

# Incoherent oscillations of respiratory sinus arrhythmia during acute mental stress in humans

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**Niizeki K, Saitoh T.** Incoherent oscillations of respiratory sinus arrhythmia during acute mental stress in humans. *Am J Physiol Heart Circ Physiol* 302: H359–H367, 2012. First published October 28, 2011; doi:10.1152/ajpheart.00746.2011.—Respiratory sinus arrhythmia (RSA) has been widely used as a measure of the cardiac vagal control in response to stress. However, RSA seems not to be a generalized indicator because of its dependency on respiratory parameter and individual variations of RSA amplitude ( $A_{RSA}$ ). We hypothesized that phase-lag variations between RSA and respiration may serve as a normalized index of the degree of mental stress. Twenty healthy volunteers performed mental arithmetic task (ART) after 5 min of resting control followed by 5 min of recovery. Breathing pattern, beat-to-beat R-R intervals, and blood pressure (BP) were determined using inductance plethysmography, electrocardiography, and a Finapres device, respectively. The analytic signals of breathing and RSA were obtained by Hilbert transform and the degree of phase synchronization ( $\lambda$ ) was quantified. With the use of spectral analysis, heart rate variability (HRV) was estimated for the low-frequency (LF) and high-frequency (HF) bands. A steady-state 3-min resting period (REST), the first 3 min (ART1), and the last 3 min (ART2) of the ART period (ranged from 6- to 19 min) and the last 3 min of the recovery period (RCV) were analyzed separately. Heart rate, systolic BP, and breathing frequency ( $f_R$ ) increased and  $\lambda$ ,  $A_{RSA}$ , and HF power decreased from REST to ART ( $P < 0.01$ ). The  $\lambda$  was correlated with normalized  $A_{RSA}$  and the HF power. The decrease in  $\lambda$  could not be explained solely by the increase in  $f_R$ . We conclude that mental stress exerts an influence on RSA oscillations, inducing incoherent phase lag with respect to breathing, in addition to a decrease in RSA.

arithmetic test; stress indicator; Hilbert transform; autonomic cardiac control

PSYCHOLOGICAL STRESS IS THOUGHT to be a major risk factor in the development of cardiovascular disease (38), and therefore quantitative and continuous evaluation of the degree of stress is needed. The relationship between psychological stress and cardiovascular responses has been studied extensively (2, 5, 6, 19, 21). In humans, cognitive stress such as mental arithmetic elicits increases in heart rate and arterial blood pressure (BP; Refs. 5, 6) and vasoconstriction in renal and splanchnic vascular beds (17, 21) mediated by the modulation of autonomic nervous activity. Heart rate variability (HRV) has been used as an indicator of autonomic nervous system function. In particular, respiratory sinus arrhythmia (RSA), the high-frequency component of HRV, has been linked to cardiac vagal tone in studies using autonomic blockade (1). Thus RSA has been recognized as an index of the vagal-cardiac nerve traffic (14). Decreased HRV (27, 31) and RSA (2, 32) have been associated

with mental stress and are signs of a low level of parasympathetic modulations. However, there is evidence that breathing frequency and tidal volume have a profound influence on RSA (18, 33), and this influence is independent from actual changes in cardiac vagal outflow (32). The magnitude of the RSA varies among individual depending on age, gender, and physical fitness (13, 22, 24). In this context, individual absolute RSA may not be suitable as a generalized stress indicator (3, 36).

In addition to RSA, cardiorespiratory phase synchronization has recently received attention (40, 41, 46). It has been shown that strong cardiorespiratory synchronization can be observed during poetry recitation, Zen meditation, or prayers that induce positive mental conditions that slow respiration (8, 9). The analysis of phase synchronization between RSA and breathing may provide a new index for evaluating the psychological state. Recently, it has been suggested that mental challenge may alter phase synchronization among hemodynamic variables and respiration (23), yet quantitative analysis for the phase relationship between RSA and respiration during mental stress has not been determined in conjunction with the autonomic indexes. In this study, we hypothesized that mental stress may distort the phase relationship between respiration and RSA, and if this is the case, phase synchronization between respiration and RSA could provide a useful and convenient measure for evaluating mental stress status. To test this hypothesis, we continuously measured R-R intervals (RRI), BP, and respiratory activity while subjects performed mental arithmetic task (ART), which has been used as a model of the defense reaction in humans.

## METHODS

**Subjects.** Thirteen men and seven women were recruited for voluntary participation in the study. The group mean age, weight, and body mass index were  $24.5 \pm 1.7$  (SE) yr,  $58.1 \pm 2.0$  (SE) kg, and  $20.9 \pm 0.5$  (SE) kg/m<sup>2</sup>, respectively. All subjects were normotensive nonsmokers and were not taking any medication. Each subject gave informed consent after verbal explanation of the experimental procedures was given. The experimental protocol was approved by the Yamagata University Institutional Ethics Committee and the study conformed to the Declaration of Helsinki.

**Experimental procedures.** Studies were performed during the day between 10:00 AM and 4:00 PM. On the day of study, subjects were not allowed to consume any food or beverages except water 1.5 h before experiment. ECG, arterial BP, and respiration were measured during mental arithmetic in the sitting position. Subjects were comfortably seated upright and wore thoracic and abdominal belts (z-RIP belts; Pro-Tech Services, Mukilteo, WA) of respiratory inductance plethysmography (RIP) to measure breathing movements. The belts were positioned around the rib cage at the level of the axilla and around the abdomen at the level of the umbilicus. The Ag-AgCl electrodes were placed on the chest for obtaining bipolar ECG leads, and a finger sensor of Finapres (model-2300; Ohmeda, Englewood, CO) was placed around the index or middle finger of the subject's left

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hand for providing a BP signal. During the data collection period, the arm of the subject was supported at the heart level. After instrumentation, the subjects rested in the sitting position for 10 min and were instructed on the procedure of the ART. After a 5-min rest period (REST) of recording, ART was started. The subjects performed ART until 100 questions were correctly answered, which was followed by 5 min of recovery (RCV).

To confirm whether ART induces sympathetic nerve activation, we measured salivary  $\alpha$ -amylase activity ( $\alpha$ -Amy), which is secreted from the salivary glands in response to sympathetic stimuli. Saliva was taken at *minute 3* (REST), *minute 6* (1 min after the initiation of ART), *minute 11* (6 min after the initiation of ART), and the RCV period (1 min before the end of experiment).

**ART.** Mental arithmetic was used to elicit mental stress. Subjects performed ART, consisting of subtraction of one- or two-digit integers from two-digit integers. Subjects were instructed to answer by operation of the mouse and to subtract as quickly and as accurately as possible. The new numbers were given after the answer was entered or when subject did not answer within 5 s. When the subjects could not give the correct answer in 5 s, an auditory alarm was given. To convey how much time is left to answer, a progress bar was indicated on the PC screen. These processes were repeated throughout the test. The ART was continued until subjects answered 100 questions correctly. The subjects competed on the completion time with each other.

**Voluntary paced breathing experiments.** It is generally known that mental stress causes increase in respiratory frequency ( $f_R$ ), and this change indirectly affects RSA. Therefore, we examined the effect of paced breathing on the index of phase synchronization ( $\lambda$ ) and magnitude of squared coherence (MSC) between RSA and respiration, and amplitude of RSA ( $A_{RSA}$ ) without mental stress (see *Data analyses*). Voluntary paced breathing (VPB) experiments were carried out on a separate day. The experimental setup was similar to that outlined for ART experiments with the exception that salivary  $\alpha$ -Amy and BP were not monitored. After signal stabilization was completed, subjects breathed spontaneously for 5 min and then breathed at a fixed frequency for another 5 min. The respiratory frequency was determined as the group-averaged value observed during ART2. The subjects were instructed to follow a respiratory pacing stimulus displayed on the PC monitor to breathe with the inspiratory duty cycle set at 40%.

**Signal acquisition.** During the experiments, ECG by means of wireless electro-cardiogram (ZM-940P; Nihon Kohden), beat-to-beat BP by means of Finapres and breathing activity by means of uncalibrated RIP were continuously recorded. After achievement of a stable BP waveform, the servo-reset mechanism of the Finapres was turned off, allowing uninterrupted data collection. All signals were digitized with

a sampling frequency of 1 kHz with a PowerLab Data Acquisition System (model-8/SP; ADInstruments, New South Wales, Australia).

Salivary  $\alpha$ -Amy activity was analyzed by a biosensor monitor (CM-2; Nipro). The saliva collecting paper was directly inserted into an oral cavity under the tongue. Immediately after saliva collecting, the test strip consisting of a reagent paper for amylase was placed onto the monitor. The time required for collection of saliva is  $\sim 60$  s, and it takes 30 s for analysis using the monitor (47).

**Data analyses.** Beat-by-beat RRI and systolic BP (SBP) and diastolic BP values were collected. The times of the R peaks were calculated using an R-wave peak detection algorithm. Then RRI was calculated as the duration between successive R peaks. The resulting RRI was resampled at 10 Hz using a spline interpolation method. Before resampling, calculated RRIs were visually inspected, and outliers were deleted if present. Respiratory movement signals were also sampled with a frequency of 10 Hz. This bivariate time series was further band-pass filtered and detrended to extract the respiration related oscillations of RRI. For this purpose, we used a finite impulse response (FIR) digital band-pass filter to remove variances  $<0.15$  and  $>0.75$  Hz, as these frequency bands cover the respiratory frequency range observed in the present study. Although use of the FIR filter alters the phase of time series linearly with the frequency, this alteration is not essential because both phases for the RRI and the respiratory signal were altered to same degree by the FIR filter, and our interest is the relative phase difference. From the oscillatory signals of RRI and respiration obtained, instantaneous amplitude and phase can be calculated in the complex unit circle based on the Hilbert transform (37). The basic theory for computing the Hilbert transform has been well described previously (15). Essentially, the Hilbert transform shifts phases of all frequency components of its input by  $-\pi/2$  radians. The analytic signal  $\zeta(t)$  can be constructed from a real input signal, and its Hilbert transform will be  $\zeta(t) = \nu(t)e^{i\phi(t)}$ , where  $\nu(t)$  and  $\phi(t)$  are the instantaneous amplitude or “envelope” and instantaneous phase of the analytic signal, respectively. Because the  $\phi(t)$  exhibits phase jumps of  $\pm 2\pi$  whenever the signal crosses zero, the  $\phi(t)$  was unwrapped to form a continuously increasing function of time. The phase evolutions at discrete  $k$ -th times  $t_k$  [RRI:  $\phi_{RRI}(t_k)$ , respiration:  $\phi_{resp}(t_k)$ ] were then used to compute time-dependent phase synchronization ( $\lambda$ ) that assesses the strength of phase locking between the respiration and RSA. To compute  $\lambda$  the phase difference  $\Psi(t_k) = [\phi_{resp}(t_k) - \phi_{RRI}(t_k)]$  modulo  $2\pi$  was defined. Then,  $\lambda$  was calculated as

$$\lambda(t_k) = \left| \frac{1}{N} \sum_{j=k-N}^k e^{i\Psi(t_kj)} \right|^2,$$

where  $N$  indicates the number of consecutive data samples to be considered in the computation. The  $\lambda$  was calculated from 300-point

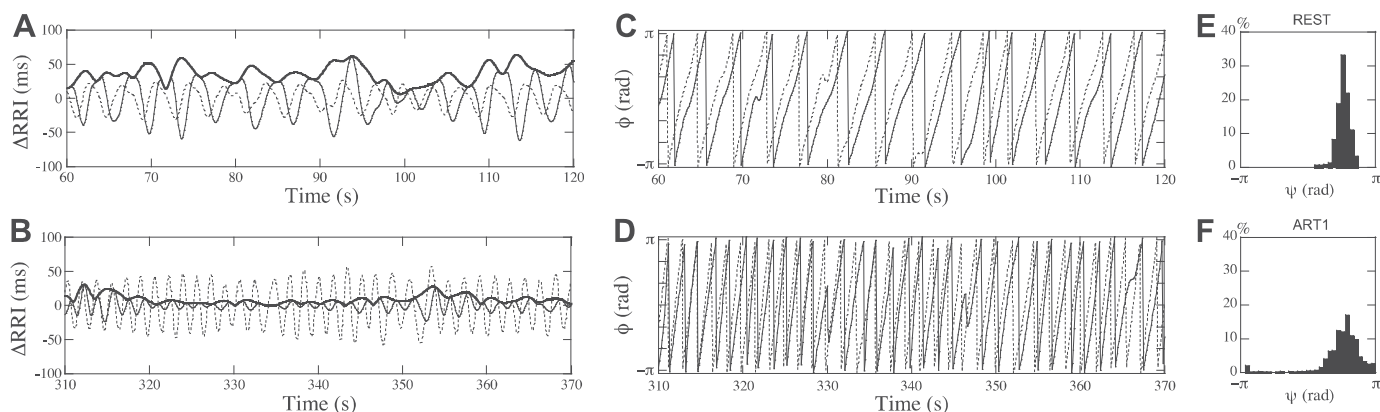


Fig. 1. Changes in R-R intervals (RRI; thin solid curve) and breathing trace (in arbitrary unit; dotted curve, upward deflections denote expiration) for a 1-min portion at rest (REST; A) and during mental arithmetic task (ART) for first 3 min (ART1; B). Bold curves indicate instantaneous amplitude of RRI. Instantaneous phases for breathing ( $\phi_{resp}$ , dotted curve) and RRI ( $\phi_{RRI}$ , solid curve) are shown for REST (C) and ART1 (D). Distributions of phase difference  $\Psi(t_k)$  during REST and ART1 are also shown (E and F).

Table 1. Completion time and percentage of questions answered correctly for mental arithmetic task for each subject

Subject Number	Arithmetic Completion Time, s	Percentage of Correct Answer, %
1	429	76.3
2	363	79.4
3	790	48.3
4	449	74.6
5	1,149	34.8
6	518	70.9
7	588	58.1
8	390	87.7
9	360	82.6
10	372	87.7
11	437	74.6
12	382	93.3
13	541	66.9
14	640	47.2
15	720	52.6
16	601	74.6
17	390	88.7
18	645	61.6
19	405	100
20	488	68
Means $\pm$ SE	533 $\pm$ 44	71.4 $\pm$ 3.9

windows (30 s) with a sliding window of 50-point. The  $\lambda$  is always  $\leq 1$ , taking a value of 1 only when the  $\Psi(t_k)$  remains constant throughout respiratory cycles, while taking a value of 0 when the  $\Psi(t_k)$  distributes randomly. We defined  $A_{RSA}$  as the average of  $\nu(t)$  of RRI time series.  $A_{RSA}$  was expressed in both absolute (ms) and normalized units ( $nA_{RSA}$ , %), which were calculated by dividing by the mean RRI. The  $f_R$  was calculated by dividing the  $\phi_{resp}(t)$  derivative by  $2\pi$  as a function of time. Before derivation,  $\phi_{resp}(t)$  was smoothed by a 30-point (3 s) rectangular window.

An example of the analysis is illustrated in Fig. 1, where parts of the filtered RRI (solid curve) and simultaneous breathing pattern (dotted curve) at REST (Fig. 1A) and during ART1 (Fig. 1B) are shown. The Hilbert transform of these traces gives the instantaneous phase of  $\phi_{RRI}(t)$  and  $\phi_{resp}(t)$  (Fig. 1, C and D) and amplitude  $\nu(t)$  or envelope for the RRI fluctuation (bold curves in Fig. 1, A and B). At REST and ART1,  $\phi_{RRI}(t)$  and  $\phi_{resp}(t)$  exhibited similar saw-tooth-like phase evolution with  $\phi_{resp}(t)$  slightly preceding the  $\phi_{RRI}(t)$ . The histogram profile of the phase difference  $\Psi(t_k)$  showed a relatively narrow distribution with SD of 0.29 radian at REST (Fig. 1E), whereas it was broad distribution with SD of 1.02 radian during ART1 (Fig. 1F).

**Frequency domain analysis.** In addition to the calculation of the time-dependent phase synchronization index  $\lambda$ , MSC between respiration and RSA was estimated using following equation to quantify the degree of association in a frequency domain:

$$MSC = \frac{|S_{xy}(f)|^2}{S_{xx}(f) \cdot S_{yy}(f)}$$

where  $S_{xy}(f)$  is the cross-power spectrum between RSA and respiration and  $S_{xx}(f)$  and  $S_{yy}(f)$  are the auto-power spectra of each signal.

Table 2. Response of salivary  $\alpha$ -amylase activity to the ART

	REST	1 min after Initiation of ART	6 min after Initiation of ART	RCV
$\alpha$ -Amy, kU/l	38 $\pm$ 6	45 $\pm$ 6	62 $\pm$ 8*	47 $\pm$ 10

REST, resting period; ART, mental arithmetic task; RCV, recovery;  $\alpha$ -Amy,  $\alpha$ -amylase. \* $P < 0.05$ , compared with REST.

MSC provides an appropriate measure of similarity between RSA and respiratory oscillations. In our study, mean value determined by averaging MSC over the frequency where respiratory autopower spectrum was  $>10\%$  of its maximum power was used as a quantitative measure of coupling. Cross- and auto-power spectral analyses were made from 1,024-point windows with 50% overlapping segments, after trend elimination and Hanning windowing of filtered RRI and respiratory signals. Estimated MSC and respiratory auto-power spectrum were smoothed by using a mean filter (kernel size = 5). Variation of the respiratory frequency was estimated with the spectral spread, expressed as full width at half maximum ( $FWH_R$ ) of the power spectral density for respiration. Changes in the  $\lambda$ , MSC and  $A_{RSA}$  were also analyzed when breathing was voluntarily paced at a similar frequency observed during the ART in each subject.

To assess autonomic activation, the low-frequency (LF; 0.04–0.15 Hz) and high-frequency (HF; 0.15–0.40 Hz) components of HRV (44) were computed by applying a fast Fourier transform by the Welch method. To evaluate HF power, we checked most respiratory power was found in the HF region. If the peak frequency of auto-power spectra of respiration was beyond the HF region, we modified the

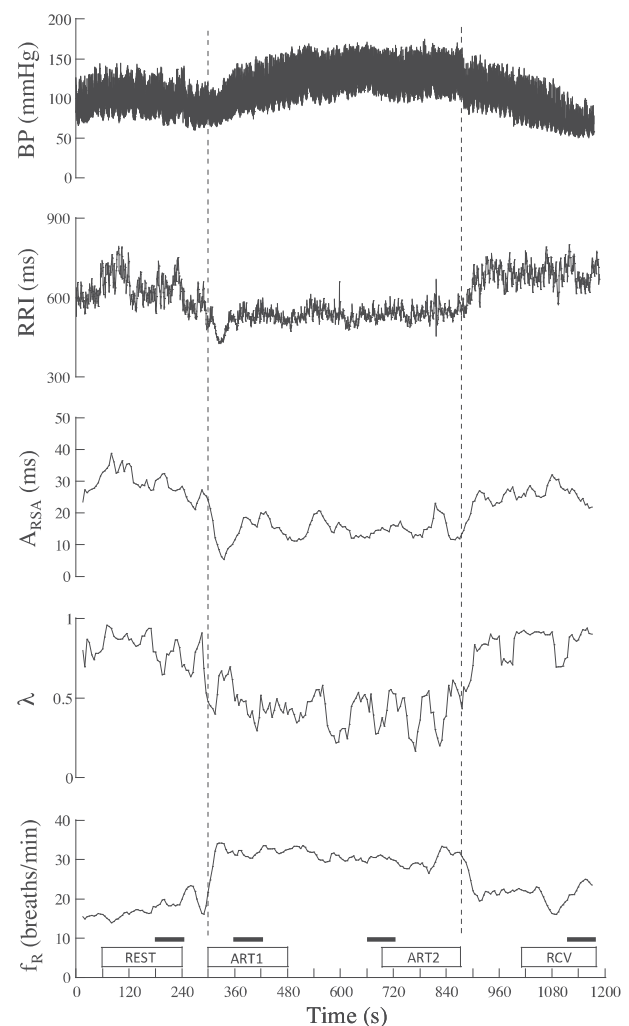


Fig. 2. Effects of ART on blood pressure (BP), RRI, amplitude of respiratory sinus arrhythmia ( $A_{RSA}$ ), phase synchronization index ( $\lambda$ ), and breathing frequency ( $f_R$ ) from 1 subject. Data were analyzed at REST, during ART1, during the last 3 min of the ART (ART2), and at recovery (RCV). Horizontal bold bars indicate sampling time of saliva. Vertical dashed lines indicate the beginning and termination of ART, respectively. Data for the same subject shown in Fig. 1 are presented.



upper boundary frequency of HF band where auto spectral power of respiration reduced to 10% of its maximum power. After trend elimination and Hanning windowing of raw RRI, power spectral density estimates were made from 1,024-point windows, with 50% overlapping segments. The total spectral power was calculated for the LF and HF frequency bands by integration of each spectral component. The LF-to-HF ratio (LF/HF), an estimate of sympathovagal balance, was also calculated. The power spectrum for each frequency component was expressed in both absolute values ( $\text{ms}^2$ ) and normalized units [ $100 \times (\text{absolute power})^{1/2}/(\text{mean RRI}), \%$ ]. This normalization has been found to eliminate the influence of basal level of cardiac sympathetic tone on HF power (13, 16). All data were analyzed off-line on a PC using self developed software in the Matlab (MathWorks).

**Statistics.** To quantify the variability of parameters, time domain measures of the mean and the SE over the 3-min resting period (REST, 1–4 min from the beginning of recording), first 3 min and last 3 min of ART period (ART1 and ART2), and the last 3 min of the RCV were separately calculated for each subject and then averaged to obtain group mean values. The effect of ART on each variable was assessed using a repeated-measures one-way ANOVA. Pair-wise comparisons with Bonferroni corrections were used to examine significant interactions. The relationship between  $\lambda$  and autonomic indexes ( $n\text{A}_{\text{RSA}}$ ,  $n\text{HF}$ ,  $n\text{LF}$ , and  $n\text{LF}/n\text{HF}$  ratio) including MSC were assessed by linear least squares regression analysis.  $P < 0.05$  was regarded as statistically significant for all comparisons.

## RESULTS

Completion time and percentage of correct answers for ART for each subject are presented in Table 1. Salivary  $\alpha$ -Amy activity at 6 min after initiation of ART significantly elevated compared with REST (Table 2), but at 1 min after initiation of ART they did not reach statistical significance. During RCV (4 min after cessation of ART), saliva  $\alpha$ -Amy activity tended to return toward the REST level.

A representative trace for cardiorespiratory variables from one subject is shown in Fig. 2. From REST to ART, a transient increase in BP was observed. At the initiation of the ART,  $f_R$  increased sharply and RRI and  $A_{\text{RSA}}$  decreased, exhibiting undershoot. A transient decrease in  $\lambda$  was also observed during the ART. After the transient period, BP, RRI, and  $A_{\text{RSA}}$

remained relatively constant throughout the ART, while  $\lambda$  showed some fluctuations. The decrease in  $\lambda$  reflects a decrease of phase synchronization between  $\phi_{\text{RRI}}(t)$  and  $\phi_{\text{resp}}(t)$ . At the cessation of ART, BP, RRI,  $A_{\text{RSA}}$ , and  $\lambda$  gradually returned to the level for the REST condition, while  $f_R$  decreased immediately to baseline level. These responses were observed for 19 subjects examined, whereas for 1 subject (*subject 5*),  $A_{\text{RSA}}$  and  $\lambda$  during the ART stayed at a level similar to those for REST, but increased  $f_R$  was still observed. Examples of power spectral density of HRV and coherence analysis are shown in Fig. 3. From REST to ART1 and to ART2, respiratory autowave spectra peaks moved from 0.28 to  $\sim 0.53$  Hz as a result of the increase in  $f_R$ . Clearly, ART led to decreases of power spectral density in the HF region of HRV and the MSC between RSA and respiration compared with REST.

On average (except the data from *subject 5*), ART caused a significant increase in HR, SBP, and  $f_R$  as well as significant decreases in  $A_{\text{RSA}}$ ,  $\lambda$ , and MSC (Table 3). Mean diastolic BP increased significantly during ART2 compared with REST and RCV. Judging from  $\text{FWH}_R$ , there was no significant change in the variation of  $f_R$  during ART compared with REST and RCV. The LF and HF powers of HRV were decreased during ART1 and ART2 compared with REST ( $P < 0.01$ ), while there was no significant change in LF/HF ratio. The normalized HRV indexes showed similar results except that  $n\text{HF}$  during ART1 did not reach statistical significance. Bivariate correlations among  $\lambda$ ,  $n\text{A}_{\text{RSA}}$ , MSC, and normalized HRV indexes ( $n\text{HF}$ ,  $n\text{LF}$ , and  $n\text{LF}/n\text{HF}$  ratio) are shown in Fig. 4. The  $\lambda$  correlated to  $n\text{A}_{\text{RSA}}$  ( $r = 0.415$ ;  $P = 0.0002$ ) and  $n\text{HF}$  ( $r = 0.371$ ;  $P = 0.001$ ) and was inversely related with  $n\text{LF}/n\text{HF}$  ( $r = -0.388$ ;  $P = 0.0005$ ), whereas no correlation was observed between  $\lambda$  and  $n\text{LF}$ . A high correlation coefficient was found between  $\lambda$  and MSC ( $r = 0.733$ ;  $P < 0.0001$ ).

We then analyzed whether the reduction in  $\lambda$  can be explained by the increased  $f_R$ . Typical example is shown in Fig. 5 in which subjects performed VPB at a similar  $f_R$  level observed during the ART. The reduction in  $A_{\text{RSA}}$  due to the increase in  $f_R$  induced by VPB was clearly seen, while the change in  $\lambda$  was much less

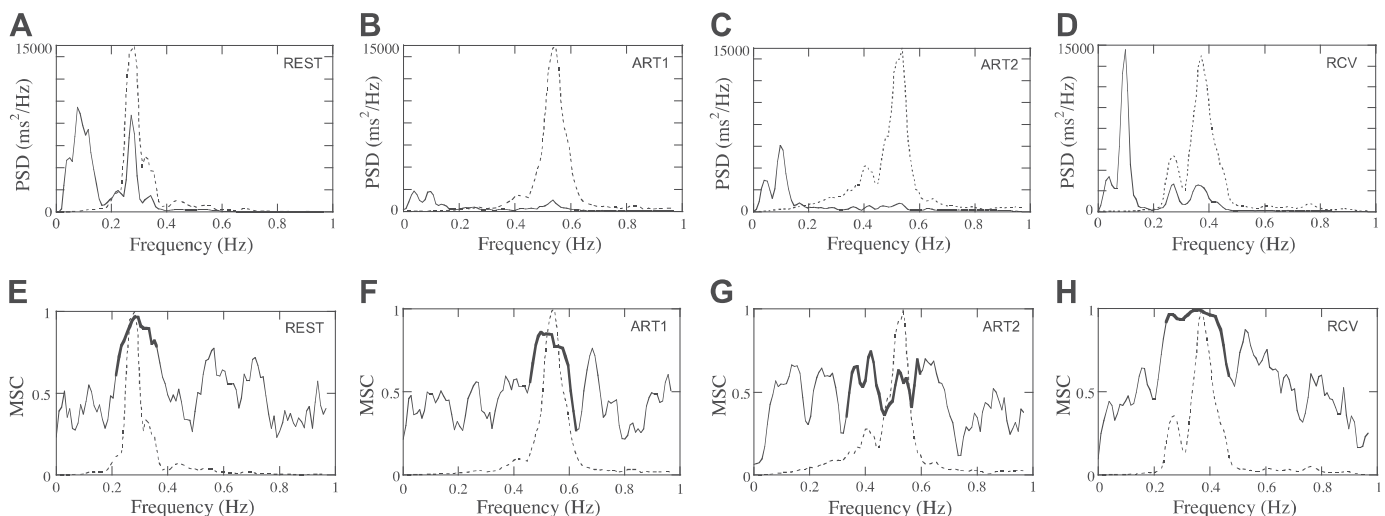


Fig. 3. Examples of power spectral density of heart rate variability (PSD; solid lines in A–D) and magnitude of squared coherence function (MSC; solid lines in E–H) between respiration and RSA for 1 subject. Normalized PSD of respiration (dashed line) are shown in A–H. As a quantitative measure of coupling, mean MSC determined by averaging the MSC over the frequency where respiratory auto-power spectrum was  $>10\%$  of its maximum power was used (thick line of the MSC function).



Table 3. Effects of ART on cardiorespiratory variables and autonomic indexes

	REST	ART1	ART2	RCV
SBP, mmHg	127 ± 4.1	143 ± 6.5†	150 ± 6.2†§	132 ± 4.5
DBP, mmHg	71.3 ± 3.0	79.0 ± 4.0	86.4 ± 4.8†§	75.4 ± 3.4
HR, beats/min	78.2 ± 3.3	90.0 ± 4.2†§	86.1 ± 3.9†§	77.1 ± 3.1
f <sub>R</sub> , breaths/min	18 ± 0.8	25 ± 0.9†§	24 ± 1.0†§	17 ± 0.9
FWH <sub>R</sub> , breath/min	4.9 ± 1.1	4.8 ± 0.7	4.0 ± 0.3	4.4 ± 0.6
A <sub>RSA</sub> , ms	25.5 ± 3.0	19.6 ± 2.6†§	19.7 ± 2.6†§	24.1 ± 2.6
nA <sub>RSA</sub> , %	3.11 ± 0.23	2.71 ± 0.24*	2.63 ± 0.21†	2.91 ± 0.22
λ	0.69 ± 0.03	0.53 ± 0.04†§	0.47 ± 0.04†§	0.69 ± 0.03
MSC	0.77 ± 0.03	0.66 ± 0.03†§	0.64 ± 0.03†§	0.74 ± 0.03
HRV				
HF, ms <sup>2</sup>	539 ± 155	334 ± 98†	319 ± 106†	462 ± 119
LF, ms <sup>2</sup>	611 ± 106	305 ± 51†§	324 ± 45†§	538 ± 77
LF/HF	1.95 ± 0.37	1.76 ± 0.40	1.87 ± 0.31	2.07 ± 0.36
Normalized HRV				
nHF, %	2.53 ± 0.20	2.23 ± 0.19	2.05 ± 0.17†	2.30 ± 0.19
nLF, %	3.03 ± 0.23	2.31 ± 0.15†	2.41 ± 0.20*	2.78 ± 0.19
nLF/nHF	1.30 ± 0.12	1.16 ± 0.12	1.28 ± 0.12	1.34 ± 0.12

Values are means ± SE ( $n = 19$ ). ART1, first 3 min of ART; ART2, last 3 min of ART; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; f<sub>R</sub>, respiratory frequency; FWH<sub>R</sub>, full-width at half maximum of power spectral density for respiration; A<sub>RSA</sub>, amplitude of respiratory sinus arrhythmia; nA<sub>RSA</sub>, normalized A<sub>RSA</sub>; λ, phase synchronization index; MSC, magnitude of squared coherence; HRV, heart rate variability; HF, high frequency; LF, low frequency; nHF, normalized HF; nLF, normalized LF; nLF/nHF, normalized LF/HF ratio. \* $P < 0.05$  and † $P < 0.01$  vs. REST; § $P < 0.01$  vs. RCV.

evident. Figure 6 summarizes the effects of f<sub>R</sub> on λ, MSC, and normalized autonomic indexes. The f<sub>R</sub> increased from 15 breaths/min of spontaneous breathing to 24 breaths/min by VPB on average. The normalized A<sub>RSA</sub> and HRV indexes as well as MSC decreased by a similar level as the increase in f<sub>R</sub> (Fig. 6, B–F), while VPB did not lead to a corresponding reduction of λ with increase in f<sub>R</sub> (Fig. 6A).

## DISCUSSION

The present study demonstrated that mental stress exerts an influence on the interaction of respiration and heart rate with increasing phase-lag variations between RSA and respiration (decreasing λ), in addition to an attenuation of RSA. These changes are accompanied by increases in f<sub>R</sub>, but this could not

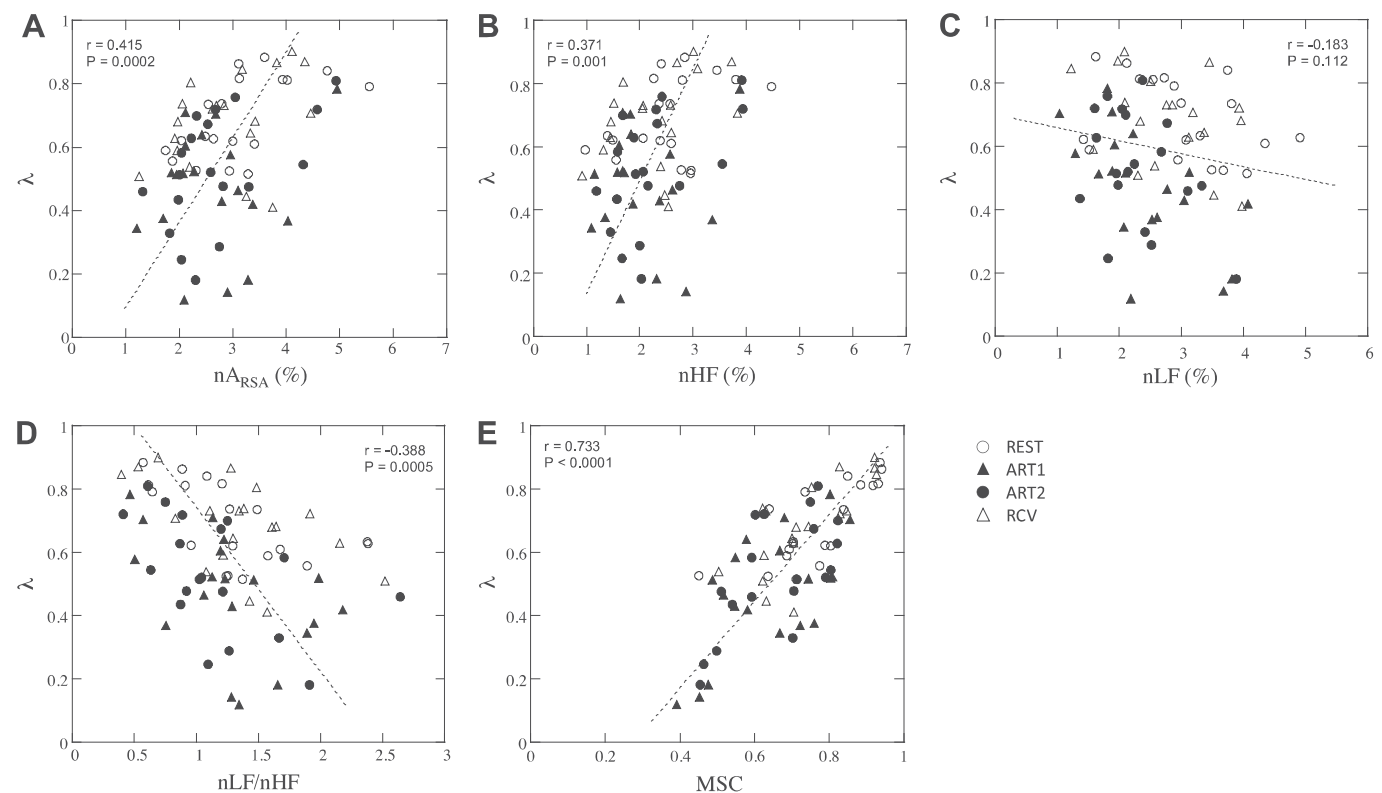


Fig. 4. Scatter plots of relations between λ and normalized (n) A<sub>RSA</sub> (A), between λ and normalized high frequency (HF) power (B), between λ and normalized low frequency (LF) power (C), between λ and normalized LF/HF (D), and between λ and MSC (E). Regression line (dotted line) and correlation coefficient for each relation are indicated.

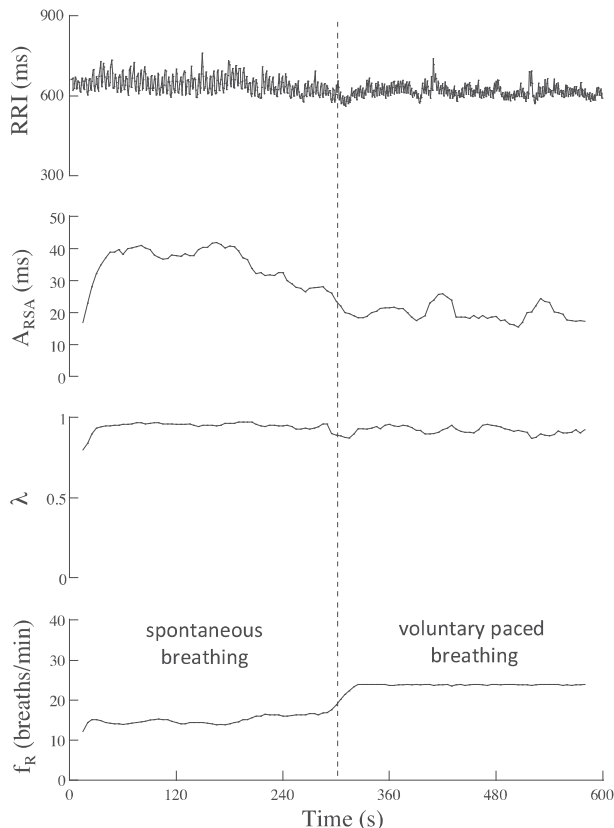


Fig. 5. Effects of voluntary paced breathing (VPB) on RRI,  $A_{RSA}$ , and  $\lambda$  from the same subject as in Fig. 2. Vertical dashed line indicates the beginning of VPB. Note that the  $\lambda$  is less influenced by  $f_R$ .

be ascribed solely to the decrease in  $\lambda$ . The  $\lambda$  showed significant positive correlation with autonomic vagal activity indexes of normalized  $A_{RSA}$  and the HF component of HRV, suggesting a possibility that  $\lambda$  may provide information about the parasympathetic status related to stress reactions in the body without interference from  $f_R$  change.

$\alpha$ -Amy activity did not increase significantly 1 min after the initiation of ART but did 6 min after the initiation of ART.  $\alpha$ -Amy activity has been suggested to be an indicator for the activity of the sympathetic-adrenal medullary system although the mechanisms for the increased activity of  $\alpha$ -Amy due to stress are not entirely understood (7, 28, 29). Chatterton et al. (7) demonstrated that the time course of  $\alpha$ -Amy activity under stress was very close to that of plasma catecholamine. Therefore, we assume that sympathetic activation was present at least 6 min after the commencement of ART.

ART decreased both absolute and normalized  $A_{RSA}$  and HF power of HRV and increased HR and SBP significantly, indicating an autonomic profile of sympathetic activation and parasympathetic inhibition. The observation of these responses follows the generally accepted consequences and results of mental stressors (4, 5, 6, 31). However, neither the LF component of HRV nor the LF/HF ratio increased during ART (Table 3). Similar to our study, several investigators have demonstrated that LF power decreases parallel to HF power during mental stress in healthy humans (25, 31, 43). Although LF power is considered to be determined by both sympathetic and parasympathetic activities (26), validity of the use of this

index has not been fully established (43). Sympathetic contribution to the LF power may be factored out in the case of typical psychological stressors. In this situation, use of the LF/HF ratio as a measure of sympathovagal balance can be misleading.

In the present study, ART increased  $f_R$  by  $\sim 35\%$  and decreased  $\lambda$  by  $\sim 32\%$  compared with REST (Table 3). This does not contradict the previous observation that the extent of cardiorespiratory synchronization increases if the breathing frequency decreases (8, 9). Lackner et al. (23) recently observed that the phase synchronization between the HF component of RRI variability and respiration decreased from rest to mental challenge. However, the mechanism underlying the stress-induced incoherent oscillations of RSA is not fully understood. Alteration of respiratory parameters can profoundly influence RSA amplitude; slow and deep breathing will amplify RSA magnitude among healthy adults, whereas faster and shallow breathing may eliminate RSA (10, 14). Also, the phase relationship between the RSA pattern and respiration was influenced by  $f_R$  (11, 42). Thus one might expect that the decrease in  $\lambda$  was solely due to the increased  $f_R$ . However, when  $f_R$  was voluntarily increased at similar levels as during ART2,  $\lambda$  decreased only 9% compared with spontaneous breathing despite that the attenuation of the normalized HRV indexes and MSC were almost the same (Fig. 6). Therefore, we do not attribute the decrease in  $\lambda$  solely to the effect of the increase in  $f_R$ . The  $f_R$  was low during spontaneous breathing of VPB experiment compared with REST ( $15 \pm 0.5$  for spontaneous breathing vs.  $18 \pm 0.8$  for ART2), which was not expected, but this would suggest elevated anxiety levels before performing ART, because each subject was aware which protocol the subject was conducting on each experimental day.

It could be inferred that an increase in the variation of  $f_R$  during ART would be responsible for the decrease in  $\lambda$ , since the transduction delay from input (respiration) to output (RRI) through efferent vagal activity varies with change in  $f_R$  (39). However,  $FWH_R$  as a measure of the variation of  $f_R$  did not show any significant difference between REST and ART. The similarity of the  $FWH_R$  suggests that the reduction in  $\lambda$  was not a consequence of breathing irregularity. The precise mechanism(s) responsible for the incoherent RSA oscillations is unclear. A possible explanation is that the enhanced sympathetic nerve activity may modulate the transduction property of cardiac vagal efferent nerve. There is evidence that sympathetic outflow influences sinoatrial node responses to vagus nerve traffic and markedly reduces RRI oscillation in the frequency range of breathing (45). The result indicates the effect of sympathetic nerve activity on RSA. The mechanism responsible for this effect was postulated to be due to the coreleased neuromodulators from cardiac sympathetic nerve terminal, which can exert an inhibitory action on the phasic vagal driving (34, 35). We therefore hypothesize that sympathetic nerve activation elicited by ART modulates dynamic transfer of vagal-cardiac nerve traffic, perhaps through the interaction of efferent vagal nerve at the sinoatrial node, which leads to breath-to-breath variations in the latency of RSA. Direct sympathetic nervous activity measurements, e.g., muscle sympathetic activity, would reveal more about the association.

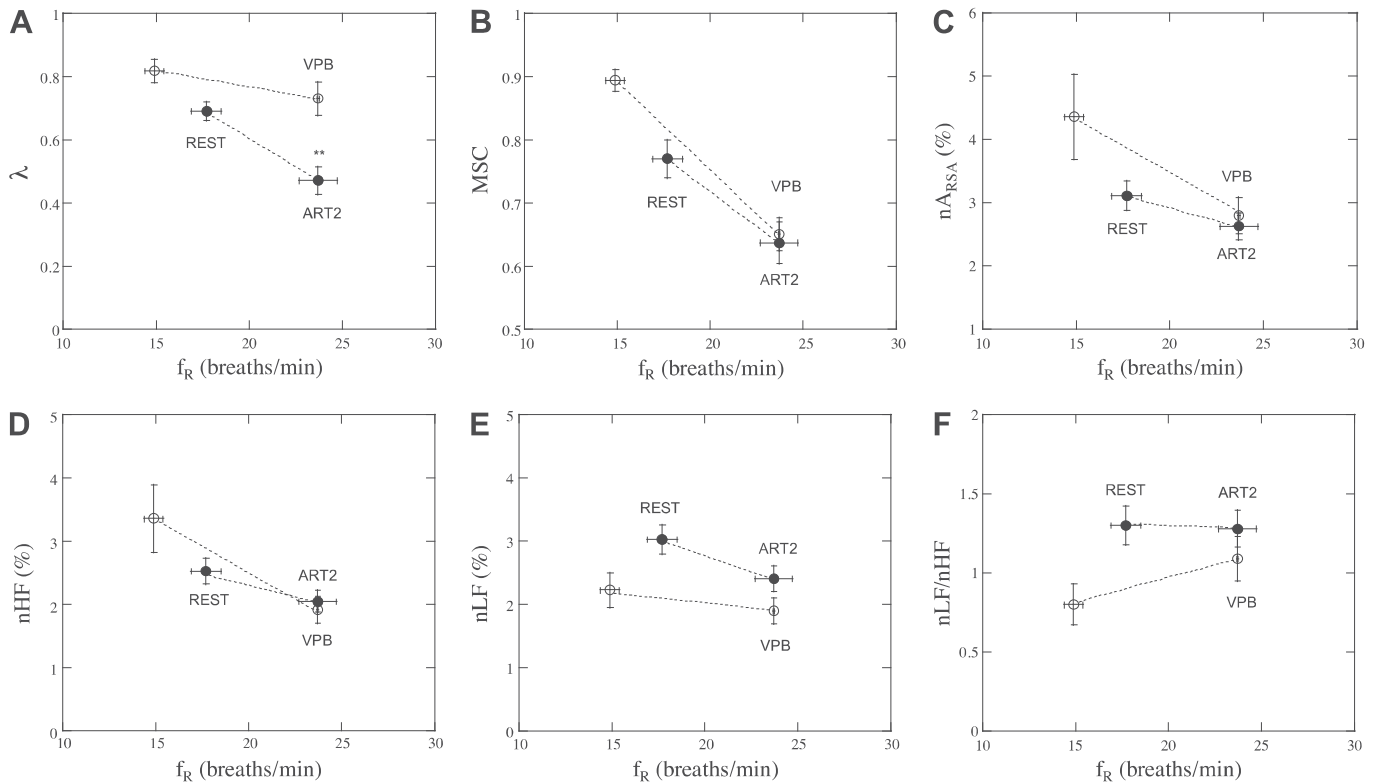


Fig. 6. Effect of breathing frequency ( $f_R$ ) on  $\lambda$  (A), MSC (B),  $nA_{RSA}$  (C), nHF (D), nLF (E), and nLF/nHF (F).  $\circ$ , Data from VPB experiments;  $\bullet$ , data from ART. Error bars indicate SE. Comparison was performed for VPB vs. ART2. \*\* $P < 0.01$  compared with the VPB experiment.

Furthermore, ART requires a high amount of cognitive effort and maintenance of concentration. The increase in  $f_R$  observed during ART was probably evoked by the influence of descending pathway from the cortex on neuronal networks of the respiratory generator. The rhythmical fluctuation of RRI was demonstrated to be closely related to changes in centrally driven phrenic nerve activity but could be dissociated from actual respiratory movements (3). Thus the possible involvement of the higher central nervous system in the decrease in  $\lambda$  cannot be ruled out.

Among the autonomic indexes we derived,  $\lambda$  showed significant positive correlations with normalized  $A_{RSA}$  ( $nA_{RSA}$ ) and the HF index (nHF) but negative correlation with nLF/nHF ratio (Fig. 4). Since it has been shown that normalized  $A_{RSA}$  or HF index can eliminate the influence of basal levels of cardiac sympathetic tone (13, 16), significant correlation between  $\lambda$  and  $nA_{RSA}$  or nHF suggests that  $\lambda$  comprises information given by stress-induced changes in those autonomic indexes, preferentially reflecting a parasympathetic nerve activity. The  $\lambda$  would dissociate with  $nA_{RSA}$  and nHF if  $f_R$  is purposely altered without mental challenge, because  $\lambda$  is not susceptible to  $f_R$  influence (Fig. 6A). In this sense,  $\lambda$  could be an alternative index that allows a better standardization in the measure of autonomic vagal nerve tonicity related to stress reactions in the body. However, caution must be used in inferring autonomic regulation from indirect measures of  $A_{RSA}$  and spectral parameters of HRV. Because  $f_R$  was increased during mental stress, we cannot distinguish whether the decreases in  $A_{RSA}$  and HF power are the result of mental stress and/or respiratory influences. RSA is a complex phenomenon the physiological origins and mechanisms of which are still a matter of debate (12,

20). Our interpretations assume that  $\lambda$  could be a marker for assessing the interaction between psychological states and autonomic control on a practical level.

As demonstrated in Fig. 4E,  $\lambda$  was closely correlated with MSC, indicating that variations of phase lag between the oscillations could be important aspect for the similarity of oscillations. However, in contrast to  $\lambda$ , MSC was also profoundly influenced by  $f_R$  (Fig. 6B). This is probably because that the MSC reflects the similarity of appearance in both time series at certain frequency regardless of the phase difference. In contrast,  $\lambda$  represents the degree of synchronization based on the analysis of phase difference between both oscillations. Computation of MSC requires successive segmental spectral analysis and the processed signal to be stationary in the analyzed temporal window, while  $\lambda$  can be calculated without these restrictions. Thus  $\lambda$  may have some advantage over spectral analyses especially when used to assess nonstationary data.

Limitations of the study have to be noted. First, we calculated  $\lambda$  from the phase difference between  $\phi_{RRI}$  and  $\phi_{resp}$  using Hilbert transformation. This approach is meaningful if the embedded signal rotates around a fixed center. Although we used a band-pass filter to eliminate the trend and therefore eliminate the variations of the center for RSA oscillations, this may not be accurate. Second, we could not use flow sensors to measure tidal volume and ventilation precisely because of the measurement of salivary  $\alpha$ -Amy activity. The effect of tidal volume on  $\lambda$  remains to be shown. Third, because all subjects in the present study were healthy young adults, our results are not generalizable beyond the study participants. As to the potential utility of  $\lambda$  for assessing mental stress, quantitative



studies are needed to determine the extent to which  $\lambda$  reflects stress-induced changes in autonomic vagal activity. A question may arise as to how changes in the  $\lambda$  may relate to physical activity. It has been shown that the immediate autonomic adjustment to moderate exercise is a vagal withdrawal. Therefore, subsequent research examining altered vagal and sympathetic tonic conditions will be of interest to reveal whether the  $\lambda$  can discriminate between mental stress and physical activity, as such discrimination is known to be difficult to differentiate by the HRV index.

In summary, we examined the effect of mental stress on phase-lag variations ( $\lambda$ ) between RSA and respiration. We found that the coherent oscillations of RSA were disrupted by mental stress. The reduction of  $\lambda$  was correlated with the decreases in normalized RSA and the HF component of HRV, suggesting the association of autonomic vagal activity. We attribute this partly to the increase in  $f_R$ , but the detailed mechanism(s) remain unknown. Further research should be performed to elucidate the informative content of coherent oscillations of RSA in physiological and clinical experimental settings.

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#### DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

#### AUTHOR CONTRIBUTIONS

Author contributions: K.N. conception and design of research; K.N. and T.S. performed experiments; K.N. analyzed data; K.N. interpreted results of experiments; K.N. prepared figures; K.N. drafted manuscript; K.N. edited and revised manuscript; K.N. and T.S. approved final version of manuscript.

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