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Contribution of tonic vagal modulation of heart rate, central respiratory drive, respiratory depth, and respiratory frequency to respiratory sinus arrhythmia during mental stress and physical exercise

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Abstract

This study tested various sources of changes in respiratory sinus arrhythmia (RSA). Twenty-two healthy participants participated in three experimental conditions (mental stress, relaxation, and mild physical exercise) that each consisted of three breathing parts (normal breathing, breathing compressed room air, and breathing compressed 5% CO_2-enriched air). Independent contributions to changes in RSA were found for changes in tonic vagal modulation of heart rate, central respiratory drive (i.e., $PaCO_2$), respiratory depth, and respiratory frequency. The relative contributions to changes in RSA differed for mental stress and physical exercise. It is concluded that uncorrected RSA will suffice to index within-subject changes in tonic vagal modulation of heart rate in most situations. However, if the central respiratory drive is expected to change, RSA should ideally be corrected for changes in $PaCO_2$, respiratory depth, and respiratory frequency.

Descriptors: Spectral HR power, CO_2, Respiration, Respiratory sinus arrhythmia, Preejection period

Heart period variability that is related to respiration is known as respiratory sinus arrhythmia (RSA). Between-subject clinical studies have demonstrated that reduced RSA is associated with cardiac disease (Bigger, Fleiss, Rolnitzky, & Steinman, 1993; Hayano et al., 1991; Lombardi et al., 1987; Saul et al., 1988; Singer et al., 1988; Tsuji et al., 1996), hypertension (Julius, Pascual, & London, 1971; Lazzeri et al., 1998; Malliani, Pagani, Lombardi, Guzzetti, & Cerutti, 1991), anxiety (Thayer, Friedman, & Borkovec, 1996; Watkins, Grossman, Krishnan, & Sherwood, 1998), and depression (see Musselman, Evans, & Nemeroff, 1998). Within-subject psychophysiological studies have demonstrated that psychological stress (Allen & Crowell, 1989; Kamphuis & Frowein, 1985; Langewitz & Ruddel, 1989) and physical exercise (Hatfield et al., 1998; Tulppo, Mäkikallio, Kakala, Seppanen, & Huikuri, 1996) reduce RSA, whereas increased RSA is associated with conditions of psychological relaxation (Skakibara, Takeuchi, & Hayano, 1994). Thus, RSA is now widely considered of great importance in both fundamental and clinical (psycho)physiological research. A major point of discussion, however, is the interpretation of RSA as an index of tonic vagal modulation of heart rate (i.e., sometimes designated as "cardiac vagal tone"). Independently of changes in tonic vagal modulation of heart rate, rapid low-tidal volume breathing will reduce the degree of RSA, whereas slow high-tidal volume breathing will increase RSA (Allen & Crowell, 1989; Grossman, Karemaker, & Wieling, 1991; Grossman & Kollai, 1993; Kollai & Mizsei, 1990; Saul, Berger, Chen, & Cohen, 1989). In addition, Al-Ani, Forkins, Townend, and Coote (1996) have demonstrated that, independently of the changes in breathing pattern, changes in the central respiratory drive can also influence RSA.

In this paper, RSA is defined to result from the phasic changes in vagal nerve activity at the cardiac sino-artrial node that are linked to the respiratory frequency (i.e., a respiratory-related phasic vagal modulation of heart rate). Tonic vagal modulation of heart rate is defined to result from the basal (tonic) firing rate of the cardiac vagal motor neurons located at the Nucleus Ambiguus (NA). This tonic firing rate of the NA motor neurons (i.e., what Porges (1995) described as the "smart" vagus) is influenced by central projections, including those from amygdalar and hypothalamic (e.g., the paraventricular nucleus) regions, and by projections from other brain stem nuclei (e.g., the nucleus tractus solitarius). As in the model of Berntson and coworkers (Berntson, Cacioppo, & Quigley, 1993; Berntson et al., 1997) we assume that this tonic vagal firing is modulated with a respiratory-related

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phasic signal by the output of the central respiratory generator (see also Porges, 1995; Taylor, Jordan, & Coote, 1999), so that tonic vagal modulation of heart rate is reflected in the level of RSA.

The output of the central respiratory generator is regulated by several complex mechanisms of which two chemo-reflex mechanisms (based on O_2 and CO_2 receptors) are the most important (Feldman & McCrimmon, 1989). Al-Ani and coworkers (1996) compared RSA during increased respiratory activity (respiratory frequency, depth of breath) evoked by (a) inhalation of 5% CO_2-enriched air, and (b) voluntary increased breathing. The authors argued that the voluntary command to breathe bypasses the central respiratory generator to have its main effect directly on the spinal respiratory motor-neuron pools. Results showed that RSA was greater during CO_2-enriched air inhalation than during voluntary hyperventilation (with similar depth and respiratory frequency). The CO_2 effect was even more pronounced when the muscarinic M1 antagonist scopolamine was administrated to enhance the vagal output to the heart. These results suggest that RSA can be influenced by changes in the central respiratory drive, independently of actual respiratory behavior and the tonic vagal modulation of heart rate. The tonic vagal firing rate of the NA motor neurons may be further modulated by a peripheral pulmonary stretch-reflex mechanism and by respiratory linked changes in baro-reflex activity (Berntson et al., 1993; Taylor et al., 1999). Empirically, baro-reflex and chemo-reflex related changes in RSA have indeed been reported (see Al-Ani et al., 1996). Finally, RSA decreases as the respiratory frequency increases as a result of a progressive decline in the frequency-transfer function of the vagal-cardiac innervation (Berntson et al., 1993; Eckberg, 1983).

In short, four major determinants of changes in RSA are recognized: (a) the tonic vagal modulation of heart rate resulting from the tonic firing rate of the NA motor neurons, (b) the central respiratory drive, (c) peripheral respiratory-related feedback from the baro-reflex and the pulmonary stretch-reflex, and (d) the vagal-cardiac frequency transfer function. Although these four determinants of RSA may be coupled during mental stress and exert a mutually enhancing influence on a reduction of RSA (lower tonic vagal modulation of heart rate, lower central respiratory drive, lower respiratory depth, higher respiratory frequency), they may be dissociated during other conditions (e.g., exercise) and/or in specific clinical groups. The present study aimed to examine the balance of the contributions of these determinants of RSA, estimated with the high frequency (HF) heart period variability power, during relaxation, mental stress, and physical exercise. It required, therefore, that these four RSA determinants were estimated and manipulated.

Tonic Vagal Modulation of Heart Rate

How to noninvasively manipulate and/or assess tonic vagal modulation of heart rate without using RSA itself? Using dual blockade as was done in the exemplary study of Berntson, Cacioppo, Quigley, and Fabro (1994) was not considered feasible, because of the unpredictable effects of cholinergic and adrenergic blockade on respiratory drive and behavior, specifically during CO_2 breathing. As an alternative, we started with the established fact that stress and exercise both reduce tonic vagal modulation of heart rate. We then made a crucial assumption that in a within-subject design, the stressor-induced changes in tonic vagal modulation of heart rate from the NA are linearly reflected in tonic changes in heart rate level after a correction for the changes in tonic sympathetic modulation of heart rate. With this assumption, we accept having introduced two possible sources of error. First, interactive effects of cardiac sympathetic and parasympathetic nerves (i.e., accentuated antagonism effects) are left unaccounted. Although previous studies suggest that the interactive effects would probably not be substantial in the physiological range of our manipulations, they are not zero (Berntson et al., 1994; Levy, 1997; Uijtdehaage & Thayer, 2000). Second, changes in heart rate caused by an independent second vagal-cardiac pathway that has its origin in the Dorsal Motor Nucleus (DMNX) are also left unaccounted. Although this DMNX-vagal contribution to heart rate is not reflected in RSA (Porges, 1995), it might (differentially) influence the absolute heart rate response to stress and exercise. The preejection period (PEP) was used to estimate tonic sympathetic modulation of heart rate (including effects of circulating catecholamines). Although absolute PEP values may be hard to interpret, within-subject changes in PEP reflect changes in myocardial contractility, which is commonly interpreted as a sensitive index of tonic sympathetic cardiac modulation (Sherwood et al., 1990).

Central Respiratory Drive

The experiment of Al-Ani and coworkers (1996) suggests that increased arterial partial pressure of CO_2 ($PaCO_2$), either through respiratory arrest or artificial inhalation of CO_2-enriched air, is able to enhance the central respiratory drive. Thus, 5% CO_2 breathing can be used to manipulate the central respiratory drive, and an estimation of the $PaCO_2$ (e.g., with the end-tidal partial pressure of CO_2 [$PetCO_2$]) can be used to quantify changes in its strength. However, because the 5% CO_2-enriched air mixture has to be inhaled from compressed air, an additional control condition is desirable in which participants inhale compressed room air under the same conditions as they inhale 5% CO_2-enriched air.

Baro-Reflex and Pulmonary Stretch-Reflex

Although both reflex loops are complex and only partially understood, the effects of the baro-reflex and stretch-reflex on RSA are due to changes in either respiratory depth or respiratory frequency (Berntson et al., 1993). Therefore, changes in respiratory depth and frequency can be used to estimate the extent of phasic modulation of the tonic firing rate of the NA motor neurons through these peripheral respiratory-related feedback mechanisms. The classical approaches to the combined measurement of respiratory parameters include intrusive techniques like spirometry and pneumotachography, or indirect estimation by means of nose clip thermistors. The present study, which employed 5% CO_2-enriched air breathing, did not allow for the use of intrusive measurement or nose thermistors. Instead, we used the continuous thoracic impedance (dZ) signal. Recent studies from different groups (Ernst, Litvack, Lozano, Cacioppo, & Berntson, 1999; de Geus, Willemsen, Klaver, & van Doornen, 1995) have shown that, after appropriate band-pass filtering, thoracic impedance can be used to obtain a reliable index of respiratory frequency. Furthermore, in a within-subject design, the spectral power of the filtered thoracic impedance signal can be used as an approximation of changes in respiratory depth (Ernst et al., 1999).

Frequency-Transfer Function

During expiration, sinoatrial ACh release from cardiac vagal nerves increases, and during inspiration it decreases. Whether these fluctuations in ACh release fully reflect respiratory-related changes in heart rate will strongly depend on the respiratory frequency. Slow changes in cardiac vagal firing will have a fuller impact than faster changes on the difference between the longest beat in expiration and the shortest beat in inspiration. In the normal breathing range,

these filter characteristics of the muscarinergic synapse have been shown to yield a fairly linear decrease in RSA with increasing respiratory frequency (Eckberg, 1983). Based on this relationship, various studies have already used the respiratory frequency as a covariate when using RSA as an index of tonic vagal modulation of heart rate in both within-subject and between-subject comparisons (Allen & Crowell, 1989; Grossman et al., 1991; Grossman & Kollai, 1993; Grossman & Wientjes, 1986; Kollai & Kollai, 1992; Kollai & Mizsei, 1990; Saul et al., 1989).

Within the perspective outlined above, the contributions of within-subject changes in tonic vagal modulation of heart rate, changes in central respiratory drive, and changes in respiratory parameters to within-subject changes in RSA can be estimated using the change scores (Δ) of HF power, change scores of interbeat interval (IBI), change scores of PEP, change scores of $PetCO_2$, change scores of respiratory power, and change scores of respiratory frequency. A path diagram of this model is shown in Figure 1.

Two main hypotheses were tested with this study: (a) Each of the four determinants has a significant influence on RSA, and (b) their relative contributions may vary across stressors (or situations). To demonstrate situation specificity, RSA and its determinants were assessed during mental stress and physical exercise. During exercise, the $PaCO_2$ (related to the central respiratory drive) increases, and, as a result, the respiratory frequency and depth also increase (see Feldman & McCrimmon, 1999). However, during mental stress, the $PaCO_2$ is more likely to decrease than to increase, while the respiratory activity generally increases (Grossman, 1983; Wientjes, 1992). Thus, the contributions of changes in the central respiratory drive and respiratory activity on changes in RSA are different during mental stress compared to physical exercise. For an optimal comparison of the two conditions, we real-time adjusted the load during exercise for each participant, to obtain an identical heart rate response during physical exercise as was found during mental stress. This procedure was followed to ensure that the two conditions were relatively similar in the tonic vagal modulation of heart rate, although they could freely differ in the contributions of the other determinants of RSA. The relaxation condition was used as a general baseline. Better understanding of the relative contribution of the determinants of RSA in various conditions should improve future interpretation of deviating RSA responses in high risk and patient groups.

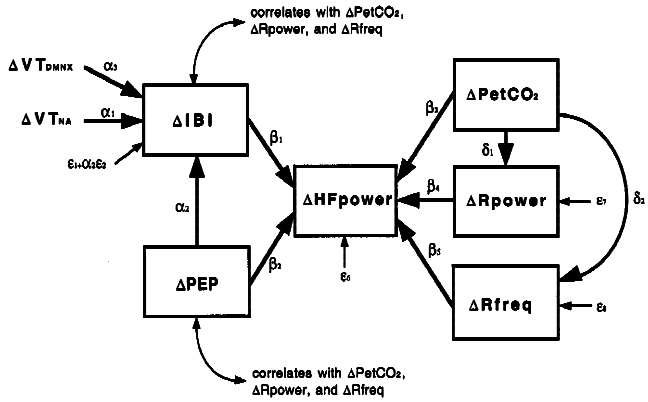

Figure 1. Path diagram depicting all contributions to changes in RSA. See the appendix for the corresponding equations. (Note that it is intuitively not immediately apparent why tonic vagal modulation of heart rate is made to influence RSA through both IBI and PEP in this path diagram. This is clarified in the equations in the appendix).

Methods

Participants

There were 30 young adults without chronic disease or health complaints invited to participate, of which 8 were excluded because they were unsuccessful in maintaining their heart rate within the requested range during the (mild) physical exercise task. The final sample consisted of 11 men (age $M = 24.0$, $SD = 5.9$) and 11 women (age $M = 20.3$, $SD = 1.1$). The study was presented as investigation of breathing patterns. The participants believed that they could win 100 Dutch guilders ($40), although all received a similar amount of 30 Dutch guilders ($12) after the experiment. All participants signed an informed consent. The study had been approved by the ethics committee of the Department of Psychology, University of Amsterdam. None of the participants used medication excepting oral contraceptives in seven women. The participants were instructed to refrain from eating, drinking (except for water), smoking, and physical exercise within 1 hr before the experiment.

Procedure

The experiment consisted of three conditions that were conducted in fixed order: (a) a mental stress task, (b) a "relaxation" condition, and (c) mild physical exercise. Each of these conditions consisted of three parts of 4 min each, again conducted in fixed order: (a) breathing normally, (b) breathing compressed room air through a face mask, and (c) breathing compressed 5% CO_2-enriched air through a face mask. All experimental sessions took place between 11 a.m. and 4 p.m., and lasted approximately 2.5 hr.

After general instructions, the recording electrodes were attached and connected to the Vrije Universiteit Ambulatory Monitoring System (VU-AMS version 4.3; see below). Next, the participants went into a waiting room for 15 min to relax, during which they quietly sat and read a magazine. Next, they entered the experimental room that was sound shielded and dimly lit. The VU-AMS was connected to an MS-DOS computer, and the participants were attached to the $PetCO_2$ recording equipment (see below). Next, the mental stress task was started on the MS-DOS computer. Intelligence test questions were presented one by one in the middle of the screen. The maximum time for each question was 60 s and the elapsed time was visible on the screen. The participants selected one of five multiple-choice responses (1 to 5) and pressed the corresponding key on the PC keyboard. A simultaneously presented reaction-time task consisted of random timed falling red and green coins on the left and right side of the screen. The participants were instructed to press the left button (located at the left side of the keyboard) when a green coin was falling on the left side, and to press the right button (located at the right side of the keyboard) when a green coin was falling on the right side. The computer acknowledged each response (or lack of response) with a brief auditory signal: a musical tone indicating a correct response and a low frequency buzz indicating error. The combined score on the intelligence and reaction-time tasks was expressed in Dutch guilders on the screen. The initial amount was 100 Dutch guilders ($40), which gradually diminished as a result of the errors made. Real bank notes were placed in front of the participants before the task started, and withdrawn when lost. Two research assistants observed the participants and their performance at close range to increase the stressfulness of the task. After 4 min, participants (additionally) had to breathe compressed room air through a face mask (4 min), and breathe compressed CO_2-enriched air through this face mask (4 min). Next, the $PetCO_2$ recording equipment was

disconnected and the participants were debriefed about the stress induction and accompanied to the waiting room.

After a new 15-min period of quiet sitting and reading, the participants reentered the experimental room for the relaxation condition. The VU-AMS was again connected to the MS-DOS computer and the participants were again attached to the $PetCO_2$ recording equipment. This condition was not different from the previous relaxation (i.e., the participants quietly sat reading a magazine) but after 4 min, they (additionally) had to breathe compressed room air through a face mask (4 min), and breathe compressed CO_2-enriched air through this face mask (4 min).

Before the final physical exercise condition, participants again relaxed in the waiting room for 15 min. After they had returned to the experimental room, the VU-AMS was again connected to the MS-DOS computer, and the participants were again attached to the $PetCO_2$ recording equipment. Next, the participants cycled on a bicycle home-trainer, which was set at minimal resistance, while watching the computer screen. A feedback procedure was used to ensure that the same increase in heart rate was obtained (for each participant) during exercise as during mental stress. The participants were instructed to cycle faster or slower in such a way that the top of the bar on the screen was as close as possible to a set-point indicated by a line. The height of the bar represented their mean heart rate over the previous 10 s, and it was updated every 4 s. Participants were kept unaware that the height of the bar reflected their current heart rate, and that the line reflected their (previous measured and saved) mean heart rate during the corresponding part of the mental stress task. The participants' body posture during this bicycle task was fairly similar to their posture during the mental stress and relaxation tasks. The physical exercise task was classified as successful when the differences (for each part) between the mean heart rate during the mental stress task and the mean heart rate during the physical exercise task was below 3 bpm. After 4 min, they again (additionally) had to breathe compressed room air through a face mask (4 min), and breathe compressed CO_2-enriched air through this face mask (4 min).

Finally, all equipment was disconnected and the electrodes were removed, participants were debriefed, paid, and sent home.

Compressed Room Air and 5% CO_2-Enriched Air Breathing

Compressed room air and CO_2-enriched air were stored in two cylinders, which were located in an adjacent room. One cylinder contained medical air and the other a mixture of medical air and CO_2. Each cylinder had its own flow regulation as well as a moisturizing device. The air flow from both cylinders was connected by a T piece to a single silicon tube with an inner diameter of 7 mm, and a length of 4 m, of which 1 m came out in the experimental room. This end was fed into a silicon air reservoir, in turn connected (via a silicon tube of 32 mm inner diameter and a length of 50 cm) to a silicon half face mask (Dräger, Combitox Nova RA). This nonleaking mask, commonly used among fire workers, had two valves that separated incoming and exhaled airflow. The flow of both cylinders could be adjusted to create a part with room air and a part with an air mixture with 5% CO_2.

Physiological Recordings

Interbeat intervals (IBIs), systolic time intervals, respiratory frequency, and a raw estimate of changes in respiratory depth (respiratory power) were measured with the Vrije Universiteit Ambulatory Monitoring System (VU-AMS version 4.3, TD-FPP, Vrije Universiteit, Amsterdam, The Netherlands). This device uses six Ag/AgCl electrodes to record the electrocardiogram and thoracic impedance (dZ). Details on the measurement procedure with the VU-AMS can be found in de Geus et al. (1995) and Willemsen, de Geus, Klaver, van Doornen, and Carroll (1996).

The $PaCO_2$ was estimated by measuring the partial pressure of CO_2 at the end of a normal expiration ($PetCO_2$). This was measured with the Capnogard $etCO_2$ Monitor (Novametrix, Walingford, CT, USA) and expressed in millimeters of mercury. A small tube was inserted in each of the participant's nostrils. The values were automatically fed into a separate MS-DOS computer that was connected to the main system for synchronization of measuring intervals.

Physiological Data Analysis

The heart period data of each participant were analyzed in segments representing 128 s. An artifact preprocessing was performed on the IBI data by detecting outliner IBI values with three methods: (a) by absolute values ($>$1,800 ms or $<$300 ms), (b) a moving average filter ($>$3 *SD* deviation from the moving mean), and (c) by visual inspection. Because artifacts cannot simply be deleted because the continuity of time would be lost, spuriously short IBIs were summed and missing beats were "created" by splitting spuriously long IBIs. The IBI mean values were computed from these corrected data. Next, uniformly spaced samples were created, and the segments were discrete Fourier transformed. Heart period variability power values were computed for the low frequency (LF) band (0.0625–0.125 Hz), and the high frequency (HF) band (0.125–0.5 Hz). The power values were $\log^{10}$ transformed to obtain normal distributions. Changes in the $\log^{10}$ transformed HF power values were used to estimate changes in RSA. In this approach, a high incidence of segments with a respiratory frequency that is systematically below 0.125 Hz could lead to gross underestimation of true spectral heart period power due to respiration. Therefore, all segments were checked for the occurrence of slow breaths. Only 4 out of 396 segments (1.01%) appeared to have a sizable portion of breaths with a frequency similar to or below 0.125 Hz. The advantage of selectively removing these, which would make our study less comparable to most current studies, was considered not to outweigh the very small error introduced.

The thoracic impedance (dZ) data (sampled at 10 Hz) were band-pass filtered by a discrete wavelet transform filter with a cubic spline function as base (0.125–0.5 Hz). Next, the respiratory power values were computed from this filtered thoracic impedance (dZ) data by computing the variance of this filtered time series. Changes in the respiratory power values were used as a (raw) estimation of changes in respiratory depth. The respiratory power values were also $\log^{10}$ transformed to obtain normal distributions. The mean respiratory frequency values were estimated from the band-pass filtered thoracic impedance (dZ) data by counting the number of up-going zero crossings and dividing this value by the time of a segment. This procedure is comparable to the method used by de Geus and coworkers (1995), who computed the mean total respiratory cycle time as the mean interval between the initiating moments of inspiration.

The dZ/dt values (sampled at 250 Hz around each R-wave) were ensemble averaged over 60 s. The B-points were manually determined for each ensemble averaged segment, and the PEP values were determined by summing a fixed Q-to-R interval of 48 ms to the R-B interval time. The 1-min ensemble averaged PEPs were pooled over two succeeding values to obtain a value for each 2-min period, similar to the other measures.

Statistical Data Analysis

For each measure, 18 repeated observations were available for each participant (three conditions with three different breathing parts of 4 min, and two observations per part). To test for within-subject condition effects, the two repeated observations within each 4-min part were averaged to yield nine within-subject values. Within-subject effects (Condition × Breathing Manipulation) were tested with repeated measures MANOVA tests using Wilks' Lambda. Follow-up paired *t* tests were performed to test for specific condition and breathing effects. These follow-up tests (a) compared relaxation with stress and exercise during normal breathing (i.e., the conditions without breathing through the face mask), and (b) tested the specific effects of breathing the compressed CO_2-enriched air mixture compared to breathing compressed room air in each of the conditions. The alpha level was set at the .05 level for all statistical tests.

Finally, a path analysis (using Lisrel V8.12a) was performed over the pooled covariance matrices that were computed for each participant over 18 repeated observations (for change scores in IBI, PEP, HF power, $PetCO_2$, respiratory power, and respiratory frequency). This path analysis tested for the relative contributions of the determinants of HF power as depicted in Figure 1. Because change scores (indicated with Δ in Figure 1) were used, the intercepts were left out of the regression equations (see the appendix), resulting in regression lines through the origin (representing the values during normal breathing in the relaxation condition). Because the true degrees of freedom (*df*) for this mixed between-within-subject analysis was not known, it was estimated halfway between the theoretical lower limit of 22, and the theoretical upper limit of 18 × 22 (i.e., $df = 209$). Using either lower or upper limit *df* did not change the pattern of results.

Results

Table 1 shows the mean and corresponding standard deviation values of all measures for all nine conditions. Figures 2 to 8 show graphs (one for each measure) with bars that represent the mean within-subject change scores between each specific condition and the relaxation condition during normal breathing.

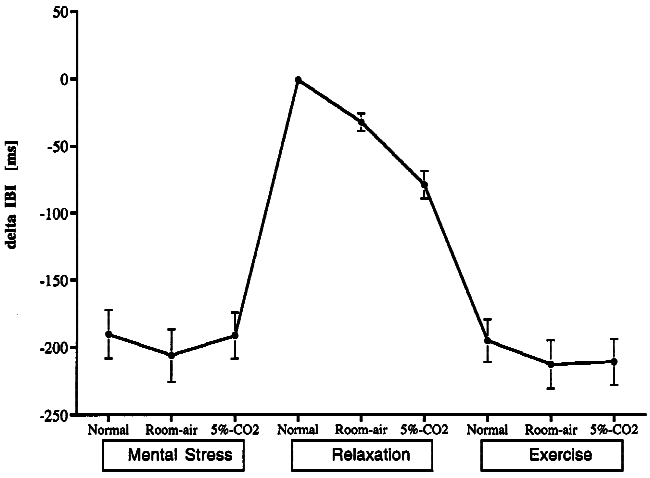

Figure 2. Mean within-subject change scores (±*SEM*) between each specific condition and the relaxation condition during normal breathing for IBI.

IBI and PEP

A significant overall condition effect was found for IBI, $F(8,13) = 20.11$, $p < .001$, and PEP, $F(8,13) = 4.73$, $p = .007$. Follow-up tests limited to the normal breathing parts revealed that, as compared to the relaxation condition, the mean IBI and PEP were significantly lower during mental stress, $t_{ibi}(21) = 10.55$, $p < .001$; $t_{pep}(21) = 5.96$, $p < .001$, and during physical exercise, $t_{ibi}(21) = 12.26$, $p < .001$; $t_{pep}(21) = 3.59$, $p = .002$. No significant difference was found between the IBI response to mental stress and the

Table 1. *Mean and Corresponding Standard Deviation Values of all Measures for All Nine Conditions*

		IBI	PEP	$PetCO_2$	HF power	LF power	R power	R freq
Mental stress								
Normal	*M*	697.24	84.06	37.26	2.73	2.69	1.61	0.269
	SD	108.15	9.75	3.49	0.39	0.36	0.15	0.044
Room air	*M*	681.54	83.61	38.01	2.76	2.64	1.70	0.251
	SD	109.39	10.17	3.38	0.42	0.42	0.19	0.048
5% CO_2	*M*	696.21	83.47	46.22	3.04	2.62	1.98	0.270
	SD	99.21	9.03	3.62	0.41	0.40	0.14	0.048
Relaxation								
Normal	*M*	887.62	90.82	37.31	3.08	3.01	1.59	0.238
	SD	98.37	11.02	3.04	0.33	0.35	0.18	0.045
Room air	*M*	855.34	91.27	37.90	3.10	2.99	1.64	0.233
	SD	93.76	10.76	3.24	0.31	0.37	0.19	0.047
5% CO_2	*M*	808.46	90.17	46.34	3.29	2.83	2.00	0.261
	SD	96.77	9.50	3.93	0.41	0.43	0.17	0.059
Physical exercise								
Normal	*M*	692.29	86.11	39.89	2.49	2.45	0.96	0.309
	SD	100.21	9.61	3.94	0.50	0.45	0.31	0.038
Room air	*M*	674.86	85.41	42.38	2.69	2.47	1.09	0.276
	SD	107.89	10.07	4.22	0.50	0.47	0.37	0.052
5% CO_2	*M*	676.68	84.76	50.37	2.93	2.50	1.02	0.284
	SD	103.31	10.65	4.28	0.59	0.55	0.33	0.063

Note: IBI = interbeat interval (ms), PEP = preejection period (ms), $PetCO_2$ = end-tidal partial pressure of CO_2 (mmHg), HF power = high-frequency heart period variability power [$\log^{10}(ms^2 + 1)$], LF power = low-frequency heart period variability power [$\log^{10}(ms^2 + 1)$], Rpower = respiratory power [$\log^{10}$(arbitrary units + 1)], Rfreq = respiratory frequency (Hz).

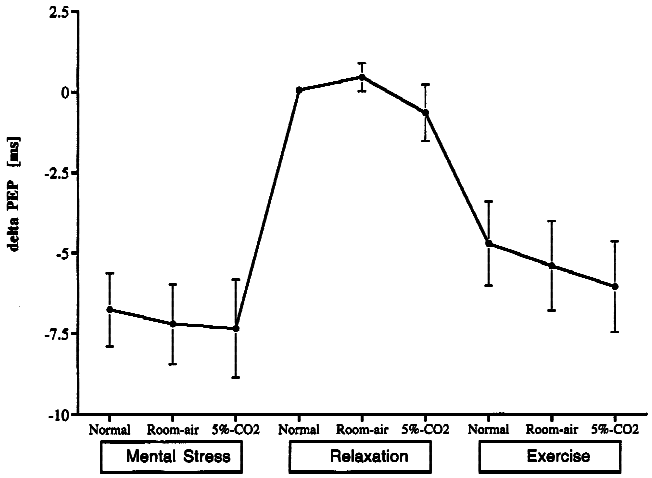

Figure 3. Mean within-subject change scores (±*SEM*) between each specific condition and the relaxation condition during normal breathing for PEP.

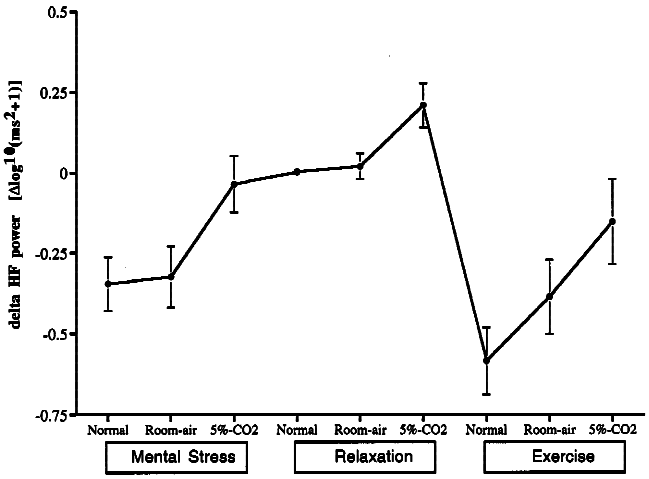

Figure 5. Mean within-subject change scores (±*SEM*) between each specific condition and the relaxation condition during normal breathing for HF power.

IBI response to physical exercise, testifying to the success of our experimental manipulation of heart rate. In spite of equal heart rate reactivity, the PEP response to mental stress was significantly larger than the PEP response to exercise, $t(21) = 2.48$, $p = .022$. Follow-up tests for differences in air mixture revealed no significant differences for IBI and PEP responses to compressed room air and compressed CO_2-enriched air mixture during mental stress or exercise. However, as compared to compressed room air, the mean IBI was significantly lower for the compressed CO_2-enriched air mixture during relaxation, $t(21) = 5.15$, $p < .001$. Thus, the $PaCO_2$ (central respiratory drive) manipulation had some effects on heart rate, but only during relaxation.

$PetCO_2$, Respiratory Power, and Respiratory Frequency

Significant overall condition effects were found for the $PetCO_2$, $F(8,13) = 108.28$, $p < .001$, respiratory power, $F(8,13) = 87.97$, $p < .001$, and respiratory frequency, $F(8,13) = 11.98$, $p < .001$. Follow-up tests limited to the normal breathing parts revealed that the mean $PetCO_2$ and respiratory power were not significantly different during mental stress as compared to relaxation, but both were significantly higher during physical exercise, $t_{PetCO2}(21) = 5.68$, $p < .001$; $t_{Rpower}(21) = 3.16$, $p = .005$. Respiratory frequency, in contrast, increased above relaxation levels during exercise, $t(21) = 6.21$, $p < .001$, as well as during mental stress, $t(21) = 2.50$, $p = .021$, although the response to exercise was significantly larger, $t(21) = 4.26$, $p < .001$. Taken together, the results for these respiratory parameters demonstrate that $PetCO_2$, respiratory power, and respiratory frequency responses varied across conditions independently of the magnitude of the heart rate response.

Follow-up tests for differences in air mixture revealed, as expected, a significantly higher mean $PetCO_2$ and respiratory power for the compressed CO_2-enriched air mixture as compared to

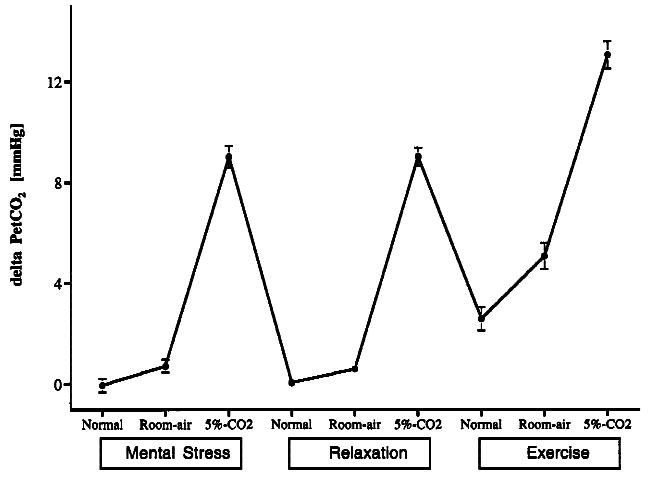

Figure 4. Mean within-subject change scores (±*SEM*) between each specific condition and the relaxation condition during normal breathing for $PetCO_2$.

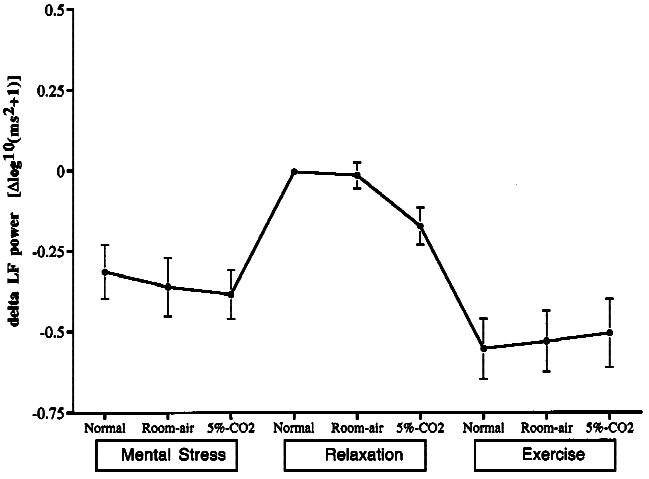

Figure 6. Mean within-subject change scores (±*SEM*) between each specific condition and the relaxation condition during normal breathing for LF power

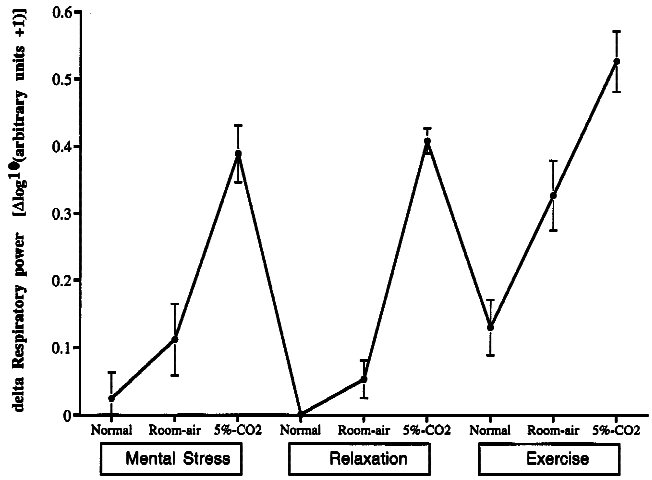

Figure 7. Mean within-subject change scores (±*SEM*) between each specific condition and the relaxation condition during normal breathing for respiratory power.

compressed room air in all three conditions, *p*s $< .001$. For respiratory frequency, no differential effects of breathing compressed room air or compressed CO_2-enriched air mixture were found during mental stress or exercise. However, during relaxation, the mean respiratory frequency was significantly higher for the compressed CO_2-enriched air mixture, $t(21) = 3.12$, $p = .005$, although the effect was due as much to a decrease in respiration frequency during room air as to an increase during CO_2-enriched air. These $PetCO_2$ results are clearly indicative of successful manipulation of $PaCO_2$ (central respiratory drive).

HF and LF Power Values

Significant overall condition effects were found for the HF, $F(8,13) = 8.03$, $p = .001$, and LF, $F(8,13) = 4.10$, $p = .012$, powers. Follow-up tests limited to the normal breathing parts revealed that the mean HF and LF powers were significantly decreased during mental stress, $t_{HF}(21) = 4.13$, $p < .001$; $t_{LF}(21) = 3.73$, $p = .001$, and exercise, $t_{HF}(21) = 6.64$, $p < .001$; $t_{LF}(21) = 5.92$, $p < .001$, as compared to relaxation. For both powers, the response to exercise was larger than the response to stress, $t_{HF}(21) = 4.08$, $p = .001$; $t_{LF}(21) = 3.32$, $p = .003$.

Follow-up tests for differences in air mixture revealed, as expected, a significantly higher mean HF power for the compressed CO_2-enriched air mixture as compared to compressed room air during relaxation, $t(21) = 3.94$, $p = .001$, as well as during mental stress, $t(21) = 4.40$, $p < .001$, and during exercise, $t(21) = 3.30$, $p = .003$. In contrast, no significant effect of CO_2-enriched air breathing was found on the response of LF power during mental stress or during exercise, and lower rather than higher LF power was found during relaxation, $t(21) = 2.40$, $p = .026$. These results demonstrate that mental stress and exercise reduced both HF and LF powers, but that the $PaCO_2$ manipulation selectively influenced HF power. The impact of the increased central respiratory drive on HF power during CO_2-enriched air breathing was very large: The normal reduction in HF power observed during mental stress and exercise almost completely disappeared.

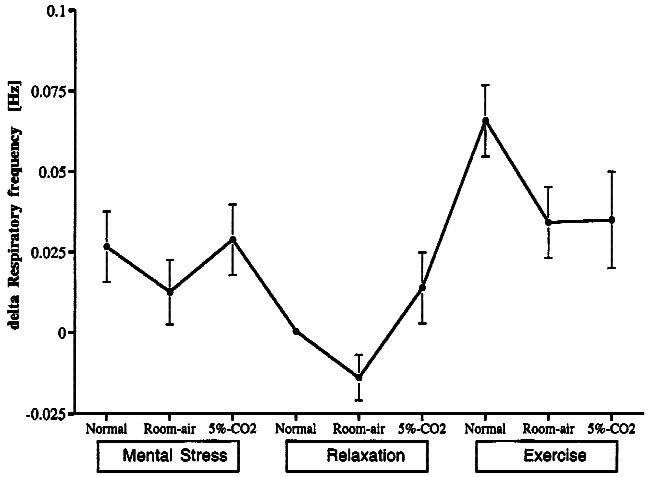

Figure 8. Mean within-subject change scores (±*SEM*) between each specific condition and the relaxation condition during normal breathing for respiratory frequency.

Path Analysis for All Contributions to the HF Power

Path analysis was performed to test for the relative contributions to within-subject changes in HF power due to changes in (a) IBI and PEP, (b) $PetCO_2$, (c) respiratory power, and (d) respiratory frequency. The model as depicted in Figure 1 resulted in an acceptable goodness of fit, $\chi^2(1) = 0.034$, $p = .84$. The total variance in the changes in HF power explained by this model was 76%. The standardized beta values are shown in Table 2. Note that in path analysis, all beta and correlation coefficients are essentially partial correlation coefficients. For example, the contribution of changes in $PetCO_2$ to changes in HF power is independent of the increase in respiratory power caused by CO_2 breathing. The results of the path analysis indicate that, apart from tonic vagal modulation of heart rate, changes in $PetCO_2$, respiratory power, and respiratory frequency had significant and independent contributions to changes in HF power. Figure 9 shows a graph with mean within-subject changes in the HF power across the various conditions: (a) uncorrected, (b) corrected for changes in $PetCO_2$, (c) corrected for changes in respiratory power, (d) corrected for changes in respiratory frequency, and (e) corrected for changes in all these determinants, using the beta values of the path analysis. Changes in this corrected HF power (see Figure 9) closely correspond to changes in IBI corrected for changes in PEP, and can be considered the most accurate estimation of within-subject changes in tonic vagal modulation of heart rate.

Table 2. *Standardized Beta-Values Corresponding with the Path-Analysis Depicted in Figure 1*

		beta-value
α_2	$\Delta PEP \rightarrow \Delta IBI$	.58**
β_1	$\Delta IBI \rightarrow \Delta HF$ power	.72**
β_2	$\Delta PEP \rightarrow \Delta HF$ power	−.23**
β_3	$\Delta PetCO_2 \rightarrow \Delta HF$ power	.21**
β_4	ΔRespiratory power $\rightarrow \Delta HF$ power	.24**
β_5	ΔRespiratory frequency $\rightarrow \Delta HF$ power	−.29**
δ_1	$\Delta PetCO_2 \rightarrow \Delta$Respiratory power	.79**
δ_2	$\Delta PetCO_2 \rightarrow \Delta$Respiratory frequency	.12

**$p < .01$ (2-tailed).

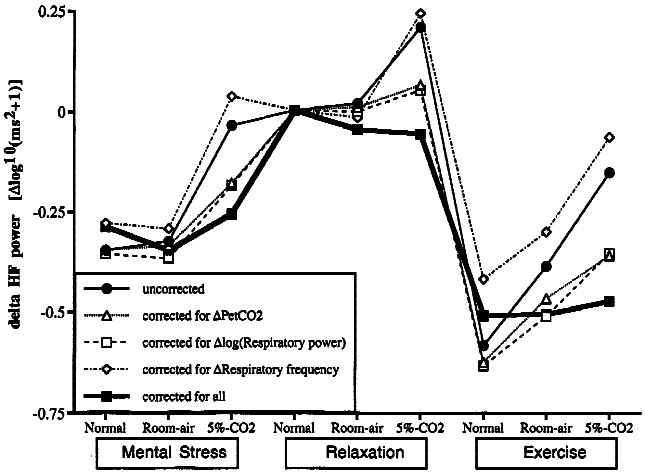

Figure 9. Mean within-subject changes in log (HF power) that are (a) uncorrected, (b) corrected for changes in $PetCO_2$, (c) corrected for changes in log(respiratory power), (d) corrected for changes in respiratory frequency, and (e) corrected for all these determinants.

Discussion

The present study confirmed our hypothesis that changes in tonic vagal modulation of heart rate, central respiratory drive, and respiratory depth and frequency each contribute to within-subject changes in RSA, measured as HF heart period variability power. Independence of the various effects on RSA was shown by the path analysis in which changes in IBI, corrected for changes in PEP, were used as a proxy for central-nervous-system-induced changes in tonic vagal modulation of heart rate. This analysis showed that the reduction in RSA during mental stress and physical exercise was only in part accounted for by changes in tonic vagal modulation of heart rate. Additional significant contributions were shown from changes in respiratory depth, changes in respiratory frequency, and changes in $PaCO_2$ (central respiratory drive). The main new finding of the present study is that situation specificity (stress vs. exercise) exists in the relative contributions of these RSA determinants.

The effects of changes in respiratory depth and frequency on RSA were as expected, and their direction confirms the previous literature (Eckberg, 1983; Grossman & Kollai, 1993; Hirsch & Bisschop, 1981; Kobayashi, 1998): Task-induced reductions in RSA are enhanced by faster breathing but reduced by deeper breathing. Increased central respiratory drive strongly, and independently, affects the normal task-induced RSA reduction, which also confirms the previous literature (Al-Ani et al., 1996). Compared to relaxation, a pronounced reduction in IBI, PEP, and RSA was found during mental stress and physical exercise. However, the task-induced reduction in RSA was only observed under normal breathing conditions. It was nullified by 5% CO_2-enriched air breathing during mental stress, and greatly reduced by 5% CO_2-enriched air breathing during physical exercise. CO_2 effects were specific to RSA: The relative increase in RSA during 5% CO_2-enriched air breathing in all three conditions was not coupled to similar effects on IBI, PEP, or LF heart period variability power.

An important consequence of our findings is that correcting within-subject changes in RSA for changes in respiratory frequency only may not always yield the theoretically optimal estimate of changes in tonic vagal modulation of heart rate. Conditions resulting in increased or decreased respiratory depth (i.e., estimated in this study with respiratory power) and/or increased or decreased $PaCO_2$ (i.e., estimated in this study with $PetCO_2$) can compromise RSA as an index of tonic vagal modulation of heart rate. Fortunately, during mental stress under normal breathing conditions, the within-subject changes in RSA corrected for respiratory frequency largely paralleled the changes in tonic vagal modulation of heart rate. In fact, this correction did not even produce a better estimator than the uncorrected RSA. Thus, in practice, uncorrected within-subject changes in RSA are acceptable to index within-subject changes in tonic vagal modulation of heart rate for most (clinical) stress studies. However, it is uncertain that this will apply to all stressors, particularly if they influence the $PaCO_2$ (e.g., as a result of hypo- or hyperventilation or as a result of a changed metabolism during exercise). Therefore, for studies that might affect the central respiratory drive, RSA should be optimally corrected for changes in respiratory depth, respiratory frequency, and $PaCO_2$ to index changes in tonic vagal modulation of heart rate.

As a result of our manipulation, a similar reduction in IBI was found during mental stress and physical exercise. However, this same heart rate response to physical exercise and mental stress was brought about by a different mix of tonic vagal and sympathetic modulation of heart rate. The reduction in PEP was larger during mental stress, whereas the reduction in HF (and LF) heart period variability power was larger during physical exercise. In line with our main findings, the differences in HF power reduction can be partially explained by different effects of exercise and stress in the central respiratory drive and respiratory behavior. The respiratory frequency (for the normal breathing conditions) increased more during physical exercise than during mental stress, whereas the respiratory depth and $PaCO_2$ increased only during physical exercise. However, inspection of the corrected HF power in Figure 9 shows that the contribution of tonic vagal modulation of heart rate for exercise and stress truly varied across situations. This is most likely explained by a fundamental difference in the neural regulation of heart rate in these two conditions. During physical exercise, tonic vagal modulation of heart rate is reduced and tonic sympathetic modulation of heart rate is enhanced by a combination of a feedforward "central command" and a feedback signal from the chemo- and mechanoreceptors in the working muscles (Potts & Mitchell, 1998; Rowell & O'Leary, 1990; Williamson, Nobrega, Winchester, Zim, & Mitchell, 1995). During stress, only the central command will be active with a relatively negligible increase in feedback from muscle activity. Because the muscle–heart reflexes largely operate through resetting of the baro-reflex (Potts & Mitchell, 1998; Potts, Shi, & Raven, 1993), their effect will be mainly parasympathetic in origin, specifically in the first minutes of exercise. Thus, it is not surprising that physical exercise, exploiting both feedforward and feedback signals, inhibited tonic vagal modulation of heart rate more strongly than stress.

Although we tried to include all relevant determinants, our model (Figure 1) did not explain the total variance in the changes in RSA (i.e., estimated as changes in the HF power in our model). The exact sources of the remaining error variance need to be established but at least three factors can be identified a priori. First, possible effects of accentuated antagonism of sympathetic and vagal activity at the sinoatrial node (Abramovich & Akselrod, 1998; Uijtdehaage & Thayer, 2000) were set to zero in our model. Second, using IBI corrected for PEP as an index of tonic vagal modulation of heart rate was not optimal. Although an error variance for IBI (ε_1 in Figure 1) was modeled, we did not estimate

(task-dependent) changes in DMNX-vagal contribution to heart rate. Finally, using PEP as measure of the tonic sympathetic modulation of heart rate when comparing exercise and stress may be flawed. During physical exercise ventricular preload increases and afterload decreases more than during mental stress (where a reverse effect may occur). This compromises PEP as an index of sympathetic beta-adrenergic influences on the heart (Sherwood et al., 1990). However, an increase in preload and a decrease in afterload should have yielded a *lowered* PEP value during exercise. Just the opposite was found. Unfortunately, preload and afterload are not the only factors to affect the validity of PEP. PEP measures the contractility of the left ventricle, which is dependent on both the amount of adrenergic neurotransmitters as well as the affinity and density of the left ventricular adrenoceptors. Density of beta-receptors on lymphocytes has been shown to change rapidly in response to adrenaline infusion, exercise, and mental stress (Graafsma et al., 1989, 1990), and the same may apply to cardiac receptors, specifically the ventricular beta-2-receptors (Muntz, Zhao, & Miller, 1994). This dynamic receptor regulation may be situation specific in that beta-receptor density may increase more strongly during exercise than mental stress (Graafsma et al., 1989, 1990).

In spite of the problems mentioned above, the converging evidence of this study clearly demonstrates that, under certain conditions, an important contribution of central respiratory drive to RSA exists that is, in part, independent of influences of tonic vagal modulation of heart rate and respiratory depth and frequency. It also demonstrates situation specificity in the relative contributions of these determinants of RSA. Although our results are strictly obtained from within-subject change scores, it seems reasonable to expect that between-subject differences in RSA may also be modified by individual differences in central respiratory drive. This may have practical implications for RSA studies including participants with deviant respiratory behavior (e.g., hyperventilation). It has already been shown that strong individual differences exist in $PetCO_2$, and that these differences represent a stable trait that is associated with increased risk for hypertension and is accompanied by a tendency to worry and experience negative emotions (Dhokalia, Parsons, & Anderson, 1998). There is a growing literature showing individual differences in uncorrected RSA to be predictive of hypertension or cardiac disease (Bigger et al., 1993; Hayano et al., 1991; Julius et al., 1971; Malliani et al., 1991; Saul et al., 1988; Singer et al., 1988; Tsuji et al., 1996) and to correlate with low psychological well-being (Musselman et al., 1998; Thayer et al., 1996; Watkins et al., 1998). Refinement of RSA, by taking into account the central respiratory drive (i.e., $PaCO_2$) in addition to respiratory frequency and depth, could help to further improve the associations and predictions found in such studies.

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APPENDIX

$$(1)\ \Delta IBI = \alpha_1 \Delta VT_{NA} + \alpha_2 \Delta ST + \alpha_3 \Delta VT_{NMXD} + \epsilon_1$$

$$\Rightarrow (2)\ \Delta IBI = \alpha_1 \Delta VT_{NA} + \alpha_2 (\Delta PEP + \epsilon_2) + \alpha_3 \Delta VT_{NMXD} + \epsilon_1$$

$$\Leftrightarrow (3)\ \Delta VT_{NA} = \frac{1}{\alpha_1} \Delta IBI - \frac{\alpha_2}{\alpha_1} \Delta PEP - \frac{\alpha_3}{\alpha_1} \Delta VT_{NMXD} - \frac{\epsilon_1}{\alpha_1} - \frac{\alpha_2 \epsilon_2}{\alpha_1}$$

$$(4)\ \Delta RSA = \gamma_1 \Delta VT_{NA} + \gamma_2 \Delta PaCO_2 + \gamma_3 \Delta Rdepth + \gamma_4 \Delta Rfreq$$

$$\Rightarrow (5)\ \Delta HFpower + \epsilon_3 = \gamma_1 \Delta VT_{NA} + \gamma_2 (\Delta PetCO_2 + \epsilon_4) + \gamma_3 (\Delta Rpower + \epsilon_5) + \gamma_4 \Delta Rfreq$$

$$(3) \rightarrow (5)\ \Delta HFpower = \beta_1 \Delta IBI + \beta_2 \Delta PEP + \beta_3 \Delta PetCO_2 + \beta_4 \Delta Rpower + \beta_5 \Delta Rfreq + \epsilon_6$$

$$(6+7)\ \Delta Rpower = \delta_1 \Delta PetCO_2 + \epsilon_7$$

$$\Delta Rfreq = \delta_2 \Delta PetCO_2 + \epsilon_8$$

$$\beta_1 = \frac{\gamma_1}{\alpha_1} \qquad \beta_2 = \frac{-\gamma_1 \alpha_2}{\alpha_1} \qquad \beta_3 = \gamma_2 \qquad \beta_4 = \gamma_3 \qquad \beta_5 = \gamma_4$$

$$\epsilon_6 = -\frac{\gamma_1}{\alpha_1} \epsilon_1 - \frac{\gamma_1 \alpha_2}{\alpha_1} \epsilon_2 - \epsilon_3 + \gamma_2 \epsilon_4 + \gamma_3 \epsilon_5 - \frac{\gamma_1 \alpha_3}{\alpha_1} \Delta VT_{NMXD}$$

$$\epsilon_7 = \delta_1 \epsilon_4 - \epsilon_5 \qquad \epsilon_8 = \delta_2 \epsilon_4,$$

where IBI = interbeat interval; VT_{NA} = tonic vagal modulation of heart rate from nucleus ambiguus; VT_{DMNX} = tonic vagal modulation of heart rate from dorsal motor nucleus; ST = tonic sympathetic modulation of heart rate; PEP = preejection period; RSA = respiratory sinus arrhythmia; $HFpower$ = logarithm of high-frequency heart period variability power; $PaCO_2$ = arterial partial pressure of CO_2; $PetCO_2$ = end-tidal partial pressure of CO_2; $Rdepth$ = respiratory depth; $Rpower$ = logarithm of respiratory power; $Rfreq$ = respiratory frequency.