sleep-wake syndrome and depression. There have, however, been various studies on the relationship between biologic rhythm and depression. Some of the evidence suggests that late arising itself may predispose to depression. Wehr et al[51] introduced the circadian-rhythm phase-advance hypothesis in which it has been inferred that, in depression, the circadian rhythm is phase advanced relative to the sleep phase. Another study suggests that an acute sleep-phase delay in some normal subjects causes depressive mood changes.[52] Our previous studies[38,39] revealed that sleep phase was delayed relative to melatonin rhythm in patients with non-24-hour sleep-wake syndrome, as compared with controls. The delay of sleep phase relative to the circadian pacemaker may be an etiologic factor of the depression that is associated with non-24-hour sleep-wake syndrome. Another possible trigger for this depression is the social disruption caused by the circadian rhythm sleep disorder.

ACKNOWLEDGEMENTS

This study was supported by a Research Grant for Nervous and Mental Disorders (14-2), the Health Science Grant (15130301) from Ministry of Health, Labor and Welfare, a Special Coordination Funds from the Ministry of Education, Culture, Sports, Science and Technology.

REFERENCES

1. Moore-Ede MC, Czeisler CA, Richardson GS. Circadian time-keeping in health and disease. I. Basic properties of circadian pacemakers. N Engl J Med 1983;309:469-76.
2. Wever RA. The circadian system of man. In: Topics in Environmental Physiology and Medicine. New York: Springer-Verlag; 1979.
3. International Classification of Sleep Disorders: Diagnostic and Coding Manual. Rochester, MN: American Sleep Disorders Association; 1997.
4. Miles LE, Raynal DM, Wilson MA. Blind man living in normal society has circadian rhythms of 24.9 hours. Science 1977;198:421-3.
5. Okawa M, Nanami T, Wada S, et al. Four congenitally blind children with circadian sleep-wake rhythm disorder. Sleep 1987;10:101-10.
6. Arendt J, Aldhous M, Wright J. Synchronisation of a disturbed sleep-wake cycle in a blind man by melatonin treatment. Lancet 1988;1:772-3.
7. Palm L, Blennow G, Wetterberg L. Correction of non-24-hour sleep-wake cycle by melatonin in a blind retarded boy. Ann Neurol 1991;29:336-9.
8. Klein T, Martens H, Dijk DJ, Kronauer RE, Seely EW, Czeisler CA. Circadian sleep regulation in the absence of light perception: chronic non-24-hour circadian rhythm sleep disorder in a blind man with a regular 24-hour sleep-wake schedule. Sleep 1993;16:333-43.
9. Lapierre O, Dumont M. Melatonin treatment of a non-24-hour sleep-wake cycle in a blind retarded child. Biol Psychiatry 1995;38:119-22.
10. Palm L, Blennow G, Wetterberg L. Long-term melatonin treatment in blind children and young adults with circadian sleep-wake disturbances. Dev Med Child Neurol 1997;39:319-25.
11. Eliott AL, Mills JN, Waterhouse JM. A man with too long a day. J Physiol 1970;212:30-1.
12. Kokkoris CP, Weitzman ED, Pollak CP, Spielman AJ, Czeisler CA, Bradlow H. Long-term ambulatory temperature monitoring in a subject with a hypernychthemeral sleep-wake cycle disturbance. Sleep 1978;1:177-90.
13. Weber AL, Cary MS, Connor N, Keyes P. Human non-24-hour sleep-wake cycles in an everyday environment. Sleep 1980;2:347-54.
14. Kamgar-Parsi B, Wehr TA, Gillin JC. Successful treatment of human non-24-hour sleep-wake syndrome. Sleep 1983;6:257-64.
15. Wollman M, Lavie P. Hypernychthemeral sleep-wake cycle: some hidden regularities. Sleep 1986;9:324-34.
16. Sugita Y, Ishikawa H, Mikami A, et al. Successful treatment for a patient with hypernychthemeral syndrome. Sleep Res 1987;16:642.
17. Eastman CI, Anagnopoulos CA, Cartwright RD. Can bright light entrain a free-runner? Sleep Res 1988;17:372.
18. Hoban TM, Sack RL, Lewy AJ, Miller LS, Singer CM. Entrainment of a free-running human with bright light? Chronobiol Int 1989;6:347-53.
19. Moriya Y, Yamazaki J, Higuchi T, Yamauchi T, Takahashi K. A case of non-24-hour sleep-wake syndrome. Jpn J Psychiatry Neurol 1990;44:189-90.
20. Sasaki T, Hashimoto O, Honda Y. A case of non-24-hour sleep-wake syndrome preceded by depressive state. Jpn J Psychiatry Neurol 1990;44:191-2.
21. Ohta T, Ando K, Iwata T, et al. Treatment of persistent sleep-wake schedule disorders in adolescents with methylcobalamin (vitamin B12). Sleep 1991;14:414-8.
22. Oren DA, Wehr TA. Hypernyctohemeral syndrome after chronotherapy for delayed sleep phase syndrome. N Engl J Med 1992;327:1762.
23. Tagaya H, Matsuno Y, Atsumi Y. A schizophrenic with non-24-hour sleep-wake syndrome. Jpn J Psychiatry Neurol 1993;47:441-2.
24. Emens JS, Brotman DJ, Czeisler CA. Evaluation of the intrinsic period of the circadian pacemaker in a patient with a non-24-hour sleep-wake schedule disorder. Sleep Res 1994;23:256.
25. Tomoda A, Miike T, Uezono K, Kawasaki T. A school refusal case with biological rhythm disturbance and melatonin therapy. Brain Dev 1994; 16:71-6.
26. McArthur AJ, Lewy AJ, Sack RL. Non-24-hour sleep-wake syndrome in a sighted man: circadian rhythm studies and efficacy of melatonin treatment. Sleep 1996;19:544-53.
27. Uchiyama M, Okawa M, Ozaki S, Shirakawa S, Takahashi K. Delayed phase jumps of sleep onset in a patient with non-24-hour sleep-wake syndrome. Sleep 1996;19:637-40.
28. Yamadera H, Takahashi K, Okawa M. A multicenter study of sleep-wake rhythm disorders: clinical features of sleep-wake rhythm disorders. Psychiatry Clin Neurosci 1996;50:195-201.
29. Nakamura K, Hashimoto S, Honma S, Honma K, Tagawa Y. A sighted man with non-24-hour sleep-wake syndrome shows damped plasma melatonin rhythm. Psychiatry Clin Neurosci 1997;51:115-9
30. Shibui K, Uchiyama M, Iwama H, Ozaki S, Takahashi K, Okawa M. Periodic fatigue symptoms due to desynchronization in a patient with non-24-hours sleep-wake syndrome. Psychiatry Clin Neurosci 1998;52:477-81.
31. Hashimoto S, Nakamura K, Honma S, Honma K. Free-running of plasma melatonin rhythm prior to full manifestation of a non-24 hour sleep-wake syndrome. Psychiatry Clin Neurosci 1998;52:264-5.
32. Hayakawa T, Kamei Y, Urata J, et al. Trials of bright light exposure and melatonin administration in a patient with non-24 hour sleep-wake syndrome. Psychiatry Clin Neurosci 1998;52:261-2.
33. Akaboshi S, Inoue Y, Kubota N, Takeshita K. Case of a mental-

ly retarded child with non-24 hour sleep-wake syndrome caused by deficiency of melatonin secretion. Psychiatry Clin Neurosci 2000;54:379-80.
34. Watanabe T, Kajimura N, Kato M, Sekimoto M, Hori T, Takahashi K. Case of a non-24 hours sleep-wake syndrome patient improved by phototherapy. Psychiatry Clin Neurosci 2000;54:369-70.
35. Morinobu S, Yamashita H, Yamawaki S, Tanaka K, Ohkawa M. Obsessive-compulsive disorder with non-24-hour sleep-wake syndrome. J Clin Psychiatry 2002;63:838-40.
36. Boivin DB, James FO, Santo JB, Caliyurt O, Chalk C. Non-24-hour sleep-wake syndrome following a car accident. Neurology 2003;60:1841-3.
37. Dagan Y, Eisenstein M. Circadian rhythm sleep disorders: Toward a more precise definition and diagnosis. Chronobiol Int 1999;16:213-22
38. Uchiyama M, Shibui K, Hayakawa T, et al. Larger phase angle between sleep propensity and melatonin rhythms in sighted humans with non-24-hour sleep-wake syndrome. Sleep 2002;25:83-8.
39. Uchiyama M, Kamei Y, Tagaya H, Takahashi K. Poor compensatory function for sleep loss in delayed sleep phase syndrome and non-24-hour sleep-wake syndrome. Sleep Biol Rhythms 2004;2:S5-6
40. Diagnostic and Statistical Manual of Mental Disorders, 3rd ed-revised. Washington: American Psychiatric Association; 1987.
41. Lockley SW, Skene DJ, Arendt J, Tabandeh H, Bird AC, Defrance R. Relationship between melatonin rhythms and visual loss in the blind. J Clin Endocrinol Metab 1997;82:3763-70.
42. Sack RL, Lewy AJ, Blood ML, Keith LD, Nakagawa H. Circadian rhythm abnormalities in totally blind people: incidence and clinical significance. J Clin Endocrinol Metab 1992;75:127-34.
43. Uchiyama M, Okawa M, Shibui K, et al. Altered phase relation between sleep timing and core body temperature rhythm in delayed sleep phase syndrome and non-24-hour sleep-wake syndrome in humans. Neurosci Lett 2000;294:101-4.
44. Aschoff J. Circadian rhythms in man. Science 1965;148:1427-32.
45. Orth DN, Besser GM, King PH, Nicholson WE. Free-running circadian plasma cortisol rhythm in a blind human subject. Clin Endocrinol 1979;10:603-17.
46. Klerman EB, Rimmer DW, Dijk DJ, Kronauer RE, Rizzo JF 3rd, Czeisler CA. Nonphotic entrainment of the human circadian pacemaker. Am J Physiol 1998; 274:R991-6.
47. Czeisler CA, Duffy JF, Shanahan TL, et al. Stability, precision, and near-24-hour period of the human circadian pacemaker. Science 1999;284:2177-81.
48. Wehr TA, Jacobsen FM, Sack DA, Arendt J, Tamarkin L, Rosenthal NE. Phototherapy of seasonal affective disorder. Time of day and suppression of melatonin are not critical for antidepressant effects. Arch Gen Psychiatry 1986;43:870-5
49. Thorpy MJ, Korman E, Spielman AJ, Glovinsky PB. Delayed sleep phase syndrome in adolescents. J Adolesc Health Care 1988;9:22-7.
50. Regestein QR, Monk TH. Delayed sleep phase syndrome: a review of its clinical aspects. Am J Psychiatry 1995;152:602-8.
51. Wehr TA, Wirz-Justice A, Goodwin FK, Duncan W, Gillin JC. Phase advance of the circadian sleep-wake cycle as an antidepressant. Science 1979 ;206:710-3.
52. Surridge DM, MacLean A, Coulter ME, Knowles JB. Mood change following an acute delay of sleep. Psychiatry Res 1987;22:149-58.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neuroscience Research 53 (2005) 123–128
Neuroscience Research
www.elsevier.com/locate/neures

Diurnal fluctuation of time perception under 30-h sustained wakefulness

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

[a] *Department of Psychophysiology, National Institute of Mental Health, National Center of Neurology and Psychiatry, 4-1-1 Ogawa-Higashi, Kodaira 187-8533, Japan*
[b] *Department of Psychiatry, Kohnodai Hospital, National Center of Neurology and Psychiatry, Kohnodai, Ichikawa 272-8516, Japan*
[c] *Japan Foundation for Neuroscience and Mental Health, Ogawa-higashi, Kodaira 187-8551, Japan*

Received 30 March 2005; accepted 10 June 2005
Available online 21 July 2005

Abstract

Previous studies have reported that time perception in humans fluctuates over a 24-h period. Behavioral changes seem to affect human time perception, so that the fluctuation in human time perception may be the result of such changes due to self-determined activities. Recently, we carried out a study in which a healthy human cohort was asked to perform simultaneously loaded cognitive tasks under controlled conditions, and found that time perception decreased linearly from morning to evening. In addition, the variations in time perception were not a consequence of behavioral changes. It remains to be elucidated whether diurnal variations in time perception are a consequence of circadian rhythm or of some homeostatic changes that are attributable to accumulated wake time. The effects of circadian rhythm on time perception were investigated in eight healthy young male volunteers by conducting 10-s time production tasks under 30-h constant-routine conditions. Core body temperature and serum melatonin and cortisol levels were measured during the course of the study. Produced time exhibited a diurnal variation and was strongly correlated with circadian variations in core body temperature and serum melatonin levels. These results suggest that human short-term time perception is under the influence of the circadian pacemaker.

Keywords: Time perception; Time production; Temperature; Human circadian; Melatonin; Warm-sensitive neurons; Sustained wakefulness

1. Introduction

Most higher organisms have two endogenous timekeeping systems (Morell, 1996): a circadian pacemaker system located in the suprachiasmatic nuclei of the hypothalamus, which is driven by a self-sustaining oscillator with a period of about 24 h and provides the time of day as the hour hand of the clock (Moore-Ede et al., 1983) and a stopwatch system, which measures the passage of a brief period as the minute hand of the clock (Gibbon and Church, 1981).

Recent progress in functional imaging techniques suggests that the cerebellum, the basal ganglia, and the prefrontal cortex are involved in the basis of time perception in normal human subjects (Harrington et al., 1998; Pouthas et al., 1999). These brain regions are likely to comprise an ensemble neural network that functions as the endogenous stopwatch system, enabling a human to perceive a brief period of time.

Animal studies have indicated that most animals use the endogenous stopwatch system to enhance survival and preserve their own species, for instance in reproduction, predation, or brooding (Gibbon et al., 1984; Terman et al., 1984). However, in humans, utilization of the wristwatch has obscured the functioning of the endogenous stopwatch system. As a result, we may only be able to observe the

* Corresponding author. Tel.: +81 42 346 2071; fax: +81 42 346 2072.
E-mail address: macoto@ncnp-k.go.jp (M. Uchiyama).

0168-0102/$ – see front matter
doi:10.1016/j.neures.2005.06.006

function of this stopwatch system when humans perceive a passage of time without referring to the watch or clock.

It has been well documented that humans can perceive the passage of time almost accurately without referring to a wrist watch (Treisman, 1963; Ivry, 1996; Aschoff, 1998; Rammsayer, 1999). Previous studies have reported that human time perception fluctuates over a 24-h period (Pöppel and Giedke, 1970; Aschoff, 1998; Campbell et al., 2001; Morofushi et al., 2001). It has also been noted that psychological status and behavioral changes affect human time perception (Watts and Sharrock, 1984; Angrilli et al., 1997), so that this fluctuation in human time perception may be the result of psychological or behavioral changes that are attributable to self-determined activities. Recently, we carried out a study in which a healthy human cohort was asked to perform simultaneously loaded cognitive tasks under controlled conditions, and found that produced time (i.e., the accuracy with which an individual can assess the passage of a set amount of time) decreased linearly from morning to evening, and that the changes in produced time over a 24-h period were not a consequence of psychological or behavioral changes (Kuriyama et al., 2003). It remains to be elucidated whether diurnal changes in produced time are a consequence of the circadian rhythm or of other influential factors, such as accumulated sleepiness or tiredness during sustained wakefulness.

Clarifying the circadian features of human time perception has been of particular interest to us. We, therefore, studied the circadian rhythm of produced time using a 10-s time-production task in healthy humans under constant-routine conditions in which a constant resting wakefulness was kept for 30 h, so that we excluded potential confounding factors that may mask the circadian variation. In our study protocol, the time production session was performed seven times every 4 h during 30-h constant-routine conditions. Simultaneously, we measured physical or mental status using a visual analogue scale (VAS), core body temperature (CBT), and serum hormone levels (melatonin (MLT) and cortisol (CRL)) to establish the output of the circadian pacemaker.

2. Materials and methods

2.1. Subjects and Protocol

Eight healthy male volunteers, aged 19–22 years (mean ± S.D., 20.3 ± 0.9 years) participated in a 3-day laboratory-based experimental session. They slept from 11:00 p.m. to 7:00 a.m. on day 2 on the first night under conditions of complete darkness (<0.1 lx). Thereafter, they began a 30-h constant-routine regimen in a time-isolation chamber. The participant was forced to stay awake quietly on a semi-recumbent bed under room light conditions (180 lx) during the constant-routine period. The effects of body movement, sleep–wake behavior, light intensity, and posture, which have potential influences on human time perception (Aschoff and Daan, 1997; Pöppel and Giedke, 1970), were minimized so that the subjects were constrained to their endogenous circadian rhythm while in the isolation chamber (Fig. 1). No time cues were given until the end of the study. The ambient temperature and humidity were controlled at 24.0 ± 0.5 °C and 60 ± 5%, respectively. Isocaloric meals (470 kcal) and nonsparkling mineral water (500 ml) were provided every 4 h. All procedures for the study were in accordance with the guidelines outlined in the Declaration of Helsinki. The experimental protocol was approved by the Intramural Research Board of the National Center of Neurology and Psychiatry, and each participant gave his informed consent to participate.

2.2. Procedure

A time production session (TPS) was conducted seven times at 4-h intervals with the aid of a computer-based program (Kuriyama et al., 2003) under 30-h constant-routine conditions (Fig. 1). The participants produced 10 s by pressing a space key on the computer. This task was repeated five times in each TPS. The maximum and minimum produced times were excluded from our analysis, and the three remaining produced times from each TPS were averaged (averaged produced time (PT)). Feedback information on

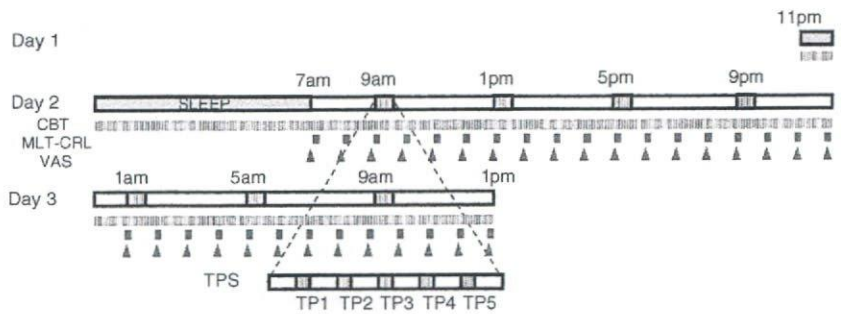

Fig. 1. Study protocol. The time production session (TPS) was conducted seven times, once each at 9:00 a.m., 1:00 p.m., 5:00 p.m., 9:00 p.m., 1:00 a.m., 5:00 a.m., and 9:00 a.m. during the constant-routine period, and five time-production trials (TP1–TP5) were included in each TPS. Core body temperature (CBT) data were measured every 2 min throughout the course of the study. Blood sampling (for analysis of melatonin (MLT) and cortisol (CRL)) and visual analogue scale (VAS) scoring were conducted hourly from 7:00 a.m. on day 2 to the end of the study.

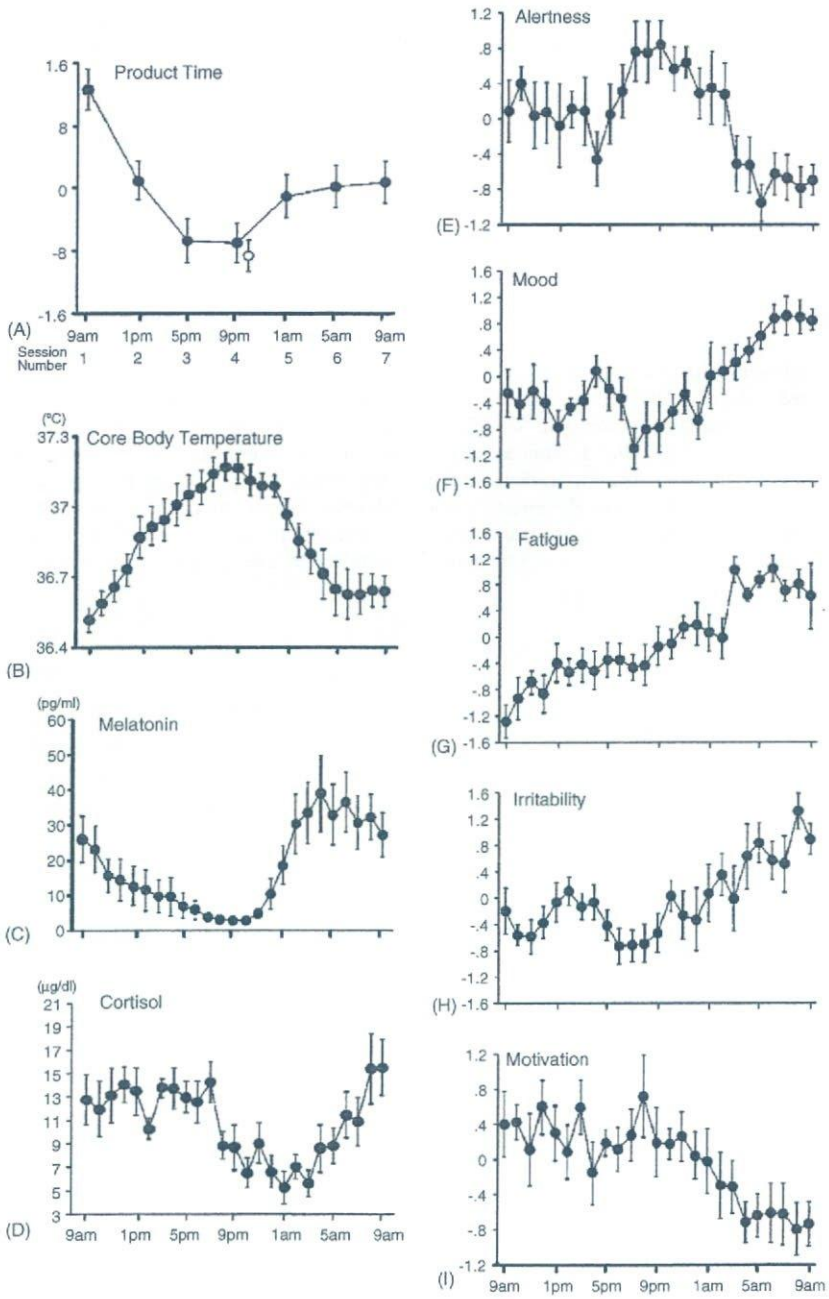

Fig. 2. Diurnal variations in standardized produced time (z-PT) (A), CBT (B), MLT (C), CRL (D), and VAS scores (E–I). Individual data points represent the mean value and bars represent the standard error of mean. The z-PT and VAS scores were standardized using Fisher's Z-transformation. Repeated-measures analysis of variance revealed a significant effect of time course for each value. The clear circle represents the z-PT (mean ± S.E., −0.97 ± 0.26) achieved during a training trial that was conducted at 9:00 p.m. on day 1. The z-PT decreased gradually during the first four sessions (sessions 1–4) and increased gradually thereafter (sessions 5–7). CBT exhibits a circadian waveform, and its pattern appears to be the reverse of the z-PT curve.

the accuracy of produced time was not given to the participants until the end of the study. The subjects were trained on the time production task prior to the study (at 9:00 pm on day 1) to eliminate any improvements achieved by acquiring the skill and to verify the accuracy of their produced time. Produced times in the training trials ranged from 9.05 to 16.51 s (mean ± S.D.: 12.19 ± 2.28; Fig. 2). The PT obtained in each TPS was standardized by using Fisher's *Z*-transformation before the subsequent analysis (*z*-PT).

2.3. Measures and analyses

CBT was measured every 2 min throughout the study and the data were stored telemetrically in a computerized monitoring system (Vital Sense, resolution 0.02 °C, Mini-Mitter Co. Inc., Bend, OR, USA). Blood samples were collected every hour through an intravenous catheter that had been placed in the participant's forearm. Serum levels of MLT and CRL were measured using radioimmunoassay kits (Bühlmann, Laboratories AG, Switzerland, and Immunotech, Beckman Coulter Inc., CA, USA, respectively); the limits of sensitivity for the assays were 2.8 pg/ml and 1.0 μg/dl, respectively. Mental status was measured every hour by using a VAS comprising five items: alertness, mood, fatigue, motivation, and irritability (Fig. 1). The VAS was presented as a 100-mm long horizontal line labeled, for example, "Very Alert" on the left and "Very Sleepy" on the right. The participant was asked to draw a vertical mark on the line at the point corresponding to their perceived present status. The VAS scores were obtained by measuring the distance of the mark from the left end of the line (higher scores being associated with more intense feelings of each state). The VAS scores were standardized by using Fisher's *Z*-transformation before the subsequent analysis. Repeated-measures analysis of variance (ANOVA) was performed to determine the time-course effect, and Pearson's correlation coefficients were calculated. Subsequently, multiple linear regression was performed to explore the determinants of diurnal PT variation. For these analyses, CBT, hormonal parameters, and VAS scores at the individual TPS were obtained by averaging the value of that time, those 1 h before

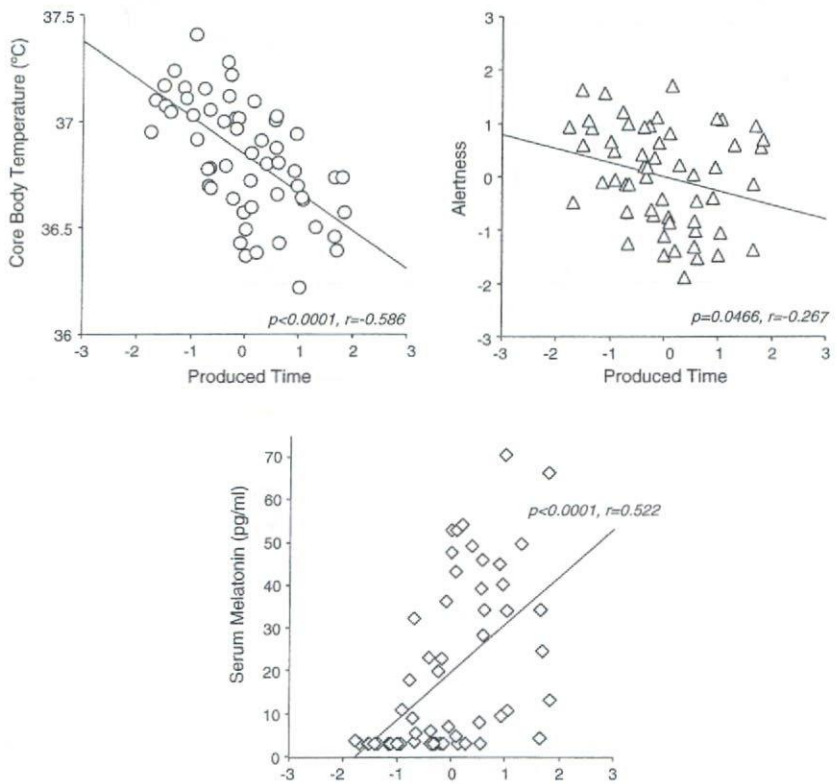

Fig. 3. Correlation between PT and CBT (A), MLT (B), and alertness (C). The results of the correlation analyses together with the regression line are shown. CBT, MLT, and alertness in individual TPSs were obtained by averaging the value of that time, and those measured 1 h before and 1 h after that time. The *z*-PT and VAS scores were standardized using Fisher's *Z*-transformation. significant correlations were found between *z*-PT and CBT ($r = -0.586$, $p < 0.0001$), MLT ($r = 0.522$, $p < 0.0001$), and alertness ($r = -0.267$, $p = 0.0466$).

and those 1 h after that time. The level of statistical significance was set at $p < 0.05$.

3. Results

The mean ± S.D. PT obtained from seven sessions was 13.35 ± 2.91 s. PT decreased markedly from the first sessions to the third session, but did not appear to decrease from the third to fourth session. Thereafter, PT increased gradually from the fourth to the last session. This diurnal variation of PT was apparent in Fig. 2, in which the z-PT data were displayed as a function of time.

To examine diurnal variation in the accuracy of time production task, we applied repeated-measures ANOVA to the z-PT data, and found a significant time-course effect ($p < 0.0001$). Repeated-measures ANOVA also revealed a significant diurnal variation in CBT ($p < 0.0001$), MLT ($p < 0.0001$), CRL ($p < 0.0001$), and VAS scores (alertness: $p < 0.0001$; mood: $p < 0.0001$; fatigue: $p < 0.0001$; irritability: $p < 0.0001$; motivation: $p = 0.0030$).

To investigate correlates influencing the accuracy of time production task, Pearson's correlation coefficients between the z-PT and other measures (CBT, MLT, CRL, and VAS scores) were calculated. The z-PT exhibited a significant correlation with CBT ($r = -0.586$, $p < 0.0001$) and MLT ($r = 0.522$, $p < 0.0001$; Fig. 3), while no significant correlation was found between z-PT and CRL.

There was a significant negative correlation between z-PT and alertness ($r = -0.267$, $p = 0.0466$; Fig. 3), while no significant correlations were found between z-PT and the other VAS scores.

Multiple linear regression analysis was performed to confirm the determinations of diurnal variation in the PT. CBT, hormonal parameters (MLT and CRL), and all VAS scores (alertness, mood, fatigue, motivation, and irritability) were included in the original model. Backward stepwise regression was conducted, and a p-value greater than 0.2 was used for variable removal. The final linear regression model included CBT ($r = -0.579$), mood ($r = -0.396$), fatigue ($r = -0.288$), and irritability ($r = -0.378$) as significant variables influencing PT. Analysis of results obtained from four of the eight measures, excluding the outlier, MLT, CRL, and two VAS items (alertness and motivation) yielded a significant coefficient of determination ($r^2 = 0.514$, $p < 0.0001$).

4. Discussion

4.1. The diurnal variation in time perception

PT was found to decrease from the morning to the evening, a finding that is consistent with our previous report (Kuriyama et al., 2003). We also found that PT increased from the evening to the morning, reaching a maximum during the morning hours and a minimum in the early evening. Several studies have reported this diurnal variation in time perception (Pöppel and Giedke, 1970; Aschoff, 1998). However, the mechanism responsible for it remains unclear, since the factors confounding variations in time perception were not controlled sufficiently in the previous studies. In the present study, factors affecting a subject's performance (i.e., mental and physical activities, food, and posture) and factors affecting endogenous oscillation (i.e., light intensity, room temperature, humidity, and sleep–wake behavior) were strictly controlled, so that our results are likely to represent an endogenous PT variation. Given that PT represents the rate of the biological stopwatch system (Morell, 1996), an increased PT, which is observed when the subject produces a longer time than a given period of time, indicates that the stopwatch system measuring the period runs slower. Likewise, a decreased PT is associated with a stopwatch system that runs faster. Our results provide evidence that the inherent rate of the stopwatch system is accelerated during the daytime and gradually returns to the morning level during the night.

4.2. Circadian oscillation of time perception

Significant correlations between z-PT and CBT or MLT were found in the present study. This suggests that the diurnal variation in PT reflects the output of the circadian pacemaker, since CBT and MLT rhythms obtained under constant-routine conditions are established markers of the circadian pacemaker (Czeisler et al., 1990, 1999). Aschoff illustrated a relatively weak correlation between produced time and rectal temperature in his study under free-running conditions, although the experimental setting was different from that used in our study (Aschoff, 1998). In contrast, we found a strong correlation between z-PT and CBT or MLT. In addition, a weak correlation between z-PT and alertness was observed. It has been reported that the circadian pacemaker has a more substantial influence on subjective alertness compared to other subjective measures (Dijk et al., 1992). Hence, this relatively weak correlation between z-PT and alertness is likely to be caused by the effects of the circadian pacemaker on the fluctuations in subjective alertness.

There is a possibility that CBT per se directly affects time perception. It has been reported that a pathological or manipulated high head temperature in humans is associated with a shortening of time perception, regardless of circadian timing (Hoagland, 1933; Hancock, 1993). Taken together, these data suggest that CBT has a direct effect on time perception, whether or not it is under the control of the circadian pacemaker. More recent animal studies have reported that the warm-sensitive neurons located in the suprachiasmatic nucleus are activated by warming (Boulant, 1998; Burgoon and Boulant, 2001). The circadian fluctuation of time perception may be mediated by a direct effect of CBT, and the existence of warm-sensitive neurons in animals supports this idea.

The multiple linear regression model revealed that CBT and mental status (mood, fatigue, and irritability) influenced PT independently, suggesting that there were factors other than the circadian rhythm affecting time perception. Recent functional imaging studies have reported that the frontal cortices, the basal ganglia, and the cerebellum are activated by repeated short-term time perception tasks (Harrington et al., 1998; Pouthas et al., 1999), suggesting that intricate neural networks are involved in the human stopwatch system. Since our study setting was not completely free from the effects of sustained wakefulness, the weak but significant influences of mood, fatigue, and irritability observed in the present study might be attributable to the effects of sustained wakefulness upon the neural networks of the human stopwatch system.

Acknowledgements

This study was supported in part by a Research Grant for Nervous and Mental Disorders (14-2), Health Science Grants (15130301 and H14 brain 003) from the Ministry of Health, Labor, and Welfare, and a Grant-in-aid for Scientific Research (13470200) from the Ministry of Education, Sports, Science, and Culture, Japan.

References

Angrilli, A., Cherubini, P., Pavese, A., Mantredini, S., 1997. The influence of affective factors on time perception. Percept. Psychophys. 59 (6), 972–982.

Aschoff, J., Daan, S., 1997. Human time perception in temporal isolation: effects of illumination intensity. Chronobiol. Int. 14 (6), 585–596.

Aschoff, J., 1998. 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.

Boulant, J.A., 1998. Hypothalamic neurons. Mechanisms of sensitivity to temperature. Ann. NY Acad. Sci. 856, 108–115.

Burgoon, P.W., Boulant, J.A., 2001. Temperature-sensitive properties of rat suprachiasmatic nucleus neurons. Am. J. Physiol. Regul. Integr. Comp. Physiol. 281 (3), R706–R715.

Campbell, S.S., Murphy, P.J., Boothroyd, C.E., 2001. Long-term time estimation is influenced by circadian phase. Physiol. Behav. 72 (4), 589–593.

Czeisler, C.A., Johnson, M.P., Duffy, J.F., Brown, E.N., Ronda, J.M., Kronauer, R.E., 1990. Exposure to bright light and darkness to treat physiologic maladaptation to night work. N. Engl. J. Med. 322 (18), 1253–1259.

Czeisler, C.A., Duffy, J.F., Shanahan, T.L., Brown, E.N., Mitchell, J.F., Rimmer, D.W., Ronda, J.M., Silva, E.J., Allan, J.S., Emens, J.S., Dijk, D.J., Kronauer, R.E., 1999. Stability, precision, and near-24-hour period of the human circadian pacemaker. Science 284 (5423), 2177–2181.

Dijk, D.J., Duffy, J.F., Czeisler, C.A., 1992. Circadian and sleep/wake dependent aspects of subjective alertness and cognitive performance. J. Sleep Res. 1 (2), 112–117.

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

Gibbon, J., Morrell, M., Silver, R., 1984. Two kinds of timing in circadian incubation rhythm of ring doves. Am. J. Physiol. 247 (6 Pt 2), R1083–R1087.

Hancock, P.A., 1993. Body temperature influence on time perception. J. Gen. Psychol. 120 (3), 197–216.

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

Hoagland, H., 1933. The physiological control of judgments of duration: evidence for a chemical clock. J. Gen. Psychol. 9, 267–287.

Ivry, R.B., 1996. The representation of temporal information in perception and motor control. Curr. Opin. Neurobiol. 6 (6), 851–857.

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 healthy human subjects. Neurosci. Res. 46 (1), 23–31.

Moore-Ede, M.C., Czeisler, C.A., Richardson, G.S., 1983. Circadian time-keeping in health and disease. Part 1. Basic properties of circadian pacemakers. N. Engl. J. Med. 309, 469–476.

Morell, V., 1996. Setting a biological stopwatch. Science 271 (5251), 905–906.

Morofushi, M., Shinohara, K., Kimura, F., 2001. Menstrual and circadian variations in time perception in healthy women and women with premenstrual syndrome. Neurosci. Res. 41 (4), 339–344.

Pöppel, E., Giedke, H., 1970. Diurnal variation of time perception. Psychol. Forsch. 34, 182–198.

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

Rammsayer, T.H., 1999. Neuropharmacological evidence for different timing mechanisms in humans. Q. J. Exp. Psychol. B 52 (3), 273–286.

Terman, M., Gibbon, J., Fairhurst, S., Waring, A., 1984. Daily meal anticipation: interaction of circadian and interval timing. Ann. NY Acad. Sci. 423, 470–487.

Treisman, M., 1963. Temporal discrimination and the indifference interval. Implications for a model of the 'internal clock'. Psychol. Monogr. 77 (13), 1–31.

Watts, F.N., Sharrock, R., 1984. Fear and time estimation. Percept. Motor Skills 59 (2), 597–598.

Brief Communication

Mouse Period1 (mPER1) Acts as a Circadian Adaptor to Entrain the Oscillator to Environmental Light/Dark Cycles by Regulating mPER2 Protein

Satoru Masubuchi,[1] Noritoshi Kataoka,[1] Paolo Sassone-Corsi,[2] and Hitoshi Okamura[1]

[1]Division of Molecular Brain Science, Department of Brain Sciences, Kobe University Graduate School of Medicine, Chuo-ku, Kobe 650-0017, Japan, and [2]Institut de Génétique et de Biologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, Université Louis Pasteur, 67404 Illkirch-Strasbourg, France

***Mouse period1* (*mPer1*) and *mPer2* are mammalian homologs of the *Drosophila* clock gene *period* that show robust oscillation in the suprachiasmatic nucleus, the mammalian master clock, and have been implicated as essential components of the core clock mechanism. Gene-targeting studies have demonstrated that *mPer2* plays a dominant function in behavioral rhythm generation, although the role of *mPer1* has not been fully clarified. Here, we report that prolongation of the lighting period (4–16 h) induces a larger-delay phase shift of the behavioral rhythm in *mPer1*-deficient (*mPer1*$^{-/-}$) mice. During the light-elongation task, mPER2 protein decay in *mPer1*$^{-/-}$ mice is slower (~4 h) than in wild-type mice, which thereby causes larger behavioral phase delay. *mPer1*$^{-/-}$ mice could not adapt to environmental light/dark cycles in long complete photoperiods with dim light or in long skeleton photoperiods. These photoperiodic conditions mimic natural environmental changes present at high latitudes, indicating that *mPer1* could operate in the adaptation of the circadian clock of nocturnal mice to large seasonal changes of environmental light/dark cycles.**

Key words: hypothalamus; suprachiasmatic; circadian; behavior; *mPer1*; *mPer2*

Introduction

Mouse period1 (*mPer1*) and *mPer2* are mammalian homologs of the *Drosophila* clock gene *period* (for review, see Dunlap, 1999) that show robust oscillation in the suprachiasmatic nucleus (SCN), the mammalian master clock (Klein et al., 1991), and have been implicated as essential components of the core clock mechanism. Gene-targeting studies have demonstrated that *mPer2* knock-out mice are arrhythmic in constant darkness (DD) (Zheng et al., 1999; Bae et al., 2001), whereas *mPer1* knock-out (*mPer1*$^{-/-}$) mice elicit a persistent rhythmicity with a slightly shorter period length (Bae et al., 2001; Cermakian et al., 2001; Zheng et al., 2001). Although it is demonstrated that *mPer1* has a crucial role for rhythm generation in peripheral clocks (Cermakian et al., 2001; Pando et al., 2002), its function in the central oscillator remains unclear.

Phase shifting by light is an essential feature of circadian rhythms (Daan and Pittendrigh, 1976a). The phase-shift profiles arising from short exposure to light do not show a difference between *mPer1*$^{-/-}$ and wild-type (*mPer1*$^{+/+}$) mice (Cermakian et al., 2001; Spoelstra et al., 2004), although a decrease in phase advances was noted just after the move to DD (Albrecht et al., 2001; Spoelstra et al., 2004). Because it is considered that the larger the duration of light exposure, the greater the phase shift that occurs (Daan and Pittendrigh, 1976b), in the present study, we adopted long light exposure as a task for revealing the role of *mPer1* to detect the difference between *mPer1*$^{-/-}$ and *mPer1*$^{+/+}$ mice. Here, we found that the magnitude of phase delays arising from long light in *mPer1*$^{-/-}$ mice was larger than that of wild-type mice, accompanying the delay of mPER2 protein decay without effecting a change at the mRNA level in the initial phase during the light exposure. The altered core clock machinery in these mice was evident in long complete photoperiods with dim light or in long skeleton photoperiods in which animals show free-running rhythms not adapting to environmental cycles.

Received Nov. 21, 2004; revised March 31, 2005; accepted March 31, 2005.

This work was supported by the Scientific Research Fund from the Ministry of Health, Labor and Welfare of Japan (H.O.), by SRF (H.O.), by grants from the Special Coordination Funds on Priority Areas of the Ministry of Education, Culture, Sports, Science and Technology of Japan (H.O.), by the Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, Centre Hospitalier Universitaire Régional, Fondation de la Recherche Médicale, Université Louis Pasteur, Human Frontier Science Program (RG-240), and Ligue contre le Cancer (P.S.-C.), and by a grant-in-aid for Encouragement of Young Scientists from the Japan Society for the Promotion of Science (S.M.). We thank W. Schwartz for discussions and H. Isejima for assistance.

Correspondence should be addressed to Dr. Hitoshi Okamura, 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.

DOI:10.1523/JNEUROSCI.4761-04.2005

 0270-6474/05/254719-06$15.00/0

Materials and Methods

Animals and behavioral rhythm monitoring. *mPer1*$^{+/+}$, heterozygous mutant (*mPer1*$^{+/-}$), and *mPer1*$^{-/-}$ mice (Cermakian et al., 2001) were bred and housed under light/dark (LD) 12 h cycles (fluorescent light, 200–300 lux). Locomotor activity was detected by passive (pyroelectric) infrared sensors (FA-05 F5B; Omron, Kyoto, Japan) (Shigeyoshi et al., 1997). Data were monitored and analyzed as described previously (Masubuchi et al., 2000) by Chronobiology kit (Stanford Software Systems, Stanford, CA). The experimental protocol of the current research was approved by the Committee for Animal Research at Kobe University.

In situ *hybridization.* *In situ* hybridization histochemistry using the free-floating sections was performed according to the method detailed

previously (Shigeyoshi et al., 1997). We used ^{33}P-radiolabeled cRNA probes for *mPer2* (Takumi et al., 1998) and albumin gene D-site-binding protein (*dbp*) (Yamaguchi et al., 2000) for the *in situ* hybridization studies. The peak value of *mPer1* $^{+/+}$ mice was adjusted to 100, and relative RNA abundance was used.

Immunocytochemistry. Immunocytochemistry was performed with the avidin–biotin–peroxidase method applied to free-floating sections (Ban et al., 1997). Serial frontal sections (30 μm thick) from the rostral end to the caudal end of the SCN were incubated for immunostaining with 1 μg/ml anti-mPER2 (affinity-purified rabbit antisera; Alpha Diagnostic International, San Antonio, TX) (Matsuo et al., 2003), which was finally visualized brown with 3,3′-diaminobenzidine tetrahydrochloride. We counted the mean number of immunoreactive cells in three sections in the middle portion of the SCN. For each point, we used four animals.

Statistical analysis. The 24 h mRNA and protein variations were statistically tested by one-way ANOVA. The effects of genotypes on the behavioral rhythm onsets were tested by two-way ANOVA. A *post hoc* Bonferroni/Dunn test was used for the comparison between the values of *Per1* $^{+/+}$, *Per1* $^{+/-}$, and *Per1* $^{-/-}$ groups at the same time points. The effects of genotypes on the entrainment daily lighting tasks (complete and skeleton photoperiod) were tested by Fisher's exact probability test.

Results

Extended light exposure induces larger phase delay of behavioral rhythm in *mPer1* $^{-/-}$ mice

mPer1 $^{+/+}$, *mPer1* $^{+/-}$, and *mPer1* $^{-/-}$ mice (Cermakian et al., 2001) entrained to an LD cycle (12 h; fluorescent light, 200–300 lux) were exposed to 0, 4, 8, 12, and 16 h of light prolongation (LP) (task LP00, LP04, LP08, LP12, and LP16, respectively) from lights off [Zeitgeber time 12 (ZT12)] at the last day of LD. Afterward, mice were kept in DD. We defined the lights-off points of each task as L00 (corresponding to ZT12), L04 (ZT16), L08 (ZT20), L12 (ZT24), and L16 (ZT28, ZT4 of the next cycle), respectively. The phase-delay effect of the prolongation of light of the last day on the behavioral rhythm was evaluated using extrapolated eye-fitted lines made by >10 d of activity onsets. Only the switch from LD to DD does not shift the onset of the extrapolated rhythm in both in *mPer1* $^{+/+}$ and *mPer1* $^{-/-}$ mice, when there was no light prolongation (LP00) (Fig. 1*A*, left). The onset of the behavioral rhythm was delayed in proportion to the increase of the duration of the light exposure (LP04, LP08, LP12) in all three groups (*mPer1* $^{+/+}$, *mPer1* $^{+/-}$, and *mPer1* $^{-/-}$ mice; one-way ANOVA; $p < 0.0001$) (Fig. 1*B*). The phase-delay effect peaked at 12 h (LP12) and then decreased at 16 h (LP16). Interestingly, depending on the genotype, the elongation of light exposure differentially affects the magnitude of the phase delay. As shown in Figure 1*A*, when mice were exposed to light prolongation for 12 h (LP12) for 1 d, the onset of activity rhythm in *mPer1* $^{-/-}$ mice was shifted by ~12 h, strikingly different from *mPer1* $^{+/+}$ mice (~5 h). The effects of elongation of light exposure on the phase delay were significantly different between genotypes (two-way ANOVA; *mPer1* $^{-/-}$ vs *mPer1* $^{+/+}$, $p < 0.0001$; *mPer1* $^{-/-}$ vs *mPer1* $^{+/-}$, $p < 0.0001$; *mPer1* $^{+/+}$ vs *mPer1* $^{+/-}$, $p < 0.01$): the behavioral phase delay is much larger in *mPer1* $^{-/-}$ than *mPer1* $^{+/+}$ ($p < 0.0001$ in LP04, LP08, LP12, and LP16 tasks) and than *mPer1* $^{+/-}$ ($p < 0.0001$ in LP08, LP12, and LP16 tasks) and slightly larger in *mPer1* $^{+/-}$ than *mPer1* $^{+/+}$ ($p < 0.0001$ in LP16 task) (Fig. 1*B*). The prolongation of light exposure (4–16 h) produces larger phase delay of behavioral rhythm in *mPer1* $^{-/-}$ mice.

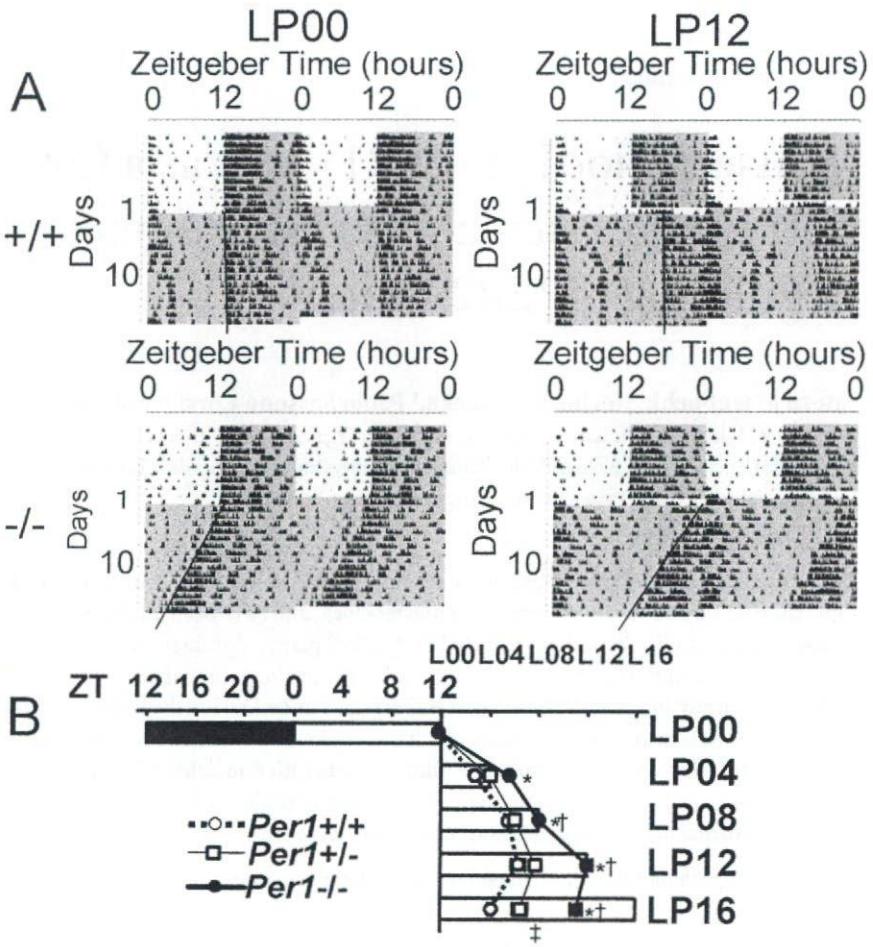

Figure 1. ***A***, Double-plotted locomotor activity rhythms of *mPer1* $^{+/+}$ mice (+/+) and *mPer1* $^{-/-}$ mice (−/−). Mice were moved to constant darkness after LD 12 h lighting cycle (LP00) and 12 h of light prolongation at the last day (day 1) of LD cycle (LP12). Locomotor activities were expressed in the histogram. Periods of darkness are indicated by gray backgrounds. ***B***, The effect of light tasks (LP00, LP04, LP08, LP12, LP16) to activity rhythms in *mPer1* $^{+/+}$, *mPer1* $^{+/-}$, and *mPer1* $^{-/-}$ mice. Extrapolated activity onsets of the first day are indicated by open circles (*mPer1* $^{+/+}$), open squares (*mPer1* $^{+/-}$), and filled circles (*mPer1* $^{-/-}$) (mean ± SEM). For each point, we used five or six animals. The filled bar is the dark phase of LD cycle; open bars are the light phase of LD cycle and light exposure time of each tasks. The values indicated by asterisks and daggers are statistically significant. *$p < 0.0001$, *mPer1* $^{+/+}$ versus *mPer1* $^{-/-}$; †$p < 0.0001$, *mPer1* $^{+/+}$ versus *mPer1* $^{+/-}$; ‡$p < 0.0001$, *mPer1* $^{-/-}$ versus *mPer1* $^{+/-}$ (Bonferroni/Dunn).

Extended light exposure does not yield differences in *mPer2* gene expression in the SCN of *mPer1* $^{-/-}$ mice

Because the SCN is the master clock that times rhythmicity at the systemic level (Schibler and Sassone-Corsi, 2002), the behavioral changes observed here must be a reflection of the alteration of the circadian core transcription/translation feedback loop within the SCN. Indeed, rapid resetting by photic cues was confirmed in the SCN *in vivo* (Best et al., 1999) and *in vitro* (Asai et al., 2001). Because *mPer2* is essential for the timing of the core feedback loop in the mammalian circadian clock (Hastings et al., 2003), we