ORIGINAL ARTICLE

Title: Instability of parasympathetic nerve function evaluated by instantaneous time-frequency analysis in patients with obstructive sleep apnea.

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Research involving Human Participants

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Abstract

The purpose was to determine whether the instability of parasympathetic nerve (PN) function is associated with fragmentation of sleep and the instability can be improved by CPAP treatment in obstructive sleep apnea (OSA). Fifty-three OSA and 50 non-OSA subjects were examined by full-PSG and pulse rate variability (PRV) was recorded simultaneously using a photoplethysmograph and evaluated by instantaneous time-frequency analysis using the complex demodulation method. PN and sympathetic nerve (SN) activity were assessed by the mean high-frequency (HF) amplitude and ratio of low-frequency (LF) and HF amplitude (LF/HF ratio), respectively. Furthermore, the shift in central frequency (CF) of the main HF peak over time was monitored continuously. The relative times over which the same main HF peak was sustained for at least 20 seconds and 5 minutes in total recording time ($\%HF_{20sec}$ and $\%HF_{5min}$) were considered as markers of PN stability. Twenty-two of 53 patients with OSA also examined under the treatment with continuous positive airway pressure (CPAP). A significant increase in mean LF/HF ratio and decrease in HF amplitude were observed in severe OSA. Furthermore, both $\%HF_{20sec}$ and $\%HF_{5min}$ were significantly decreased not only in mild-to-moderate OSA but also in severe OSA, and $\%HF_{20sec}$ was the strongest independent determinant for arousal index. Treatment with CPAP significantly decreased the LH/HF ratio and increased both $\%HF_{20sec}$ and $\%HF_{5min}$. These findings suggest that the stability of PN function is impaired by arousal due to repeated apnea and hypopnea in OSA, and that CPAP therapy

improves SN activity and PN dysfunction.

Keywords

Autonomic nerve function, obstructive sleep apnea, heart rate variability, photoplethysmograph, continuous positive airway pressure (CPAP)

Introduction

In patients with obstructive sleep apnea (OSA), the frequently repeated periods of apnea and hypopnea during sleep result in sleep disturbance, periodic hypoxemia, and hypercapnia, and large swings of intrathoracic pressure, which may result in increased sympathetic nerve (SN) activity [1-3]. The status of increased SN activity may contribute to the development of comorbidities, including hypertension [4], myocardial infarction [5], and cerebrovascular disease [6], and adversely affect prognosis [7, 8]. Conversely, parasympathetic nerve (PN) activity was suggested to be suppressed. There is a close and positive association between slow wave sleep and PN activity [9], and a decrease or lack of slow wave sleep may result in suppressed PN activity during sleep. Therefore, monitoring of autonomic nerve (AN) function during sleep in OSA may be useful to predict the onset of comorbidities.

Analysis of heart rate variability (HRV) is a simple and non-invasive method to assess AN function, generally evaluated by power spectrum analysis of HRV [10, 11]. The ratio of the power of low-frequency bundle (LF) and high-frequency bundle (HF), i.e., the LF/HF ratio, represents SN activity and the power of HF represents PN activity. It has been demonstrated that the LF/HF ratio is increased in central sleep apnea (CSA) and OSA [11, 12]. However, the power of very low frequency (VLF) and LF has been demonstrated to be affected by cyclic variation of heart rate (CVHR) [13] due to the repeated periods of apnea in OSA [14, 15]. On the other hand, the HF domain has been shown to be simply associated with the PN discharge, as the sympathetic nerve and CVHR cannot transmit neural signals at frequencies

above 0.15 Hz [16]. However, PN discharge is also governed by neural integration of the reflex from pulmonary stretch receptors [10]. Therefore, obstructive apnea may lead to incomplete inspiratory gating and may affect HF bundle [17, 18]. Recently, it has been demonstrated that instantaneous time-frequency analysis of HRV using a method of complex demodulation (CD) allows estimation of transitional changes of PN function during sleep in patients with OSA [19, 20]. Yamaguchi et al. [21] used this method and reported that the stability of PN activity during sleep in OSA was impaired but improved following treatment with continuous positive airway pressure (CPAP).

In this study, peak-to-peak intervals (PPI) of volume pulse wave were recorded using a photoelectric plethysmograph instead of electrocardiography (ECG), and the pulse rate variability (PRV) was evaluated by instantaneous time-frequency analysis using the CD method. This study was performed to determine whether the instability of PN function is associated with fragmentation of sleep and may be useful as a marker of decreased quality of sleep in OSA, which can be improved by CPAP treatment.

Materials and Methods

Subjects

Fifty-three patients with OSA were recruited from among all patients admitted to Shinshu University Hospital between April 2011 and March 2016. OSA was diagnosed in accordance with international classification of sleep disorders by American Academy of Sleep Medicine [22]. The subjects who showed

arrhythmia more than 10% of total pulse rate and atrial fibrillation, were diagnosed as diabetes mellitus, heart failure, renal failure, and impaired cognitive function by a medical specialist, had sleep disorders classified by American Academy of Sleep Medicine except OSA, and were taking beta-agonists, beta-antagonists, or anticholinergic agents were excluded from the study. The fifty healthy volunteers without any daytime sleepiness and sleep disorders, and with apnea-hypopnea index (AHI) < 10 events/hour, participated in this study. This study was conducted in accordance with the International Conference on Harmonisation-Good Clinical Practice and the Declaration of Helsinki (2008), and was approved by the institutional research ethics committee of Shinshu University School of Medicine (No. 2099). All subjects were given an adequate explanation of the study and provided written informed consent.

Protocol

All subjects were examined by full-polysomnography (PSG), and simultaneously the PPIs of volume pulse wave were recorded overnight using a photoelectrical plethysmograph (Denso Co., Ltd, Kariya, Japan) attached to the wrist, and nocturnal AN functions were evaluated by instantaneous time-frequency analysis of PRV using a CD method [19, 20]. Furthermore, patients that consented to CPAP treatment were examined again during treatment with CPAP.

Analysis of autonomic nerve function

In this study, we evaluated PRV in the frequency domain using a photoelectric plethysmograph instead of HRV analyzed from electrocardiography, The PRV technique using the same device was already validated against the traditional HRV [23]. The sampling frequency of the electric plethysmograph was 20 Hz. When a peak-to-peak interval (PPI) of volume pulse wave due to isolated/sporadic premature supraventricular/ventricular contraction or artifact was markedly different from the just before and after PPI, it is eliminated from the analysis automatically. The frequency spectra of the PPI data were estimated for the range between 0 and 0.40 Hz and divided into two components depending on their central frequencies (CF); one domain between 0.04 and 0.15 Hz was labeled as the band with LF and the other between 0.15 and 0.40 Hz as the band with HF. The LF/HF ratio was used as a marker of the SN discharge to the cardiac sinus node [10]. The mean values of HF amplitude were used as markers of PN discharge [16]. Furthermore, nocturnal PN dysfunction was also evaluated as the stability of PN discharge according to the method reported by Yamaguchi et al. [21] using HRV LOG Analysis Pro-DSA software (NoruPro Light Systems Inc., Tokyo, Japan). Briefly, the HF domain with the maximum instantaneous amplitude was defined as the main HF peak and was used as a surrogate marker of PN discharge. The shift in CF of the main HF peak over time was monitored continuously based on the density spectrum array (DSA) map for main HF peak constructed with a time scale of 1 s and a frequency resolution of 0.002 Hz. When the CF of the main HF peak was shifted by more than ±0.014 Hz, corresponding to approximately ±5% of that of respiratory sinus arrhythmia (RSA), we assumed that the PN discharge was

significantly altered from the previous state, i.e., disruption of the PN discharge. In OSA, the duration of apnea and hypopnea was relatively short period and the increased breathing effort following the end of the respiratory event increased the amplitude of RSA during the first few seconds, but the increased HF power was dispersed at some peaks. The default of 0.014 Hz was the best way to detect this phenomenon. When the main HF peak lasted for at least 20 s or 5 minutes without any disruption on the HF-DSA map, the PN function was considered to be stable or very stable. As an index of stability of PN function, we calculated the relative times over which the same main HF peak was sustained for at least 20 s and 5 minutes in total recording, which was represented as $\%HF_{20sec}$ and $\%HF_{5min}$, respectively.

Polysomnography examination

Overnight full-laboratory PSG was examined using a digital polygraph (EEG-9200; Teijin Pharma Co., Ltd., Tokyo, Japan) with a clinical technician in attendance. Standard PSG montages were used as follows: C4-A2, C3-A1, O2-A1, and O1-A2 electroencephalography (EEG); left and right electrooculography; submental electromyography; a nasal cannula to measure nasal pressure; a thermistor to monitor nasal and oral flow; movement sensors for left and right tibialis anterior muscles; respiratory effort by thoracoabdominal inductive plethysmography; ECG; finger pulse oximetry; a neck microphone to record snoring; and a sensor on a thoracic belt to evaluate body posture. Data were recorded overnight for 9 hours from 21:00 to 06:00 the next morning. PSG data were analyzed using Polysmith ver. 7.0

software (Nihon Kohden Co., Ltd., Tokyo, Japan) and the analysis was carried out both automatically and manually in accordance with the recommendations by the American Academy of Sleep Medicine [24].

Statistical analysis

Data are presented as means ± standard error or ratios with percentages as appropriate. The data distribution of the variables in the various groups was first assessed with Bartlett's test. When the data for the variables showed a normal distribution, they were compared by one-way analysis of variance (ANOVA), followed by multiple comparisons with the Tukey–Kramer method. When the data for the variables did not show a normal distribution, the variables were compared with the Kruskal–Wallis test, followed by multiple comparisons among groups with the nonparametric Tukey–Kramer method. Simple correlations between variables were examined by calculating Pearson's product correlation coefficient. Multiple stepwise linear regression analysis was performed to identify which variables were significant determinants for AN activity and PN instability. Comparisons of variables before and under treatment with CPAP were performed using the paired *t* test. A value of $p \leq 0.15$ was used to identify candidate variables, and then variables were removed from the regression model if $p > 0.1$. All statistical analyses were performed using Windows-compatible software (StatFlex version 6; Artech Co., Ltd., Osaka, Japan). In all analyses, $p < 0.05$ was taken to indicate statistical significance.

Results

Table 1 shows the characteristics and the PSG data in both groups. There were no significant differences in age or male:female ratio between groups. The mean body mass index (BMI) was higher in the OSA group than the non-OSA group. In the OSA group, four, 13, 19, and 17 subjects were classified as having mild, moderate, severe, and very severe OSA, respectively, and they showed nocturnal hypoxemia, higher arousal index, decreased sleep efficiency, and decreased slow wave sleep (SWS). Of the parameters of AN function, the mean LF/HF ratio was increased and HF amplitude was significantly decreased in severe OSA compared to the control group. Both the $\%HF_{20sec}$ and $\%HF_{5min}$ were significantly decreased not only in severe OSA but also in mild to moderate OSA compared with the control group (Table 1 and Fig. 1), indicating the instability of PN function in OSA.

Table 2 shows the correlation coefficients of simple linear regression analysis among the parameters of AN function and age and findings regarding PSG in control subjects. The LF/HF ratio showed significant negative correlations with the parameters of PN activity and PN stability, and a weak negative correlation with %SWS. The mean HF amplitude showed a strong negative correlation with age ($r = –0.62$), a positive correlation with %SWS ($r = 66$), and a weak negative correlation with nocturnal hypoxemia (Fig. 2). The $\%HF_{20sec}$ and $\%HF_{5min}$ showed negative correlations with age and %SWS, and $\%HF_{20sec}$ showed a weak negative correlation with AHI. Multiple stepwise regression analysis in OSA indicated that the regression model for the LF/HF ratio among age, sex, BMI, sleep efficiency, % SWS, %REM, AHI,

arousal index, CT90, was significant (r = 0.48, $p = 0.0005$) and consisted of sex (stdβ = 0.48, $p = 0.0005$), that for HF amplitude was significant (r = 0.70, $p < 0.0001$) and consisted of age (stdβ = –0.23, $p = 0.14$) and %SWS (stdβ = 0.53, $p = 0.0012$), and that for $\%HF_{20sec}$ was significant (r = 0.52, $p = 0.0007$) and consisted of age (stdβ = –0.31, $p = 0.0268$) and sex (stdβ = –0.32, $p = 0.0201$).

Table 3 shows the correlation coefficients of simple linear regression analysis among the parameters of AN function and age and findings regarding PSG in patients with OSA. There were significant but weak positive correlations between the mean LF/HF and AHI or arousal index, and between mean HF amplitude and sleep efficiency. On the other hand, the $\%HF_{20sec}$ and $\%HF_{5min}$ showed significant negative correlations with AHI (r = –0.44 and –0.37, respectively) and arousal index (r = –0.48 and –0.41, respectively) (Fig. 3). Multiple stepwise regression analysis revealed that the regression model for the LF/HF ratio was significant (r = 0.38, $p = 0.02$) and consisted of AHI (stdβ = 0.37, $p = 0.009$) and age (stdβ = –0.23, $p = 0.10$), that for HF amplitude was significant (r = 0.36, $p = 0.03$) and consisted of age (stdβ = –0.22, $p = 0.10$) and sleep efficiency (stdβ = 0.23, $p = 0.09$), and that for $\%HF_{20sec}$ was significant (r = 0.48, $p = 0.0003$) and consisted of arousal index (stdβ = –0.48, $p = 0.10$).

Twenty-two of 53 patients with OSA examined continuous positive airway pressure (CPAP) titration and then again evaluated the AN function under the treatment with CPAP. Treatment with CPAP significantly improved the increased LF/HF ratio from 1.26 ± 0.09 to 1.06 ± 0.06 ($p < 0.01$), the decreased $\%HF_{20sec}$ from 26.1% ± 3.6% to 44.3% ± 4.4% ($p < 0.01$), and $\%HF_{5min}$ from 4.3% ± 1.5% to 12.0% ± 2.7% ($p <$

0.05) together with improvement in AHI from 56.0 ± 5.7 events/hour ($p < 0.01$) to 14.1 ± 2.5 events/hour, apnea index from 43.0 ± 6.8 events/hour to 3.3 ± 1.2 events/hour, and arousal index from 46.4 ± 4.6 events/hour to 23.8 ± 3.8 events/hour ($p < 0.01$) (Fig. 4).

Discussion

Severe OSA patients with AHI > 30 events/hour showed significant increases in LF/HF ratio and decreases in mean HF amplitude. On the other hand, the $\%HF_{20sec}$ and $\%HF_{5min}$, representing PN stability, were significantly decreased not only in severe OSA but also in mild to moderate OSA. Multiple stepwise linear regression analysis indicated that %SWS was the most important independent determinant of PN activity in non-OSA control subjects. In the OSA group, AHI and arousal index were the most important independent determinants of SN activity and PN instability, respectively. Treatment with CPAP significantly decreased the LH/HF ratio and increased $\%HF_{20sec}$ and $\%HF_{5min}$. These findings suggest that PN function may be correlated with SWS in non-OSA subjects; however, patients with OSA showed not only decreased PN activity but also instability of PN function, which may reflect arousal induced by repeated apnea and hypopnea.

Analysis of HRV has been used to evaluate autonomic nerve function. However, in OSA patients, CVHR due to periodic apnea and hypopnea affects HRV [13-15]. In addition, the large swing of intrathoracic pressure due to respiratory effort against upper airway obstruction may affect HRV through baroreceptors

in the cardiovascular system [25, 26]. In addition, there are problems in the analytical method. The time resolution of classical power spectrum analysis on HRV, including the fast Fourier transform (FFT) algorithm or the autoregressive approach (AR), is low and requires at least 100 heart beats thus requiring approximately 2 minutes to obtain the data necessary for definitive analysis of the frequency domains contained in the R-R intervals of the heartbeat [27, 28]. On the other hand, the CD method used in the present study enabled measurement of transitional changes in instantaneous amplitude of the target frequency domain from short-time recordings lasting for 6.7 s, corresponding to approximately 7 beats [19, 20]. The CD method can differentiate the quantitative changes in PN discharge during morbid apnea and hypopnea episodes lasting 10 s or longer. However, even with the CD method, analysis of the LF domain for autonomic nerve activity cannot provide definitive results in OSA because the LF domain is affected by CVHR. Therefore, it is difficult to conclude that the SN activity, represented by the LF/HF ratio, is definitively increased in severe OSA. On the other hand, the HF domain has been shown to be simply associated with the PN discharge, as sympathetic nerves and CVHR cannot transmit neural signals at frequencies above 0.15 Hz [16]. The CD method can improve the time resolution of the HF domain, and is also able to evaluate HF peak stability. It has been demonstrated that there is a close correlation between SWS and HF power [29], and the HF power while awake or sleeping is associated with quality of sleep. Higher resting HF is associated with higher subjective and objective sleep quality, and HF in stages N2 and N3 of sleep is correlated with better subjective sleep quality [30]. In addition, patients with

major depression and primary insomnia exhibit significant reductions in HF power both while awake and while sleeping, and this association is mainly driven by the presence of poor sleep [31]. In OSA, it has been reported that the relative power of HF and normalized power of HF are significantly negatively correlated with AHI [32]. However, Jurysta et al. [33] demonstrated that there were no significant differences in the values of LF/HF ratio, HF power, or normalized HF and LF while awake, in REM sleep, and in non-REM sleep among control subjects and those with mild to moderate OSA and severe OSA. It has been suggested that measurement of the frequency domain of HRV during or immediately after OSA is problematic, because the apnea-hypopnea events per se cause marked alterations in HRV [34]. An increase in heart rate is found when arousals are detected at the end of the respiratory event and the amplitude of the respiratory sinus arrhythmia may increase during the first few seconds because of the hyperpnea after a respiratory event. The close correlation between %SWS and the mean HF amplitude in non-OSA control subjects was a reasonable finding in the present study. However, a significant correlation was not observed in the OSA group due to the reduced amount of SWS. Interestingly, the mean HF amplitude was decreased only in severe OSA. However, the instability of PN function was also observed even in mild to moderate OSA, and was more profound in severe OSA correlated with the evidence of increased arousal. The modulation of CF of main HF peak may correspond to the alteration of respiratory sinus arrhythmia due to arousal tachycardia and hyperpnea after a respiratory event.

It has been reported that 3 months of CPAP treatment has beneficial effects on ANS function among

uncomplicated OSA patients free from diseases or medications significantly affecting LF power, HF power, and the LF/HF ratio, and the effect of CPAP persisted into the daytime suggesting improvement SN activity [35]. Yamaguchi et al. [21] demonstrated that CPAP significantly decreased the instantaneous amplitude of the main HF peak in NREM sleep, but increased both $\%HF_{20sec}$ in NREM and REM sleep. Consistent with their study, CPAP treatment significantly decreased LF/HF ratio and increased $\%HF_{20sec}$ and $\%HF_{5min}$ corresponding to improvement in AHI and arousal in the present study, but a significant change in the mean HF amplitude was not observed. Therefore, evaluation of the stability of PN function, represented by the indexes of $\%HF_{20sec}$ and $\%HF_{5min}$, using instantaneous time-frequency analysis is useful for assessment of sleep quality in OSA.

Due to its easy applicability, pulse waves has been proposed as a surrogate of ECG for the analysis of HRV. The pulse frequency demodulation (PFDM) technique using the photoelectric plethysmograph was already validated against the traditional HRV [23] and provides a reliable assessment of PRV. Given the popularity of pulse wave equipment, we can obtain daily assessment of sleep quality easily, which may contribute to the health promotion.

In conclusion, to evaluate the instability of PN function at night in OSA, we examined the modulation effect of the obstructive apneic events on the cardiac sinus node by instantaneous time-frequency analysis of PRV based on the CD method. The stability of PN function was impaired corresponding with arousals due to repeated apnea-hypopnea, and CPAP treatment improved the instability of PN function. These

findings suggest that evaluation of the instability of PN function by the present method may be useful for assessing sleep quality and treatment efficacy in OSA.

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Compliance with Ethical Standards

The authors did not receive any specific grants from funding agencies in the public, commercial, or not-for-profit sectors, and report no conflicts of interest in this work.

This study was conducted in accordance with the International Conference on Harmonisation-Good Clinical Practice and the Declaration of Helsinki (2008), and was approved by the institutional research ethics committee of Shinshu University School of Medicine (No. 2099). All subjects were given an adequate explanation of the study and provided written informed consent.

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Table 1. Characteristics and data of polysomnography and autonomic nerve function in healthy control subjects (Control) and patients with obstructive sleep apnea (OSA) consisting of two subgroups: non-severe group (AHI < 30 events/hour) and severe group (AHI ≥ 30 events/hour).

	Control	Total OSA	Non-severe OSA	Severe OSA
Number	50	53	17	36
Age, years	44.4 ± 2.7	49.7 ± 1.9	42.6 ± 3.1	53.1 ± 2.3 *†
Male/Female	35/15	44/9	17/0	27/9
BMI, kg/m^2	23.2 ± 0.5	26.3 ± 0.5 **	25.7 ± 1.0 *	26.7 ± 0.6 **
TST, min	385.0 ± 11.4	361.2 ± 12.7	398.0 ± 20.9	343.8 ± 15.2 †
Sleep efficiency,%	84.1 ± 1.5	76.2 ± 2.2 *	83.5 ± 4.0	72.7 ± 2.5 **††
SWS,%	6.2 ± 1.2	1.5 ± 0.5 **	3.2 ± 1.1	0.7 ± 0.4 **††
REM,%	16.9 ± 1.0	12.5 ± 0.9 **	16.5 ± 1.2	10.6 ± 1.0 **††
AHI, events/h	6.4 ± 0.5	47.6 ± 3.7 **	17.5 ± 1.1 **	61.8 ± 3.5 **††
AI, events/h	18.1 ± 1.1	44.9 ± 3.2 **	22.7 ± 1.4 *	55.3 ± 3.4 **††
Mean SpO_2,%	95.6 ± 0.3	94.3 ± 0.3 **	95.5 ± 0.2	93.8 ± 0.4 **††
Lowest SpO_2,%	89.2 ± 0.7	77.7 ± 1.7 **	82.8 ± 2.6 *	75.3 ± 2.0 **†
CT90,%	0.4 ± 0.1	8.4 ± 1.9 **	1.1 ± 0.5 *	11.9 ± 2.6 **††

LF/HF ratio	0.96 ± 0.04	1.22 ± 0.06 **	1.15 ± 0.11	1.26 ± 0.07 **
HF amp, ms	30.2 ± 2.1	24.7 ± 1.3	27.1 ± 2.7	23.5 ± 1.3 *
$\%HF_{20sec}$,%	56.9 ± 2.7	36.3 ± 2.7 **	42.5 ± 4.6 **	33.3 ± 3.2 **
$\%HF_{5min}$,%	22.4 ± 2.4	8.4 ± 1.4 **	12.0 ± 3.2 *	6.8 ± 1.5 **

Values are means ± SEM. *$p < 0.05$ and **$p < 0.01$ vs. control subjects; $^{\dagger}p < 0.05$ and $^{\dagger\dagger}p < 0.01$ vs. mild to moderate OSA.

BMI, body mass index; TST, total sleep time; SWS, slow wave sleep; AHI, apnea-hypopnea index; AI, arousal index; CT90, cumulative time with SpO_2 less than 90%; LF, low frequency; HF, high frequency; LF/HF ratio; a marker of sympatho-vagal balance; HF amp, mean HF amplitude (a marker of parasympathetic nerve activity); $\%HF_{20sec}$ and $\%HF_{5min}$, the relative times over which the same main HF peak was sustained for at least 20 s and 5 minutes in total recording time (markers of stability of parasympathetic nerve function).

Table 2. Correlation coefficients of simple linear regression analysis between parameters of autonomic nerve function and age and the findings on polysomnography in control subjects.

	LF/HF ratio	HF amp	$\%HF_{20sec}$	$\%HF_{5min}$
Age, years	0.16	-0.62 **	-0.42 **	-0.29 *
AHI, events/h	0.09	-0.25	-0.30 *	-0.23
AI, events/h	0.09	-0.27	-0.21	-0.25
Sleep effiency,%	0.06	0.13	0.15	0.07
SWS,%	-0.34 *	0.66 **	0.30 *	0.31 *
REM,%	-0.08	0.15	0.03	-0.01
CT90,%	0.01	-0.30 *	-0.28	-0.22
LF/HF ratio	-	-0.50 **	-0.42 **	-0.46 **
HF amp, ms	-0.50 **	-	0.43 **	0.43 **
$\%HF_{20sec}$,%	-0.42 **	0.43 **	-	0.86 **
$\%HF_{5min}$,%	-0.46 **	0.43 **	0.86 **	-

Values are mean ± SEM. AHI, apnea-hypopnea index; AI, arousal index; SWS, slow wave sleep; CT90, cumulative time with SpO_2 less than 90%; LF, low frequency; HF, high frequency; LF/HF ratio; a marker of sympatho-vagal balance; HF amp, mean HF amplitude (a marker of parasympathetic nerve activity); $\%HF_{20sec}$ and $\%HF_{5min}$, the relative times over which the same main HF peak was sustained for at least 20

s and 5 minutes in total recording time (markers of stability of parasympathetic nerve function).

Table 3. Correlation coefficients of simple linear regression analysis between parameters of autonomic nerve function and age and the findings on polysomnography in patients with OSA.

	LF/HF ratio	HF amp	$\%HF_{20sec}$	$\%HF_{5min}$
Age, years	0.12	-021	0.03	-0.06
AHI, events/h	0.30 *	-0.25	-0.44 **	-0.37 **
AI, events/h	0.30 *	-0.20	-0.48 **	-0.41 **
Sleep efficiency,%	-0.19	0.26 *	0.18	0.12
SWS,%	0.01	0.11	-0.02	-0.02
REM,%	-0.16	0.06	0.18	0.11
CT90,%	0.07	-0.24	-0.16	-0.13
LF/HF ratio	-	-0.43 **	-0.64 **	-0.35 *
HF amp, ms	-0.47 **	-	0.47 **	0.32 *
$\%HF_{20sec}$,%	-0.64 **	0.47 **	-	0.81 **
$\%HF_{5min}$,%	-0.35 *	0.31 *	0.81 **	-

Values are mean ± SEM. AHI, apnea-hypopnea index; AI, arousal index; SWS, slow wave sleep; CT90, cumulative time with SpO_2 < 90%; LF, low frequency; HF, high frequency; LF/HF ratio; a marker of sympatho-vagal balance; HF amp, mean HF amplitude (a marker of parasympathetic nerve activity); $\%HF_{20sec}$ and $\%HF_{5min}$, the relative times over which the same main HF peak was sustained for at least 20

s and 5 minutes in total recording time (markers of stability of parasympathetic nerve function).

Figure Legends

Figure 1. Comparison of the stability of parasympathetic nerve function overnight in control subjects and patients with obstructive sleep apnea (OSA).

$\%HF_{20sec}$ and $\%HF_{5min}$, the relative times over which the same main HF peak was sustained for at least 20 s and 5 minutes in total recording time (markers of stability of parasympathetic nerve function).

Figure 2. Relationship between mean HF amplitude and age or % of slow wave sleep in control subjects. HF, high frequency; TST, total sleep time.

Figure 3. Relationship between $\%HF_{20sec}$ and apnea-hypopnea index (AHI) or arousal index (AI) in the patients with OSA.

$\%HF_{20sec}$, the relative times over which the same main HF peak was sustained for at least 20 s and 5 minutes in total recording time (markers of stability of parasympathetic nerve function).

Figure 4. Comparison of $\%HF_{20sec}$ and $\%HF_{5min}$ in patients with OSA before and during treatment with continuous positive airway pressure (CPAP).

Both $\%HF_{20sec}$ and $\%HF_{5min}$ were significantly increased ($p < 0.01$ and $p < 0.05$, respectively) and the

instability of parasympathetic nerve function improved.

$\%HF_{20sec}$ and $\%HF_{5min}$, the relative times over which the same main HF peak was sustained for at least 20 s and 5 minutes in total recording time (markers of stability of parasympathetic nerve function).

Fig. 1

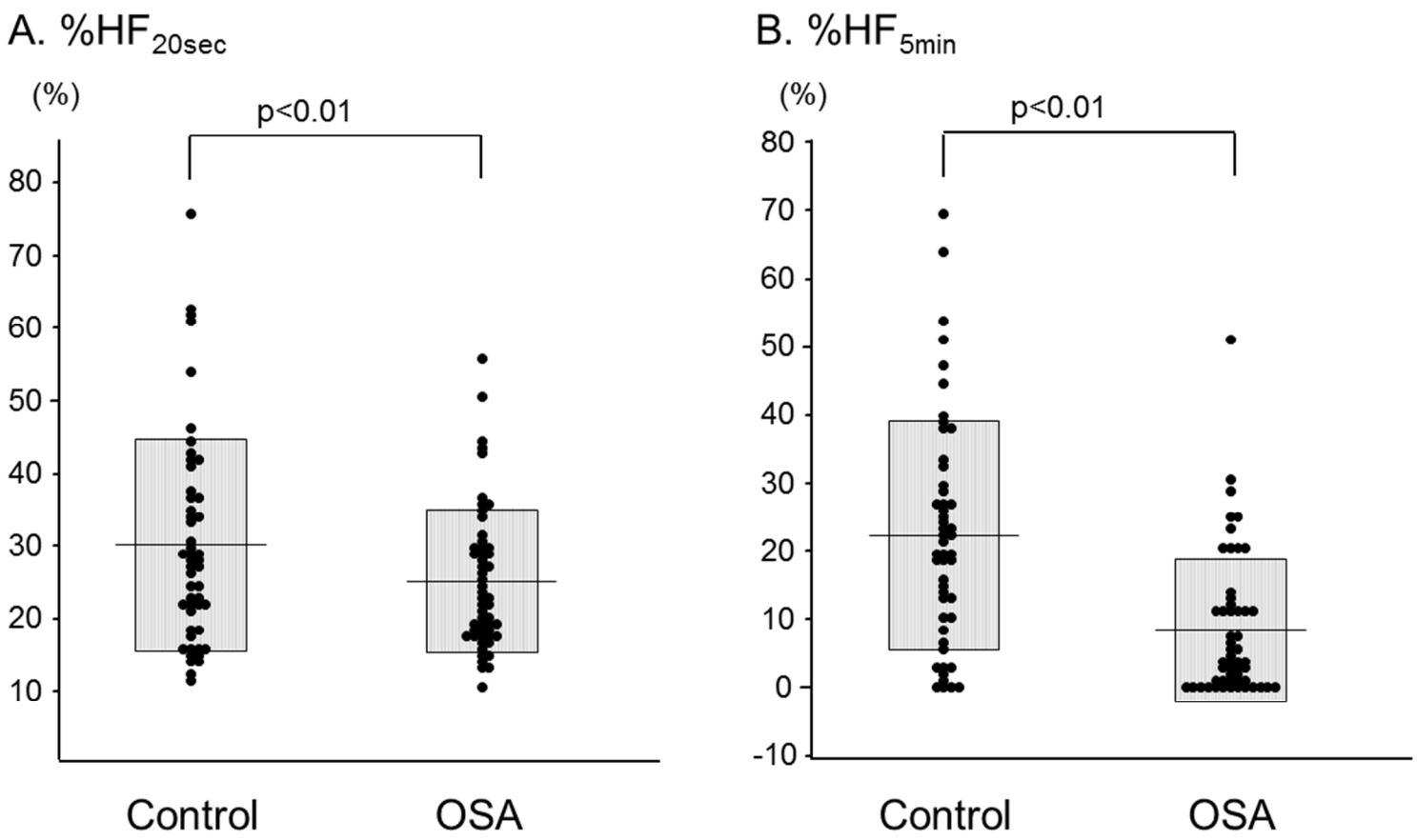

A. %HF20sec
(%)
p<0.01
80
70
60
50
40
30
20
10
Control
OSA
B. %HF5min
(%)
p<0.01
80
70
60
50
40
30
20
10
0
-10
Control
OSA

Fig. 2

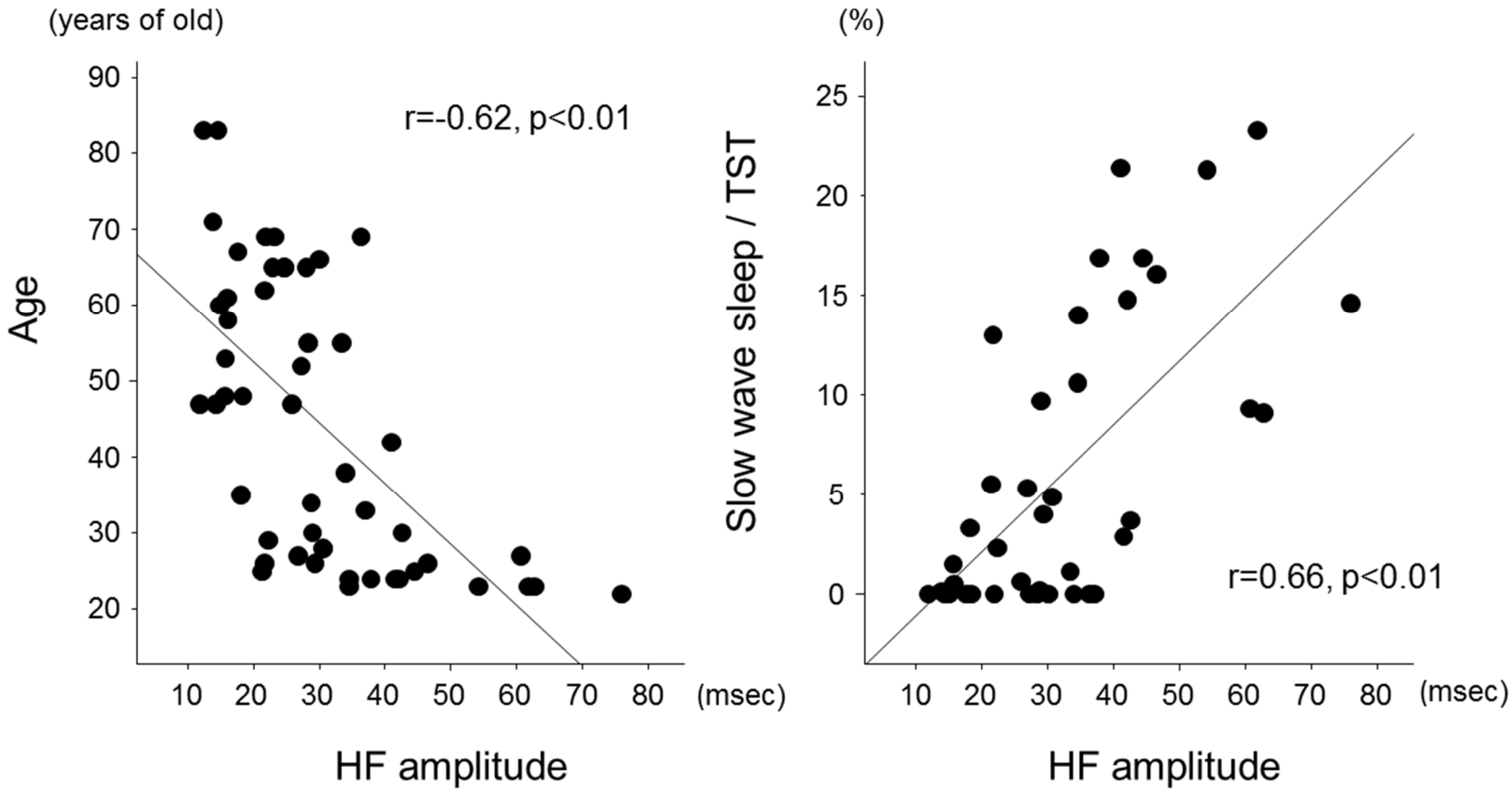

(years of old)
Age
r=-0.62, p<0.01
HF amplitude
(msec)
(%)
Slow wave sleep / TST
r=0.66, p<0.01
HF amplitude
(msec)

Fig. 3

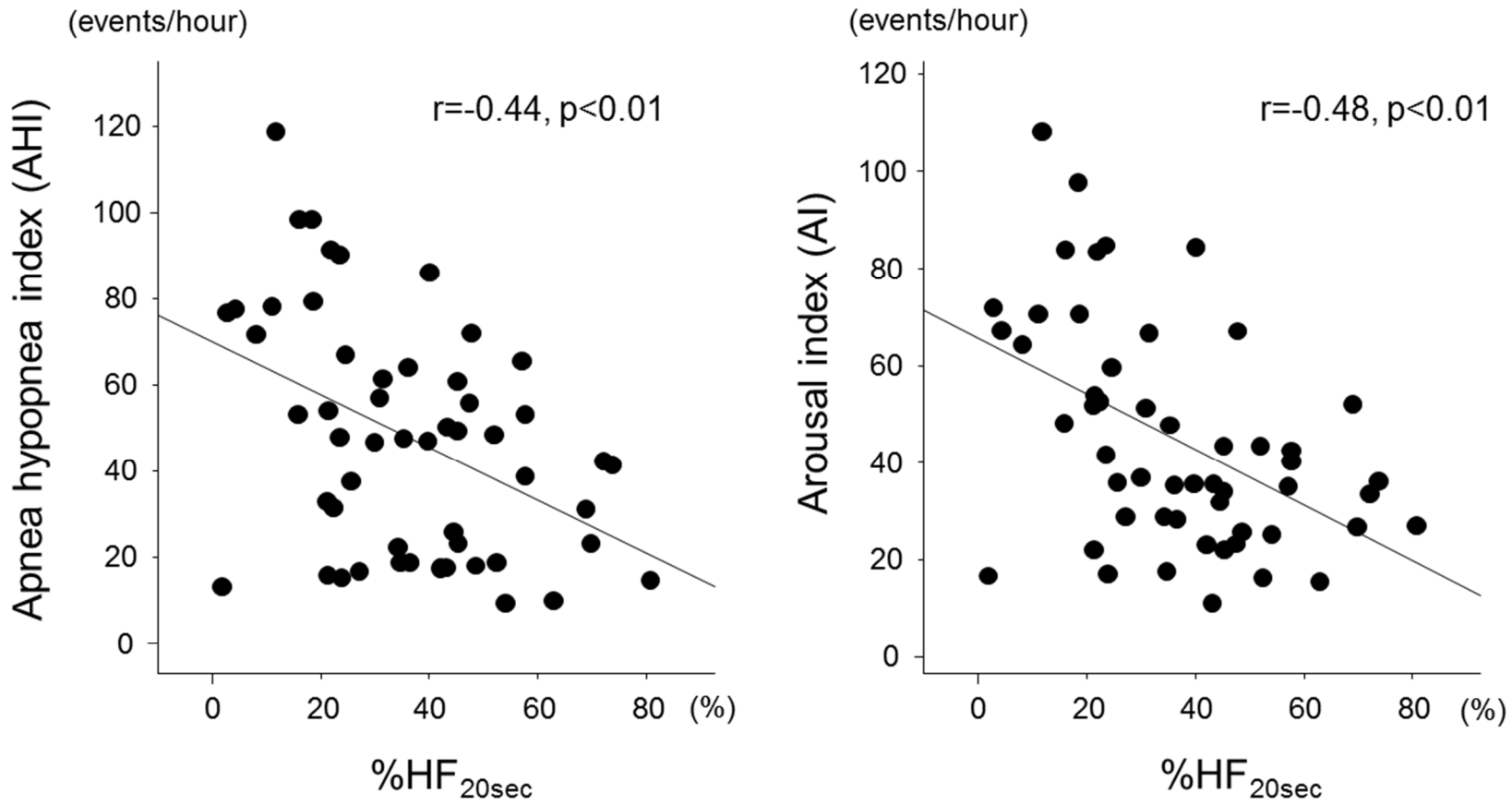
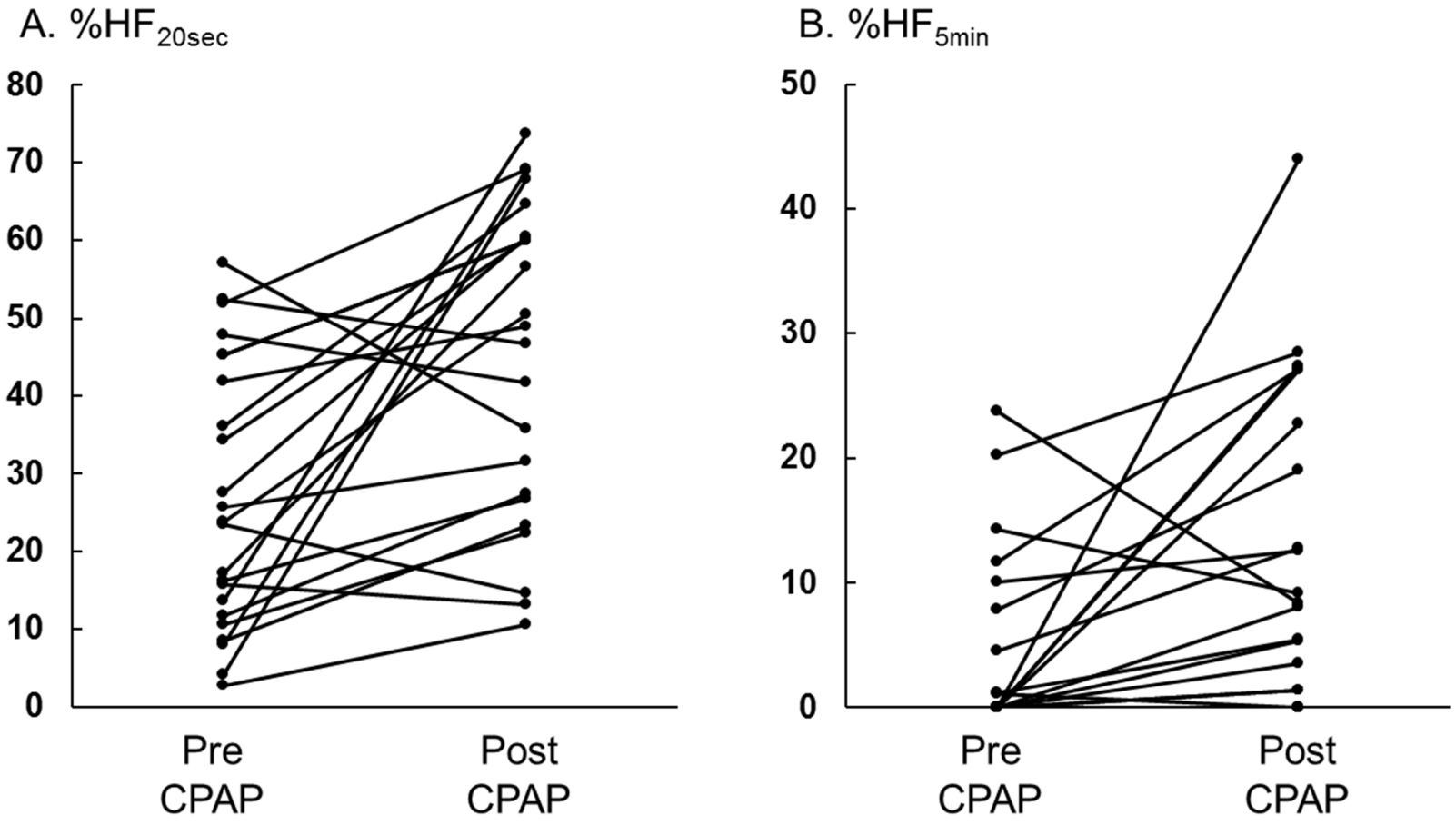

(events/hour)
Apnea hypopnea index (AHI)
r=-0.44, p<0.01
120
100
80
60
40
20
0
0
20
40
60
80
(%)
%HF20sec
(events/hour)
Arousal index (AI)
r=-0.48, p<0.01
120
100
80
60
40
20
0
0
20
40
60
80
(%)
%HF20sec

Fig. 4


A. %HF20sec
80
70
60
50
40
30
20
10
0
Pre CPAP
Post CPAP
B. %HF5min
50
40
30
20
10
0
Pre CPAP
Post CPAP