Moreover, significant differences between genotypes in relative pupillary constriction were found not only under blue light of high intensity, but also under green light of high intensity. Action spectra of ipRGCs [11] and results of several studies [33,34] have shown that green regions of light are effective for driving melanopsin, although melanopsin excitement is greater under blue than green light exposure. In this respect, our findings indicate that the melanopsin sensitivity of the TC + CC genotype was larger than that of the TT genotype when melanopsin was strongly stimulated.

It is notable that under the red light conditions, there was no significant difference between genotype groups in pupil size and pupil constriction, regardless of photic intensity. This is consistent with our hypothesis and supports our findings for genotype differences in PLR, because the red lights (λ_{peak} 632 nm) used in our study were expected to be correlated with activation of M- and L- cones (λ_{max} 534 and 563 nm) [25], not melanopsin. In support of this, human beings lacking the outer retina (i.e., blind but with normal melanopsin) were barely able or unable to detect long-wave light [32,35]. Hence, we predicted that there would be no significant differences between genotypes under the red light conditions.

Interestingly, there are geographic or ethnic differences in allele frequency of I394T. According to the International HapMap Project, C allele frequencies of I394T are 34.2% in Europeans, 27.8% in Chinese, 17% in Japanese, and 14.2% in Nigerians. It is not clear what caused these allele frequency differences, but it is obvious that the C allele frequency in Europeans is larger than that in people living in lower-latitude regions. Given that I394T genotype groups with the C allele were more sensitive to high-intensity lights than were the TT genotype group in this study, it would be interesting to determine whether the C allele is associated with biological adaptation in a photic environment. In addition, there is evidence to suggest ethnic differences in seasonal affective disorder [36], which is assumed to be increased as a result of the short photoperiod in winter [37]. Roecklein *et al.* [38] showed that an SNP of the melanopsin gene (P10L) was associated with prevalence of seasonal affective disorder. Although this study indicates a functional connection between *OPN4* gene polymorphism and a non-image-forming process, there was not sufficient physiological evidence. In future work, the functional differences between *OPN4* gene polymorphisms, including I394T and P10L, should be examined with other ethnic groups.

In terms of a selection of experimental photic stimuli for exciting melanopsin, there are ongoing debatable problems. For instance, our results showed that genotype differences in pupil size did not always appear under high-intensity lights. There are some claims that established photometric measures are inappropriate for quantifying effective light exposure for melanopsin [13], and a new measurement named 'melanopic illuminance' (m-lux) has been suggested to predict the sensitivity of melanopsin to lights [39,40]. Measurement of melanopic illuminance might be helpful to explain our findings in this study or our future work more precisely.

We used steady-state pupil response in this study, but there is an efficient method to assess ipRGC-driven pupil photoresponses called the post-illumination pupillary response (PIPR) [11,41]. This is a response after light offset, which means it is unknown whether PIPR represents the ipRGC-driven pupillary response to continuous light exposure, namely a real light environment, that we focused on in this study.

We determined an association between PLR and *OPN4* genotype groups in this study, indicating that the melanopsin sensitivity could be different, depending on the genotype of

I394T. However, we still do not know the functional differences of the *OPN4* polymorphism (I394T) in other non-image-forming processing and how much the genotype differences in PLR could influence other irradiance responses. For example, ipRGCs also interact with light-induced melatonin suppression in human beings [42,43], and it has been reported that pupil size is correlated with melatonin suppression [17]. Further, human circadian phase could be shifted by exposure to high-intensity light and short-wavelength light [44,45], suggesting involvement of ipRGCs in human sleep-wake patterns. In addition, researchers in the field of physiological anthropology, which concerns human environmental adaptation, have revealed the influence of light on human physiological responses [46-49]. To validate our findings, it is necessary to determine the relationship between I394T and such physiological responses.

Conclusions

In conclusion, human melanopsin gene polymorphism I394T functionally interacts with PLR depending on light intensity and, particularly, wavelength. Our findings suggest that, under a light condition that strongly excites melanopsin (high intensity and short wavelength), the pupillary light response of C allele subjects (TC + CC) is more sensitive to light than TT subjects. Further, we found that green light of high intensity, even though it activates melanopsin less than blue light, is also effective in eliciting a functional *OPN4* genetic difference in PLR.

Abbreviations

ANOVA, analysis of variance; ipRGCs, intrinsically photosensitive retinal ganglion cells; LED, light-emitting diode; OPN, olivary pretectal nucleus; PIPR, post-illumination pupillary response; PLR, pupillary light reflex; RGB, red-green-blue; SNP, single nucleotide polymorphism.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SL collected experimental data, performed statistical analysis and wrote the manuscript. SL and SH participated in the design of the study. SL and AH carried out the molecular genetic analysis. KM, ST and TM revised the manuscript. SH supervised the study and helped to draft the manuscript. All authors read and approved the final manuscript.

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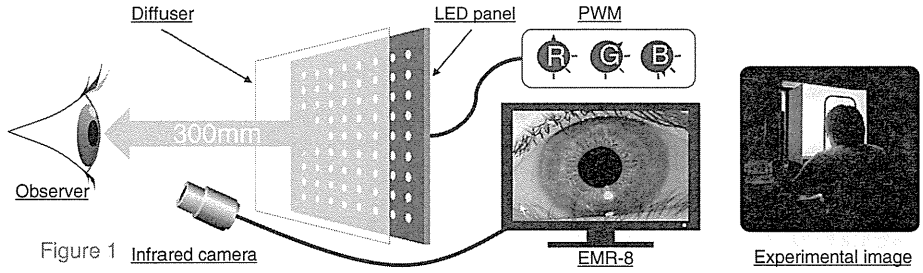

Figure 1

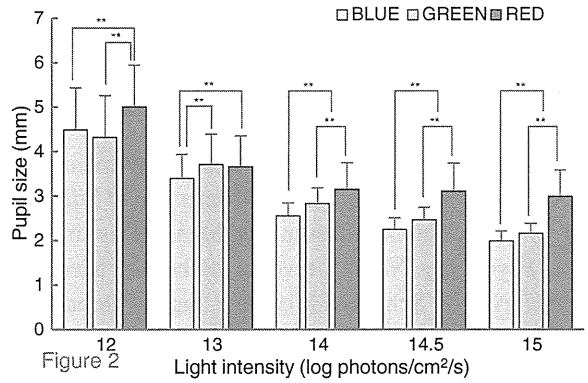

Figure 2

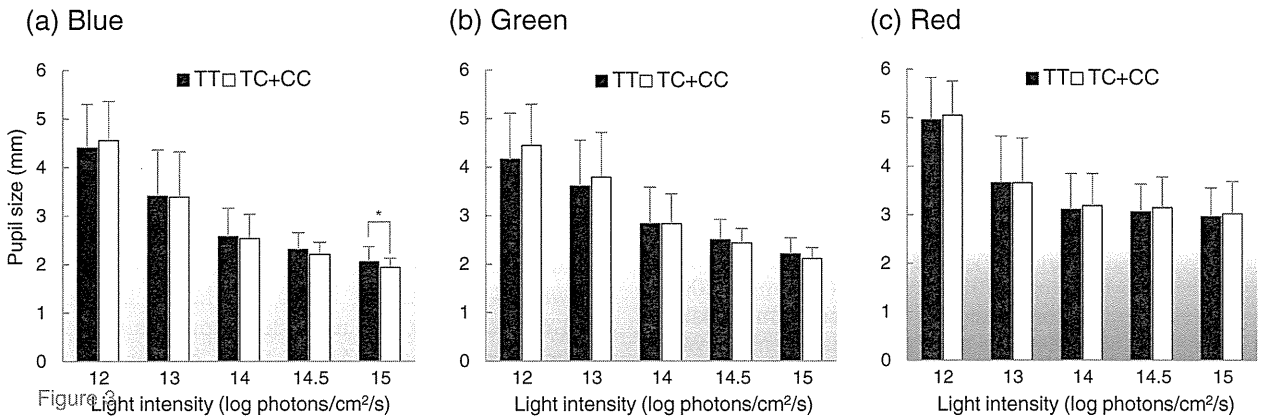

Figure 3

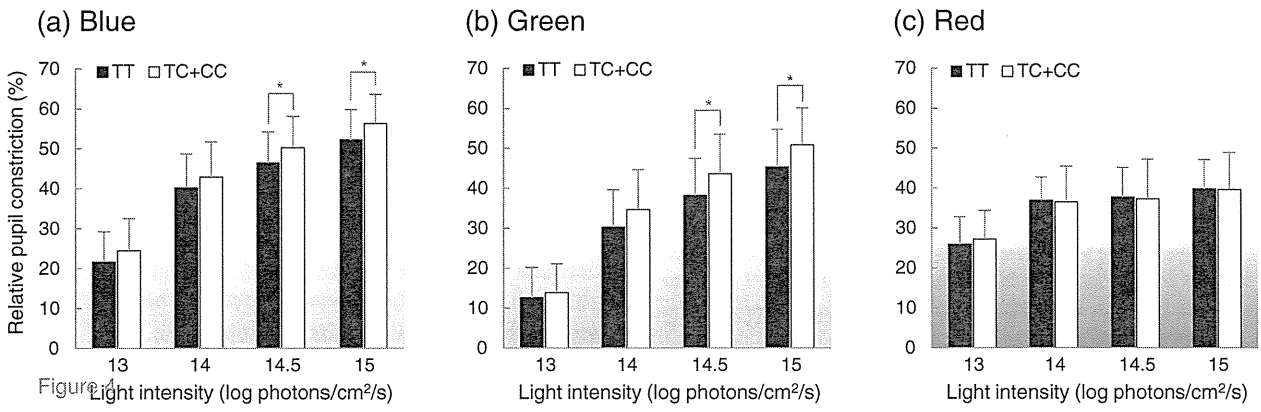

Figure 4

J Occup Health 2013; 55: 307–311

Journal of Occupational Health

Brief Report

A Cross-sectional Study of the Association between Working Hours and Sleep Duration among the Japanese Working Population

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Abstract: A Cross-sectional Study of the Association between Working Hours and Sleep Duration among the Japanese Working Population: Tadahiro OHTSU, *et al.* Department of Public Health, Showa University School of Medicine, Japan—Objectives: This study aimed to clarify the association between long working hours and short sleep duration among Japanese workers. **Methods:** We selected 4,000 households from across Japan by stratified random sampling and conducted an interview survey of a total of 662 participants (372 men; 290 women) in November 2009. Logistic regression analyses were performed using "sleep duration <6 hours per day" as a dependent variable to examine the association between working hours/overtime hours and short sleep duration. **Results:** When male participants who worked for ≥7 but <9 hours per day were used as a reference, the odds ratio (OR) for short sleep duration in those who worked for ≥11 hours was 8.62 (95% confidence interval [CI]: 3.94–18.86). With regard to overtime hours among men, when participants without overtime were used as a reference, the OR for those whose period of overtime was ≥3 hours but <4 hours was 3.59 (95% CI: 1.42–9.08). For both men and women, those with long weekday working hours tended to have a short sleep duration during weekdays and holidays. **Conclusions:** It is essential to avoid working long hours in order to prevent short sleep duration.
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Key words: Holiday, Overtime hours, Sleep duration, Weekday, Working hours

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Van der Hulst reviewed studies published between January 1996 and June 2001 on the association between long working hours and health and identified 6 studies that had used sleep hours as one of the outcome measures[1]. All of those studies had been conducted in Japan; one used a longitudinal design[2], and the others used a cross-sectional design[3–7]. Among them, only one focused on the association between sleep and working hours[7].

In November 2010, Kobayashi *et al.* conducted a systematic review of studies published in 1998 or later on the role played by sleep duration in the association between working hours and cerebrovascular/cardiovascular diseases[8]. They found only two reports on the association between working hours and sleep duration (a cohort study in the UK[9] and a large-scale cross-sectional study in Australia[10]) and recommended further studies on this issue[8].

Here we provide new data regarding the association between long working hours and short sleep duration, although our approach was limited by being a cross-sectional survey with a small sample size.

Materials and Methods

The subjects of this study were selected as follows. A total of 4,000 households were randomly selected across the country, and 2,206 adults were home when the researchers visited the households; 1,224 of them (539 men and 685 women; response rate: 55.5%) agreed to participate in the interview survey[11]. Of these, 662 (372 men; 290 women) were employed, and their data were analyzed. The duration of the survey was 1 month (November 2009). Approval was obtained from the Ethics Committee for Epidemiological Studies of Nihon University School of Medicine before the study began.

The survey included the following items: (1) sex, age, years of schooling completed (junior high/high school/college or university) and size of the city of residence (19 large cities/other cities/towns or villages); (2) working hours and overtime (extra working) hours per weekday; and (3) sleep duration per day on weekdays (workdays) and holidays (Sundays or days off). Regarding (2) and (3), participants were requested to select an answer about their status in the past month from among the categorical answer options provided. We did not ask about work patterns or burden of housework.

In our statistical analyses of working and overtime hours per weekday and sleep duration per day on weekdays and holidays, the composition in each category was calculated based on sex. The univariate logistic regression analyses used "sleep duration <6 hours per day" as a dependent variable and working hours or overtime hours as an explanatory variable and were performed for weekdays and holidays according to sex. Each model was adjusted for age class, years of schooling completed and size of the city of residence. The significance level was set at 5% (two-sided), and the IBM SPSS Statistics 20 software package was used for statistical analysis.

In Japan, overtime is defined as extra working hours that exceed 40 hours per week, excluding breaks, but including working hours on holidays (according to the Ministry of Health, Labour and Welfare integrated measures for prevention of health problems caused by overwork).

Results

Working hours, overtime hours and sleep duration are shown in Table 1 (classified according to sex). A large difference was observed between men and women with regard to working hours and overtime hours per weekday. The proportions of the participants with <6 hours' sleep per day on weekdays and holidays were 34.8% and 16.8%, respectively, among men and 44.3% and 27.0%, respectively, among women. Thus, significant differences were observed between men and women for sleep duration on weekdays and holidays. The average ages (standard deviation) of the men and women were 45.0 (13.7) and 45.3 (12.6) years, respectively, and no significant age-related difference was observed (Mann-Whitney U test; p=0.491).

Table 1. Gender-based working hours, overtime hours and sleep duration

	Men	Women	p value[b]
	n=372[a]	n=290[a]	
Working hours per weekday			<0.001
<5 h	5.1	27.2	
≥5 h, <7 h	10.8	22.6	
≥7 h, <9 h	51.4	36.2	
≥9 h, <11 h	21.9	6.3	
≥11 h	10.8	7.7	
Overtime hours[c] per weekday			<0.001
None	41.3	71.8	
<2 h	30.9	23.2	
≥2 h, <3 h	9.9	2.5	
≥3 h, <4 h	6.6	0.7	
≥4 h	11.3	1.8	
Sleep duration on weekdays[d]			0.002
<6 h	34.8	44.3	
≥6 h, <7 h	40.4	40.8	
≥7 h, <8 h	19.1	13.5	
≥8 h	5.7	1.4	
Sleep duration on holidays[e]			<0.001
<6 h	16.8	27.0	
≥6 h, <7 h	28.4	39.1	
≥7 h, <8 h	33.2	22.8	
≥8 h, <9 h	17.8	6.6	
≥9 h	3.8	4.5 (%)	

[a]In each section, the response "I do not know" was excluded from the statistical analyses. [b]χ^2 test. [c]Extra working hours. [d]Workdays. [e]Sundays or days off.

The results of the logistic regression analyses using "sleep duration <6 hours per day" as a dependent variable are shown in Table 2. When participants working ≥7 but <9 hours per day were used as a reference, the odds ratio (OR) for "sleep duration <6 hours per day" on weekdays was significantly higher among those working ≥9 hours per weekday. In the same group of participants, the OR for holidays was also significantly high. With regard to overtime hours, when participants without overtime were used as a reference, the OR for having "sleep duration <6 hours per day" on

Table 2. Logistic regression analyses using "sleep duration <6 hours per day" as a dependent variable[a]

	Sleep duration on weekdays[b]				Sleep duration on holidays[c]			
Explanatory variables	n[d]	AOR	95% CI	*p* value	n[d]	AOR	95% CI	*p* value
Men								
Working hours per weekday								
<7 h	58	1.67	0.83–3.36	0.152	57	3.69	1.59–8.55	0.002
≥7 h, <9 h	190	1.00	Reference		190	1.00	Reference	
≥9 h, <11 h	81	2.76	1.57–4.86	<0.001	81	2.71	1.27–5.79	0.010
≥11 h	40	8.62	3.94–18.86	<0.001	40	5.59	2.43–12.86	<0.001
Overtime hours[e] per weekday								
None	150	1.00	Reference		150	1.00	Reference	
<2 h	111	0.91	0.52–1.62	0.757	111	0.55	0.25–1.18	0.123
≥2 h, <3 h	36	1.05	0.46–2.37	0.912	35	0.28	0.06–1.28	0.101
≥3 h, <4 h	24	3.59	1.42–9.08	0.007	24	2.02	0.73–5.62	0.179
≥4 h	41	3.46	1.64–7.30	0.001	41	1.45	0.62–3.41	0.396
Women								
Working hours per weekday								
<5 h	78	1.13	0.60–2.10	0.709	78	1.45	0.71–2.94	0.308
≥5 h, <7 h	65	1.58	0.83–3.03	0.167	65	1.46	0.70–3.06	0.318
≥7 h, <9 h	104	1.00	Reference		104	1.00	Reference	
≥9 h	40	2.51	1.17–5.39	0.018	40	2.23	0.97–5.12	0.060
Overtime hours[e] per weekday								
None	203	1.00	Reference		203	1.00	Reference	
<2 h	66	1.58	0.88–2.82	0.125	66	1.31	0.69–2.49	0.417
≥2 h	14	0.68	0.21–2.20	0.520	14	0.71	0.18–2.76	0.620

[a]Working hours and overtime hours were used as explanatory variables (univariate analysis). Each model was adjusted for age class, years of schooling completed and size of the city of residence. [b]Workdays. [c]Sundays or days off. [d]In each section, the response "I do not know" was excluded from the statistical analyses. [e]Extra working hours. AOR, adjusted odds ratio; CI, confidence interval.

weekdays was 3.59 (95% confidence interval [CI]: 1.42–9.08) among those working ≥3 but <4 hours overtime and 3.46 (95% CI: 1.64–7.30) among those working ≥4 hours overtime, indicating significantly high ORs. No significant OR was observed regarding holidays. Among women, the OR for "sleep duration <6 hours per day" on weekdays was significantly higher among those working ≥9 hours per day and that for holidays among the same group was 2.23 (95% CI: 0.97–5.12). There was no significant OR with regard to overtime hours.

Discussion

The results of this study show that the OR for "sleep duration <6 hours per day" was significantly higher among men working ≥9 hours per day or ≥3 hours overtime. The overall total of overtime was equivalent to >60 hours per month. In Japan, an amendment to the relevant law in 2005 made it obligatory for overworked workers to receive health guidance via an interview with a physician[12]. According to this legislation, 80 hours overtime per month (approximately 4 hours overtime per day) would prevent workers from sleeping the required total of approximately 6 hours per day[13]. The results of our study suggest a need to review this claim and are therefore noteworthy. Kageyama and colleagues reported a significant negative association between ≥60 hours overtime per month in the previous 3 months and sleep length on weekdays among Japanese white-collar workers[7].

Almost half of the women in this study worked <7 hours per day, and most appeared to be part-time workers. For this group of women, the OR for having "<6 hours sleep" was high among those working ≥9 hours, as seen in men. In addition, although more than 70% of women did not work overtime, the proportions of those with <6 hours sleep on weekdays and holidays were higher than in men. The burden of doing housework in addition to employed work may explain this result. From our results, it is unclear to

what extent long working hours were associated with short sleep duration among women.

A cohort study conducted in the UK reported that the OR for short sleep duration (<7 hours) was 3.24 (95% CI: 1.45–7.27) among subjects working >55 hours per week when those with 35–40 working hours per week were used as a reference[9]. In addition, large-scale cross-sectional studies conducted in Australia[10] and the USA[14] indicated that short sleep duration was associated with working long hours. The findings of these overseas studies appear to support our results, although the classifications of working hours and sleep duration differed.

In the present study, both men and women with long weekday working hours tended to have a short sleep duration (<6 hours) on weekdays as well as holidays. Two possible explanations for this are (1) that people working long hours on weekdays spend holidays attending to personal matters that cannot be taken care of on weekdays and (2) that they became accustomed to a short sleep duration. Kageyama and colleagues reported that the amount of overtime was positively correlated with the amount of time spent sleeping on the nights before holidays[4]; however, a later report stated that sleep length before holidays was inversely correlated with overtime[7]. This latter result concurs with that of our study.

This study had some limitations. First, the response rate was not particularly high (55.5%). It is possible that only respondents with enough spare time tended to participate in this survey. Thus, working hours may have been underestimated. In addition, as the ORs in Table 2 for both male and female participants working <7 hours per day were >1, selection bias caused by the low response rate would have led to underestimation of the present findings. Second, the types of jobs varied, and we did not investigate items that might have affected sleep duration, such as presence/absence of shift work, commuting time and family composition. Third, questions on whether participants worked full-time or part-time and had housework burdens such as child-rearing and nursing care were not asked; these issues are particularly relevant to women. Belenky and colleagues stated in a recent report that occupational sleep medicine is a new field within sleep medicine[15]. We hope to design a survey investigating sleep problems in terms of occupational health, in which the aforementioned limitations will be corrected.

This study examined the associations between working hours and sleep duration. Long working hours were associated with short sleep duration (<6 hours). In men, the OR for short sleep duration was significantly higher among participants with ≥3 hours of overtime per day. In addition, sleep duration was short on weekdays as well as on holidays among people with long working hours. It is essential to avoid working long hours in order to prevent short sleep duration.

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Association between Heart Rate Variability, Blood Pressure and Autonomic Activity in Cyclic Alternating Pattern during Sleep

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Study Objectives: Cyclic alternating pattern (CAP) is frequently followed by changes in heart rate (HR) and blood pressure (BP), but the sequential associations between CAP and autonomic nerve activity have not been studied. The study aimed to reveal the precise changes in heart rate variability (HRV) during phase A of the CAP cycle.

Design: Polysomnography was recorded according to the CAP Atlas (Terzano, 2002), and BP and electrocardiogram were simultaneously recorded. The complex demodulation method was used for analysis of HRV and evaluation of autonomic nerve activity.

Setting: Academic sleep laboratory.

Participants: Ten healthy males.

Measurements and Results: The increase in HR (median [first quartile – third quartile]) for each subtype was as follows: A1, 0.64 (-0.30 to 1.69), A2, 1.44 (0.02 to 3.79), and A3, 6.24 (2.53 to 10.76) bpm (A1 vs. A2 $P < 0.001$, A1 vs. A3 $P < 0.001$, A2 vs. A3 $P < 0.001$). The increase in BP for each subtype was as follows: A1, 1.23 (-2.04 to 5.75), A2, 1.76 (-1.46 to 9.32), and A3, 12.51 (4.75 to 19.94) mm Hg (A1 vs. A2 $P = 0.249$, A1 vs. A3 $P < 0.001$, A2 vs. A3 $P < 0.001$). In all of phase A, the peak values for HR and BP appeared at 4.2 (3.5 to 5.4) and 8.4 (7.0 to 10.3) seconds, respectively, after the onset of phase A. The area under the curve for low-frequency and high-frequency amplitude significantly increased after the onset of CAP phase A ($P < 0.001$) and was higher in the order of subtype A3, A2, and A1 ($P < 0.001$).

Conclusions: All phase A subtypes were accompanied with increased heart rate variability, and the largest heart rate variability was seen in subtype A3, while a tendency for less heart rate variability was seen in subtype A1.

Keywords: Cyclic alternating pattern, heart rate variability, blood pressure, complex demodulation method, autonomic nerve activation

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INTRODUCTION

Arousal reactions are important for clarifying the physiological and pathological mechanisms of natural sleep and sleep disorders. After a report published in 1992 by the ASDA (American Sleep Disorders Association) on EEG arousals, EEG arousals have been used as a marker of sleep fragmentation.[1] According to the ASDA report, an EEG arousal is an abrupt shift in EEG frequency, which may include theta, alpha, and/or EEG frequencies greater than 16 Hz. Asynchronous waves maintained for 3 seconds were defined as arousals, but high-amplitude slow waves, such as K complexes (KC) and delta bursts, were not counted as arousals. On the other hand, high-amplitude slow waves are often observed before the appearance of the asynchronous low-voltage mixed waves in NREM sleep. These periodic EEG complexes were defined as cyclic alternating patterns (CAP) by Terzano in the 1980s.[2]

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CAP is considered a marker of sleep instability and has been used for the evaluation of sleep in various sleep disorders and sleep changes with hypnotics.[3-16] A CAP cycle, which is the minimum unit of CAP, consists of two phases: phase A and B. During phase A, high voltage waves appear and diminish synchronously. The period following phase A, in which low amplitude EEG is present, is defined as phase B. Phase A is scored within a CAP sequence only if it preceded and/or follows another phase A within the 2-60 seconds temporal range. In addition, phase A is divided into three subtypes (A1, A2, A3) on the basis of the ratio of the synchronous high-amplitude slow wave period to the whole duration of phase A (A1: > 80%, A2: 50% to 80%, A3: < 50%).[2,17] Subtype A1 is frequently observed in the first part of the sleep cycle, in slow wave sleep, and subtypes A2 and A3 appear before the onset of REM sleep in healthy volunteers.

The components of arousal reactions include not only the changes in EEG frequency but also autonomic nerve activity involved with HR, BP, and skeletal muscle tension.[18] In NREM sleep, external stimuli induce not only high-amplitude slow wave components in EEG (e.g., K complexes [KC]), but also autonomic nerve reactions, such as the increase of HR and BP.[19,20] These autonomic nerve activities are also observed in the occurrences of KC and delta bursts with no external stimuli.[19,21-23] EEG shifts followed by autonomic nerve activity also appear before or simultaneously with leg movements in periodic leg movements (PLM).[3,24-27] Thus, EEG shifts,

Table 1—Demographic data (n = 10)

	Median	IQR
Age, y	21.0	4.0
Height, cm	175.0	7.4
Weight, kg	66.4	10.8
BMI, kg/m^2	21.5	2.5
PSQI GS	3.5	2.0
ESS	4.5	4.0

IQR, interquartile range; BMI, body mass index; PSQI GS, Pittsburgh Sleep Quality Index Global Score; ESS, Epworth Sleepiness Scale

including both synchronous high-amplitude slow wave and asynchronous components, are considered to have significant associations with the autonomic nerve and skeletal muscle activities in physiological and pathological conditions.

Heart rate variability (HRV) is often used for evaluating autonomic nerve activity.[28] There are a number of reports that have previously analyzed HRV in CAP using frequency analysis.[29,30] According to these reports, the balance of autonomic nerve activity is important, and the results indicate sympathetic nerve dominance during CAP sequences versus non-CAP sequences, even for the same sleep stages. However, detailed analysis of the autonomic nerve activity involved with the increased heart rate in KC and delta bursts has not been reported. Furthermore, the duration of phase A including KC and delta bursts typically lasts for 2 to 10 seconds. However, the frequency analysis logically needs data from at least 40 seconds.[28] Thus, it is impossible to assess the short, rapid autonomic nervous reactions by the standard frequency analysis. Therefore, we continuously measured both HR and BP using nocturnal polysomnography, and applied the complex demodulation method (CDM) for sequential analysis of HRV.[31,32] The CDM makes it possible to analyze the time course of amplitude in a specific frequency band and to evaluate the autonomic nerve activity with high time resolution.

This study aims to reveal the relationship between CAP and the time course of HRV, and study the physiological significance of CAP.

METHODS

Subjects

We evaluated 10 healthy males with a median age (IQR; interquartile range) of 21.0 (4.0) years. Exclusion criteria included the following: habitual drinker, habitual smoker, having physical or psychiatric diseases. Habitual sleep state was evaluated by the Pittsburgh Sleep Quality Index (PSQI).[33,34] For the PSQI, the median (IQR) global score was 3.5 (2) points, which matched the average global score of the general Japanese population.[35] Subjective daytime sleepiness was assessed by the Epworth Sleepiness Scale (ESS).[36] The median (IQR) of ESS was 4.5 (4.0) points, which was equivalent to that of healthy Japanese volunteers (Table 1).

All subjects gave written informed consent, which was conducted with the approval of the Ethics Committee of Akita University School of Medicine.

Polysomnography

Polysomnography was conducted for 8 h, in accordance with the habitual sleep time of each subject. To determine the sleep stages and the CAP parameters, both unipolar induction electrodes (C3-A2, C4-A1, O1-A2, O2-A1) and bipolar induction electrodes (Fp1-F3, F3-C3, C3-P3, P3-O1, Fp2-F4, F4-C4, C4-P4, and P4-O2) were attached to each subject. Electrodes were also attached to obtain electromyograms of the chin and anterior tibialis muscles, electroculograms, and ECGs. To measure the flow of air, airflow sensors were attached to the nose and mouth using the thermocouple method. To record breathing movements, respiratory effort sensors were attached to the chest and abdomen using the piezoelectric method. Body position sensors, snore sensors, and pulse oximeters were attached to record body position, snoring, and arterial oxygen saturation, respectively, of each subject.

The Neurofax EEG-1524 (Nihon Kohden Corporation, Tokyo, Japan) was used to record digital electroencephalographs. The data obtained were then imported and recorded on a computer using the BioSignal Acquisition System (NoruPro Light Systems, Tokyo, Japan). The sampling rates for recording were as follows: EEG, 1000 Hz; electromyograms, 200 Hz; snore sensors, 200 Hz; ECG, 1000 Hz; breathing movements, body position sensors, pulse oximeters, and pressure sensors, 100 Hz.

NightOwl Professional (NoruPro Light Systems) was used for throughout the analysis of sleep stages; 1 epoch was defined as 30 seconds. All evaluations were based on the criteria by Rechtschaffen and Kales.[37] EEG arousals and periodic limb movements were scored according to the AASM Scoring Manual.[38]

Blood Pressure Measurement

Portapres Model-2 (Finapres Medical Systems BV, Amsterdam, Netherlands) was used for consecutive blood pressure measuring, using plethysmography. Cuffs were fixed on the first and second fingers of the left hand, and BP measurement was alternated between the 2 fingers every hour. Pressure wave data obtained were imported by analog output with a sampling rate of 100 Hz and were recorded on an electroencephalograph by digital input.

CAP, Heart Rate, and Blood Pressure Analysis

For the evaluation of CAP, PSG data were visually scored by T.Y., A.K., and H.K., based on the scoring rules written by Terzano,[2] with the aid of CAP analysis software (NoruPro Light Systems). CAP analysis was also used for analyzing variations in heart rate and systolic pressure for each CAP subtype.

RR intervals were calculated in accordance with the peaks of R waves in lead II of the electrocardiogram. RR intervals < 300 ms or > 1700 ms were excluded in order to eliminate the influence of artifacts. In regards to BP, systolic blood pressure was detected by the peaks of pulse waves based on intermittent automatic calibration waves of a sphygmomanometer for 3 s with 90 s intervals. The BP data obtained during calibration were excluded for analysis.

The variations in heart rate and blood pressure were analyzed from 15 s before to 60 s after the onset of phase A. The moving averages were calculated using datum points with 0.1 second intervals; average values between 1.5 s before to

Table 2—Polysomnography findings

	Median	IQR
Total recording time, min	481.3	11.9
Total sleep time, min	456.8	37.9
Sleep efficiency, %	94.9	6.9
WASO , min	9.8	18.4
Sleep latency, min	4.5	10.3
Time in each stage, min		
REM	71.3	38.5
Stage 1	60.3	28.0
Stage 2	241.0	29.4
Slow wave sleep	56.3	34.1
Movements	6.3	2.9
Percent of TST, %		
REM	16.1	7.0
Stage 1	13.1	6.4
Stage 2	54.0	2.6
Slow wave sleep	12.2	8.2
Movements	1.4	0.6
REM latency, min	73.8	24.3
AHI , n/h	0.1	0.3
Arousal index, n/h	15.7	8.0
PLMS index, n/h	1.2	4.0

IQR, interquartile range; WASO, wake after sleep onset; TST, total sleep time; Movements, major body movements; AHI, apnea-hypopnea index; PLMS, periodic limb movements of sleep.

Table 3—CAP parameters

	Median	IQR
CAP Rate, %	36.4	21.6
CAP Time, min	140.4	82.1
CAP Cycle, n	334.0	212.3
A1, n	218.5	92.5
Ratio, %	73.2	23.3
CAP index, n/h	35.8	14.7
A2, n	62.0	77.8
Ratio, %	18.2	11.2
CAP index, n/h	10.0	12.8
A3, n	36.0	38.8
Ratio, %	11.0	12.2
CAP index, n/h	5.7	6.2

IQR, interquartile range; CAP, cyclic alternating pattern; CAP Rate was calculated as the ratio of total CAP sequence time to whole non-REM sleep time; CAP Time was calculated as total CAP sequence time; CAP Cycle indicates total CAP cycle counts; CAP Ratio represents the percentage of the number of CAP cycle counts for each subtype; CAP index represents the number of CAP cycle counts for each subtype per hour of NREM.

1.5 s after the datum points were calculated, and the amount of change was analyzed on the basis of the average 5 s prior to the onset of phase A.

HRV Analysis

We applied the CDM[31,32] for sequential analysis of autonomic nerve activity. Hayano proposed and established the use CDM for assessment of frequency shifts in HR and BP variability.[32] CDM is suited for continuous assessment of time-dependent changes in amplitude in the rhythmic components of predefined frequency bands. The RR intervals of the ECG were analyzed from 15 s before to 60 s after the onset of phase A using the CDM. The amplitude values for the low-frequency content (LF: 0.04 to 0.15 Hz) and the high-frequency content (HF: 0.15 to 0.4 Hz) were calculated continuously.

The amplitude value for the HF content is considered an indicator of parasympathetic nerve activity, while the amplitude value for the LF content reflects both sympathetic and parasympathetic nerve activity.[28] The area under the curve (AUC) of the LF and HF amplitude values was calculated for the first 20 s of CAP. In order to compare the changes before and after the CAP, the AUC for the 5 seconds leading to the CAP onset were quadrupled and compared with that of values for the 20 s after the CAP onset.

Statistics

PASW Statistics version 17.02 for Windows (SPSS Japan Inc., Tokyo, Japan) was used for statistical processing. Most data sets in this study did not indicate normal distribution. The data of HRV in subtype A3 indicated logarithmic normal distribution, but the others did not. Thus, data were shown as median (IQR) or median (first quartile – third quartile), and nonparametric statistics were applied: Kruskal-Wallis H statistic was used for comparisons among the 3 groups in CAP subtypes. Scheffe test was used for multiple comparisons. Wilcoxson signed rank test was used for comparing the AUC of the LF and HF amplitude values before and after the CAP onset. The level of significance was set at 0.05 for each test.

RESULTS

According to the sleep parameters, the sleep structures of our subjects were considered normal, and sleep related respiratory disorders and/or periodic limb movement disorders were not found (Table 2).

CAP parameters (Table 3) had large individual variations; the median CAP rate (IQR) was 36.4% (21.6%) and the median CAP cycle counts was 334.0 (212.3). Some subjects have higher CAP rates than those reported in healthy subjects from a similar age group.[39] Three of the 10 subjects had a CAP > 50%, and in 2 of the 3 subjects, the percentage of the number of subtype A2 and A3 was > 50%. The higher CAP values in this study might be due to influences on the sleep quality by the attachments of cuffs on fingers and a monitor device on the arm.

The number of phase As totaled 3527 in 10 subjects. R waves of ECG were well detected, and HRV could be calculated in 3262 of the phase As. Changes in BP could be evaluated without the influence of finger change and/or intermittent automatic calibration in 2474 of the phase As.

HR increased immediately after the beginning of CAP. The increase in HR for each subtype was as follows: A1, 0.64 (-0.30 to 1.69); A2, 1.44 (0.02 to 3.79); and A3, 6.24 (2.53 to 10.76) bpm ($H = 516.9$, $df = 2$, $P < 0.001$, A1 *vs.* A2 $P < 0.001$,

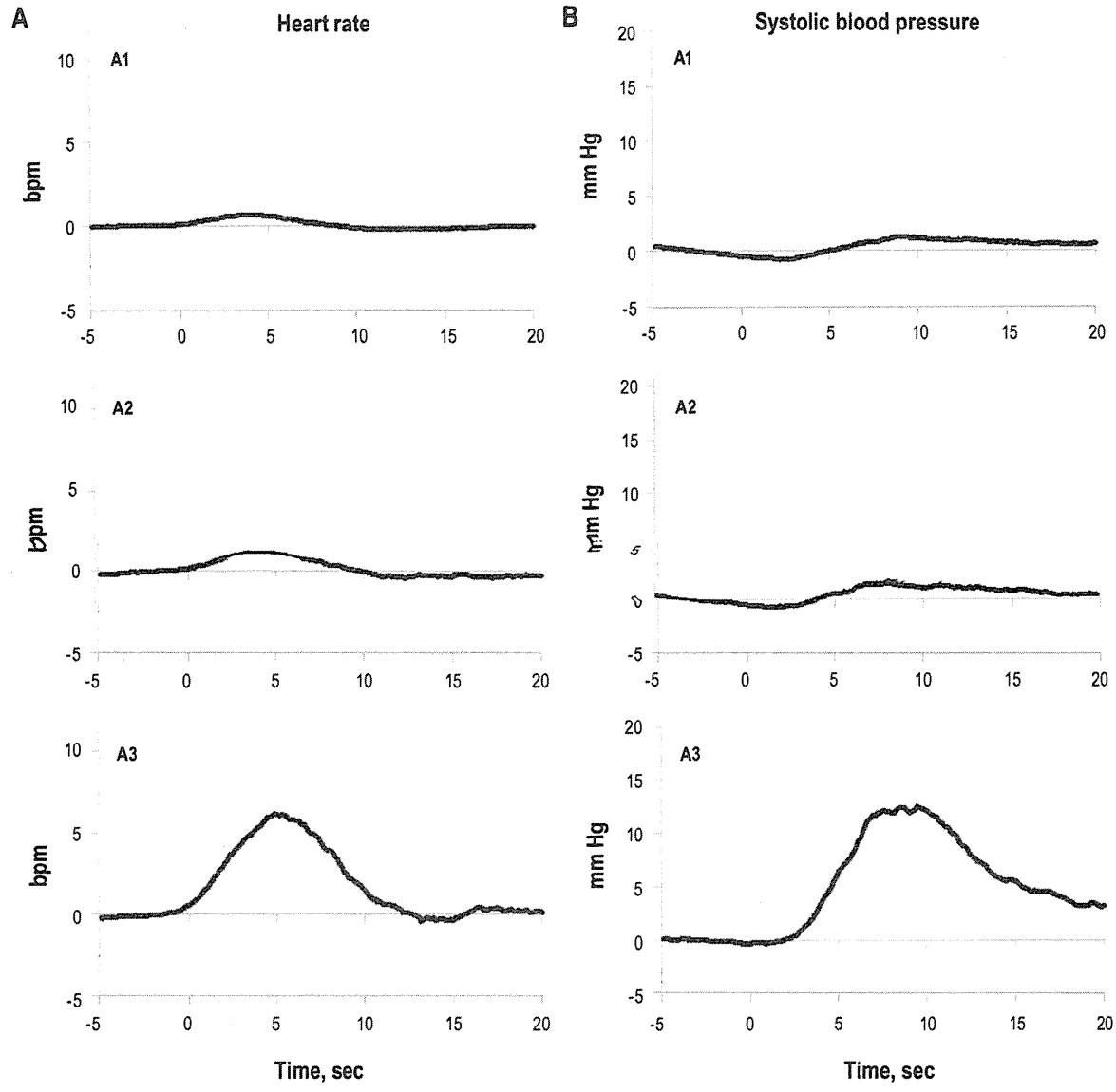

Figure 1—The time course of heart rate and systolic blood pressure changes before and after the onset of CAP. The median value (black line) of each CAP subtype was calculated **(A, B)** and graphed. Gray shadows indicate interquartile range of heart rate and systolic blood pressure. Zero seconds indicates the onset of CAP. The peak time of systolic blood pressure is delayed by approximately 4 seconds compared with that of heart rate. The increase in both heart rate and systolic pressure is higher in the order of subtype A3, A2, and A1.

A1 *vs.* A3 $P < 0.001$, A2 *vs.* A3 $P < 0.001$). In all of phase A, the peak values for HR appeared at 4.2 (3.5 to 5.4) s after the onset of phase A (Figure 1A).

BP transiently decreased after the onset of phase A, and then gradually increased. The decrease in BP for each subtype was as follows: A1, -1.40 (-3.10 to -0.07); A2, -1.44 (-3.15 to -0.24); A3, -0.89 (-2.82 to 0.46) mm Hg ($H = 11.1$, $df = 2$, $P = 0.004$, A1 *vs.* A2 $P = 0.719$, A1 *vs.* A3 $P = 0.294$, A2 *vs.* A3 $P = 0.162$). The nadir values for BP appeared at 1.5 (0.0 to 2.8) sec after the onset of phase A. The increase in BP for each subtype was as follows: A1, 1.23 (-2.04 to 5.75); A2, 1.76 (-1.46 to 9.32); and A3, 12.51 (4.75 to 19.94) mm Hg ($H = 201.7$, $df = 2$, $P < 0.001$, A1 *vs.* A2 $P = 0.249$, A1 *vs.* A3 $P < 0.001$, A2 *vs.* A3 $P < 0.001$). The time courses in all subtypes of CAP were similarly observed. Concerning the variations in HR and BP, the magnitude of subtype A3 was the largest, and the magnitude of subtype A1 was the smallest among the 3 CAP subtypes. In all subtypes of phase A, the peak values for BP appeared at 8.4 (7.0 to 10.3) s after the onset of phase A (Figure 1B).

As a result of evaluation of autonomic nerve activity using the CDM, we observed that the amplitude of LF had 2 peaks within 10 s after the onset of phase A. The AUC of LF for the 20 s after the onset of phase A was significantly higher than before the onset of phase A in all CAP subtypes. As for the AUC for LF amplitude before *vs* after the onset of phase A for each subtype, A1 was 491.3 (318.4 to 759.4) *vs.* 559.0 (387.9 to

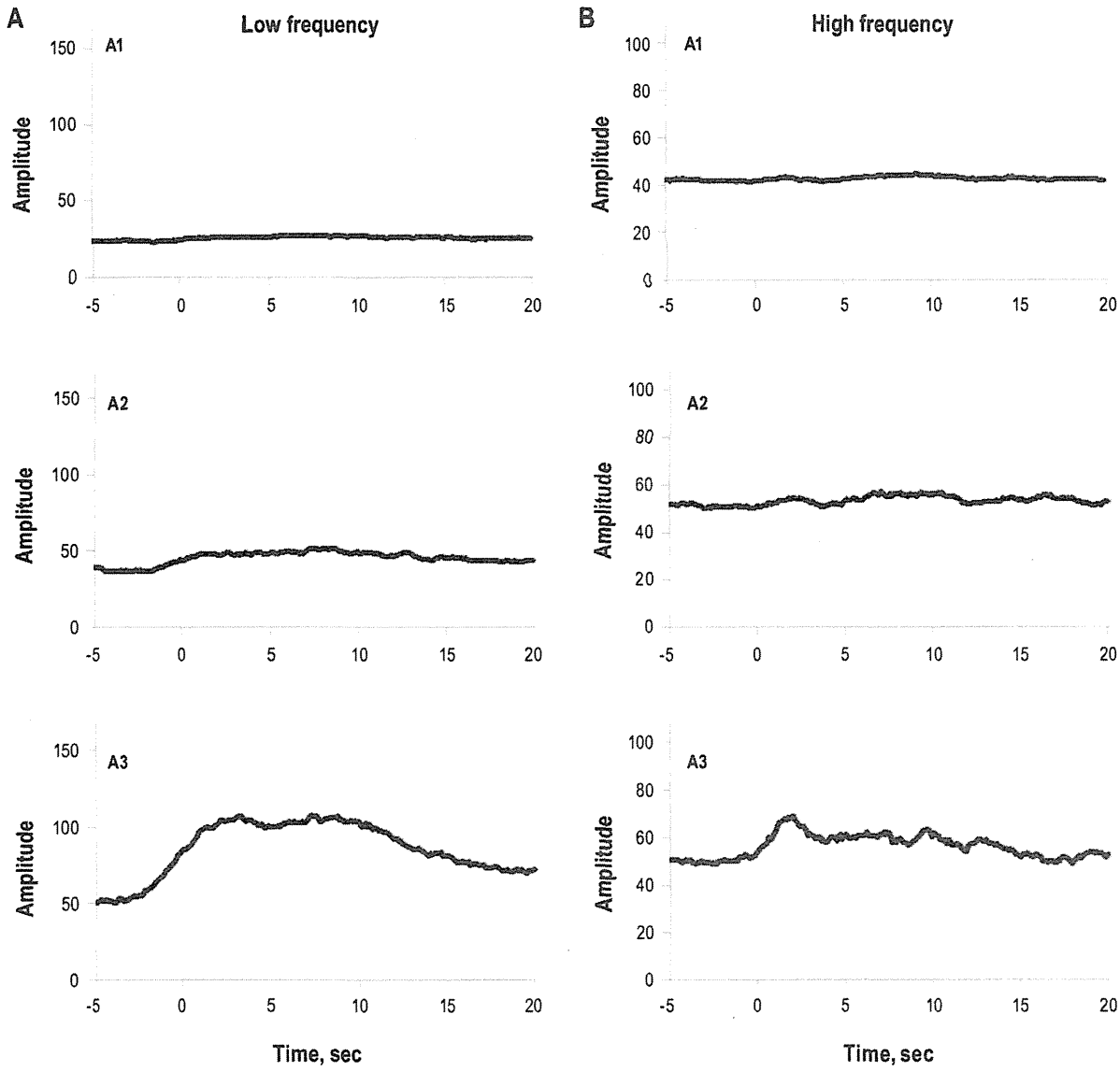

Figure 2—Comparison of time course in heart rate variability before and after the onset of CAP phase A. The median value (black line) of each CAP subtype was calculated **(A, B)** and graphed. Gray shadows indicate interquartile range of low frequency (LF: 0.04 to 0.15 Hz) and high frequency (HF: 0.15 to 0.4 Hz). Zero seconds indicates the onset of phase A. The amplitude of LF was the highest in subtype A3, followed by subtypes A2 and A1.

878.1) ($P < 0.001$); A2 was 787.1 (502.1 to 1299.1) *vs.* 1044.0 (644.3 to 1688.2) ($P < 0.001$); and A3 was 1226.9 (772.5 to 1903.9) *vs.* 2087.1 (1373.1 to 2744.0) ($P < 0.001$). The AUC for LF amplitude before the onset of phase A had significant differences among the 3 subtypes ($H = 548.3$, $df = 2$, $P < 0.001$, A1 *vs.* A2 $P < 0.001$, A1 *vs.* A3 $P < 0.001$, A2 *vs.* A3 $P < 0.001$); it was the highest in A3 and the lowest in A1. Similarly, the AUC for LF amplitude after the onset of phase A had significant differences among the 3 subtypes; it was higher in the order of A3, A2, and A1 ($H = 895.7$, $df = 2$, $P < 0.001$, A1 *vs.* A2 $P < 0.001$, A1 *vs.* A3 $P < 0.001$, A2 *vs.* A3 $P < 0.001$; Figure 2A).

The HF amplitude values changed more smoothly than those for LF. The HF amplitude values in every CAP subtype were significantly higher than those before the onset of phase A for the first 20 s after the onset of phase A. The AUC for HF amplitude before *vs* after the onset of phase A for each subtype was as follows: A1, 872.6 (631.2 to 1146.7) *vs.* 924.0 (679.4 to 1175.3) ($P < 0.001$); A2, 1054.7 (779.6 to 1401.2) *vs.* 1186.1 (910.2 to 1466.3) ($P < 0.001$); A3, 1037.5 (778.6 to 1443.5) *vs.* 1367.0 (1032.1 to 1672.4) ($P < 0.001$). The AUC for HF amplitude value had significant differences before the onset of phase A among the 3 CAP subtypes ($H = 136.1$, $df = 2$, $P < 0.001$), but the value of A3 was not significantly different to that of A2 (A1 *vs.* A2 $P < 0.001$, A1 *vs.* A3 $P < 0.001$, A2 *vs.* A3 $P = 0.999$). Concerning the AUC for HF amplitude after the onset of phase A, it was higher in the order of A3, A2, and A1 ($H = 398.9$, $df = 2$, $P < 0.001$, A1 *vs.* A2 $P < 0.001$, A1 *vs.* A3 $P < 0.001$, A2 *vs.* A3 $P < 0.001$) (Figure 2B).

DISCUSSION

Time Course of Heart Rate, Blood Pressure, And Heart Rate Variability after the Onset of CAP

To our knowledge, this is the first manuscript that analyzes the time course of HRV for each CAP subtype using a high time resolution CDM. In healthy male subjects, the HR started to increase at the onset of CAP. BP transiently decreased after the onset of phase A, and then gradually increased. The result of HRV analysis using the CDM indicates that the amplitude of LF was larger than that of HF, and its time course showed two peaks, with the latter peak corresponding to the peak time of blood pressure. Although time courses of increases in HR, BP, and HRV are similar among the three CAP subtypes, the magnitudes of the variations were larger in the order of subtype A3, A2, and A1, demonstrating that the degree of HR, BP, and HRV varied among subtypes of CAP, and that A3 induced the most prominent effects followed by A2. We thus attempted to interpret and discuss the data in detail.

Why Does Blood Pressure Increase Later than Heart Rate after the Onset of CAP?

Concerning the responsiveness of HR and BP (i.e., the time until HR and BP peaked), our findings coincided with previous reports of K complex (KC), HR, and BP changes. HR began to increase at the appearance of KC, and reached the peak value on the third beat, whereas BP increase was relatively delayed and reached the peak value on the sixth beat.[19,21]

The reaction time is determined by the network conduction velocity of the autonomic nervous system and the responsiveness of the end effectors. The reaction time is considered the same between individuals. It can explain why the BP increased later than the peak HR, based on the differences between the reaction times of the end effectors. The parasympathetic nerve activity has a rapid reaction time system (approximately 10^{-3} seconds) by the ion-channel type reaction. On the other hand, the cardiac sympathetic nerve activity has a slower reaction time system (from 10^{-1} to 10^{0} seconds) and is characterized by long reaction time duration, which is due to a series of reactions induced by the intracellular second messengers occurring through G protein-coupled receptors. The rise of blood pressure is affected by the increase of HR, the heightened vascular resistance due to the arteriole shrinking, and the cardiac contractile force. Thus, it may take approximately 8 seconds to reach the peak blood pressure as a consequence all of these vital reactions.

Muscle sympathetic nerve activity (MSNA) is a sympathetic impulse activity, which induces vascular shrinking by controlling the vascular smooth muscle in skeletal muscle and also contributes to the regulation of blood pressure. Previous studies using microneurography reported that MSNA started to rise at the second beat (approximately 1.2 s after the appearance of KC).[20] The time lapse of MSNA from KC may partly explaine the decrease of BP after the onset of phase A.

Comparison of this Study with the Time Course of Autonomic Nerve Activity Induced by PLM

Although our study reports the time course of HRV for each CAP subtype for the first time, sequential measuring of HRV to clarify of the autonomic nerve activity in PLM has been previously reported.[27] In a report by Guggisberg, the time course of LF showed two peaks at 2 and 6 seconds after the onset of PLM, and the power of LF was higher in the latter peak.[27] Interestingly, the delta power of EEG started to increase approximately 2 seconds before the onset of PLM. Sforza also reported a similar increase of delta power of EEG.[26] Moreover, reports showed CAP subtypes A2 and A3 were frequently observed in patients with PLM, and the delta power of EEG appeared prior to or in concurrence with the emergence of PLM.[11,25] Considering the time difference (2 s) between the emergences of PLM and the slow wave, the estimated time peak of LF will be 4 and 8 seconds after the occurrence of slow wave; these values coincided with our findings.

It was not clear why the time course of LF had two peaks and reached the maximum level at 8 seconds after the onset of CAP. Previous studies reported that MSNA showed a transient activation at 1.2 seconds after KC appearance.[20] This transient activation is considered the first peak of LF after the onset of CAP in our study. It was reported that MSNA was activated transiently after the onset of KC, then returned to baseline, and was suppressed at the sixth beat where the BP reached a peak.[21] However, our data and those of Guggisberg showed that the power/amplitude values for LF represented the latter peaks in these periods where MSNA were suppressed. Therefore, it is uncertain whether the latter peak of LF really reflected the sympathetic nerve activity. Baroreceptor reflex leads to the increase of the cardiac parasympathetic nerve activity. In this period, the power/amplitude values for HF actually rose. As the parasympathetic nerve activity is also reflected in the power of LF, the increase of LF power after the onset of CAP, especially in the latter peak, may represent the parasympathetic nerve activity rather than the sympathetic nerve activity.

On the other hand, the amplitude of HF increased approximately 8 seconds after the onset of phase A. Guggisberg reported that the power of HF slightly increased for a little while after the emergence of PLM, and peaked at about 6 seconds after the emergence of PLM (approximately 8 s after the occurrence of delta power of EEG).[27] It is believed that these results reflect the increase of the cardiac parasympathetic nerve activity induced by the baroreceptor reflex.

In our study, the amplitude increase of HF was observed approximately 3 seconds after the onset of phase A. The same amplitude of HF was not observed in Guggisberg's report. This may be due to the difference in the CAP occurrences; we analyzed spontaneous CAP, while Guggisberg specifically analyzed CAP induced by PLM. Further studies will be needed to clarify the difference between CAP induced spontaneously and secondarily.

Study Limitations

Although the CDM has a higher time resolution than frequency domain analysis, the amplitude values for LF are influenced by the ± 8 seconds of the evaluation point; those for HF are also influenced by the ± 3 seconds around the point. Thus, the transient changes in LF before the onset of phase A could be affected by the subsequent changes.

The power/amplitude value for HF indicates parasympathetic nerve activity, and the power/amplitude value for LF reflects both sympathetic nerve activity and parasympathetic nerve

activity. There are some studies evaluating the predominant state of sympathetic nerve using the ratio of HF to LF power/amplitude value. But these procedures are often controversial, because the time range required for measuring is different between the amplitude values for LF and HF. Thus, we should note that HRV analysis is an indirect assessment method of autonomic nerve activity.

Moreover, it should be noted that parasympathetic nerve activity is reflected not only in the HF region. Parasympathetic nerve activity reflects respiratory sinus arrhythmia (RSA). If respiratory frequency is more than 9/min, RSA is recognized in the HF region. If respiratory frequency decreases below 9/min, RSA is recognized in the LF region. The minimum of respiratory frequency was 10.8/min in this study. Thus, we believe that we could successfully assess the RSA reflected in the HF region.

As for statistical analysis, the values of measurements almost represented nonparametric distributions despite logarithmic transformation. Thus, we could not employ a suitable analysis method taking sleep stages, sleep cycles, and factors between individuals into consideration, because of use of nonparametric analysis. In this study CAP parameters had large individual differences. Therefore, more subjects with enough CAP events are needed to assess HRV that takes the influences of sleep stages and sleep cycles into consideration.

Relationship between the Autonomic Network and CAP

In regard to the amplitude before the onset of phase A, HF was similar among the three CAP subtypes. In terms of LF, subtype A3 was the largest, and subtype A1 was the smallest. In the study using the low resolution brain electromagnetic tomography (LORETA), Ferri revealed the distinct difference in the areas of the cortical generators between subtype A1 and A3; subtype A1, anterior frontal regions; A3, the parietal-occipital areas.[40] It was also reported that the amount of CAP subtype A2 and A3 was highly correlated to the arousal index.[39] However, that of subtype A1 was not. Subtype A1 instead correlated positively with the percentages of slow wave sleep, in which a tendency of parasympathetic nerve activity dominancy was frequently observed.

Our results suggest the functional interaction between the central autonomic network and the thalamo-cortical network,[41] which is related to the occurrence of high-amplitude slow waves. The central autonomic network[42] includes the limbic system and the area from the hypothalamus to the medulla oblongata and the midbrain, which regulates autonomic nerve activity. Future studies that anatomically clarify the connections between the central autonomic network and the thalamo-cortical system are needed, as well as studies that more directly evaluate the autonomic nerve activity in the occurrence of CAP, such as by MSNA measuring.

In conclusion, this is the first report that describes the sequential time course of HRV around the occurrence of CAP. We simultaneously observed rapid and transient HRV and CAP, and the largest HRV was seen in subtype A3. Since the sleep-wake controlling system has a high association with the regulation of autonomic nerve activity and is responsible for the maintenance of this behavioral state, further clarification of functional significances of the findings is warranted to understand the physiological significance of sleep and the pathological mechanisms of sleep related disorders.

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DISCLOSURE STATEMENT

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