and mental comorbidities, sub-clinical levels of psychiatric conditions (perhaps more common in those with insomnia)[29] may also contribute to a hrQOL decrements. Regardless of the reasons, a clear pattern of worse outcomes was observed for clinical insomniacs despite being under treatment.

The results also provide useful information as to what factors are associated with hrQOL among patients with insomnia, something not previously investigated. Specifically, behavioral factors (such as smoking and exercise) and comorbidity variables were among the strongest predictors of hrQOL among clinical insomniacs. Particularly, psychiatric comorbidities were the strongest predictors of all. Although a somewhat hypothetical exercise, the regression equation results suggest that through alleviating the effects of mental health comorbidities and promoting health behaviors related to sleep hygiene (smoking cessation, alcohol abstinence, and regular exercise), the health utility scores can approach that of good sleepers. Although we focused on malleable health-related factors, it is possible other variables may also be associated with health utilities (such as employment). These results have a number of clinical implications. Given past research has suggested that 40% of patients with insomnia have comorbid psychiatric illness,[29] physicians should give particular emphasis to patients with poor behavioral profiles and psychiatric comorbidities as their hrQOL is likely to be poorest and most in need of intervention. Also, given the observed relationship between insomnia and an increased risk of depression,[5] intervening even among clinical insomniacs with sub-clinical psychiatric symptoms may help to prevent future mood disorders in this population.

On the whole, these findings suggest that a combination of addressing the insomnia symptoms and taking mental health and behavior factors into consideration might maximize the hrQOL benefit to the patient. Similarly, these results suggest significant unmet needs with respect to insomnia treatments. A significant burden of insomnia remains even for those treated. The physical hrQOL (PCS) burden of insomnia reported in our current study was comparable (if not slightly larger) to that of diabetes while the mental burden was greater than diabetes, hypertension, obesity, and neuropathic pain underscoring the importance from a public health perspective of improved management.[30,31] Aside from affecting patients' day-to-day functioning and hrQOL, insomnia has clear effects on society which could, potentially, be mitigated by optimizing treatments.

Limitations

All data were self-reported and no verification of an insomnia diagnosis or treatment usage was available. The NHWS did not include information on non-pharmacological treatment (eg, cognitive behavioral therapy, sleep routines, etc) which could be relevant to include in future studies. The study was cross-sectional so causality between insomnia, treatments, comorbidities, health behaviors, and hrQOL is only hypothesized. Although the NHWS is demographically representative, it is unclear the extent to which this analytical sample generalizes to the various insomnia subpopulations in Japan.

Disclosure

This study was funded by MSD KK, Japan. The authors have no other conflicts of interest to disclose.

References

1. Ohayon MM, Smirne S. Prevalence and consequences of insomnia disorders in the general population of Italy. *Sleep Med.* 2002;3(2):115–120.
2. American Psychiatric Association, editors. *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision*. Washington DC: American Psychiatric Association; 2000.
3. American Academy of Sleep Medicine, editors. *The International Classification of Sleep Disorders. Diagnostic and Coding Manual*. 2nd ed. Illinois: American Academy of Sleep Medicine; 2005.
4. Mellinger GD, Balter MB, Uhlenhuth EH. Insomnia and its treatment. Prevalence and correlates. *Arch Gen Psychiatry*. 1985;42(3):225–232.
5. Ford DE, Kamerow DB. Epidemiologic study of sleep disturbances and psychiatric disorders. An opportunity for prevention? *JAMA*. 1989;262(11):1479–1484.
6. Kim K, Uchiyama M, Okawa M, Liu X, Ogihara R. An epidemiological study of insomnia among the Japanese general population. *Sleep*. 2000;23(1):41–47.
7. Morin CM, LeBlanc M, Daley M, Gregoire JP, Mérette C. Epidemiology of insomnia: prevalence, self-help treatments, consultations, and determinants of help-seeking behaviors. *Sleep Med*. 2006;7(2):123–130.
8. Buysse DJ, Angst J, Gamma A, Ajdacic V, Eich D, Rössler W. Prevalence, course, and comorbidity of insomnia and depression in young adults. *Sleep*. 2008;31(4):473–480.
9. Hysing M, Pallesen S, Stormark KM, Lundervold AJ, Sivertsen B. Sleep patterns and insomnia among adolescents: a population-based study. *J Sleep Res*. 2013;22(5):549–556.
10. Kim WH, Kim BS, Kim SK, et al. Prevalence of insomnia and associated factors in a community sample of elderly individuals in South Korea. *Int Psychogeriatr*. 2013;25(10):1729–1737.
11. Furihata R, Uchiyama M, Takahashi S, et al. The association between sleep problems and perceived health status: A Japanese nationwide general population survey. *Sleep Med*. 2012;13(7):831–837.
12. Akashiba T, Kawahara S, Akahoshi T, et al. Relationship between quality of life and mood or depression in patients with severe obstructive sleep apnea syndrome. *Chest*. 2002;122(3):861–865.
13. Bonnet MH, Arand DL. 24-Hour metabolic rate in insomniacs and matched normal sleepers. *Sleep*. 1995;18(7):581–588.
14. Simon GE, VonKorff M. Prevalence, burden, and treatment of insomnia in primary care. *Am J Psychiatry*. 1997;154(10):1417–1423.
15. Sivertsen B, Overland S, Neckelmann D, et al. The long-term effect of insomnia on work disability: the HUNT-2 historical cohort study. *Am J Epidemiol*. 2006;163(11):1018–1024.
16. Ozminkowski RJ, Wang S, Walsh JK. The direct and indirect costs of untreated insomnia in adults in the United States. *Sleep*. 2007;30(3):263–273.

17. Daley M, Morin CM, LeBlanc M, et al. Insomnia and its relationship to health-care utilization, work absenteeism, productivity and accidents. *Sleep Med*. 2009;10(4):427–438.
18. Bolge SC, Doan JF, Kannan H, Baran RW. Association of insomnia with quality of life, work productivity, and activity impairment. *Qual Life Res*. 2009;18:415–422.
19. Ishak WW, Bagot K, Thomas S, et al. Quality of life in patients suffering from insomnia. *Innov Clin Neurosci*. 2012;9(10):13–26.
20. Sasai T, Inoue Y, Komada Y, Nomura T, Matsuura M, Matsushima E. Effects of insomnia and sleep medication on health-related quality of life. *Sleep Med*. 2010;11(5):452–527.
21. Liu GG, DiBonaventura MD, Yuan Y, et al. The burden of illness for patients with viral hepatitis C: evidence from a national survey in Japan. *Value Health*. 2012;15(Suppl 1):S65–S71.
22. Charlson ME, Pompei P, Ales KL, et al. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis*. 1987;40(5):373–383.
23. Fukuhara S, Bito S, Green J, Hsiao A, Kurokawa K. Translation, adaptation, and validation of the SF-36 Health Survey for use in Japan. *J Clin Epidemiol*. 1998;51(11):1037–1044.
24. Fukuhara S, Ware JE Jr, Kosinski M, Wada S, Gandek B. Psychometric and clinical tests of validity of the Japanese SF-36 Health Survey. *J Clin Epidemiol*. 1998;51(11):1045–1053.
25. Brazier JE, Roberts J. The estimation of a preference-based measure of health from the SF-12. *Med Care*. 2004;42(9):851–859.
26. Ohayon M. Epidemiological study on insomnia in the general population. *Sleep*. 1996;19(3 Suppl 3):S7–S15.
27. Walsh JK, Ustun TB. Prevalence and health consequences of insomnia. *Sleep*. 1999;22:S427–S436.
28. Weyerer S, Dilling H. Prevalence and treatment of insomnia in the community: results from the Upper Bavarian Field Study. *Sleep*. 1991;14(5):392–398.
29. Frey DJ, Ortega JD, Wiseman C, Farley CT, Wright KP Jr. Influence of zolpidem and sleep inertia on balance and cognition during nighttime awakening: a randomized placebo-controlled trial. *J Am Geriatr Soc*. 2011;59(1):73–81.
30. Baglioni C, Battagliese G, Feige B, et al. Insomnia as a predictor of depression: a meta-analytic evaluation of longitudinal epidemiological studies. *J Affect Disord*. 2011;135(1–3):10–19.
31. DiBonaventura M, Fukuda T, Stankus AP. The prevalence and burden of comorbid hypertension and obesity among patients with type 2 diabetes in Japan. Presented at: ISPOR Asia-Pacific Conference; Sep 2012; Taipei, Taiwan.

Supplementary materials

Table S1 Regression results predicting mental component summary scores

	b	SE	95% LCL	95% UCL	P
Intercept	42.369	0.856	40.691	44.047	0.000
Clinical insomniac	−13.721	0.270	−14.250	−13.191	0.000
Male	−0.007	0.133	−0.266	0.253	0.960
Hokkaido	−1.035	0.743	−2.492	0.422	0.164
Tohoku	−1.680	0.742	−3.133	−0.226	0.023
Kanto	−1.102	0.704	−2.482	0.278	0.118
Chubu	−1.201	0.714	−2.600	0.198	0.092
Kinki	−0.899	0.709	−2.289	0.492	0.205
Chugoku	−0.776	0.742	−2.231	0.680	0.296
Shikoku	−0.568	0.791	−2.118	0.981	0.472
Kyushu	−0.389	0.728	−1.816	1.038	0.593
Okinawa (reference)	–	–	–	–	–
High school or less	−0.025	0.132	−0.283	0.233	0.847
Two-year university	−0.032	0.172	−0.370	0.306	0.852
Four-year university	–	–	–	–	–
Annual income: <¥3 MM	−1.272	0.194	−1.653	−0.891	0.000
Annual income: ¥3 to <¥5 MM	−0.465	0.172	−0.802	−0.128	0.007
Annual income: ¥5 to <¥8 MM	−0.520	0.170	−0.852	−0.188	0.002
Annual income: ¥8 MM or more	−1.045	0.227	−1.490	−0.600	0.000
Annual income: decline to answer (reference)	–	–	–	–	–
Employed	0.949	0.132	0.690	1.208	0.000
Not employed and looking for work	−1.709	0.347	−2.389	−1.028	0.000
Not employed and not looking for work (reference)	–	–	–	–	–
National health insurance	0.838	0.450	−0.045	1.721	0.063
Social insurance	1.165	0.449	0.285	2.046	0.009
Late stage elderly insurance	−1.256	0.657	−2.543	0.031	0.056
None of the above	−1.493	0.534	−2.538	−0.447	0.005
Other insurance (reference)	–	–	–	–	–
Current smoker	−0.406	0.144	−0.689	−0.124	0.005
Alcohol use	−0.359	0.128	−0.609	−0.109	0.005
Regular exercise	1.510	0.117	1.281	1.740	0.000
BMI: underweight	−0.562	0.189	−0.931	−0.192	0.003
BMI: normal weight	−0.282	0.157	−0.589	0.026	0.073
BMI: obese	−1.243	0.294	−1.819	−0.667	0.000
BMI: unknown (reference)	–	–	–	–	–
Age	0.154	0.004	0.146	0.162	0.000
CCI	−1.290	0.129	−1.543	−1.037	0.000

Abbreviations: CCI, Charlson comorbidity index; BMI, body mass index; SE, standard error, LCL, 95% lower confidence level; UCL, 95% upper confidence level.

Table S2 Regression results predicting physical component summary scores

	b	SE	95% LCL	95% UCL	*P*
Intercept	56.807	0.587	55.656	57.957	0.000
Clinical insomniac	−4.788	0.185	−5.151	−4.424	0.000
Male	0.352	0.091	0.174	0.530	0.000
Hokkaido	−0.350	0.510	−1.349	0.649	0.492
Tohoku	−0.452	0.508	−1.449	0.545	0.374
Kanto	−0.109	0.483	−1.056	0.837	0.821
Chubu	−0.079	0.489	−1.039	0.880	0.871
Kinki	−0.188	0.486	−1.141	0.765	0.699
Chugoku	−0.486	0.509	−1.484	0.512	0.340
Shikoku	−0.325	0.542	−1.388	0.737	0.548
Kyushu	0.201	0.499	−0.777	1.180	0.687
Okinawa (reference)	–	–	–	–	–
High school or less	−0.230	0.090	−0.407	−0.053	0.011
Two-year university	−0.082	0.118	−0.314	0.149	0.486
Four-year university	–	–	–	–	–
Annual income: <¥3 MM	−0.460	0.133	−0.721	−0.199	0.001
Annual income: ¥3 to <¥5 MM	−0.251	0.118	−0.482	−0.020	0.033
Annual income: ¥5 to <¥8 MM	0.008	0.116	−0.220	0.235	0.949
Annual income: ¥8 MM or more	0.211	0.156	−0.094	0.515	0.176
Annual income: decline to answer (reference)	–	–	–	–	–
Employed	−0.290	0.091	−0.468	−0.113	0.001
Not employed and looking for work	−0.853	0.238	−1.320	−0.386	0.000
Not employed and not looking for work (reference)	–	–	–	–	–
National health insurance	0.851	0.309	0.246	1.457	0.006
Social insurance	1.012	0.308	0.408	1.616	0.001
Late stage elderly insurance	−1.195	0.450	−2.078	−0.312	0.008
None of the above	−0.434	0.366	−1.151	0.283	0.235
Other insurance (reference)	–	–	–	–	–
Current smoker	−0.262	0.099	−0.456	−0.068	0.008
Alcohol use	−0.361	0.087	−0.532	−0.189	0.000
Regular exercise	1.046	0.080	0.888	1.203	0.000
BMI: underweight	−0.111	0.129	−0.365	0.142	0.390
BMI: normal weight	−1.314	0.108	−1.525	−1.103	0.000
BMI: obese	−1.062	0.201	−1.457	−0.667	0.000
BMI: unknown (reference)	–	–	–	–	–
Age	−0.063	0.003	−0.068	−0.057	0.000
CCI	−2.380	0.089	−2.554	−2.207	0.000

Abbreviations: CCI, Charlson comorbidity index; BMI, body mass index; SE, standard error, LCL, 95% lower confidence level; UCL, 95% upper confidence level.

Table S3 Regression results predicting health utilities

	b	SE	95% LCL	95% UCL	*P*
Intercept	0.751	0.012	0.728	0.774	0.000
Clinical insomniac	−0.146	0.004	−0.153	−0.139	0.000
Male	0.009	0.002	0.005	0.012	0.000
Hokkaido	−0.004	0.010	−0.024	0.016	0.722
Tohoku	−0.012	0.010	−0.032	0.008	0.222
Kanto	−0.002	0.010	−0.021	0.017	0.816
Chubu	−0.004	0.010	−0.024	0.015	0.648
Kinki	−0.002	0.010	−0.021	0.017	0.857
Chugoku	−0.004	0.010	−0.024	0.016	0.694
Shikoku	−0.001	0.011	−0.022	0.021	0.960
Kyushu	0.006	0.010	−0.014	0.025	0.567
Okinawa (reference)	–	–	–	–	–
High school or less	−0.003	0.002	−0.007	0.000	0.060
Two-year university	−0.001	0.002	−0.006	0.003	0.564
Four-year university	–	–	–	–	–
Annual income: <¥3 MM	−0.017	0.003	−0.023	−0.012	0.000
Annual income: ¥3 to <¥5 MM	−0.009	0.002	−0.013	−0.004	0.000
Annual income: ¥5 to <¥8 MM	−0.007	0.002	−0.012	−0.002	0.003
Annual income: ¥8 MM or more	−0.010	0.003	−0.016	−0.004	0.002
Annual income: decline to answer (reference)	–	–	–	–	–
Employed	0.002	0.002	−0.001	0.006	0.225
Not employed and looking for work	−0.021	0.005	−0.030	−0.011	0.000
Not employed and not looking for work (reference)	–	–	–	–	–
National health insurance	0.011	0.006	−0.001	0.023	0.083
Social insurance	0.017	0.006	0.005	0.029	0.006
Late stage elderly insurance	−0.033	0.009	−0.051	−0.015	0.000
None of the above	−0.021	0.007	−0.036	−0.007	0.004
Other insurance (reference)	–	–	–	–	–
Current smoker	−0.005	0.002	−0.009	−0.002	0.006
Alcohol use	−0.004	0.002	−0.007	0.000	0.040
Regular exercise	0.018	0.002	0.015	0.021	0.000
BMI: underweight	−0.004	0.003	−0.009	0.001	0.088
BMI: normal weight	−0.013	0.002	−0.017	−0.008	0.000
BMI: obese	−0.015	0.004	−0.023	−0.008	0.000
BMI: unknown (reference)	–	–	–	–	–
Age	0.001	0.000	0.000	0.001	0.000
CCI	−0.031	0.002	−0.034	−0.028	0.000

Abbreviations: CCI, Charlson comorbidity index; BMI, body mass index; SE, standard error, LCL, 95% lower confidence level; UCL, 95% upper confidence level.

Nakazaki et al. Journal of Physiological Anthropology 2014, 33:31
http://www.jphysiolanthropol.com/content/33/1/31

Journal of PHYSIOLOGICAL ANTHROPOLOGY

METHODOLOGY ARTICLE Open Access

Validity of an algorithm for determining sleep/wake states using a new actigraph

Kyoko Nakazaki[1], Shingo Kitamura[1], Yuki Motomura[1], Akiko Hida[1], Yuichi Kamei[2], Naoki Miura[3] and Kazuo Mishima[1*]

Abstract

Background: This study aimed to develop an algorithm for determining sleep/wake states by using chronological data on the amount of physical activity (activity intensity) measured with the FS-750 actigraph, a device that can be worn at the waist, allows for its data to be downloaded at home, and is suitable for use in both sleep research and remote sleep medicine.

Methods: Participants were 34 healthy young adults randomly assigned to two groups, A (n =17) and B (n =17), who underwent an 8-hour polysomnography (PSG) in the laboratory environment. Simultaneous activity data were obtained using the FS-750 attached at the front waist. Sleep/wake state and activity intensity were calculated every 2 minutes (1 epoch). To determine the central epoch of the sleep/wake states (x), a five-variable linear model was developed using the activity intensity of Group A for five epochs (x_{-2}, x_{-1}, x, x_{+1}, x_{+2}; 10 minutes). The optimal coefficients were calculated using discriminant analysis. The agreement rate of the developed algorithm was then retested with Group B, and its validity was examined.

Results: The overall agreement rates for group A and group B calculated using the sleep/wake score algorithm developed were 84.7% and 85.4%, respectively. Mean sensitivity (agreement rate for sleep state) was 88.3% and 90.0% and mean specificity (agreement rate for wakeful state) was 66.0% and 64.9%, respectively. These results confirmed comparable agreement rates between the two groups. Furthermore, when applying an estimation rule developed for the sleep parameters measured by the FS-750, no differences were found in the average values between the calculated scores and PSG results, and we also observed a correlation between the two sets of results. Thus, the validity of these evaluation indices based on measurements from the FS-750 is confirmed.

Conclusions: The developed algorithm could determine sleep/wake states from activity intensity data obtained with the FS-750 with sensitivity and specificity equivalent to that determined with conventional actigraphs. The FS-750, which is smaller, less expensive, and able to take measurements over longer periods than conventional devices, is a promising tool for advancing sleep studies at home and in remote sleep medicine.

Keywords: polysomnography, actigraphy, sleep/wake scoring algorithm, validation, sleep estimation

Background

Polysomnography (PSG) is regarded as the gold standard in the objective evaluation of sleep/wake states. On the one hand, PSG can distinguish between sleep and wake states and determine the depth of sleep with high accuracy, while on the other hand, evaluating sleep in natural settings with it is difficult because people are mainly evaluated in a laboratory setting while wearing surface electrodes to monitor their brain waves. In addition, investigations for diagnosing circadian rhythm sleep disorder or to ascertain an individual's sleep habits require continuous monitoring of sleep/wake patterns over the course of a few weeks or months, which is not possible with PSG.

Methods other than PSG for monitoring sleep/wake states include both subjective and objective measurement methods. Although subjective measurement methods such as self-kept sleep diaries are easy to administer, they are less accurate than objective measurement methods since record keeping is retrospective and sleep state misperceptions are

* Correspondence: mishima@ncnp.go.jp
[1]Department of Psychophysiology, National Institute of Mental Health, National Center of Neurology and Psychiatry, 4-1-1 Ogawa-higashi, Kodaira, Tokyo 187-8553, Japan
Full list of author information is available at the end of the article

possible. The objective method of actigraphy has been attracting recent attention as it can make continuous recordings over long periods in natural settings and places little burden on the subject. Actigraphy uses an actigraph, a lightweight device that can be attached to the wrist or waist, to continuously monitor the amount of physical activity (activity intensity) of the subject. This device can determine the sleep/wake states in epochs of a few seconds to a few minutes by processing the activity data using a unique algorithm. It is reported that the agreement rate between PSG and actigraphy for sleep/wake state determination is in the range of 85% to 96% [1-6]. Actigraphy is a useful method for evaluating an individual's sleep/wake pattern over the long term, and it has been widely used in clinical studies targeting patients with insomnia or circadian rhythm sleep disorder, as well as in research studies targeting healthy general populations including children [7-10]. Although actigraphy is less expensive compared to PSG, the equipment it requires is still relatively expensive, which has prevented it from being introduced in epidemiological studies targeting large numbers of participants and from being widely used in clinical practice. Its use is also restricted by the fact that a specific interface is needed to download the device's recorded data, which requires participants to regularly visit a specific research facility or facility specializing in sleep medicine. It is expected that solving these issues will promote its use in large-scale studies and also in remote sleep medicine, and consequently expand the scope of use of actigraphy in research and clinical settings.

The FS-750, distributed recently by Estera Corporation, is an inexpensive device that is worn less obtrusively at the waist than other actigraphs worn at the wrist. It also produces fewer artifacts from movements limited to the upper extremities. Another plus is that the FS-750 can determine the subject's posture (that is, standing, inverted, supine, lateral (left and right), and prone) from information about the direction the device is facing, which means it can be applied for sleep/wake state determination. The memory capacity is 40 days and it functions for about 6 months with commercially available batteries (CR2032). It uses near field communication (NFC) technology to transmit data and can download its data to a PC using a commercially available NFC reader/writer device.

In this study, to determine whether the FS-750 can be used for monitoring sleep/wake states, we developed an algorithm that estimates sleep/wake states using data on the amount of physical activity (activity intensity) measured with the FS-750 and tested its validity. In addition, following previous studies [2,11], we developed an estimation rule to optimize the scoring of sleep parameters by the FS-750 and then examined its effectiveness.

Methods

Subjects

Forty-one young adults (31 men, 10 women; mean age 22.0 ± 1.8 years) participated in this study. Questionnaire and medical examination results confirmed that the participants had no serious mental, physical, or sleep disorders, were not working nightshifts constantly, had not traveled to a destination with a >6-hour time difference within the 3 months prior to the study, and were not taking medications that might affect the experimental results. To avoid the possibility that abnormal movements of the extremities or trunk would affect the estimation of sleep/wake (S/W) states, data from participants with a periodic limb movement index (PLMI) score of ≥5 on PSG (five participants) and those with an apnea and hypopnea index (AHI) score >15/hour (one participant) were excluded. In addition, data from a participant who had extreme difficulty falling asleep (sleep latency: 207 min) was excluded from the analysis. This left 34 participants (25 men, 9 women; mean age 21.9 ± 1.7 years) as the targets for analysis, and they were randomly assigned to Group A (17 participants) and Group B (17 participants).

This study was approved by the Ethics Committee of the National Center of Neurology and Psychiatry, and written consent was obtained from all participants.

Polysomnography

The study was conducted in the sleep laboratory unit of the National Institute of Mental Health, National Center of Neurology and Psychiatry.

The participants arrived at 19:00 and after PSG electrodes and the FS-750 were attached, they went to bed at 24:00. Wake-up time was set at 8:00 (maximum 8 hours of sleep), and the participants were directed that if they woke earlier than 8:00 they should not get up and should try to get as much sleep as possible until the lights were turned on. Inside the unit, the temperature was maintained at 25°C and humidity at 50% relative humidity (RH).

PSG recording was made using a Neurofax digital EEG system (EEG-1200, Nihon Koden, Tokyo, Japan) and included an electroencephalogram (EEG) with a conventional montage (F_3, F_4, C_3, C_4, O_1, O_2) based on the contralateral mastoid (A_1, A_2), an electrooculogram (EOG) at the outer canthus of each eye, a submental electromyogram (EMG), an electrocardiogram (ECG), an EMG at the left and right tibialis anterior muscles, and monitoring of respiratory signals (oronasal airflow, movements of the chest wall and abdomen, and O_2 saturation of arterial blood). During the recordings, EEG, EOG, EMG, and ECG signals were digitized at 200 Hz, and the signal was filtered using a high-pass filter with the following time constants: EEG 0.3 s, EOG 0.03 s, submental EMG 0.03 s,

and ECG 1.0 s. The signal was filtered using a low-pass filter with the following data: EEG 60 Hz, EOG 60 Hz, submental EMG 60 Hz, and ECG 60 Hz.

EEG recordings from a monopolar lead at C_3 were assessed visually in 30-second epochs according to the method of Rechtschaffen and Kales [12] and categorized into one of the following sleep stages: Stage Wake, Stage REM, Stage 1, Stage 2, Stage 3, or Stage 4. Four consecutively judged sleep stages (1 epoch =30 seconds) were reclassified as sleep (sleep epochs determined by PSG, S_{PSG}) or wake (wake epochs determined by PSG, W_{PSG}) state every 2 minutes so they corresponded with the activity intensity data measured by the FS-750 (1 epoch =2 minutes). In accordance with a previous study [5], if there were two or more occurrences of Stage Wake in four continuous epochs, this was defined as W_{PSG}; otherwise, S_{PSG}. S_{PSG} was subdivided into Stage REM, Stage 1, Stage 2, Stage 3, and Stage 4 according to the sleep stage that was most frequent in the four consecutive epochs. However, for epochs that contained different sleep stages occurring at the same frequency, this was categorized according to the order of Stage REM, Stage 1, Stage 2, Stage 3, and Stage 4. (For example, if there were two occurrences of Stage REM and two of Stage 1, S_{PSG} was sub-categorized as Stage REM.)

Activity recording with the FS-750

Activity during the night while the lights were off was recorded with the FS-750 (Estera Corporation, Saitama, Japan), which is worn at the waist. This small and light, rectangular device (external dimensions: 75 × 33.5 × 10.8 mm (width × height × depth); weight, 26 g including the battery) records the amount of activity by using an internal three-axis accelerometer (electrostatic capacity sensor). Every 0.125 seconds, the number of times that acceleration exceeded a reference value was summed, and the value was recorded as the activity value over 2-minute bins. The activity intensity is calculated from the activity value as a value from 0 to 31 (32 levels). An activity intensity of 0 means the subject did not move, and larger values indicate higher amounts of activities.

Formulation of an algorithm for sleep/wake scoring

To develop an algorithm for the FS-750 that determines the S/W states, following previous studies [5], a five-dimensional linear model was hypothesized that utilizes activity intensity at an evaluation epoch as well as two epochs before and two epochs after (total 10 minutes). Using the activity intensity at 4 minutes and 2 minutes before the evaluation epoch, at the evaluation epoch, and at 2 minutes and 4 minutes after the epoch (x_{-2}, x_{-1}, x, x_{+1}, x_{+2}), each with a weighting coefficient (a_{-2}, a_{-1}, α, a_{+1}, a_{+2}), the following equation gives composite variable z, which is the discriminant score:

$$z = a_{-2}x_{-2} + a_{-1}x_{-1} + \alpha x + a_{+1}x_{+1} + a_{+2}x_{+2}$$

Here, S_{PSG} (=0) and W_{PSG} (=1), which are obtained by PSG (one epoch =2 minutes), are taken as a baseline. Score z classifies the activity intensity obtained from the corresponding epoch by the FS-750 into sleep (S_{ACT}) and wake (W_{ACT}). The above equation was obtained by discriminant analysis using the data set containing activity intensity data and PSG data (total 4080 epochs) for the 17 participants in Group A. Because data during the 4 minutes before and after the evaluation epoch were needed for the development of an algorithm, activity intensity data staring 4 minutes before and ending 4 minutes after the PSG recording were used in the study.

Sleep/wake agreement rate, sensitivity, specificity

Using the S/W scoring algorithm developed, the overall agreement rate, sensitivity, and specificity were calculated for the entire recording period as well as for each sleep stage (Stage Wake, Stage REM, Stage 1, Stage 2, and Stage 3 + 4) for each participant in both groups. The overall agreement rate indicates how closely each and all epoch determinations (S_{PSG}, W_{PSG}) by PSG match the activity intensity score (S_{ACT}, W_{ACT}) for each corresponding epoch. The agreement rate for each sleep stage determined by PSG (Stage Wake, Stage REM, Stage 1, Stage 2, and Stage 3 + 4) is the percentage of how closely the activity intensity score (S_{ACT}, W_{ACT}) matches that calculated for each sleep stage. Sensitivity is the ratio of S_{ACT} to S_{PSG} during the entire recording period. Specificity is the ratio of W_{ACT} to W_{PSG} during the entire recording period.

Optimizing the definitions of sleep parameters

Sleep latency (SL), total sleep time (TST), wake after sleep onset (WASO), and sleep efficiency (SE) were calculated using the S/W data obtained from PSG and activity intensity data for each 2-min epoch [13]. Calculation was performed using the S/W data obtained during time in bed (TIB), where TIB was defined as the recording period, starting at 0:00 when the lights were turned off and ending at 8:00 when the lights were turned on next morning.

The definitions of sleep parameters calculated from the PSG data are as follow:

SL_{PSG} - The interval between the time lights were turned off and the time of the first epoch when any of the sleep stages appeared (sleep-onset time).
TST_{PSG} - The total period of time when sleep (S_{PSG}) appeared, from the time of sleep-onset to the time when lights were turned on.

$WASO_{PSG}$ - The time in bed (TIB) from which SL_{PSG} and TST_{PSG} were subtracted.
SE_{PSG} - The ratio of TST_{PSG} to TIB.

The definitions of sleep parameters calculated from the activity intensity data are as follow:

SL_{ACT} - The interval between the time lights were turned off and the time of the first S_{ACT} (sleep-onset time) among the sleep states that appeared continuously for more than *n* epoch for the first time after the lights were turned off. *n* ranged from 1 to 15 (2 to 30 minutes), and SL_{ACT} was calculated for each. When S_{ACT} appeared continuously for more than *n* epochs for the first time after the lights were turned off, the epochs between lights-out and the first S_{ACT} were defined as W_{ACT}.
$WASO_{ACT}$ - The total time of W_{ACT} that appeared continuously for more than *n* epochs after sleep onset. *n* ranged from1 to 10 (2 to 20 minutes), and $WASO_{ACT}$ was calculated for each. When W_{ACT} appeared continuously for more than *n* epochs, the epochs were defined as W_{ACT}.
TST_{ACT} - The amount of time in TIB from which SL_{ACT} and $WASO_{ACT}$ were substracted.
SE_{ACT} - The ratio of TST_{ACT} to TIB.

For the calculation of SL_{ACT} and $WASO_{ACT}$, the values obtained from the criteria applied above to the values of SL_{PSG} and $WASO_{PSG}$ were compared, and the epoch numbers that would optimize the calculated results were sought. The optimization rules were to minimize the difference between average parameter values obtained by PSG for the 34 participants and the average parameter values obtained from the S/W algorithm so that the difference was not significant, and the intraclass correlation coefficient (ICC) was considered significant when ICC was ≥0.4 [14].

Statistics

Unpaired t-tests were used to compare, between Group A and Group B, the sensitivity, specificity, and agreement rates for both the entire recording period and each sleep stage. Paired t-tests were performed to compare sleep parameters determined from PSG and activity intensity data as well as sensitivity, specificity, and agreement rates obtained before and after the application of the optimization rules. Furthermore, ICC was calculated to analyze the correlation (agreement rate) between sleep parameters determined from PSG and activity intensity data. All data are expressed as mean ± SE. All statistical analysis was performed with IBM SPSS Statistics version 22.0. Statistical significance was set at $P < 0.05$.

Results

Sleep/wake scoring algorithm

The S/W scoring algorithm below was obtained by performing discriminant analysis using the activity intensity and PSG data (total 4,080 epochs) obtained from the 17 participants in Group A.

$$z = 0.24669x_{-2} + 0.2562x_{-1} + 0.408771x + 0.155046x_{+1} + 0.136728x_{+2}$$

Here, z ≥1 denotes wake (W_{ACT}) and z <1 denotes sleep (S_{ACT}). x_{-2}, x_{-1}, x, x_{+1}, and x_{+2} indicate the activity intensity at 4 minutes before the evaluation epoch, at 2 minutes before, at the evaluation epoch, at 2 minutes after the evaluation epoch, and at 4 minutes after, respectively.

Validity of the sleep/wake scoring algorithm

Table 1 shows the agreement rate, sensitivity, and specificity for each group between the S/W scoring algorithm using the activity intensity data and the S/W states determined visually from the PSG data. The agreement rates for the entire recording period for Group A and Group B were 84.7 ± 3.0% and 85.4 ± 2.8%, respectively, with no significant difference between the two groups ($t(32) = -0.157$, $P = 0.876$). Similarly, there was no significant difference between the two agreement rates calculated for each sleep stage.

Likewise, there was no significant difference in sensitivity and specificity of S/W determination between the groups. Therefore, equivalent score accuracies were obtained not only for the data in Group A, which were calculated using the S/W algorithm, but also for the data in Group B, which were independent sample sets.

Optimizing the calculation of sleep parameters with the FS-750

Since there were no significant difference between the agreement rates for Group A and Group B for the entire recording period or each sleep stage, the data were analyzed by merging the data for both groups. Among the

Table 1 Accuracy of sleep/wake determination using the FS-750 actigraph

		Group A[a]	Group B[a]	*t*	*p*
Agreement rates (%)	Overall	84.7 ± 3.0	85.4 ± 2.8	−0.157	0.876
	Stage W	66.0 ± 6.3	64.9 ± 7.1	0.114	0.910
	Stage 1	65.5 ± 6.5	65.2 ± 5.0	0.034	0.973
	Stage 2	91.5 ± 2.0	93.4 ± 1.2	−0.791	0.435
	Stage 3 + 4	99.0 ± 0.6	98.2 ± 0.7	0.884	0.383
	Stage REM	84.3 ± 5.3	82.4 ± 3.7	0.303	0.764
Sensitivity (%)		88.3 ± 2.8	90.0 ± 1.5	−0.522	0.605
Specificity (%)		66.0 ± 6.3	64.9 ± 7.1	0.114	0.910

[a]Values are expressed as mean ± SE.

four sleep parameters, total sleep time (TST) and sleep efficiency (SE) are dependent on the values of sleep latency (SL) and wake after sleep onset (WASO), and therefore the optimization of SL_{ACT} and $WASO_{ACT}$ was attempted.

For the continuous number of epochs n =1 to 11 based on the definition of SL_{ACT} (see the Methods), there was no significant difference between SL_{ACT} and SL_{PSG}. The difference was minimal when n was set to six epochs (Figure 1.) There was a significant intraclass correlation (ICC) between SL_{ACT} and SL_{PSG} when n was set between 6 and 11 (P <0.05), with ICC being ≥0.4 when n was 7 to 8. Therefore, the number of continuous epochs was chosen to be seven for SL_{ACT}.

The optimization of $WASO_{ACT}$ was examined setting SL_{ACT} to seven epochs. In the range of one to six continuous epochs based on the definition of $WASO_{ACT}$ (see the Methods), there was no significant difference between $WASO_{ACT}$ and $WASO_{PSG}$. The difference was minimal when n was set to four epochs (Figure 2). For a range between one and seven epochs, there was a significant intraclass correlation between $WASO_{ACT}$ and $WASO_{PSG}$ (P <0.01), with ICC being ≥0.4 in the corresponding range. As a result, the number of continuous epochs chosen for $WASO_{ACT}$ was four.

The sleep parameters calculated by PSG and the activity intensity using the most optimal criteria (SL_{ACT}: n =7 epochs, $WASO_{ACT}$: n =4 epochs) are shown in Table 2. For each item, no significant difference was seen between the sleep parameters calculated using the PSG data and those calculated using the activity intensity, and there was a significant intraclass correlation between the two.

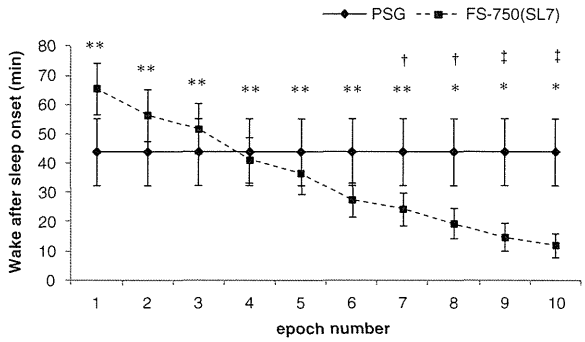


Figure 2 Optimization of wake after sleep onset determined by the FS-750 ($WASO_{ACT}$). $WASO_{ACT}$ is the total time that wake epochs determined by the FS-750 (W_{ACT}) appeared continuously for more than n epochs after sleep onset. The horizontal axis shows the n defined above. The vertical axis shows wake after sleep onset (min) defined for each n. *P < .05 and **P < .01, significant intraclass correlation between wake after sleep onset determined by polysomnography ($WASO_{PSG}$) and $WASO_{ACT}$. †P < .05 and ‡P < .01, significant difference between $WASO_{PSG}$ and $WASO_{ACT}$ (paired t-test). Values are expressed as mean ± SE.

Table 3 shows overall agreement rates, sensitivity (agreement rate for sleep state), and specificity (agreement rate for wakeful state) before and after the optimization of SL_{ACT} and $WASO_{ACT}$. Despite a reduction in specificity between before optimization (SL_{ACT}: n =1 epochs, $WASO_{ACT}$: n =1 epochs) and after optimization (SL_{ACT}: n =7 epochs, $WASO_{ACT}$: n =4 epochs), overall agreement rates and sensitivity were improved significantly.

Discussion

It became clear that by using the S/W scoring algorithm developed in this study, the sleep/wake states of healthy

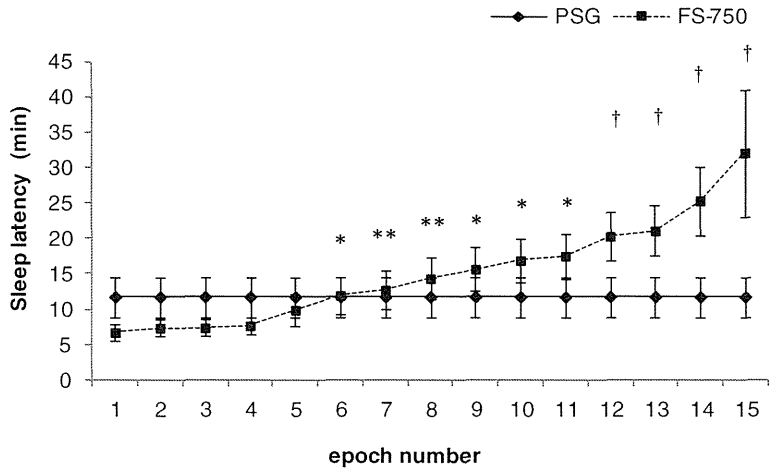


Figure 1 Optimization of sleep latency determined by the FS-750 (SL_{ACT}). SL_{ACT} is the interval between the time lights were turned off and the time of the first S_{ACT} (sleep-onset time) among the sleep states that appeared continuously for more than n epoch for the first time after the lights were turned off. The horizontal axis shows the n defined above. The vertical axis shows sleep latency (min) defined for each n. *P < .05 and **P < .01, significant intraclass correlation between sleep latency determined by polysomnography (SL_{PSG}) and SL_{ACT}. †P < .05, significant difference between SL_{PSG} and SL_{ACT} (paired t-test). Values are expressed as mean ± SE.

Table 2 Optimized sleep parameters determined by the FS-750 actigraph

		Polysomnography	FS-750					
			Before optimization			After optimization		
		Mean ± SE	Mean ± SE	*t*	ICC	Mean ± SE	*t*	ICC
Sleep latency	min	11.8 ± 2.9	6.7 ± 1.1	1.766	0.124	12.7 ± 2.7	−0.310	0.403**
Wake after sleep onset	min	44.1 ± 11.5	69.5 ± 8.8	−2.360*	0.419**	41.2 ± 7.9	0.271	0.427**
Total sleep time	min	424.2 ± 13.0	403.8 ± 8.7	1.716	0.411**	426.1 ± 8.0	−0.165	0.412**
Sleep efficiency	%	88.4 ± 2.7	84.1 ± 1.8	1.716	0.411**	88.8 ± 1.7	−0.166	0.412**

*$P < .05$, **$P < .01$.

adults could be determined from the FS-750 actigraph data with sensitivity and specificity equivalent to that determined with conventional actigraph data. Although the agreement rates in Stage Wake and Stage 1 were relatively low (about 65%), the agreement rates were high for stages 2 to 4 (≥90%). As a result, the overall agreement rate for the entire recording period was equivalent to that obtained using conventional actigraphs (about 85%) [1-6,15]. The S/W scoring algorithm was developed in this study by using discriminant analysis that optimized differentiation between the sleep/wake states measured simultaneously by PSG. However, it should be pointed out that it would be difficult to determine the sleep states from the activity intensity data alone because mixtures of stages occur, such as when subjects are awake but motionless (silent awake) or during the early sleep-onset period when they are in light sleep and make some body movements. Accordingly, the agreement rate in Stage 1 or Stage Wake is generally lower compared to the agreement rate in other stages. According to a previous study [5], there is a tendency for the agreement rate to decrease during the light sleep stage, and the FS-750 shows a similar trend. The sleep/wake determination method thus appears to have acceptable determination accuracy given that the agreement rate for determining wakefulness by the FS-750 (specificity about 65%) is more than equivalent to that of conventional actigraphs [2-5,15,16].

The method was also optimized for calculating the sleep parameters SL, WASO, TST, and SE from the sleep/wake data for 2-minute epochs recorded by the FS-750. As a result, the values of these parameters could be predicted from the data obtained by the FS-750 and were very close to the values obtained by PSG (with no significant difference between them); there was also a significant intraclass correlation between the two. In addition, overall agreement rates were improved significantly after optimization. When looking at the values of the sleep parameters predicted by the FS-750 before the optimization (that is, when the number of continuous epochs for SL_{ACT} and $WASO_{ACT}$ were defined as $n = 1$), there was a tendency to underestimate SL and overestimate WASO (Figure 1, Table 2). By examining the criteria that minimized the differences between using PSG and activity intensity data, it was determined that SL_{ACT} ≥7 epochs (≥14 minutes of continuous sleep state determination) and $WASO_{ACT}$ ≥4 epochs (≥8 minutes of continuous wake state determination) were optimal to apply to the estimation rule for sleep parameters. Large individual differences are apparent in the activity intensity data, as actigraphy tends to misjudge a subject who moves a lot while sleeping (restless sleep) as awake and, conversely, misjudges a subject who is awake but motionless (silent awake) as sleeping. In fact, application of the optimization rules improved sensitivity significantly. On the other hand, as this rule attaches importance to the duration, because a short wake state

Table 3 Overall agreement rates, sensitivity, and specificity for sleep/wake states before and after the application of optimization rules

		Before optimization[a]	After optimization[a]	*t*	*p*
Agreement rates (%)	Overall	85.0 ± 2.0	88.4 ± 2.1	−6.087	<0.001
	Stage W	65.4 ± 4.7	56.5 ± 5.1	2.814	0.008
	Stage 1	65.4 ± 4.1	77.1 ± 3.9	−5.615	<0.001
	Stage 2	92.4 ± 1.2	96.2 ± 0.9	−7.159	<0.001
	Stage 3 + 4	98.6 ± 0.5	99.4 ± 0.4	−3.128	0.004
	Stage REM	83.3 ± 3.2	90.3 ± 2.8	−6.651	<0.001
Sensitivity (%)		89.1 ± 1.6	93.7 ± 1.4	−8.816	<0.001
Specificity (%)		65.4 ± 4.7	56.5 ± 5.1	2.814	0.008

[a]Values are expressed as mean ± SE (n =34).

(<8 minutes) is misjudged as sleep, specificity is reduced. Specificity and sensitivity can be considered trade-offs, and as such the rules developed in this study are among the most appropriate evaluation indices for maximizing overall agreement rates.

There are some limitations to this study in that the evaluation epoch periods and the wearing positions of the device are different from those used in the previous studies and the sleep/wake patterns of restless sleep, silent awake, and short wake state are difficult to classify. Moreover, 30-second PSG data were re-scored as data for 2-minute epochs in this study to enable the algorithm to be developed. A longer epoch is more likely to contain different sleep/wake states and therefore tends to have lower agreement rates. Indeed, we forcibly converted data for each epoch determined by FS-750 (1 epoch =2 minutes) from 34 subjects into data for the corresponding four 30-second epochs, and compared the obtained results (30-second FS750 scores) with 30-second PSG scores. The overall agreement rate (85.0% versus 84.8%) and specificity (65.4% versus 59.9%) were significantly lower when comparing 30-second FS-750 scores and 30-second PSG scores than when comparing the corresponding 2-minute epoch score sets. On the other hand, there was no significant change in sensitivity (89.1% versus 89.2%). These results suggest that judgment errors related to the relatively low time resolution (2 minutes) need to be taken into consideration when using the FS-750 actigraph in clinical and research studies.

Additionally, the subjects of this study were healthy young adults. The relationship between sleep conditions and activity intensity might be different for elderly individuals who have difficulty achieving good quality sleep as well as for those with sleep disturbances [17]. As a next step, the accuracy of sleep/wake determination by the FS-750 needs to be verified in a larger sample and in various populations so that its utility can be determined. Its use by individuals with medical conditions and in other age groups should be further investigated. In the meantime, however, given that the FS-750 has a large memory that can store recordings over a long period, as well as capabilities to download the recordings at home, the device should help to push forward objective sleep evaluations for the elderly who find it difficult to visit healthcare facilities regularly, for individuals with circadian sleep disturbances who need longer term sleep studies, and for those who live in remote areas. As such, it is anticipated that the device will be utilized widely in both epidemiology studies and clinical practice.

Conclusions

This study verified the accuracy of the sleep/wake states determined from the data obtained by the FS-750, an inexpensive device that has capabilities to download its data at home and which is a promising tool to use in large epidemiological studies and in remote sleep medicine. In addition, the algorithm developed can determine the sleep/wake states in 2-minute epochs using the activity intensity data measured by the FS-750. The agreement rate between the results calculated with the FS-750 and those calculated with PSG was high (85%), and the sensitivity and specificity of measurement using the FS-750 were equivalent to those determined with conventional actigraphs. The device's application to future sleep research and sleep medicine is expected.

Abbreviations

AHI: apnea and hypopnea index; ECG: electrocardiogram; EEG: electroencephalogram; EMG: electromyogram; EOG: electrooculogram; NFC: near field communication; PLMI: periodic limb movement index; PSG: polysomnography; RH: relative humidity; S_{ACT}: sleep epochs determined by the FS-750; SE: sleep efficiency; SL: sleep latency; SL_{ACT}: sleep latency determined by the FS-750; SL_{PSG}: sleep latency determined by PSG; S_{PSG}: sleep epochs determined by PSG; S/W: sleep/wake; TIB: time in bed; TST: total sleep time; W_{ACT}: wake epochs determined by the FS-750; WASO: wake after sleep onset; $WASO_{ACT}$: wake after sleep onset determined by the FS-750; $WASO_{PSG}$: wake after sleep onset determined by PSG; W_{PSG}: wake epochs determined by PSG.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

KN conducted the study, performed the statistical analysis, and drafted the manuscript. SK conceived and designed the study and contributed to drafting the manuscript. YM, AH, and YK conducted the measurement of data and contributed to drafting the manuscript. NM contributed to the discriminant analysis. KM organized and supervised the study, and contributed to drafting the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This study was supported by a Grant-in-Aid for the Strategic Research Program for Brain Sciences (Understanding of molecular and environmental bases for brain health) from the Ministry of Education, Culture, Sports, Science and Technology of Japan and an Intramural Research Grant (23-3) for Neurological and Psychiatric Disorders from the National Center of Neurology and Psychiatry. We thank Ms. Sayaka Kohtoh and Mr. Yujiro Taguchi at KISSEI COMTEC Co. for assisting with data analysis.

Author details

[1]Department of Psychophysiology, National Institute of Mental Health, National Center of Neurology and Psychiatry, 4-1-1 Ogawa-higashi, Kodaira, Tokyo 187-8553, Japan. [2]National Center Hospital, National Center of Neurology and Psychiatry, 4-1-1 Ogawa-higashi, Kodaira, Tokyo 187-8551, Japan. [3]Structural Materials Sector, Materials Science Research Laboratory, Central Research Institute of Electric Power Industry, 2-6-1 Nagasaka, Yokosuka, Kanagawa 240-0196, Japan.

Received: 20 February 2014 Accepted: 5 September 2014
Published: 4 October 2014

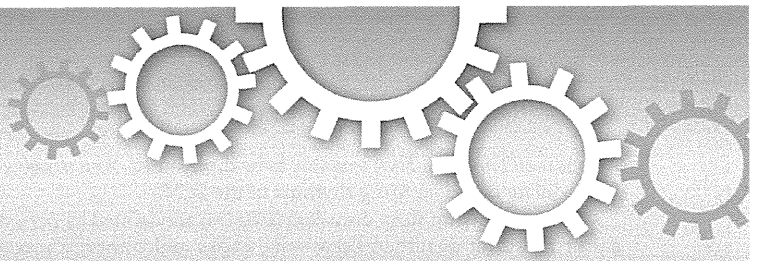
References

1. Webster JB, Kripke DF, Messin S, Mullaney DJ, Wyborney G: **An activity-based sleep monitor system for ambulatory use.** *Sleep* 1982, 5:389–399.
2. Cole RJ, Kripke DF, Gruen W, Mullaney DJ, Gillin JC: **Automatic sleep/wake identification from wrist activity.** *Sleep* 1992, 15:461–469.
3. Jean-Louis G, Kripke DF, Cole RJ, Assmus JD, Langer RD: **Sleep detection with an accelerometer actigraph: comparisons with polysomnography.** *Physiol Behav* 2001, 72:21–28.

4. Jean-Louis G, Kripke DF, Mason WJ, Elliott JA, Youngstedt SD: **Sleep estimation from wrist movement quantified by different actigraphic modalities.** *J Neurosci Methods* 2001, **105**:185–191.
5. Enomoto M, Endo T, Suenaga K, Miura N, Nakano Y, Kohtoh S, Taguchi Y, Aritake S, Higuchi S, Matsuura M, Takahashi K, Mishima K: **Newly developed waist actigraphy and its sleep/wake scoring algorithm.** *Sleep Biol Rhythms* 2009, **7**:17–22.
6. Sadeh A, Sharkey KM, Carskadon MA: **Activity-based sleep-wake identification: an empirical test of methodological issues.** *Sleep* 1994, **17**:201–207.
7. Ancoli-Israel S, Cole R, Alessi C, Chambers M, Moorcroft W, Pollak CP: **The role of actigraphy in the study of sleep and circadian rhythms.** *Sleep* 2003, **26**:342–392.
8. Morgenthaler T, Alessi C, Friedman L, Owens J, Kapur V, Boehlecke B, Brown T, Chesson A Jr, Coleman J, Lee-Chiong T, Pancer J, Swick TJ: **Practice parameters for the use of actigraphy in the assessment of sleep and sleep disorders: an update for 2007.** *Sleep* 2007, **30**:519–529.
9. Acebo C, Sadeh A, Seifer R, Tzischinsky O, Hafer A, Carskadon MA: **Sleep/wake patterns derived from activity monitoring and maternal report for healthy 1- to 5-year-old children.** *Sleep* 2005, **28**:1568–1577.
10. Iwata S, Iwata O, Matsuishi T: **Sleep patterns of Japanese preschool children and their parents: implications for co-sleeping.** *Acta Paediatr* 2013, **102**:e257–262.
11. Benson K, Friedman L, Noda A, Wicks D, Wakabayashi E, Yesavage J: **The measurement of sleep by actigraphy: direct comparison of 2 commercially available actigraphs in a nonclinical population.** *Sleep* 2004, **27**:986–989.
12. Rechtschaffen A, Kales A (Eds): *A Manual of Standardized Terminology, Technique and Scoring System for Sleep Stages of Human Subjects.* Washington, D. C: Public Health Service; 1968.
13. Iber C, Ancoli-Israel S, Chesson A, Quan S: *The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications.* Westchester, illinois: American Academy of Sleep Medicine; 2007.
14. Landis JR, Koch GG: **The measurement of observer agreement for categorical data.** *Biometrics* 1977, **33**:159–174.
15. Kogure T, Shirakawa S, Shimokawa M, Hosokawa Y: **Automatic sleep/wake scoring from body motion in bed: validation of a newly developed sensor placed under a mattress.** *J Physiol Anthropol* 2011, **30**:103–109.
16. de Souza L, Benedito-Silva AA, Pires ML, Poyares D, Tufik S, Calil HM: **Further validation of actigraphy for sleep studies.** *Sleep* 2003, **26**:81–85.
17. Kushida CA, Chang A, Gadkary C, Guilleminault C, Carrillo O, Dement WC: **Comparison of actigraphic, polysomnographic, and subjective assessment of sleep parameters in sleep-disordered patients.** *Sleep Med* 2001, **2**:389–396.

doi:10.1186/1880-6805-33-31
Cite this article as: Nakazaki *et al.*: Validity of an algorithm for determining sleep/wake states using a new actigraph. *Journal of Physiological Anthropology* 2014 **33**:31.

SCIENTIFIC REPORTS



OPEN

SUBJECT AREAS:
CIRCADIAN RHYTHMS AND SLEEP
GENETIC ASSOCIATION STUDY

Received
9 May 2014

Accepted
19 August 2014

Published
9 September 2014

Correspondence and requests for materials should be addressed to K.M. (mishima@ncnp.go.jp)

Screening of Clock Gene Polymorphisms Demonstrates Association of a *PER3* Polymorphism with Morningness–Eveningness Preference and Circadian Rhythm Sleep Disorder

Akiko Hida[1], Shingo Kitamura[1], Yasuko Katayose[1], Mie Kato[1], Hiroko Ono[1], Hiroshi Kadotani[2], Makoto Uchiyama[3], Takashi Ebisawa[4], Yuichi Inoue[5,6], Yuichi Kamei[7], Masako Okawa[8], Kiyohisa Takahashi[8] & Kazuo Mishima[1]

[1]Department of Psychophysiology, National Institute of Mental Health, National Center of Neurology and Psychiatry, Kodaira, Tokyo 187-8553, Japan, [2]Department of Psychiatry, Shiga University of Medical Science, Otsu, Shiga 520-2192, Japan, [3]Department of Psychiatry, Nihon University School of Medicine, Itabashi, Tokyo 173-8610, Japan, [4]Department of Psychiatry, Tokyo Metropolitan Police Hospital, Nakano, Tokyo 164-8541, Japan, [5]Department of Somnology, Tokyo Medical University, Shinjuku, Tokyo 160-8402, Japan, [6]Yoyogi Sleep Disorder Center, Shibuya, Tokyo 151-0053, Japan, [7]Department of Laboratory Medicine, National Center Hospital, National Center of Neurology and Psychiatry, Kodaira, Tokyo 187-8551, Japan, [8]Japan Foundation for Neuroscience and Mental Health, Kodaira, Tokyo 187-8551, Japan.

A system of self-sustained biological clocks controls the 24-h rhythms of behavioral and physiological processes such as the sleep–wake cycle. The circadian clock system is regulated by transcriptional and translational negative feedback loops of multiple clock genes. Polymorphisms in circadian clock genes have been associated with morningness–eveningness (diurnal) preference, familial advanced sleep phase type (ASPT), and delayed sleep phase type (DSPT). We genotyped single-nucleotide polymorphisms in circadian clock genes in 182 DSPT individuals, 67 free-running type (FRT) individuals, and 925 controls. The clock gene polymorphisms were tested for associations with diurnal preference and circadian rhythm sleep disorder (CRSD) phenotypes. The *PER3* polymorphism (rs228697) was significantly associated with diurnal preference and the FRT phenotype. The minor allele of rs228697 was more prevalent in evening types than in morning types (sex-adjusted odds ratio (OR), 2.483, Bonferroni-corrected $P = 0.012$) and in FRT individuals compared with the controls (age- and sex-adjusted OR, 2.021, permutated $P = 0.017$). Our findings support the notion that *PER3* polymorphisms could be a potential genetic marker for an individual's circadian and sleep phenotypes.

Sleep–wake cycles are regulated by two components, homeostatic drive and circadian drive[1]. Sleep and wakefulness occur sequentially, and sleep propensity increases gradually with extended wakefulness and decreases rapidly during sleep. Sleep propensity is under the control of sleep homeostasis, and sleep timing is under the control of circadian clocks. The circadian clock system regulates daily behavioral and physiological rhythms such as body temperature, hormone secretion, blood pressure, metabolism, and cognitive performance besides sleep/wakefulness. These rhythms are generated by the central circadian oscillator located in the suprachiasmatic nucleus (SCN) of the hypothalamus and are entrained by environmental cues (e.g., light–dark cycles)[2,3]. The molecular mechanism of the circadian clock system involves transcription-translation negative feedback loops of multiple clock genes and post-transcriptional and post-translational modification and degradation of clock proteins[4,5]. The transcription factors BMAL1 and CLOCK form heterodimers, which activate transcription of *Cryptochrome* (*Cry*) and *Period* (*Per*) by binding to E-box motifs in their promoter regions. CRY and PER proteins gradually accumulate in the cytoplasm. Phosphorylation of CRY and PER is regulated by casein kinase I (CKI). CRY, PER, and CKI proteins form complexes that translocate to the nucleus and interact with the BMAL1–CLOCK heterodimers, thereby inhibiting transcription of the *Cry* and *Per* genes. Although the circadian

function of *Timeless* (*Tim*) remains to be determined, *Tim* is known to modulate neuronal firing rhythms in the SCN[6].

Circadian rhythm sleep disorders (CRSDs) are defined by persistent or recurrent disturbed sleep–wake cycles and comprise several subtypes: advanced sleep phase type (ASPT), delayed sleep phase type (DSPT), and free-running type (FRT). ASPT is characterized by extremely early involuntary sleep timing, DSPT by significantly delayed sleep timing, and FRT by sleep timing that occurs with a 30-min to 1-h delay each day. CRSD is thought to result from impairment of the circadian clock system[7–9]. Also, circadian characteristics vary greatly among individuals[10,11]. The inter-individual differences in daily activity/sleep time are known as morningness–eveningness (diurnal) preference. The morning type manifests earlier timings for sleep and physiological rhythms than the intermediate type, and still earlier than the evening type[12,13]. Individual diurnal preference is morning type during childhood, which subsequently switches to evening type during adolescence before starting to return to morning type in adulthood[14,15]. Males show evening preference while females show more morning preference, but this gender-related difference disappears in the elderly[11,16]. The diurnal preference is commonly assessed by self-reported questionnaires, the Horne–Östberg Morningness–Eveningness Questionnaire (MEQ)[10] and the recently developed Munich ChronoType Questionnaire (MCTQ)[11]. The diurnal preference and CRSD phenotypes are thought to be influenced by genetic factors. For example, polymorphisms in the *CLOCK*, *NPAS2*, *PER2*, and *TIM* genes are associated with diurnal preference[17,18], sleep timing[19], and sleep disorders[20–22]. Furthermore, the *PER3* gene has polymorphisms in the promoter region[23], missense polymorphisms that result in amino acid substitution and a variable number tandem repeat (VNTR) consisting of either 4 or 5 repeated 54-bp sequences encoding 18 amino acids (*PER3*^{4} or *PER3*^{5})[24]. These *PER3* polymorphisms are associated with diurnal preference and/or DSPT[23–25]. Although the results of some other studies are inconsistent with the associations[26,27], a number of genetic studies have shown that genetic factors significantly contribute to individual differences in circadian and sleep phenotypes[28–30].

Missense mutations in the *PER2* and *CKIδ* genes have been found in large pedigrees with familial ASPT[31,32]. These amino acid substitutions in PER2 and CKIδ reduce the phosphorylation level of PER2, thereby shortening the intrinsic circadian period (τ) and giving rise to the familial ASPT phenotype. These findings indicate that clock gene polymorphisms, especially missense polymorphisms, may alter the function of these genes, thereby modifying diurnal preference and sleep–wake patterns. To investigate how genetic variations impact circadian and sleep phenotypes, we genotyped single-nucleotide polymorphisms (SNPs) in a number of circadian clock genes in controls and DSPT and FRT patients and tested these SNPs for associations with diurnal preference in controls and in patients with the CRSD phenotype. Our control subjects' diurnal preference was assessed by the Horne–Östberg MEQ[10]. Age-adjusted MEQ scores of 16–41 denote evening types, 42–58 denote intermediate types, and 59–86 denote morning types. The DSPT and FRT patients were diagnosed according to the International Classification of Sleep Disorders 2 (ICSD-2)[33].

Results

Association between *PER3* and diurnal preference. The 925 controls consisted of 245 morning types (79 men and 166 women; mean ± SD age-adjusted MEQ score: 62.81 ± 3.77), 594 intermediate types (163 men and 431 women; mean ± SD age-adjusted MEQ score: 51.13 ± 4.31), and 86 evening types (32 men and 54 women; mean ± SD age-adjusted MEQ score: 38.23 ± 3.55). Men showed more evening preference than women ($\chi^2 = 9.077$, $P = 0.011$; adjusted residual = ±2.3). The allele frequency of the 9 SNPs in 6 genes was compared among morning, intermediate, and evening types (Table 1). As shown in Table 1, only the SNP rs228697 in *PER3*

Table 1 | Genotype and minor allele frequencies of 9 SNPs in 6 genes in morning, intermediate, and evening types

				M (N = 245)			I (N = 594)			E (N = 86)			M	I	E	
Gene	SNP	Allele (A/a)	Variation (amino acid)	AA	Aa	aa	AA	Aa	aa	AA	Aa	aa	MAF			P (χ2)
CLOCK	rs1801260	T/C		0.714	0.269	0.016	0.714	0.263	0.024	0.64	0.302	0.058	0.151	0.155	0.209	0.162
NPAS2	rs2305160	G/A	A/T	0.629	0.322	0.049	0.677	0.288	0.035	0.628	0.291	0.081	0.21	0.179	0.227	0.163
PER1	rs2585405	C/G	P/A	0.339	0.502	0.159	0.347	0.461	0.192	0.349	0.512	0.14	0.41	0.423	0.395	0.75
PER2	rs2304672	C/G		0.873	0.127	0	0.904	0.094	0.002	0.872	0.128	0	0.063	0.049	0.064	0.411
	rs934945	G/A	G/E	0.478	0.396	0.127	0.476	0.441	0.082	0.465	0.43	0.105	0.324	0.303	0.32	0.662
PER3	rs228697	C/G	P/A	0.898	0.102	0	0.833	0.163	0.003	0.791	0.186	0.023	0.051	0.085	0.116	0.01*
	rs2640909	T/C	M/T	0.812	0.184	0.004	0.771	0.214	0.015	0.767	0.198	0.035	0.096	0.122	0.134	0.238
TIM	rs774047	A/G	Q/R	0.363	0.449	0.188	0.347	0.463	0.19	0.372	0.43	0.198	0.412	0.422	0.413	0.927
	rs2291739	C/T	P/L	0.347	0.445	0.208	0.301	0.49	0.209	0.326	0.442	0.233	0.431	0.454	0.453	0.679

A, major allele; a, minor allele; M, morning type; I, intermediate type; E, evening type; N, number; *P*, *P* value for the difference in MAF among M, I and E;
*, $P < 0.05$.

was significantly associated with diurnal preference ($\chi^2 = 9.157$, $P = 0.010$). The major allele C of rs228697 was more common in morning types than in evening types, and the minor allele G of rs228697 was more common in evening types than in morning types (sex-adjusted odds ratio (OR), 2.483; 95% confidence interval (CI), 1.339–4.603; Bonferroni-corrected $P = 0.012$; crude $P = 0.004$). Subjects with the G-positive genotype (CG, GG) for rs228697 showed lower age-adjusted MEQ scores than those with the G-negative genotype (CC) for rs228697 (mean ± SEM age-adjusted MEQ score: 51.70 ± 0.68 vs 53.26 ± 0.29; F(1, 922) = 4.561; $P = 0.033$).

Association between *PER3* and FRT. We then assessed the probability of the 9 SNPs producing DSPT and FRT phenotypes (Table 2). The SNP distributions did not differ between the controls and DSPT individuals. However, the rs228697 distribution significantly differed between controls and FRT individuals. The frequency of the G allele for rs228697 was significantly increased in FRT individuals compared with controls (age- and sex-adjusted OR, 2.021; 95% CI, 1.160–3.524; permutated $P = 0.017$; crude $P = 0.011$). The G-positive genotype (CG, GG) for rs228697 was more prevalent in FRT individuals (CG, GG, 0.284) than in controls (CG, GG, 0.154) with an age- and sex-adjusted OR of 2.253 (95% CI, 1.233–4.118; $P = 0.008$). Therefore, the *PER3* SNP rs228697 was significantly associated with the FRT phenotype in this cohort.

Discussion

We found that the G allele of rs228697 in *PER3* was more common in evening types than in morning types and in FRT individuals than in controls using a very large sample of control individuals and CRSD patients. These findings are in accordance with previous reports[23–25] suggesting genetic associations between *PER3* polymorphisms and diurnal preference and/or CRSD phenotypes. A *PER3* haplotype defined by the G allele of rs10462020 and the C allele of rs10462021 has been shown to be related to DSPT[24]. Furthermore, the 4-repeat allele of *PER3* VNTR (*PER3*^{4}) has been associated with extreme evening preference and DSPT, whereas the 5-repeat allele of *PER3* VNTR (*PER3*^{5}) has been associated with extreme morning preference[25]. In addition, the polymorphisms in the *PER3* promoter have been associated with DSPT[23]. Although the SNPs rs10462020 and rs10462021 were excluded from further analysis due to the low minor allele frequency (MAF) < 0.05, and the *PER3*^{4} and *PER3*^{5} alleles as well as the previously described polymorphisms in the *PER3* promoter, were not directly investigated in this study, the present and previous findings strongly suggest that *PER3* polymorphisms can provide potential biomarkers for estimating individual diurnal preference and CRSD phenotypes.

Morning types and evening types have been reported to differ in homeostatic sleep regulation and neurobehavioral functions in response to sleep fragmentation and sleep deprivation[29]. Mongrain et al. have demonstrated that the morning type exhibits a higher initial level and faster dissipation rate of sleep pressure than the evening type[34]. These results suggest that diurnal preference may reflect individual differences in both homeostatic and circadian regulation. Moreover, there are inter-individual differences in the impairment of neurobehavioral functions (attention, decision making, etc.) in response to sleep deprivation and sleep restriction[35–37]. A number of studies indicate that genetic traits contribute to individual differences in sleep homeostasis, circadian rhythms, and cognitive performance[28,38]. Individuals homozygous for *PER3*^{5} demonstrate more morning preference, greater sleep propensity at baseline and after sleep deprivation, and a lower level of cognitive performance than those homozygous for *PER3*^{4} [39,40]. The results of our previous study have implied that an altered expression profile of *PER3* may reflect deteriorated homeostatic sleep drive in the elderly[41]. Additionally, Archer et al. have reported that the polymorphisms in the *PER3*

Table 2 | Genotype and minor allele frequencies of 9 SNPs in 6 genes in controls, DSPT, and FRT individuals

				Control (N = 925)			DSPT (N = 182)			FRT (N = 67)			Control	DSPT	FRT		
Gene	SNP	Allele (A/a)	Variation (amino acid)	AA	Aa	aa	AA	Aa	aa	AA	Aa	aa	MAF	MAF	permutated P ($\chi 2$)	MAF	permutated P ($\chi 2$)
CLOCK	rs1801260	T/C		0.707	0.268	0.025	0.665	0.297	0.038	0.687	0.284	0.03	0.159	0.187	0.218	0.172	0.715
NPAS2	rs2305160	G/A	A/T	0.659	0.297	0.043	0.698	0.264	0.038	0.612	0.343	0.045	0.192	0.17	0.355	0.216	0.502
PER1	rs2585405	C/G	P/A	0.345	0.477	0.178	0.335	0.538	0.126	0.403	0.418	0.179	0.417	0.396	0.489	0.388	0.535
PER2	rs2304672	C/G		0.893	0.106	0.001	0.918	0.077	0.005	0.866	0.119	0.015	0.054	0.044	0.666	0.075	0.539
	rs934945	G/A	G/E	0.476	0.428	0.096	0.516	0.401	0.082	0.448	0.507	0.045	0.31	0.283	0.518	0.299	0.950
PER3	rs228697	C/G	P/A	0.846	0.149	0.004	0.846	0.143	0.011	0.716	0.284	0	0.079	0.082	0.941	0.142	0.017*
	rs2640909	T/C	M/T	0.782	0.204	0.014	0.775	0.198	0.027	0.657	0.343	0	0.116	0.126	0.765	0.172	0.088
TIM	rs774047	A/G	Q/R	0.354	0.456	0.19	0.407	0.467	0.126	0.284	0.522	0.194	0.418	0.36	0.058	0.455	0.527
	rs2291739	C/T	P/L	0.316	0.474	0.211	0.363	0.489	0.148	0.313	0.478	0.209	0.448	0.393	0.082	0.448	1

A, major allele; a, minor allele; N, number; MAF, minor allele frequency; *P*, *P* value for the difference in MAF between DSPT or FRT patients and controls;
*, $P < 0.05$.

Table 3 | Genotype and minor allele frequencies of 30 SNPs in 7 genes in 925 controls

Gene	SNP	Allele (A/a)	Variation (amino acid)	AA	Aa	aa	MAF	*P* (HWE)	Genotyping Assay ID
CLOCK	rs34897046	C/G	S/C	1	0	0	0	1	C__25595098_10
	rs6855837	C/A	L/I	1	0	0	0	1	C__29101689_10
	rs3762836	A/G	H/R	0.981	0.019	0	0.01	1	C__27479322_10
	rs1801260	T/C		0.707	0.268	0.025	0.159	1	C___8746719_20
CRY2	rs2863712	T/G	W/G	1	0	0	0	1	C__16079564_10
NPAS2	rs34628006	A/G	T/A	1	0	0	0	1	C__25757964_10
	rs2305160	G/A	A/T	0.659	0.297	0.043	0.192	0.243	C__15976652_10
	rs11541353	C/T	S/L	1	0	0	0	1	C___2153849_10
	rs58728948	G/A	A/T	1	0	0	0	1	C__25757546_10
PER1	rs2585405	C/G	P/A	0.345	0.477	0.178	0.417	0.592	C__16260899_10
	rs3027193	G/A	R/H	1	0	0	0	1	C__15770159_10
PER2	rs2304672	C/G		0.893	0.106	0.001	0.054	0.472	C___2129919_1_
	rs35572922	G/T	A/S	1	0	0	0	1	C__25973284_20
	rs4429421	G/A	V/I	0.991	0.009	0	0.004	1	C__27970170_10
	rs35333999	G/A	V/I	1	0	0	0	1	C__25992030_10
	rs35998480	T/A	F/Y	1	0	0	0	1	C__25958587_10
	rs934945	G/A	G/E	0.476	0.428	0.096	0.31	1	C___8740718_20
PER3	rs10462020	T/G	V/G	0.928	0.07	0.002	0.037	0.731	C__25956444_10
	rs35687686	C/T	R/W	1	0	0	0	1	C__25970031_10
	rs228696	C/T	L/P	1	0	0	0	1	C_____10225_10
	rs35899625	T/G	L/W	1	0	0	0	1	C__25967284_10
	rs228697	C/G	P/A	0.846	0.149	0.004	0.079	0.606	C_____10224_10
	rs2640909	T/C	M/T	0.782	0.204	0.014	0.116	0.96	
	rs2640905	C/G	S/C	1	0	0	0	1	C__16268919_10
	rs35802556	C/T	T/I	1	0	0	0	1	
	rs10462021	A/G	H/R	0.928	0.07	0.002	0.037	0.731	
	rs35072750	A/G	T/A	1	0	0	0	1	C__25963142_20
TIM	rs774027	T/A	L/I	0.359	0.453	0.188	0.415	0.048	C___8340562_10
	rs774047	A/G	Q/R	0.354	0.456	0.19	0.418	0.064	C___3134218_10
	rs2291739	C/T	P/L	0.316	0.474	0.211	0.448	0.215	C__15966257_10

A, major allele; a, minor allele; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; *P*, *P* value for HWE.

promoter are associated with DSPT and that these polymorphisms have an effect on its expression level[23]. The data reported here showed that the *PER3* SNP rs228697 was associated with diurnal preference and FRT phenotype in our large sample of CRSD patients and controls. These findings suggest that the *PER3* gene may play a functional role in homeostatic sleep and/or circadian clock systems. Furthermore, *PER3* polymorphisms may predict individual differences in vulnerability to sleep deprivation and restriction.

The SNP rs228697 (C/G) corresponds to the SNP in exon 17 of the *PER3* gene and causes the amino acid substitution (P864A)[24]. Although the amino acid substitution of proline (P) to alanine (A) does not make a significant difference to polarity or hydrophobicity, the amino acids P and A differ in their hydropathy index. Accordingly, the P/A substitution could alter the secondary structure and/or phosphorylation status of *PER3* leading to dysregulation of the homeostatic sleep and circadian clock systems. It is intriguing that P864A is located in potential Src homology (SH)3 domains in PER3. SH3 domains are found in many proteins in an array of signaling pathways that regulate the cytoskeleton, the Ras gene family, and the Src kinase family, as well as many other signaling cascades[42,43]. The proline-rich motif X-P-X-X-P is defined as the minimal consensus sequence of the SH3-binding sites. X is often an aliphatic amino acid[44]. The P864A substitution would disrupt two potential SH3-binding motif domains in PER3 (LPDPP(864) and PP(864)VCP), which could alter the binding interaction between PER3 and its partner protein(s), thereby disturbing the function of PER3 in the homeostatic and circadian regulation of sleep. CKI interacts with the scaffolding protein NCK that consists of SH2 and SH3 domains[45]. As CKI is known to regulate PER protein stability[46,47], it is interesting to speculate that the interaction between CKI and PER3 may be partially mediated via the scaffolding afforded by the interactions between the SH3 domain in NCK and the putative SH3 ligand in PER3. However, how this allelic variation of *PER3* modifies sleep and circadian phenotypes remains to be determined.

FRT is defined by sleep timing that occurs with a 30-min to 1-h delay each day. Therefore, prolongation of τ has been considered a critical factor for determining the FRT phenotype[7–9]. Free-running patterns in sleep–wake cycles are often observed in blind individuals[48]. Because totally blind individuals are not capable of perceiving photic signals, some may show free-running sleep patterns as a consequence of the loss of photic entrainment. Thus, the pathophysiology of sighted individuals with FRT might also be associated with an impaired photic entrainment mechanism as well as with prolonged τ. We recently reported that sighted FRT patients have a longer τ than intermediate types, but not when compared with evening types[49]. Moreover, the results of the present study demonstrate that extreme evening preference and FRT are associated with the same polymorphism in the *PER3* gene. Based upon these findings, there may be a genetic trait or traits shared by individuals with extreme evening preference and FRT phenotypes. In contrast, DSPT was not associated with any polymorphisms in the *PER3* gene or other clock genes investigated in this study. It appears likely that this results from the heterogeneity of the DSPT population. Not only circadian clock defects, but also psychiatric problems and/or consequent reduction of social (non-photic) entrainment are thought to be causative factors for the development of DSPT phenotypes[50].

There are some limitations to this study. It would be difficult to conduct a replication study using another sample of patients due to the extremely low prevalence of CRSDs in the Japanese population (0.13%)[51]. Other groups have shown associations of *PER3* and diurnal preference and/or CRSD phenotypes using their samples[23–25]. We did not control for medical treatments in our subjects, such as medication and light therapy, although these treatments would only alter the medical condition of patients and not their diagnoses.

Table 4 | Sequences for primers and TaqMan probes in *PER3*

Gene	SNP	Allele (A/a)	Forward primer	Reverse primer	TaqMan probes	
PER3	rs2640909	T/C	5′-CGCCTCCCATGAAGAATCCA-3′	5′-TGGCAGTAGGATGGGATGGA-3′	5′-FAM-CAATCCCATGGACAGTG-NFQ-3′	5′-VIC-AATCCCGTGGACAGTG-NFQ-3′
	rs35802556	C/T	5′-GAAGAGCCCATCTGGAGAATGAT-3′	5′-GGTACCTGGTATGTCATGAGAATGC-3′	5′-FAM-CTCAGGTGTCTGCCG-NFQ-3′	5′-VIC-CTCAGGTATCTGCCG-NFQ-3′
	rs10462021	A/G	5′-GAAGACCTGGAAAAGCTAGAAAGTATGA-3′	5′-ACCTTAGCCAGCTCCTCCTTT-3′	5′-FAM-CCAGTTTTCTCATGGGCA-NFQ-3′	5′-VIC-CCAGTTTTCTCGTGGGCA-NFQ-3′

A, major allele; a, minor allele.

In conclusion, our findings corroborate the involvement of *PER3* in regulating the homeostatic sleep and/or circadian systems. The *PER3* gene has the potential to serve as a biomarker for evaluating genetic traits of sleep and circadian phenotypes and as a research target to understand the mechanism underling the pathophysiology of FRT. Life styles have dramatically changed during the past century. Shift work and jet lag induce acute sleep deprivation and chronic sleep restriction, which is associated with deteriorated neurobehavioral performance[35,52]. Furthermore, a misalignment between endogenous circadian rhythms and sleep–wake cycles known as internal desynchronization is thought to cause sleep shortage and circadian rhythm disturbance, leading to elevated risks for autonomic disorders, cardiovascular diseases, metabolic diseases, and mood disorders[53–55]. Therefore, predicting sleep and circadian phenotypes may help develop interventions to improve quality of life and potentially prevent various diseases.

Methods

Subjects. The study population consisted of 182 DSPT individuals (111 men and 71 women; mean ± SD age: 26.68 ± 9.25 years), 67 FRT individuals (48 men and 19 women; mean ± SD age: 26.72 ± 9.79 years), and 925 controls (274 men and 651 women; mean ± SD age: 36.45 ± 12.10 years). Patient subjects and controls were all unrelated, sighted Japanese men and women who were recruited at medical and research institutes on mainland Japan. None of the controls had a history of sleep disorders or psychosis. The DSPT and FRT patients were diagnosed by trained psychiatrists according to the International Classification of Sleep Disorders II (ICSD-II, 1990, 1997)[33]. The diagnostic criteria for DSPT are (1) inability to fall asleep and wake up spontaneously at the desired time; (2) persistent delayed phase of the major sleep episode in relation to the desired time for sleep; (3) symptoms present for at least 1 month; and (4) sleep of normal quality and duration when not required to maintain a conventional sleep–wake schedule. The criteria for FRT are (1) insomnia or excessive sleepiness related to misalignment between the endogenous circadian rhythm and the 24-h light–dark cycle; (2) chronic sleep–wake cycle with a longer period than 24 h; and (3) symptoms present for at least 1 month. Ten DSPT patients had comorbidities as follows: bipolar, one; major depression, three; pervasive developmental disorder, two; seasonal affective disorder, two; sleep apnea syndrome, two. Seventeen FRT patients had comorbidities as follows: bipolar, one; major depression, one; seasonal affective disorder, one. The protocol was approved by the respective institutional ethical review boards. All subjects provided written informed consents. The present study was conducted according to the principles of the Declaration of Helsinki.

Markers and genotyping. A total of 30 SNPs in 7 genes, *CLOCK*, *CRY2*, *NPAS2*, *PER1*, *PER2*, *PER3*, and *TIM*, were selected (Table 3). These genes have been previously validated in some populations (http://www.ncbi.nlm.nih.gov/snp/). Overall, 28 SNPs resulted in amino acid substitutions, and 2 SNPs, rs1801260 and rs2304672, were in the 5′ UTR of *CLOCK* and *PER2*, respectively. The SNP rs1801260 was associated with evening preference in a Japanese population[18] and rs2304672 was found in a Japanese pedigree of familial ASPT[21]. Thirty SNPs including rs1801260 and rs2304672 were genotyped in 925 Japanese controls. The Hardy–Weinberg equilibrium (HWE) was tested for each marker. The SNPs showing monomorphism with low frequency (MAF < 0.05) or not in HWE ($P < 0.05$) were excluded from further analysis. Therefore, 9 SNPs in 6 genes, *CLOCK*, *NPAS2*, *PER1*, *PER2*, *PER3*, and *TIM*, were genotyped in 249 CRSD individuals.

Blood samples were collected from all subjects and used for DNA isolation. Genomic DNA was extracted from leukocytes with the QIAamp DNA Blood Midi Kit (QIAGEN K.K., Tokyo, Japan). Genotyping was performed by a TaqMan SNP Genotyping assay (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. The genotyping assay IDs for 27 SNPs are listed in Table 3 and the primers and TaqMan probes for rs2640909, rs35802556, and rs10462021 are listed in Table 4.

Self-assessment. The Japanese version of the Horne–Östberg MEQ[10] was administered to assess subjects' diurnal preference; this tool has been validated in a Japanese population[56]. Because diurnal preference changes with age, MEQ scores were adjusted by age (age-adjusted MEQ score, MEQ score + 0.3512 × (39.212 − age))[57]. Age-adjusted MEQ scores of 16–41 denote evening types, 42–58 denote intermediate types, and 59–86 denote morning types. Thus, lower MEQ scores indicate evening preference.

Statistical analysis. HWE was estimated using Haploview 4.1 (http://www.broad.mit.edu/mpg/haploview/)[58]. The chi-squared test was performed to compare the allele frequency and genotype distribution for each SNP marker among diurnal preference groups (morning, intermediate, and evening types). The sex-adjusted ORs and 95% CIs with Bonferroni correction were calculated to evaluate the rs228697 frequency across the three diurnal preference groups. One-way analysis of variance adjusted for sex was performed to compare age-adjusted MEQ scores between subjects with genotype CG or GG for rs228697 and those with genotype CC for rs228697. The chi-squared and permutation tests (N = 10,000) were performed to evaluate the difference in allele frequency between patients (DSPT or FRT) and controls using Haploview 4.1. The age- and sex-adjusted ORs and 95% CIs were calculated to evaluate the rs228697 frequency between FRT patients and controls. $P < 0.05$ was considered to be statistically significant. Statistical analysis was performed using SPSS ver.11.5.1J (SPSS Japan Inc., Tokyo, Japan), unless otherwise stated.

1. Daan, S., Beersma, D. G. & Borbely, A. A. Timing of human sleep: recovery process gated by a circadian pacemaker. *Am J Physiol* **246**, R161–183 (1984).
2. Yamazaki, S. *et al.* Resetting central and peripheral circadian oscillators in transgenic rats. *Science* **288**, 682–685 (2000).
3. Yoo, S. H. *et al.* PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. *Proc Natl Acad Sci U S A* **101**, 5339–5346 (2004).
4. Reppert, S. M. & Weaver, D. R. Coordination of circadian timing in mammals. *Nature* **418**, 935–941 (2002).
5. Lowrey, P. L. & Takahashi, J. S. Genetics of circadian rhythms in Mammalian model organisms. *Adv Genet* **74**, 175–230 (2011).
6. Barnes, J. W. *et al.* Requirement of mammalian Timeless for circadian rhythmicity. *Science* **302**, 439–442 (2003).
7. Okawa, M. & Uchiyama, M. Circadian rhythm sleep disorders: characteristics and entrainment pathology in delayed sleep phase and non-24-h sleep-wake syndrome. *Sleep Med Rev* **11**, 485–496 (2007).
8. Ebisawa, T. Analysis of the molecular pathophysiology of sleep disorders relevant to a disturbed biological clock. *Mol Genet Genomics* **288**, 185–193 (2013).
9. Hida, A., Kitamura, S. & Mishima, K. Pathophysiology and pathogenesis of circadian rhythm sleep disorders. *J Physiol Anthropol* **31**, 7 (2012).
10. Horne, J. A. & Ostberg, O. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *Int J Chronobiol* **4**, 97–110 (1976).
11. Roenneberg, T., Wirz-Justice, A. & Merrow, M. Life between clocks: daily temporal patterns of human chronotypes. *J Biol Rhythms* **18**, 80–90 (2003).
12. Duffy, J. F., Dijk, D. J., Hall, E. F. & Czeisler, C. A. Relationship of endogenous circadian melatonin and temperature rhythms to self-reported preference for morning or evening activity in young and older people. *J Invest Med* **47**, 141–150 (1999).
13. Baehr, E. K., Revelle, W. & Eastman, C. I. Individual differences in the phase and amplitude of the human circadian temperature rhythm: with an emphasis on morningness-eveningness. *J Sleep Res* **9**, 117–127 (2000).
14. Carskadon, M. A., Wolfson, A. R., Acebo, C., Tzischinsky, O. & Seifer, R. Adolescent sleep patterns, circadian timing, and sleepiness at a transition to early school days. *Sleep* **21**, 871–881 (1998).
15. Roenneberg, T. *et al.* A marker for the end of adolescence. *Curr Biol* **14**, R1038–1039 (2004).
16. Adan, A. & Natale, V. Gender differences in morningness-eveningness preference. *Chronobiol Int* **19**, 709–720 (2002).
17. Katzenberg, D. *et al.* A CLOCK polymorphism associated with human diurnal preference. *Sleep* **21**, 569–576 (1998).
18. 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* **133B**, 101–104 (2005).
19. Gamble, K. L. *et al.* Shift work in nurses: contribution of phenotypes and genotypes to adaptation. *PLoS One* **6**, e18395 (2011).
20. Iwase, T. *et al.* Mutation screening of the human Clock gene in circadian rhythm sleep disorders. *Psychiatry Res* **109**, 121–128 (2002).
21. Satoh, K., Mishima, K., Inoue, Y., Ebisawa, T. & Shimizu, T. Two pedigrees of familial advanced sleep phase syndrome in Japan. *Sleep* **26**, 416–417 (2003).
22. Serretti, A. *et al.* Insomnia improvement during antidepressant treatment and CLOCK gene polymorphism. *Am J Med Genet B Neuropsychiatr Genet* **137B**, 36–39 (2005).
23. Archer, S. N. *et al.* Polymorphism in the PER3 promoter associates with diurnal preference and delayed sleep phase disorder. *Sleep* **33**, 695–701 (2010).
24. Ebisawa, T. *et al.* Association of structural polymorphisms in the human period3 gene with delayed sleep phase syndrome. *EMBO Reports* **2**, 342–346 (2001).
25. Archer, S. N. *et al.* A length polymorphism in the circadian clock gene Per3 is linked to delayed sleep phase syndrome and extreme diurnal preference. *Sleep* **26**, 413–415 (2003).
26. Robilliard, D. L. *et al.* The 3111 Clock gene polymorphism is not associated with sleep and circadian rhythmicity in phenotypically characterized human subjects. *J Sleep Res* **11**, 305–312 (2002).
27. Osland, T. M., Bjorvatn, B. R., Steen, V. M. & Pallesen, S. Association study of a variable-number tandem repeat polymorphism in the clock gene PERIOD3 and chronotype in Norwegian university students. *Chronobiol Int* **28**, 764–770 (2011).
28. Takahashi, J. S., Hong, H. K., Ko, C. H. & McDearmon, E. L. The genetics of mammalian circadian order and disorder: implications for physiology and disease. *Nature Rev Genet* **9**, 764–775 (2008).
29. Goel, N., Basner, M., Rao, H. & Dinges, D. F. Circadian rhythms, sleep deprivation, and human performance. *Prog Mol Biol Transl Sci* **119**, 155–190 (2013).
30. Barclay, N. L. & Gregory, A. M. Quantitative genetic research on sleep: a review of normal sleep, sleep disturbances and associated emotional, behavioural, and health-related difficulties. *Sleep Med Rev* **17**, 29–40 (2013).
31. Toh, K. L. *et al.* An hPer2 phosphorylation site mutation in familial advanced sleep phase syndrome. *Science* **291**, 1040–1043 (2001).
32. Xu, Y. *et al.* Functional consequences of a CKIdelta mutation causing familial advanced sleep phase syndrome. *Nature* **434**, 640–644 (2005).
33. American Academy of Sleep Medicine. *ICSD-2. The International Classification of Sleep Disorders 2nd ed.: Diagnostic and Coding Manual*, (Westchester, IL, 2005).
34. Mongrain, V., Carrier, J. & Dumont, M. Circadian and homeostatic sleep regulation in morningness-eveningness. *J Sleep Res* **15**, 162–166 (2006).
35. King, A. C., Belenky, G. & Van Dongen, H. P. Performance impairment consequent to sleep loss: determinants of resistance and susceptibility. *Curr Opin Pulm Med* **15**, 559–564 (2009).
36. Van Dongen, H. P., Baynard, M. D., Maislin, G. & Dinges, D. F. Systematic interindividual differences in neurobehavioral impairment from sleep loss: evidence of trait-like differential vulnerability. *Sleep* **27**, 423–433 (2004).
37. Van Dongen, H. P., Maislin, G. & Dinges, D. F. Dealing with inter-individual differences in the temporal dynamics of fatigue and performance: importance and techniques. *Aviat Space Environ Med* **75**, A147–154 (2004).
38. Goel, N. Genetics of Sleep Timing, Duration and Homeostasis in Humans. *Sleep Med Clin* **6**, 171–182 (2011).
39. Viola, A. U. *et al.* PER3 polymorphism predicts sleep structure and waking performance. *Curr Biol* **17**, 613–618 (2007).
40. Maire, M. *et al.* Sleep ability mediates individual differences in the vulnerability to sleep loss: Evidence from a PER3 polymorphism. *Cortex* **52**, 47–59 (2014).
41. Hida, A. *et al.* Expression profiles of PERIOD1, 2, and 3 in peripheral blood mononuclear cells from older subjects. *Life Sci* **84**, 33–37 (2009).
42. Pawson, T. & Schlessingert, J. SH2 and SH3 domains. *Curr Biol* **3**, 434–442 (1993).
43. Pawson, T. Protein modules and signalling networks. *Nature* **373**, 573–580 (1995).
44. Alexandropoulos, K., Cheng, G. & Baltimore, D. Proline-rich sequences that bind to Src homology 3 domains with individual specificities. *Proc Natl Acad Sci U S A* **92**, 3110–3114 (1995).
45. Lussier, G. & Larose, L. A casein kinase I activity is constitutively associated with Nck. *J Biol Chem* **272**, 2688–2694 (1997).
46. Akashi, M., Tsuchiya, Y., Yoshino, T. & Nishida, E. Control of intracellular dynamics of mammalian period proteins by casein kinase I epsilon (CKIepsilon) and CKIdelta in cultured cells. *Mol Cell Biol* **22**, 1693–1703 (2002).
47. Shirogane, T., Jin, J., Ang, X. L. & Harper, J. W. SCFbeta-TRCP controls clock-dependent transcription via casein kinase 1-dependent degradation of the mammalian period-1 (Per1) protein. *J Biol Chem* **280**, 26863–26872 (2005).
48. Lockley, S. W. *et al.* Relationship between melatonin rhythms and visual loss in the blind. *J Clin Endocrinol Metab* **82**, 3763–3770 (1997).
49. Kitamura, S. *et al.* Intrinsic circadian period of sighted patients with circadian rhythm sleep disorder, free-running type. *Biol Psychiatry* **73**, 63–69 (2013).
50. Shirayama, M. *et al.* The psychological aspects of patients with delayed sleep phase syndrome (DSPS). *Sleep Med* **4**, 427–433 (2003).
51. Yazaki, M., Shirakawa, S., Okawa, M. & Takahashi, K. Demography of sleep disturbances associated with circadian rhythm disorders in Japan. *Psychiatry Clin Neurosci* **53**, 267–268 (1999).
52. Van Dongen, H. P., Maislin, G., Mullington, J. M. & Dinges, D. F. The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. *Sleep* **26**, 117–126 (2003).
53. Dinges, D. F. *et al.* Cumulative sleepiness, mood disturbance, and psychomotor vigilance performance decrements during a week of sleep restricted to 4–5 hours per night. *Sleep* **20**, 267–277 (1997).
54. Scheer, F. A., Hilton, M. F., Mantzoros, C. S. & Shea, S. A. Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proc Natl Acad Sci U S A* **106**, 4453–4458 (2009).
55. Puttonen, S., Harma, M. & Hublin, C. Shift work and cardiovascular disease - pathways from circadian stress to morbidity. *Scand J Work, Environ Health* **36**, 96–108 (2010).
56. Ishihara, K., Saitoh, T., Inoue, Y. & Miyata, Y. Validity of the Japanese version of the Morningness-Eveningness Questionnaire. *Percept and Motor Skills* **59**, 863–866 (1984).
57. Hida, A. *et al.* Individual traits and environmental factors influencing sleep timing: a study of 225 Japanese couples. *Chronobiol Int* **29**, 220–226 (2012).
58. Barrett, J. C., Fry, B., Maller, J. & Daly, M. J. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21**, 263–265 (2005).

Acknowledgments

We thank Kentaro Nozaki, Naoko Ayabe, Chihaya Osawa, and Keiko Hiyama for their assistance. Part of this study is the result of "Understanding of Molecular and Environmental Bases for Brain Health" carried out under the Strategic Research Program for Brain Sciences from the Ministry of Education, Culture, Sports, Science and Technology of Japan. This study was supported by Grants-in-Aid for Scientific Research (#21390335, #22791161, and #24621015) from the Japan Society for the Promotion of Science, an Intramural Research Grant (#23-3) for Neurological and Psychiatric Disorders from the National Center of Neurology and Psychiatry, and a grant from the Takeda Research Foundation.

Author contributions

A.H. and K.M. designed the research. A.H., S.K., Y.K., M.K., H.O., H.K., M.U., T.E., Y.I., Y.K., M.O., K.T. and K.M. performed the research. A.H., S.K., M.K. and H.O. analyzed the data. A.H. and K.M. wrote the manuscript. All authors reviewed the manuscript.

Additional information

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Hida, A. *et al.* Screening of Clock Gene Polymorphisms Demonstrates Association of a *PER3* Polymorphism with Morningness–Eveningness Preference and Circadian Rhythm Sleep Disorder. *Sci. Rep.* **4**, 6309; DOI:10.1038/srep06309 (2014).

Motomura et al. BMC Neuroscience 2014, 15:97
http://www.biomedcentral.com/1471-2202/15/97

BMC Neuroscience

RESEARCH ARTICLE Open Access

Sleepiness induced by sleep-debt enhanced amygdala activity for subliminal signals of fear

Yuki Motomura[1,2,3,4], Shingo Kitamura[1], Kentaro Oba[1,4], Yuri Terasawa[1,4,5], Minori Enomoto[1], Yasuko Katayose[1], Akiko Hida[1], Yoshiya Moriguchi[1,4], Shigekazu Higuchi[6] and Kazuo Mishima[1*]

Abstract

Background: Emotional information is frequently processed below the level of consciousness, where subcortical regions of the brain are thought to play an important role. In the absence of conscious visual experience, patients with visual cortex damage discriminate the valence of emotional expression. Even in healthy individuals, a subliminal mechanism can be utilized to compensate for a functional decline in visual cognition of various causes such as strong sleepiness. In this study, sleep deprivation was simulated in healthy individuals to investigate functional alterations in the subliminal processing of emotional information caused by reduced conscious visual cognition and attention due to an increase in subjective sleepiness. Fourteen healthy adult men participated in a within-subject crossover study consisting of a 5-day session of sleep debt (SD, 4-h sleep) and a 5-day session of sleep control (SC, 8-h sleep). On the last day of each session, participants performed an emotional face-viewing task that included backward masking of nonconscious presentations during magnetic resonance scanning.

Results: Finally, data from eleven participants who were unaware of nonconscious face presentations were analyzed. In fear contrasts, subjective sleepiness was significantly positively correlated with activity in the amygdala, ventromedial prefrontal cortex, hippocampus, and insular cortex, and was significantly negatively correlated with the secondary and tertiary visual areas and the fusiform face area. In fear-neutral contrasts, subjective sleepiness was significantly positively correlated with activity of the bilateral amygdala. Further, changes in subjective sleepiness (the difference between the SC and SD sessions) were correlated with both changes in amygdala activity and functional connectivity between the amygdala and superior colliculus in response to subliminal fearful faces.

Conclusion: Sleepiness induced functional decline in the brain areas involved in conscious visual cognition of facial expressions, but also enhanced subliminal emotional processing via superior colliculus as represented by activity in the amygdala. These findings suggest that an evolutionally old and auxiliary subliminal hazard perception system is activated as a compensatory mechanism when conscious visual cognition is impaired. In addition, enhancement of subliminal emotional processing might cause involuntary emotional instability during sleep debt through changes in emotional response to or emotional evaluation of external stimuli.

Keywords: Sleepiness, Nonconscious, Unconscious, Subliminal, Emotion, Fearful face, Amygdala

Background

Perceptual information that elicits emotional responses is partially processed without surfacing to the conscious mind, and the subcortical brain region is thought to play various roles in this process. Even a subliminal emotional stimulus can elicit a specific physiological response. For example, the amygdala, the brain area responsible for emotional cognition, is activated even when the subject is not aware of any emotional stimulus due to backward masking of a brief visual stimulus by a different emotional stimulus presented immediately following the target stimulus [1-3] or due to binocular rivalry [4,5]. Subcortical regions such as the superior colliculus, pulvinar, and basal ganglia mediate the signal transduction pathway responsible for such physiological responses. Although it has been conventionally thought that signals were transmitted

* Correspondence: mishima@ncnp.go.jp
[1]Department of Psychophysiology, National Institute of Mental Health, National Center of Neurology and Psychiatry, 4-1-1 Ogawa-Higashi, Kodaira, Tokyo 187-8553, Japan
Full list of author information is available at the end of the article