A Novel Method Predicting Parasympathetic Nerve Dysfunction in Subjects with Apneic Events - Instantaneous Time-Frequency Analysis

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ABSTRACT

The pathophysiological aspects of parasympathetic nerve (PN) function during sleep in patients with morbid apneas/hypopneas studied by classical methods, including power-spectrum and/or time-domain analysis on heart rate variability (HRV), are highly controversial. This controversy is attributed to methodological problems such as poor time resolution in classical methods. The present review describes a reliable method for investigating the PN function in patients with apneas/hypopneas, which has recently been elaborated and named "instantaneous time-frequency analysis (ITF)". The ITF was established based on the complex demodulation (CD) algorism, which enables one to measure the transitional change in amplitude of a target frequency domain in a practically continuous manner. Among high frequency (HF) domains between 0.15 and 0.40 Hz contained in R-R intervals of electrocardiogram tracing (representative of HRV), the HF domain with the maximum

amplitude was used as an approximate measure of PN activity. Based on density-spectrum-array map for main HF peak constructed with time scale of 1 sec and frequency resolution of 0.002 Hz (HF-DSA map), shift in central frequency of main HF peak over time was continuously monitored. When the main HF peak with the same central frequency lasted for more than 20 seconds or 5 minutes on HF-DSA map, the PN function was assumed “stable” or “very stable”, respectively. The ITF allowed elucidation of the qualitative and quantitative abnormalities in PN function in patients with obstructive sleep apnea (OSA), while it did not evaluate PN abnormalities in patients with central sleep apnea (CSA). Furthermore, the ITF allowed detection of the effects of various confounding factors on PN function in OSA patients, i.e., PN activity was inhibited by aging and obesity, while PN stability was distorted by apneas/hypopneas. To establish the clinical importance of ITF, normal reference values regarding the activity and stability of PN function were also reported in the present review.

Key words: power-spectrum analysis; time-domain analysis; complex demodulation method; parasympathetic nerve activity; parasympathetic nerve stability; obstructive sleep apnea; central sleep apnea; continuous positive airway pressure (CPAP) treatment

1. OVERVIEW

The autonomic nerve dysfunction occurring in subjects with apneic events (sleep apnea syndrome (SAS)) has been demonstrated to be closely implicated in the pathogenesis of a variety of cardiovascular diseases, including systemic hypertension, heart failure, myocardial infarction, stroke, and so on [1,8]. Unfortunately, however, no reliable method that allows a precise estimation of the autonomic nerve dysfunction (i.e., the sympathetic and parasympathetic nerve abnormalities) in subjects with SAS has been established [54,55]. A classical study performed by Somers et al. [47], who measured the sympathetic nerve activity using direct intra-neural recordings of efferent sympathetic discharges to muscle blood vessels (microneurography), demonstrated that sympathetic burst frequency was significantly augmented during both the non-rapid eye movement (NREM) period and the rapid eye movement (REM) period in patients with SAS. This finding has been confirmed by measurements of plasma and urine norepinephrine levels [1] as well as by electrophysiological studies [31,32]. On the other side, the results reported for the efferent parasympathetic nerve discharge traveling to the cardiac sinus node in patients with SAS studied by the power-spectrum analysis on heart rate variability (HRV) have been highly conflicting. Khoo et al. [23] showed that the parasympathetic-nerve-evoked modulation on HRV was inhibited, while Gula et al. [12] reported its enhancement, in patients with SAS. Vanninen et al. [52] indicated that the parasympathetic modulation on HRV was unchanged during night in patients with SAS. These confused findings on the parasympathetic nerve function in patients with SAS are considered to be attributed to the methodological concerns.

Many methods, including the fast Fourier transform (FFT) algorithm [17], the autoregressive approach (AR) [23], and the maximum entropy method (MEM) [54], have been applied for quantitatively assessing the autonomic nerve function in the body. These methods are mainly based on the power-spectrum analysis for HRV, which reflects the efferent autonomic nerve discharges. In addition, the time-domain analysis on HRV has also been used as a semi-quantitative tool for estimating the autonomic nerve function [48,54]. As described below, however, these classical methods have a couple of serious defects when applied to patients experiencing morbid apneas and hypopneas during sleep.

1. Although the classical methods analyzing HRV have been widely used for measuring the sympathetic nerve activity in subjects with various diseases

[11,35], many authors [18,19,33,46] have found difficulty in estimating the sympathetic nerve activity in terms of the power-spectrum analysis or the time-domain analysis on HRV. This is ascribed to the difference in the signal transduction within the β receptor activated by the sympathetic nerve discharge and that within the muscarinic receptor related to the parasympathetic nerve discharge [16]. In consequence, the sympathetic nerve system cannot transmit the signals with frequencies > 0.15 Hz, while the parasympathetic nerve system can impart the signals with frequencies up to 1 Hz [6] (Fig. 1). These facts certainly indicate that the low frequency (LF) components (≤ 0.15 Hz) contained in HRV are simultaneously formed by the sympathetic nerve discharge as well as the parasympathetic nerve discharge, if any. On the other hand, the high frequency (HF) components (> 0.15 Hz) in HRV are taken to be mediated simply by the parasympathetic nerve discharge [2,6,13,39,43,44,53]. Although the LF band or the LF/HF ratio has been used as a surrogate measure predicting the sympathetic nerve function [11,35], the above-mentioned findings securely suggest that the LF-associated parameters, i.e., the LF band and LF/HF ratio, are gravely contaminated with both of the sympathetic and parasympathetic nerve discharges. These facts suggest that only the parasympathetic nerve function, but not the sympathetic nerve function, can be reliably evaluated from the classical power-spectrum and/or time-domain analysis. However, attention should be paid to the fact that the difficulty in predicting the sympathetic nerve function from the HRV analysis holds true for the novel method referring to as the “instantaneous time-frequency analysis” extensively discussed in the next section, as well. Based on these considerations, the present review simply focusses on the parasympathetic nerve function in subjects with SAS, which can be predicted from analyzing the HF domains in HRV.

Fig. 1

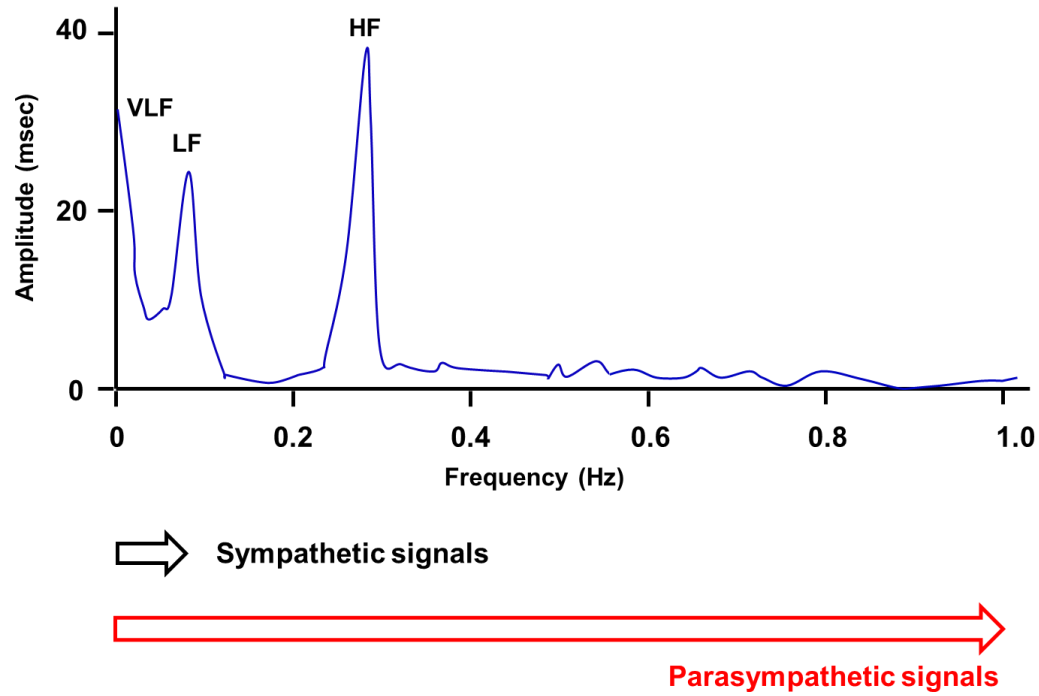

Fig.1 Frequency transmission of sympathetic and parasympathetic nerve signals depicted in the figure showing the results obtained by the classical power-spectrum analysis with autoregressive approach

Vertical axis: amplitude of the frequency domain, horizontal axis: frequency. VLF: component with very low frequency (≤ 0.04 Hz), LF: component with low frequency (> 0.04 Hz but ≤ 0.15 Hz), HF: component with high frequency (> 0.15 Hz).

2. The time resolution of the classical power-spectrum analysis such as the FFT algorism or the AR is low, requiring at least 2-min electrocardiogram (ECG) tracing (corresponding to about 100 heart beats) for obtaining the data necessary for a definitive analysis determining the HF domains contained in HRV [54,55]. Within 2 min, however, a patient with morbid apneas sometimes experiences a rapid change in breathing pattern from normal respiration to apnea and *vice versa*. As the parasympathetic nerve discharge is significantly influenced by the breathing pattern, the transitional change in the parasympathetic nerve discharge in a patient with severe apneic events is no longer followed by the classical power-spectrum analysis with a low time

resolution [54,55]. Furthermore, it should be noted that the time resolution of the time-domain analysis is substantially lower than that of the power-spectrum analysis [54], which suggests that in comparison with the power-spectrum analysis, it is much more difficult to estimate the parasympathetic nerve function in patients with SAS from the time-domain analysis. Therefore, the present review only takes up the power-spectrum analysis.

3. The classical power-spectrum analysis calculates the power ($msec^2$) by integrating the area beneath each frequency component (FFT) or by summing the square of each amplitude (msec) within a certain range of high frequency domains (AR) for estimating the mean parasympathetic nerve activity. However, apneic events forcibly yield many noises [54], including harmonic spectra and complicated spectra evoked by asynchronous increments and decrements of R-R intervals (RRI) of an ECG tracing, none of which should be ascribed to the parasympathetic nerve discharge. These noises, which are unrelated to the parasympathetic nerve discharge, are included in the power by the FFT algorism or in the square of each amplitude by the AR as if they were generated by the parasympathetic nerve discharge. These facts indicate that the classical power-spectrum analysis obscures or misses the veritable behavior of the parasympathetic nerve discharge around the time points of apneic events (see section 3.3 for further details).
4. Although the stability is as important as the activity for assessing the parasympathetic nerve dysfunction, it cannot be estimated from the analysis based on the classical methods, including the power-spectrum analysis and the time-domain analysis. To overcome these difficulties involved in the classical methods, Yamaguchi et al. [54] have recently elaborated a novel method with the high resolution for time and frequency, which enables one to reliably measure a rapid change in the parasympathetic nerve discharge in a virtually continuous fashion. This novel method has been termed “instantaneous time-frequency analysis”, which was established as the basis of the complex demodulation method [14,15]. The instantaneous time-frequency analysis allows one to detect the activity as well as the stability of the parasympathetic nerve function under many diseased conditions, including apneas and hypopneas.

The subsequent sections describe the following matters; (1) basic concept of instantaneous time-frequency analysis, (2) electrophysiological interpretation of various frequency domains in patients with obstructive sleep apnea (OSA) or with central sleep apnea (CSA), (3) impacts of various confounding factors on parasympathetic nerve function in patients with OSA, and (4) normal reference values for activity and stability of parasympathetic nerve function.

2. BASIC concept of instantaneous time-frequency analysis

2.1 Complex demodulation (CD) method

The frequency spectra held in HRV represented by RRI of an ECG tracing were estimated for the range between zero and 0.40 Hz. These frequency spectra were divided into three components depending on their central frequencies, i.e., the spectral domain with the central frequency of less than 0.04 Hz, that between 0.04 and 0.15 Hz, and that of more than 0.15 Hz but less than 0.40 Hz. They were labeled as the bands with very low frequency (VLF), low frequency (LF), and high frequency (HF), respectively [10,27]. In the classical power-spectrum analysis, VLF has been taken as a parameter reflecting a direct-current component of RRI with no rhythmic oscillation, whereas LF band or LF/HF ratio as a surrogate marker of the sympathetic nerve discharge to the cardiac sinus node [27]. However, the electrophysiological significance of VLF is controversial [10]. As argued in the overview section, LF band and LF/HF ratio are now considered to be mediated by both sympathetic and parasympathetic nerve discharges [9,10,19,33]. On the other side, HF band has been certified to be simply associated with the parasympathetic nerve discharge, because the sympathetic nerve cannot transmit a neural signal with frequency over 0.15 Hz [6]. These facts indicate that only the HF-related parasympathetic nerve function can be reliably estimated as far as the HRV data are analyzed.

The CD method [14,15] enables one to measure a transitional change in instantaneous amplitude (msec) of a target frequency spectrum from a short-time tracing of ECG recorded for at least 6.7 sec (corresponding to about 7 heart beats) when the behavior of HF spectra is brought into focus. Thereby, the CD method is able to differentiate the qualitative and/or quantitative change in the parasympathetic nerve discharge so far as it happens several seconds apart. Applying the CD method, the instantaneous amplitude of each HF band with central frequency ranging from 0.15 to 0.40 Hz was calculated at the interval of 0.002 Hz and the time scale of one second (Fig. 2). The value of 0.002 Hz is the limit of

frequency resolution in the CD method. The instantaneous amplitude was taken to be equal to the peak height of a given HF band. The arithmetic mean of instantaneous amplitudes of all HF bands was used to identify the mean level over the HF region at a certain time point. This was defined as the M (msec) and the HF bands whose instantaneous amplitudes exceeded the M were explored. Among the HF bands with instantaneous amplitudes above the M, the HF band having the maximum instantaneous amplitude (MaxA) meeting the requirement of MaxA≥ 2･M was searched and defined as "main HF peak". It should be noted, however, that instantaneous amplitudes of HF bands existing in the vicinity of the LF-HF boundary (0.15 Hz) are occasionally overestimated due to contamination with LF components. To exclude the main HF peak overestimated by superimposed LF ("false" main HF peak), the mean frequency (MF) over the HF region was estimated. The MF was defined as the junction-frequency at which the sum of instantaneous amplitudes of HF bands existing between the lower boundary (0.15 Hz) and the MF was just equal to that between the MF and the upper boundary (0.40 Hz). When the central frequency of the main HF peak was located at the position of ±0.03 Hz apart from the MF, it was taken as the "false" main HF peak and was no longer used for analyzing the parasympathetic nerve function.

Fig. 2

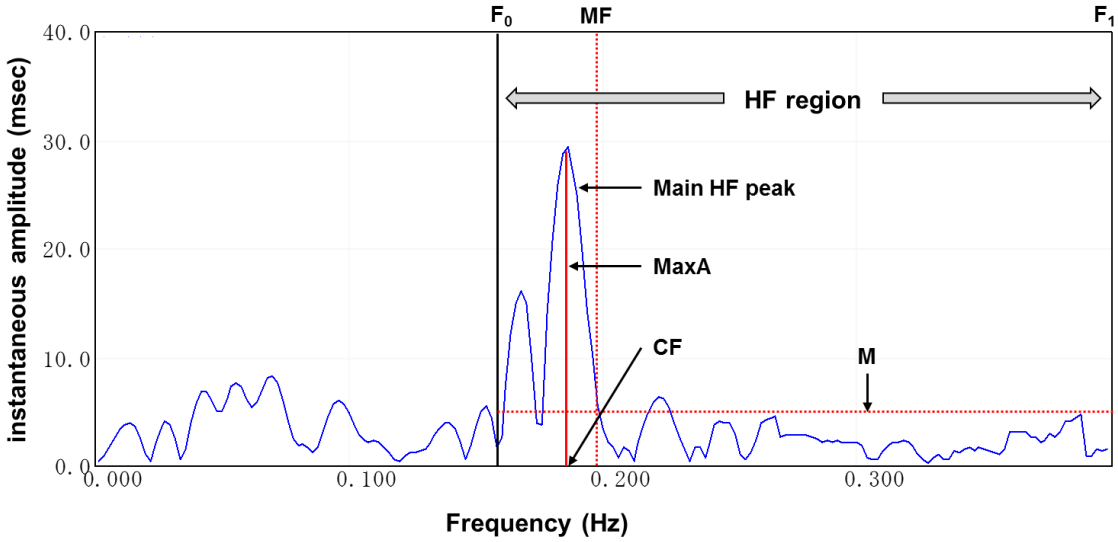

Fig. 2 Determination of the main peak in the HF region with CD method
Vertical axis: instantaneous amplitude of the frequency domain at a certain time. Horizontal axis: frequency. F_0: 0.15 Hz, F_1: 0.40 Hz. M: mean level of instantaneous amplitudes for all HF bands. MF: mean frequency dividing the sum of instantaneous amplitudes of all HF bands into two equal parts. CF: central frequency of a given HF band. MaxA: maximum instantaneous amplitude of the main HF peak satisfying the criteria of MaxA≥ 2·M. The CF of the main HF peak is located within ±0.03 Hz from the MF. Adopted from reference 54 (Yamaguchi et al. *Sleep Med* 2014; 15:33-41).

2.2 Quantitative parameter predicting parasympathetic nerve activity

The efferent parasympathetic nerve discharge transmitted to the cardiac sinus node is governed by the neural integration of cardiovascular center and respiratory center as well as the reflex from pulmonary stretch receptors [27]. Under a condition with normal respiration without apneas and hypopneas, pulmonary stretch receptors are activated by increasing lung volume during inspiration, leading to inhibition of the parasympathetic nerve discharge (inspiratory gating), while the parasympathetic nerve discharge is augmented by decreasing lung

volume during expiration [7,51]. Since the parasympathetic nerve discharge during inspiration is inhibited nearly to zero [7,22], the instantaneous amplitude of the main HF peak can be taken as the parameter varying in parallel with the average level of parasympathetic nerve activity (Figs. 2, 3). However, this does not tell the truth under conditions with abnormal respiration such as apneas and hypopneas, at which the lung volume is substantially small due to no airflow, though the thoracic motion is preserved. This leads to an incomplete inspiratory gating, and thus, the parasympathetic nerve activity may not reach zero in the thoracic-motion-judged inspiratory phase during apneas and hypopneas, resulting in that the instantaneous amplitude of the main HF peak is compulsively lowered even when the parasympathetic nerve activity is maintained at the same level. It is difficult, therefore, to take the instantaneous amplitude of the main HF peak as the parameter changing perfectly parallel with the parasympathetic nerve activity in patients with SAS. Strictly speaking, the main HF peal should be considered as the parameter reflecting the extent of parasympathetic-nerve-evoked modulation on the cardiac sinus node [16]. However, as there was no definitive parameter that reliably predicts the parasympathetic nerve activity in patients with SAS, the instantaneous amplitude of the main HF peak was used as a measure predicting the parasympathetic nerve activity in patients with SAS in a first approximation [54,55].

2.3 Quantitative parameters predicting parasympathetic nerve stability

The parasympathetic-nerve-elicited RRI variation is synchronized with inspiratory and expiratory lung volumes under a condition with normal respiration, generating the respiratory sinus arrhythmia (RSA) having the main HF peak with a relatively constant central-frequency at around 0.25 Hz [17,56]. However, since the integrated neural controls for parasympathetic nerve discharge, including the inspiratory gating, are expected to be disturbed at time points with apneic episodes, the central frequency of the main HF peak under apneic conditions may occasionally be shifted from that observed for the RSA with normal respiration. This is considered as a phenomenon giving a sign of instability of the parasympathetic nerve function under conditions with apneic episodes. Furthermore, it is anticipated that instability of the parasympathetic nerve function leads to a change in central frequency of the main HF peak with time. Therefore, the density spectrum array (DSA) map describing the transitional change in central frequency of the main HF peak was

constructed with time scale of one second and frequency resolution of 0.002 Hz. This was defined as the HF-DSA map, which allows one to estimate the time-dependent positional variation of the main HF peak in a practically continuous manner (Figs. 3, 4, 5). When the central frequency of the main HF peak was shifted by more than ±0.014 Hz, corresponding to ±5% of that of RSA, the parasympathetic nerve discharge was assumed to be significantly altered from the previous state, i.e., disruption of the parasympathetic nerve discharge. When the main HF peak lasted for at least 20 seconds with no disruption, the parasympathetic nerve function was considered to be kept at "stable state". The time of the main HF peak lasted for more than 20 seconds was defined as HF_{20sec}. The relative time of HF_{20sec} during NREM or REM, denoted by $\%HF_{20sec}$, was calculated with the equation of $100 \cdot HF_{20sec}$/(total time of NREM or REM) and used for judging the stability of the parasympathetic nerve function in respective sleep states [54,55]. Furthermore, when the main HF peak lasted for more than 5 minutes with no disruption (HF_{5min}), the parasympathetic nerve function was taken to be "very stable" [54,55]. Again, the very stable condition of parasympathetic nerve function was assessed from the relative time of HF_{5min} against the total time of NREM or REM ($\%HF_{5min}$).

Fig. 3

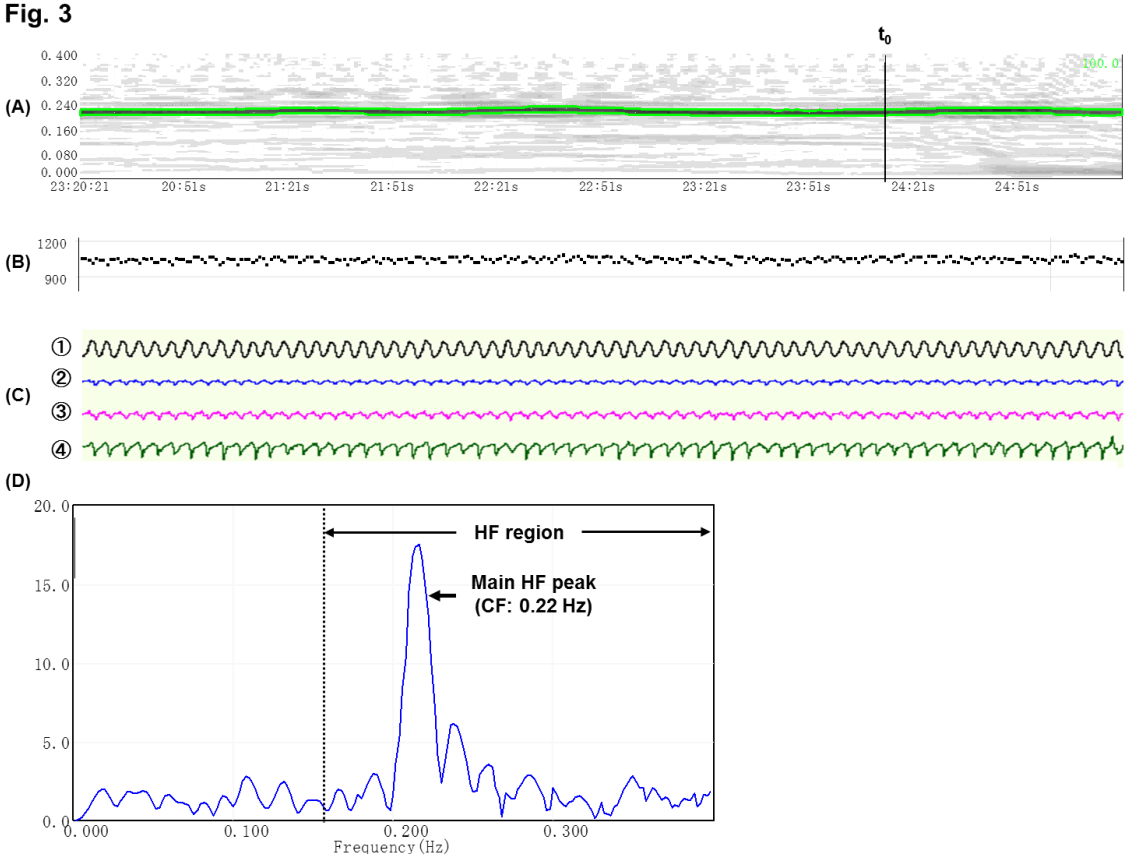

Fig. 3 Instantaneous time-frequency analysis for the subject with normal respiration during NREM sleep

(A):HF-DSA map during NREM with normal respiration (observation time: 5 min). Vertical axis: frequency. Horizontal axis: time (min : sec). Green band with black line at the center denotes the stable HF band with CF variation within ±0.014 Hz.(B): RRI change (msec) with time.(C): PSG data in which; ① oral airflow measured with thermocouples, ② nasal airflow by nasal air pressure, ③ and ④ respiration-induced thoracic and abdominal motions recorded by piezoelectric belt sensors, respectively.(D): frequency spectra at time t_0 on the HF-DSA map. Vertical axis: instantaneous amplitude (msec). Horizontal axis: frequency (Hz). Dotted line: the portion at 0.15 Hz.

Fig. 4

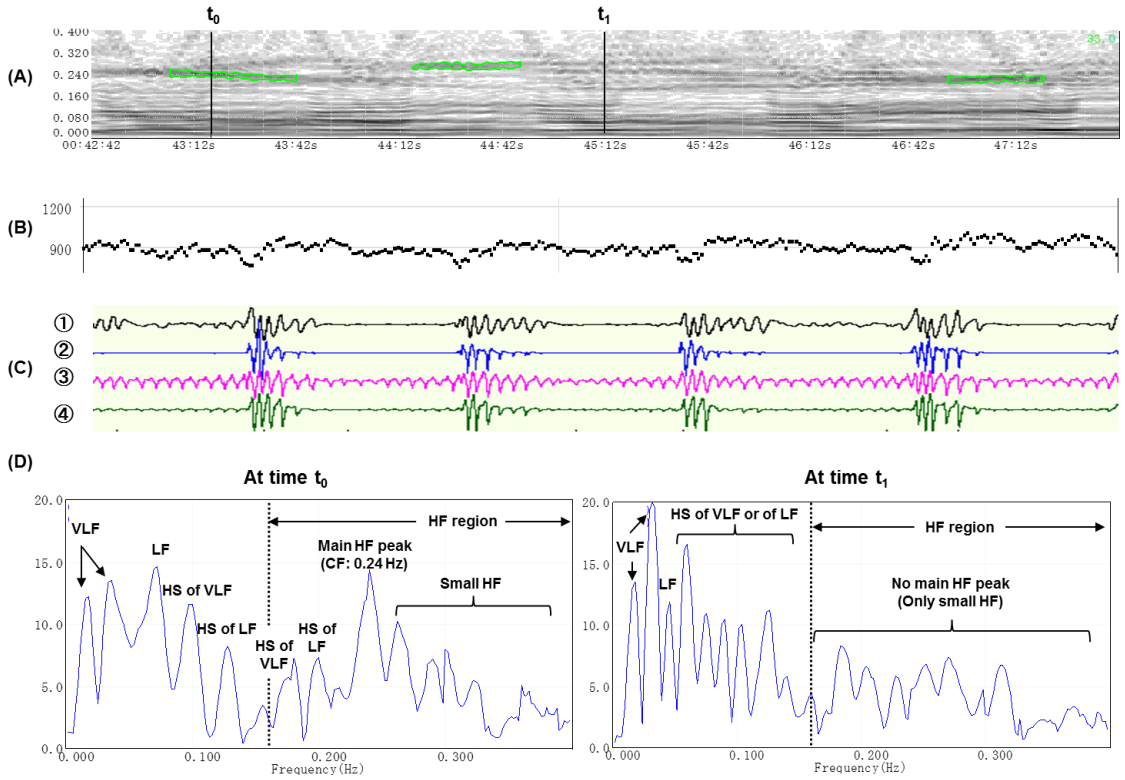

Fig. 4 Instantaneous time-frequency analysis for the subject with severe OSA during NREM sleep

Data were harvested from the subject B with overall AHI at 57.3/hr.(A): HF-DSA map for the period with repeated apneas during NREM. Vertical and horizontal axes are the same as defined in Fig. 3.(B): RRI (msec).(C): PSG data. Symbols are the same as those designated in Fig. 3.(D): frequency spectra at time t_0 (left panel) and at time t_1 (right panel) on the HF-DSA map. Dotted line: the portion at 0.15 Hz. VLF: component with very low frequency, LF: component with low frequency. HS: harmonic spectra generated by VLF or LF. Small HF: HF bands formed in the vicinity of the main HF peak. Adopted from reference 54 (Yamaguchi et al. *Sleep Med* 2014; 15:33-41).

Fig. 5

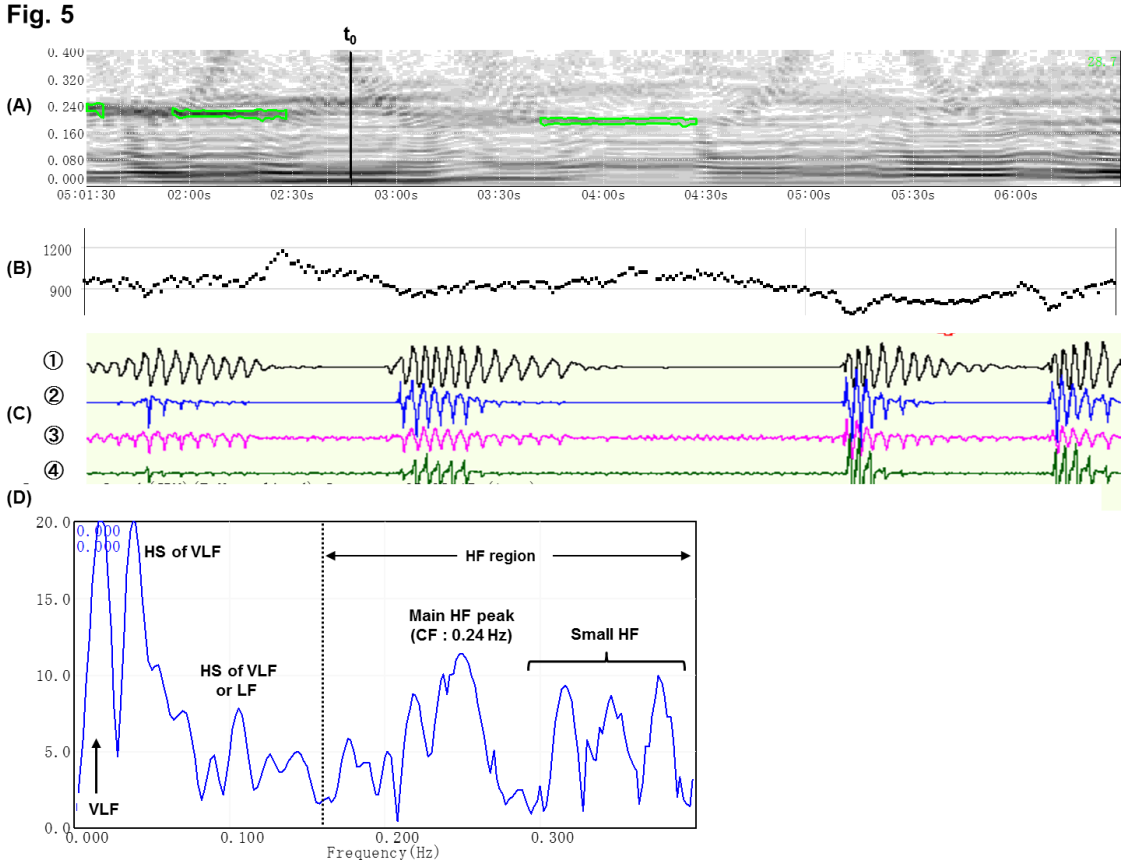

Fig. 5 Instantaneous time-frequency analysis for the subject with severe OSA during REM sleep

Data were obtained from the subject B. (A): HF-DSA map for the period with repeated apneas during REM. (B): RRI (msec). (C): PSG data. (D): frequency spectra at time t_0 on the HF-DSA map. Symbols are the same as those denoted in Fig. 3. Adopted from reference 54 (Yamaguchi et al. *Sleep Med* 2014; 15:33-41).

3. ELECTROPHYSIOLOGICAL interpretation of various frequency domains in patients with sleep apnea

3.1 Implication of HF bands in patients with obstructive sleep apnea (OSA)

In a normal subject with no morbid apnea and hypopnea, the main HF peak with central frequency at 0.22 Hz was found to be lasting for a long time without disruption (Fig. 3). There were no significant noises induced by the harmonic spectra and/or the complicated spectra evoked by asynchronous increments and decrements of RRI, which are universally detected in a subject with SAS (see below).

In a patient with severe OSA (subject B with overall AHI: 57.3 events/hr), the main HF peak with central frequency at 0.24 Hz sustained for 40 sec in combination with

small HF bands was detected during NREM (Fig. 4). The small HF bands may be ascribed to the asynchronous increments and decrements of RRI caused by the unstable inspiratory gating during apnea. At the time t_1 with obstructive apnea during NREM, the main HF peak was not identified, which suggests that the parasympathetic-nerve-elicited HF peak at this time was not so large as could be detected by the CD method and buried under the small HF bands. The HF-DSA map (Fig. 4) demonstrated three main HF peaks lasting for 30~40 sec, in which the first and third main HF peaks had the central frequency at 0.24 Hz. On the other side, the second main HF peak had the central frequency at 0.26 Hz, significantly shifting from the other two peaks. Furthermore, no sustained main HF peak was found at other time points, indicating that the parasympathetic nerve function would be very unstable and frequently disrupted during this observation period in which obstructive apneas broke out repeatedly.

At the time point of t_0 with obstructive apnea during REM in the subject B, the main HF peak with central frequency at 0.24 Hz surrounded by small HF bands was detected, though this HF peak was not lasting even for several seconds (Fig. 5). Again, the small HF bands may be attributed to the asynchronous interments and decrements of RRI.

3.2 Implication of very low frequency (VLF) and LF bands in OSA patients

In the subject B, prominent VLF and/or LF bands were investigated at the time with obstructive apnea regardless of the sleep state of NREM or REM (Figs. 4, 5). Although the possibility that the LF band with frequency around 0.10 Hz contains the neural signal coming from the sympathetic nerve cannot be eliminated [25,27], other explanation may be more persuasive. Taking the VLF and LF bands observed at time t_1 during NREM in this patient as the example (Fig. 4), the underlying mechanisms generating these bands were thought over. The special regard that should be noted is that any spectral band, particularly VLF or LF band, forcibly yields harmonic spectral domains at positions given by multiples of the original frequency; i.e., the original frequency multiplied by integer "n" (n=2, 3, 4⋯). Thus, the harmonic spectra elicited by the VLF emerge in the VLF and/or LF regions, while those by the LF come out in the LF and/or HF regions, suggesting that some of the small HF bands are contaminated with the LF-evoked harmonic spectra. Going back to the right panel of Fig. 4, there seemed to be two original VLF bands with central frequencies at 0.017 Hz and 0.029 Hz (the 1st and 2nd bands from the left). The 3rd band at 0.044 Hz was taken to be the original LF. The 5th and

7th bands might be the harmonic spectra of the 1st VLF, whereas the 4th and 8th bands might be those of the 2nd VLF. The 6th band with frequency at 0.085 Hz was considered to be the harmonic spectrum originated from the LF having frequency at 0.044 Hz. The VLF band with central frequency at 0.017 Hz reflects the oscillatory RRI changing with a time scale of about 60 sec. Looking at the trace of RRI, the 60-sec cycle of RRI change may be yielded by the recurrence of respiration, which quickly increases the output of respiratory center causing the increase in inspiratory lung volume. The augmented output of respiratory center inhibits the burst of parasympathetic nerve neurons. Simultaneously, the increased inspiratory lung volume enhances the inspiratory gating, both of which sharply shorten the RRI. The VLF at 0.029 Hz and the LF at 0.044 Hz correspond to the RRI changes with cycles of about 30 and 20 seconds, respectively. These may be related to the interval of resumed respiration. During the resumption of breathing, the thoracic motion is augmented, reached to the maximum, and decreased thereafter; leading to transitional change in the inspiratory lung volume. This lets the inspiratory gating be altered with time, generating the RRI change with cycle of 30 or 20 seconds. Although the 8th band having central frequency at 0.12 Hz was considered to be the harmonic spectrum of the 2nd VLF with 0.029 Hz, the possibility that this LF band reflects the RRI change with a time scale of about 8 seconds was not excluded.

3.3 Comparison between instantaneous time-frequency analysis and classical power-spectrum analysis in OSA patients

To examine the drawback of the classical power-spectrum analysis while investigating the autonomic nerve discharge in the apneic subject, the power spectra around the time t_1 during NREM were calculated in a patient with OSA using a software serving for analysis with the maximum entropy method (MEM) (Fig. 6). The MEM calculation showed the two domains with LF at 0.063 Hz and HF at 0.265 Hz, these being obtained by integrating the area beneath many frequency components existing in the LF and HF regions, respectively. If each domain is formed by the single frequency component, the MEM-derived LF or HF domain can be taken as the parameter containing the information emitted from the autonomic nerves. This may hold true while normal respiration is securely maintained. As elucidated by the instantaneous time-frequency analysis with the CD method, however, there exist many frequency domains in LF and HF regions at this time point with apnea, most of which are not

ascribed directly to the autonomic nerve discharge but to harmonic spectra and/or asynchronous variations of RRI. It is difficult, therefore, to quantitate the autonomic nerve discharge in patients with morbid OSA from the LF and HF domains calculated from the classical power-spectrum analysis.

Fig. 6

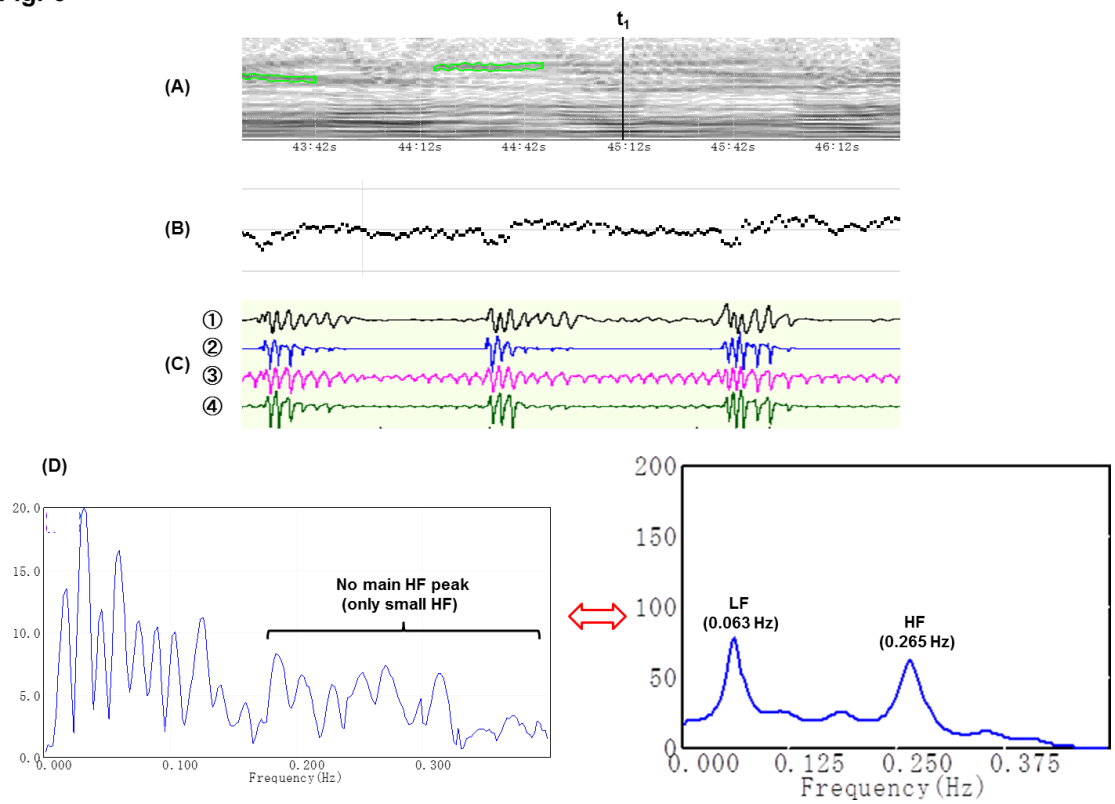

Fig. 6 Comparison between instantaneous time-frequency analysis and power-spectrum analysis

Data were obtained at the period with severe obstructive apnea (time t_1) in NREM. (A): HF-DSA map for the period with repeated apneas. (B): RRI (msec). (C): PSG data. (D): distribution of instantaneous amplitude (msec) along frequency axis decided with the CD method (left panel) and distribution of power spectrum (msec^2) along frequency axis determined with the MEM (right panel). Power spectrum for LF or HF was, respectively, calculated averaging the integrated area under all the bands existing in the LF region or in the HF region over 30 seconds at the center of time t_1. Adopted from reference 54 (Yamaguchi et al. *Sleep Med* 2014; 15:33-41).

3.4 Instantaneous time-frequency analysis for the patient with central sleep apnea

In a patient with central sleep apnea (CSA), the VLF band with central frequency at about 0.025 Hz (the 1st band from the left) was found at time t_0 in NREM sleep (Fig. 7). The formation of this VLF may be ascribed to the oscillatory RRI changing with a time scale of about 30~40 sec, which may reflect the disappearance and recurrence of respiration. The 2nd band with central frequency at about 0.050 Hz, which located in the LF region, is taken to be the harmonic spectrum formed by the first VLF. Although there were many small bands in LF and HF regions, the main HF peak corresponding to the parasympathetic nerve discharge was not detected. The small bands investigated in LF and HF regions are considered to be yielded by an asynchronous RRI change with a time scale ranging from 3 to 10 seconds, which are induced by abnormal thoracic motions during irregular respiration. The fact specifically mentioned in patients with CSA is that it is not possible to detect the main HF peak in both sleep stages of NREM and REM, which suggests that the main HF peak standing for the parasympathetic nerve discharge is conspicuously inhibited in CSA patients or the main HF peak is buried in many small HF bands. The greatly diminished output from the respiratory center during central apneas augments the burst of parasympathetic nerve neurons. In addition, the decreased output of the respiratory center may inhibit the change in lung volumes, which leads to an incomplete inspiratory gating and thus augments the parasympathetic nerve discharge at a time point with apneic events in CSA. On the other side, the output of respiratory center may be augmented during the recurrence of respiration, which quickly increases the inspiratory lung volume, resulting in the enhanced inspiratory gating. These two factors may restrain the parasympathetic nerve discharge at a time point of resumed respiration in CSA. These findings suggest that in CSA, the increased parasympathetic nerve discharge at a time point with central apneic events is counterbalanced with the decreased parasympathetic nerve discharge during a recurring respiration. The above-mentioned facts led one to conclude that it may be extremely difficult to precisely estimate the parasympathetic nerve function in patients with CSA even when the newly developed instantaneous time-frequency analysis is applied.

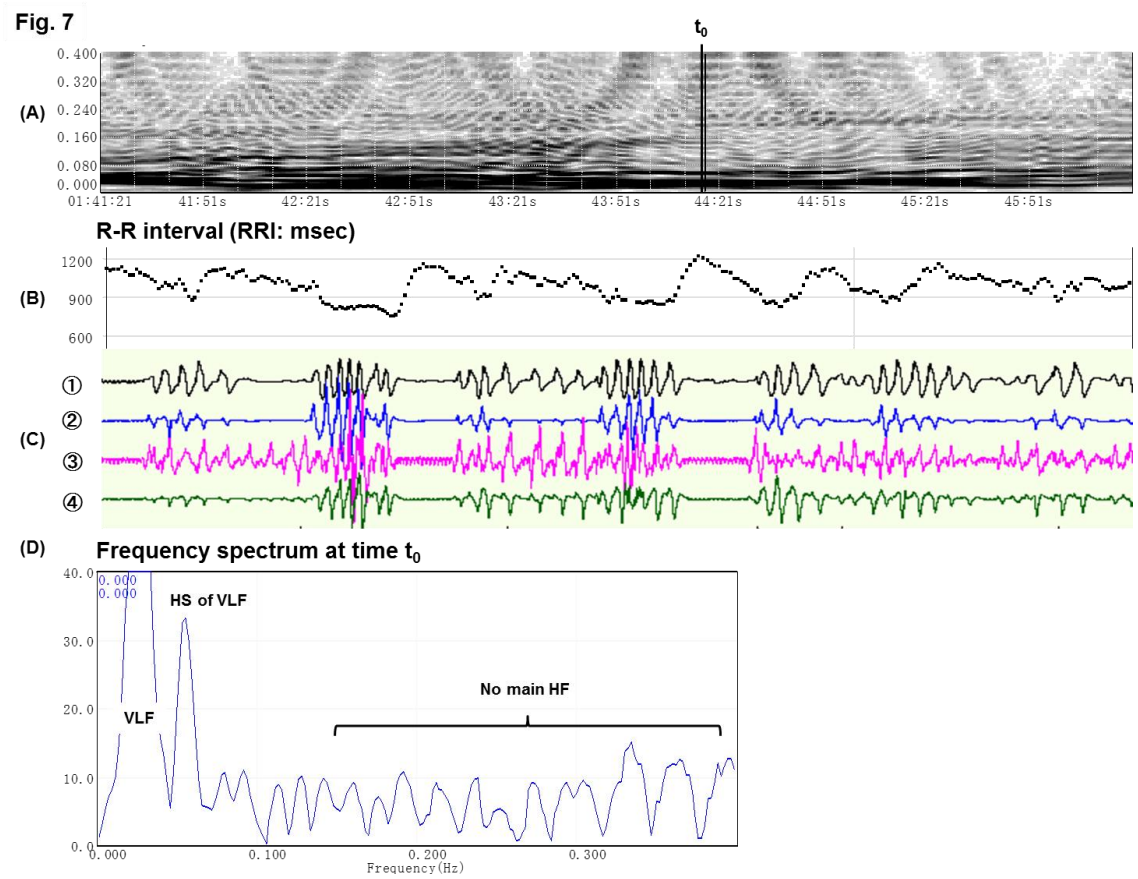

Fig. 7 Instantaneous time-frequency analysis for the subject with central sleep apnea (CSA) during NREM

Data were obtained from the subject C with CSA (overall AHI: 31.1events/hr). (A): HF-DSA map for the period with repeated apneas during NREM. (B): RRI (msec). (C): PSG data. (D): frequency spectra at time t_0 on the HF-DSA map. Symbols are the same as those denoted in Fig. 3.

3.5 Effect of continuous airway pressure (CPAP) treatment on parasympathetic nerve dysfunction in OSA patients

CPAP significantly decreased the instantaneous amplitude of the main HF peak and increased both $\%HF_{20sec}$ and $\%HF_{5min}$ in NREM (Figs. 8, 9). However, the CPAP-elicited changes in these parameters were smaller in REM than in NERM, i.e., the $\%HF_{20sec}$ was increased, but the $\%HF_{5min}$ was not, in REM after CPAP treatment.

Comparing the parameters related to the parasympathetic nerve function between NREM and REM, all the parameters, including instantaneous amplitude of the main HF peak, $\%HF_{20sec}$, and $\%HF_{5min}$, were significantly larger in NREM than those in REM under conditions with and without

CPAP treatment (Figs. 8, 9). Merging these findings together, the functional state of the parasympathetic nerve during sleep in OSA patients is inferred as follows; (1) during NREM, the parasympathetic nerve activity is enhanced while the stability is impeded, (2) during REM, the parasympathetic nerve activity is not enhanced but the stability is more impaired than that in NREM, and (3) CPAP treatment obviously improves the OSA-elicited parasympathetic nerve dysfunction in both NREM and REM. These findings are qualitatively consistent with those reported by Chrysostomakis et al. [8], who demonstrated that CPAP lowered the vagal tone at night in OSA patients based on the results harvested from the time domain analysis on HRV, but inconsistent with the older studies, in which the vagal tone in OSA patients was shown to be blunted at daytime [17] or during sleep [23].

Fig. 8

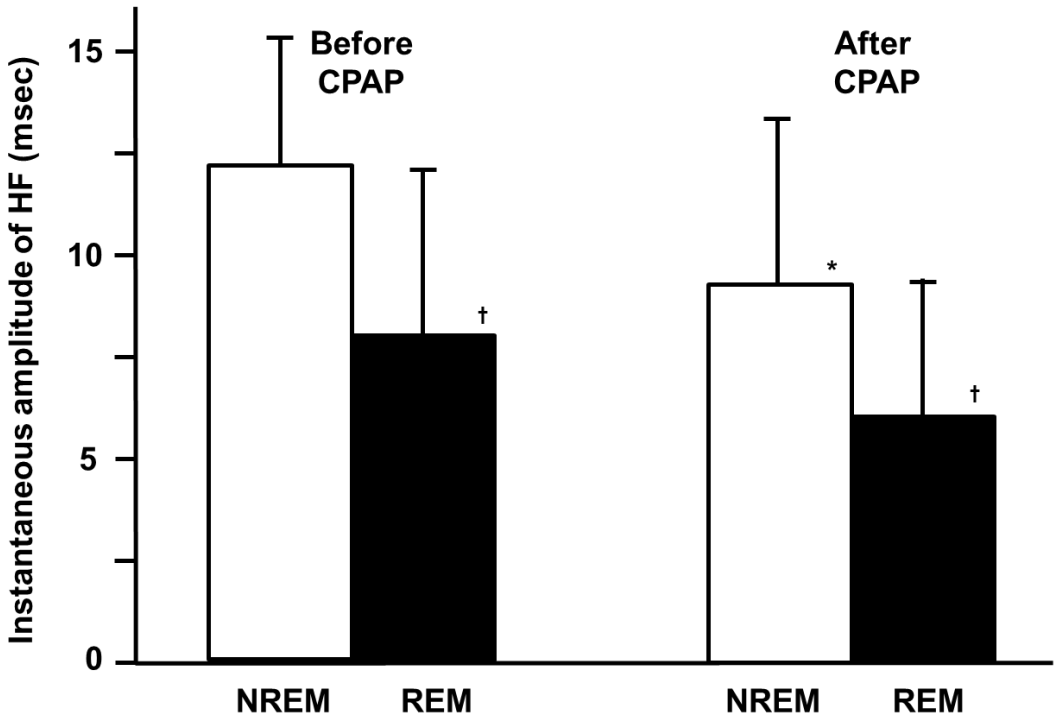

Fig. 8 Instantaneous amplitude of main HF peak in NREM and REM before and after CPAP

Data were obtained from 30 patients with OSA and presented as means ± SD. Vertical axis: instantaneous amplitude of main HF peak (msec). †: smaller than the value of NREM. *: smaller than the value before CPAP. Adopted from reference 54 (Yamaguchi et al. *Sleep Med* 2014; 15:33-41).

Fig. 9

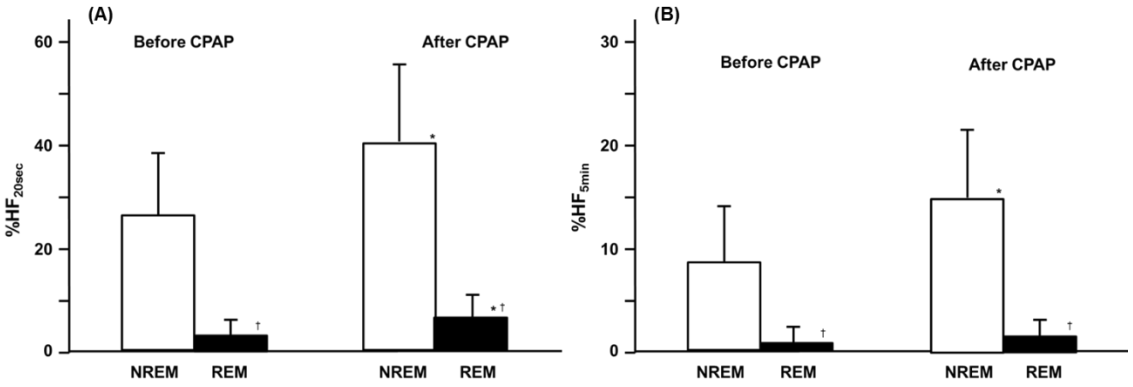

Fig. 9. $\%HF_{20sec}$ and $\%HF_{5min}$ in NREM and REM before and after CPAP
(A): $\%HF_{20sec}$. (B): $\%HF_{5min}$. Values were presented as means ± SD (n=30). †: smaller than the value of NREM. *: larger than the value before CPAP. Adopted from reference 54 (Yamaguchi et al. *Sleep Med* 2014; 15:33-41).

4 IMPACTS of various confounding factors on parasympathetic nerve function in OSA patients

Several decades ago, Hrushesky et al. [20] and Shannon et al. [45] analyzed the parasympathetic-nerve-related modulatory effects on the cardiac sinus node and revealed that these effects are substantially inhibited by aging. However, these investigations were performed in awake subjects with normal respiration and not in sleeping subjects with morbid apneas. Based on the time-domain analysis of HRV, Song et al. [48] studied the effect of age on the time-domain variables contained in HRV in male subjects with OSA, demonstrating that aging would play an important role in blunting the parasympathetic-nerve-eliciting modulatory

effect on the cardiac sinus node in these subjects, as well. However, they [48] did not measure the HRV-related time-domain variables in females with OSA. Based on these historical backgrounds, Yamaguchi et al. [55] attempted to clarify the gender-specific impacts of apnea, age, anthropometric factors (height, body weight, and body mass index (BMI)), and life-long cigarette consumption on the parasympathetic nerve dysfunction during REM and NREM sleep in patients with OSA using the complex demodulation method. Two-hundred-sixty adult patients with OSA (197 males and 63 females) were studied for elucidating the impacts of various factors on the parasympathetic nerve function [55].

4.1 Effect of apneas on modifying parasympathetic nerve function

Yamaguchi et al. [55] found that the parasympathetic nerve function in both NREM and REM was more stable in females than that in males. Although this was supported in part by the fact that the aggravating effect of apnea/hypopnea index (AHI) on the parasympathetic nerve stability was missing during REM in females, it is not easy to explain why such a gender-specific difference in the parasympathetic nerve stability occurs in OSA patients. Furthermore, they [55] identified that AHI during NREM or increased BMI during REM would respectively act as the decisive factor for making the parasympathetic nerve system unstable in females. However, the parasympathetic nerve stability was simply determined by AHI during both NREM and REM in males (Fig. 10). Supporting these findings, the previous study conducted by Yamaguchi et al. [54] demonstrated that apneic events made the parasympathetic nerve function during both NREM and REM substantially unstable in OSA patients consisting mainly of males and this instability was improved when apneas were decreased by CPAP treatment.

In addition to the aggravating effect on the stability of the parasympathetic nerve function, apneas enhanced the parasympathetic nerve activity, but this was observed only during REM in male OSA (Fig. 10). It is not clear why the apnea-related enhancement of parasympathetic nerve activity did not exist during REM in female OSA and during NREM in both male and female OSA. These findings are partly in accordance with those reported by Park et al. [37], who showed that AHI was importantly correlated with time-domain indices of HRV reflecting parasympathetic nerve function in male OSA. However, Park et al. [37] did not analyze the influence of AHI on parasympathetic nerve function in female OSA. Furthermore, they [37] did not estimate the important role of AHI in eliciting the

instability of the parasympathetic nerve function.

Fig. 10

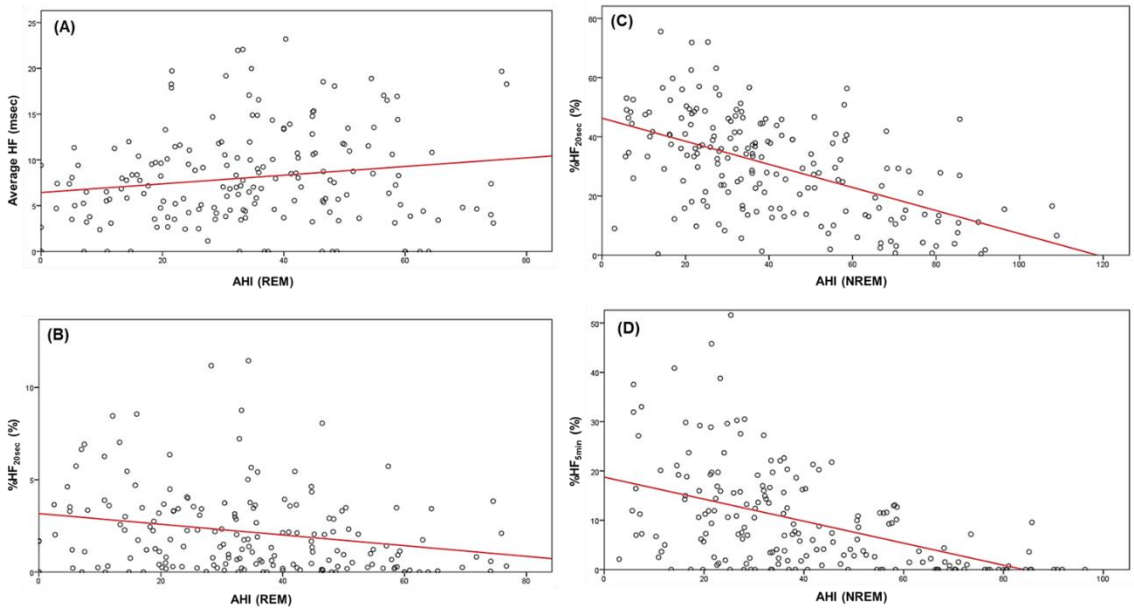

Fig. 10 Impacts of AHI on parasympathetic nerve function during REM and NREM in males Vertical axis: average value of instantaneous amplitudes of the main HF peaks (average HF) in (A), $\%HF_{20sec}$ in (B) and (C), and $\%HF_{5min}$ in (D). Abscissa: AHI in REM or NREM. Red line: regression line determined with least-squares minimization. (A): Effect of AHI on the average HF in REM. The partial correlation coefficient between AHI and average HF was 0.210 (p= 0.005). (B): Effect of AHI on the stability of parasympathetic nerve function ($\%HF_{20sec}$) in REM. The partial correlation coefficient between AHI and $\%HF_{20sec}$ was -0.177 (p= 0.017). (C): Effect of AHI on $\%HF_{20sec}$ in NREM. The partial correlation coefficient between AHI and $\%HF_{20sec}$ was -0.518 (p< 0.0001). (D): Effect of AHI on $\%HF_{5min}$ in NREM. The partial correlation coefficient between AHI and $\%HF_{5min}$ was -0.461 (p< 0.0001). Adopted from reference 55 (Yamaguchi et al. *PLoS ONE* 2014; 9: e92808).

4.2 Effect of aging on modifying parasympathetic nerve function

Differing from AHI, aging exerted no influence on the parasympathetic nerve stability. However, it acted as a universal factor in the diminishment of the parasympathetic nerve activity irrespective of

sleep stage and gender; i.e., little gender-specific difference in the effect of aging on the activity and stability of parasympathetic nerve function (Fig. 11). The importance of aging for blunting the parasympathetic nerve activity in subjects with normal respiration was argued by many authors [20,21,34,42,45,49,50], attaining a consistent conclusion that the cardiac modulation by the parasympathetic nerve system declined with age. Reardon and Malik [42] considered that the age-dependent reduction in the parasympathetic nerve activity was ascribed to the decreased responsiveness of the autonomic nerve system to external stimuli with age. Based on the time-domain analysis of HRV, Jensen-Urstad et al. [21] showed that the association between age and time-domain variables reflecting the parasympathetic nerve activity was somewhat weak in healthy females in comparison with that in males; i.e., the gender-specific difference in the effect of age on the diminution of the parasympathetic nerve activity in subjects with normal respiration. Although the methodological difference exits between the two studies, the integration of the findings reported by Jensen-Urstad et al. [21] and Yamaguchi et al. [55] may suggest that the age-dependent diminishment of the parasympathetic nerve activity is weak in healthy women but exaggerated in women with OSA. The time-domain analysis for HRV conducted by Song et al. [48] demonstrated that the HRV indices corresponding to the parasympathetic nerve activity sensitively responded to age in OSA patients, being highly consistent with the tendency observed by Yamaguchi et al. [55].

Fig. 11

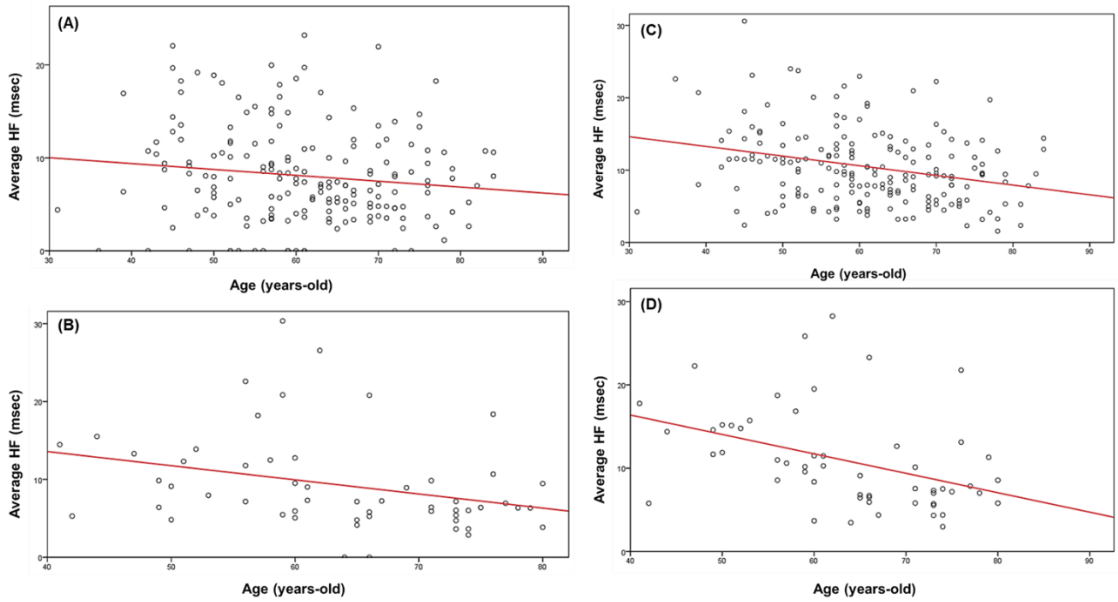

Fig. 11 Effects of age on the parasympathetic nerve activity during REM and NREM in both genders

Vertical axis: average value of instantaneous amplitudes of the main HF peaks (average HF). Abscissa: age. Red line: regression line. (A): During periods of REM in males with a partial correlation coefficient between age and average HF of -0.149 (p= 0.045). (B): During REM in females with a partial correlation coefficient between age and average HF of -0.220 (p= 0.048). (C): During NREM in males with a partial correlation coefficient between age and average HF of -0.295 (p< 0.0001). (D): During NREM in females with a partial correlation coefficient between age and average HF of -0.357 (p= 0.009). Adopted from reference 55 (Yamaguchi et al. *PLoS ONE* 2014; 9: e92808).

4.3 Effect of BMI and smoking habit on modifying parasympathetic nerve function

BMI, but not height, significantly blunted the parasympathetic nerve activity during REM in males and during NREM in both males and females (Fig. 12). Furthermore, BMI made the parasympathetic nerve function considerably unstable during REM sleep in females but not in males. These facts suggest that, contrary to the effect of age, BMI modifies the parasympathetic nerve function in a gender-specific manner. The

importance of BMI or obesity for reducing the parasympathetic nerve activity in subjects without apneic events was addressed by several authors [24,28,29,38,41], resulting in that BMI or obesity should be taken as a factor depressing the parasympathetic nerve activity. Nagai et al. [30] and Molfino et al. [28] conceived that reduction of parasympathetic nerve activity as body size increased might represent a defensive mechanism against fat deposition. Qualitatively the same trend concerning the inhibitory effect of BMI or obesity on the parasympathetic nerve activity was reported in the patients with myocardial infarction [38] and those with OSA [3]. Although the findings obtained in the study for OSA patients by Yamaguchi et al. [55] are evidently consistent with those reported by other authors, Yamaguchi et al. [55] elucidated the effect of BMI on the parasympathetic nerve system in a more detailed fashion; i.e., the dependence of the BMI effect on gender and sleep stage as well as the separate effect of BMI on the parasympathetic nerve activity and on the parasympathetic nerve stability.

Although Barutcu et al. [4] revealed that cigarette smoking had a significant impact on depressing the parasympathetic nerve activity in heavy smokers, Zhang et al. [58] did not obtain the evidence for reliably supporting the decreased parasympathetic nerve activity when acutely exposing cigarette smoke to construction workers. On the other side, there was no authentic study shedding light on the significant role of smoking habit in modifying the parasympathetic nerve function in OSA patients. Yamaguchi et al. [55] found no influence of life-long cigarette consumption on the parasympathetic nerve function, including its activity and stability, being seemingly agreed with the findings reported by Zhang et al. [58].

Fig. 12

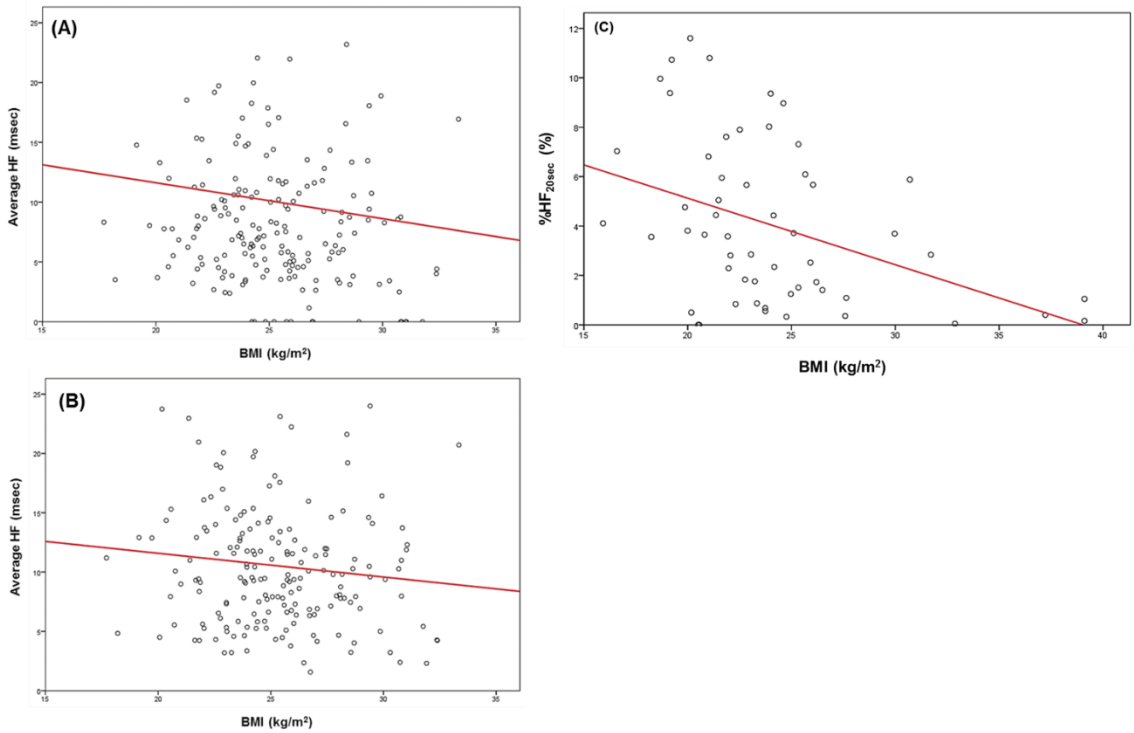

Fig. 12 Effects of BMI on the parasympathetic nerve activity during REM and NREM in males and on the stability of parasympathetic nerve function during REM in females
Vertical axes: average HF in (A) and (B), and $\%HF_{20sec}$ in (C). Red line: regression line. (A): The partial correlation coefficient between BMI and average HF during REM sleep in males was -0.187 (p= 0.012). (B): The partial correlation coefficient between BMI and average HF during NREM in males was -0.174 (p= 0.019). (C): The partial correlation coefficient between BMI and $\%HF_{20sec}$ during REM in females was -0.369 (p= 0.007). Adopted from reference 55 (Yamaguchi et al. *PLoS ONE* 2014; 9: e92808).

5 NORMAL reference values for activity and stability of parasympathetic nerve function

The normal reference values predicting the activity and stability of nighttime parasympathetic nerve function measured in terms of the instantaneous time-frequency analysis have not been reported so far. Therefore, we attempted to decide them based on the HRV data harvested from the adult subjects with normal respiration (n= 61, age≥ 20 years old, average BMI= 22.8 kg/m^2, AHI< 10 events/hr), in whom the number of

males and females was equivalent (males: 30, females: 31). Since some investigators have shown that the parasympathetic nerve activity is modified in patients with COPD [5,26,36,40,57], we excluded the adults with a significant airway obstruction ($FEV_1/FVC< 70\%$ after inhalation of a short-acting β_2 bronchodilator). As described in the above section, we certified that gender, age, and BMI, but not smoking history, had a substantial impact on the parasympathetic nerve function in patients with OSA. Therefore, we first began with inspecting the issue of whether the same aspect holds true in the case of normal subjects without SAS, as well. We found that average HF peak, $\%HF_{20sec}$, and $\%HF_{5min}$ in either sleep of NREM or REM were not different between normal males and normal females. These findings indicate that the difference in gender does not play a role in modifying the parasympathetic nerve discharge during NREM and REM sleep in normal subjects. Therefore, we joined the parasympathetic nerve function data obtained from normal males with those from normal females for the subsequent estimation of modulatory effect of age, BMI, or smoking history on parasympathetic nerve function. We confirmed the following facts in normal subjects concerning the activity and stability of parasympathetic nerve system, both of which were predicted as the basis of the instantaneous time-frequency analysis: (1) age acts as a decisive factor against the parasympathetic nerve activity during either sleep of NREM or REM, whereas BMI or smoking history exerts no impact on the parasympathetic nerve activity; and (2) the stability of parasympathetic nerve function is insensitive to any confounding factor, including age, BMI, or smoking history. Many authors [24, 28,29,38,41] demonstrated that BMI plays a role for depressing the parasympathetic nerve activity in obese subjects, which seems to be inconsistent with the findings that we obtained. This inconsistency is simply explained by the fact that in the study for deciding the normal reference values of the parasympathetic nerve activity, the percentage of obese subjects in the selected cohort was only 6%, indicating that our cohort would be insensitive to detecting the effect of BMI on the parasympathetic nerve activity. Furthermore, it should be noted that obese subjects may not be suitable when attempting to decide the normal reference value for the parasympathetic nerve activity. This is because obesity generally augments the formation of obstructive apneas and/or hypopneas, which exerts a significant influence on the parasympathetic nerve function [54,55]. Therefore, we concluded that the normal reference value for the parasympathetic nerve activity in either sleep

of NREM or REM, which is decided in terms of the instantaneous time-frequency analysis, is described by the gender-independent, age-dependent linear regression equation, whereas the normal reference values for the stability of parasympathetic nerve function are defined as the gender- and age-independent constants (Table 1).

Table 1. Regression equation predicting the reference value of average main HF peak and the reference values of $\%HF_{20sec}$ and $\%HF_{5min}$ in either sleep of NREM or REM

	NREM sleep	REM sleep
Regression equation for average HF peak (msec) (activity)	$26.79 – 0.270 \cdot age$	$22.53 – 0.248 \cdot age$
Reference mean of $\%HF_{20sec}$ (%) (stability)	55.0 ± 17.3	5.6 ± 4.1
Reference mean of $\%HF_{5min}$ (%) (stability)	27.1 ± 15.5	0.4 ± 0.9

Values are the means ± SD. The applicable age for the regression equation ranges from 20 to 85 years.

6 CONCLUSIONS

The present review certified that the classical methods, including the power-spectrum analysis and the time-domain analysis, are not applicable for precisely estimating the parasympathetic nerve function in patients with apneas and hypopneas. To conquer the difficulties involved in the classical methods, Yamaguchi et al. [54,55] established the novel method that allows one to predict a variety of electrophysiological aspects concerning the parasympathetic nerve function in patients with OSA. The newly developed method can measure the approximate activity and the stability of parasympathetic nerve system in OSA patients. However, it is difficult to estimate the parasympathetic nerve function in patients with CSA even when the newly developed instantaneous time-frequency analysis is applied. Furthermore, the present review elucidated the important roles of various confounding factors in modifying the activity and stability of parasympathetic nerve system in OSA patients. To establish the clinical applicability and usefulness of the instantaneous time-frequency analysis for estimating the parasympathetic nerve function in patients with apneas and hypopneas, the normal reference values for the instantaneous time-frequency analysis, including the main HF peak (the surrogate measure of the parasympathetic nerve activity) and $\%HF_{20sec}$ as well as $\%HF_{5min}$

(the stability measures of parasympathetic nerve function), were also reported in the present review.

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