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ORIGINAL ARTICLE

Heart period sensitivity to forced oscillations in ventilatory pressure

S. R. Quint · B. V. Vaughn

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Abstract Heart rate variability analysis is a dynamic method to estimate the autonomic control over the cardiac cycle. Although dysfunction in this control system may appear spontaneously, other deficits may require provocation of the system. In this article we describe a non-invasive method to perturb the autonomic influences on the cardiac cycle. We recorded the ECG and respiratory pressure of ten healthy volunteers while introducing a random forced oscillation pressure wave onto spontaneous respiration. The heart period time series was determined and the power spectra for the 0.05–0.15, 0.15–0.3 and 0.05–0.4 Hz bands were calculated. The random input did not alter mean heart rate. However, the segments with the forced oscillation input demonstrated, on average, a tenfold increase in spectral power averaged across all subjects, with a maximum observed effect of 100-fold increase in power. This increase in power correlated with the respiratory frequency. This study demonstrates that random noise ventilation, such as used in respiratory forced oscillation impedance estimates, significantly alters the autonomic input to cardiac cycle variability in wake subjects.

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S. R. Quint (✉) · B. V. Vaughn
Joint Department of Biomedical Engineering,
UNC-CH School of Medicine and NCSU College of Engineering,
Chapel Hill, NC 27599, USA
e-mail: quint@bme.unc.edu

B. V. Vaughn
Department of Neurology, UNC-CH School of Medicine,
Chapel Hill, NC 27599, USA
e-mail: vaughnb@neurology.unc.edu

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Introduction

The study of heart period variability (HPV) and heart rate variability (HRV) has been applied to understand the role of the autonomic nervous system in a wide variety of subjects and diseases over the past three decades. Autonomic activity is responsible for the preponderance of variation in the interval between heart beats at frequencies above 0.04 Hz (Akselrod et al. 1981; Berger et al. 1989; Parati et al. 1995; Koh et al. 1998). For detection of alterations in HRV, the ECG is usually observed without perturbing the system. This approach relies upon the abnormality to appear spontaneously. One difficulty of interpreting changes in HRV is the vast array of potential influences on the autonomic nervous system. Stimuli of both physical and mental origin may alter the autonomic regulation of the heart. However, it may be possible to elicit a response to a specific input from which an abnormal autonomic state may be revealed. Many tests of the autonomic nervous system, which generally require subject cooperation (Akselrod et al. 1981; Berger et al. 1989; Koh et al. 1998), utilize respiratory perturbation as a means to determine functional status of the autonomic system. We have applied a method for introducing an input to volunteer subjects, which is widely used in estimation of respiratory impedance, does not require subject participation for the input generation, and has a profound effect on HPV. A forced oscillatory input superimposed on voluntary respiration may reveal non-spontaneously occurring system abnormalities. In addition, this input, widely used in respiratory impedance estimation, may have autonomic respiratory effects which, in turn, may alter respiratory impedance.

However, the autonomic effects of this input for estimation of pulmonary impedance have not been investigated. For this study we have investigated the effects of the forced oscillation method for estimating pulmonary impedance on autonomic activity, as characterized by widely used analysis of HRV (Akselrod, AL-Ani, Dizon, Goldsmith, Messenheimer, Perini, Puig, Quint, Vaughn). The objectives of this research are to illustrate the potential autonomic activation by method of forced oscillations, as demonstrated by a profound amplification of HRV, and to suggest the possibility of applications derived from this method in studies of HRV in health disease and exercise conditioning.

Methods

Ten male volunteers were selected for this study, who ranged in age from 22 to 29 years, with a mean age of 26 years. Physical condition of the subjects varied from a sedentary lifestyle to daily exercise. All ten subjects in this investigation were healthy males, and none of the participants were on medications, nor were known to have any medical issues affecting the autonomic nervous system. Subjects were relaxed and in a sitting position in a closed quiet room during the recording of heart period (HP) and respiratory pressure. The respiratory input device was positioned in front of each subject, who then breathed spontaneously through the mouth apparatus as shown in Fig. 1. Respiration occurred through this apparatus during both CONTROL and RANDOM conditions. A low pressure instrumentation-type ventilation fan was used to continuously remove expired air from the dead space in the device, and tubing which was both short in length (10 cm) and of large diameter (3 cm) was used to minimize the resistance to breathing (Fig. 1). During CONTROL data collection the subjects were unable to distinguish any difference from their normal breathing, other than the presence of the mouth apparatus. During the RANDOM condition, a noise pressure wave with approximately Gaussian amplitude about mean zero and a Poisson distribution in time was imposed on spontaneous respiratory pressure. This was accomplished using a microprocessor-generated speaker position input (Fig. 1). The bandwidth of the Random signal, generated at the speaker, is shown in Fig. 2. These methods were selected as the forced oscillation methods used in many respiratory impedance estimation procedures (Eyles, Pimmel, Hantos, Michaelson, Landser), and the same equipment used for pulmonary impedance estimation research by Pimmel et al. A more detailed description of this apparatus has been presented for use in the determination of respiratory impedance (Pimmel et al. 1977). The resulting pressure input to respiration had a peak-to-peak amplitude of approximately 10 cm H_2O (Fig. 3a) and a half-power bandwidth of 3–25 Hz (Fig. 2) as measured at the mouth apparatus using a Validyne model MP45 differential pressure transducer with the reference port open to atmosphere (Pimmel et al. 1977; Eyles et al. 1982). This pressure wave produced a definite sensation of pulsation in the subject's chest, which was reported as not uncomfortable. The subjects were asked to relax and breathe naturally during both CONTROL and RANDOM conditions. This study was sanctioned by the Office of Human Research Ethics at UNC-CH.

A preliminary study was performed in six subjects to determine the transient response and accommodation to the input. The effect of the input on the HP time-series data was evaluated by inspection of the R–R interval plots of HP for transient portions of the data. These preliminary data indicated that the response to the random input showed a start-up transient lasting up to 30 s and/or accommodation to the

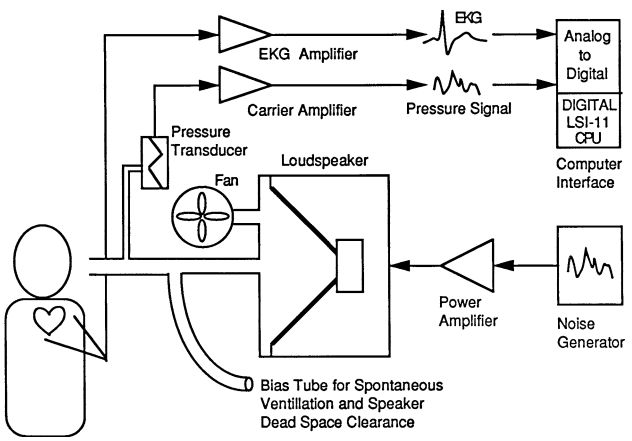

Fig. 1 The experimental setup, including the white-noise input to the subject and the EKG and pressure measurement and sampling

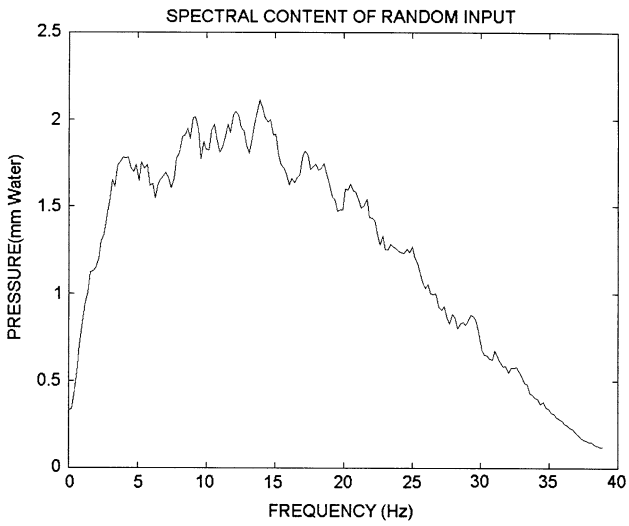

Fig. 2 Amplitude spectrum of the white-noise pressure input to spontaneous respiration of the subject, measured at the pressure transducer as shown in Fig. 1

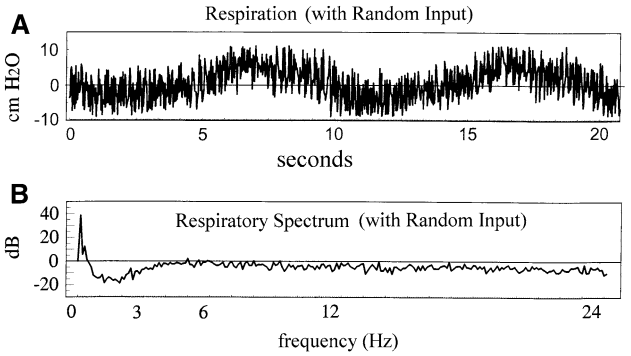

Fig. 3 **a** 20 s of the respiratory signal, as measured at the transducer (Fig. 1), including the random signal superimposed on the spontaneous respiration of one of the subjects. **b** The corresponding power spectrum (decibels) of the signal shown in **a**

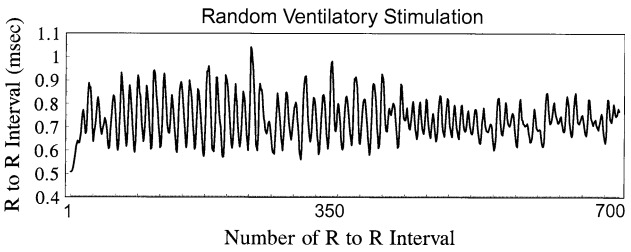

Fig. 4 Heart period from subject 1, from the onset of pseudorandom input through 700 heart beats (520 s). Accommodation of the subject to the random input can be observed to begin after about 450 beats (334 s)

input after 5½ to 12 min (Fig. 4). The experimental design for the ten subjects in this study was based on the results of the preliminary study.

In each of the ten subjects the spontaneous ECG (CONTROL) was recorded and digitized for 5 min, followed by four (RANDOM) collection periods of 5 min each. During each RANDOM collection period the random pressure wave was superimposed on spontaneous respiration, and the ECG and input airway pressure were recorded. Each RANDOM recording period began 30 s after the input was applied to the subject, and the four sequential recording periods were separated by 5 min periods without input. These rest periods separating the (RANDOM) periods differed from the (CONTROL) only in that no data was recorded. Input airway pressure, as measured at the mouthpiece, was sampled at 50 Hz. Heart period was automatically determined from the digitized ECG at 2 ms resolution (500 Hz sample rate) with a computer algorithm identification of the R–R interval. The accuracy of this measure was verified by visual inspection of the marked R wave on the digitized waveform (Messenheimer et al. 1989; Quint et al. 1990; Vaughn et al. 1995) and by inspection of the derived R–R interval record of HP (Fig. 5). All multiply marked beats were corrected and missed beats were marked by

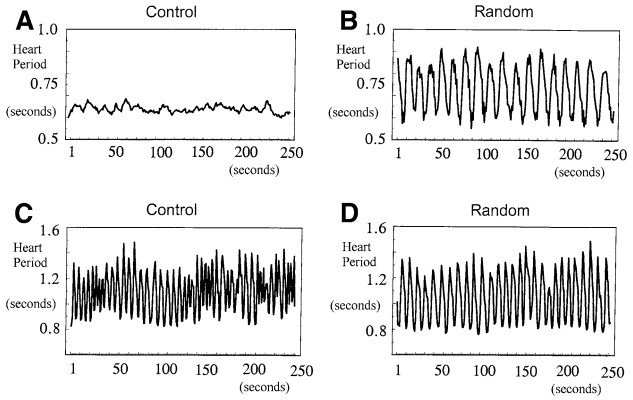

Fig. 5 The heart period time series are shown for two subjects preceding (*left* **a** and **c**) and following (*right* **b** and **d**) random stimulation. **a** and **b** (Subject 4) are typical of the pre- and post-stimulation heart period records for all subjects (showing a dramatic increase in HPV), excepting subject 3. The pre- and post-stimulation heart period records for subject 3, the only subject engaged in rigorous physical conditioning, are shown in **c** and **d**. For subject 3, while the amplitude of the HP time series oscillations is unchanged, the bandwidth HPV narrows dramatically from pre- to post-stimulation to approximate a single sinusoid (Fig. 6d)

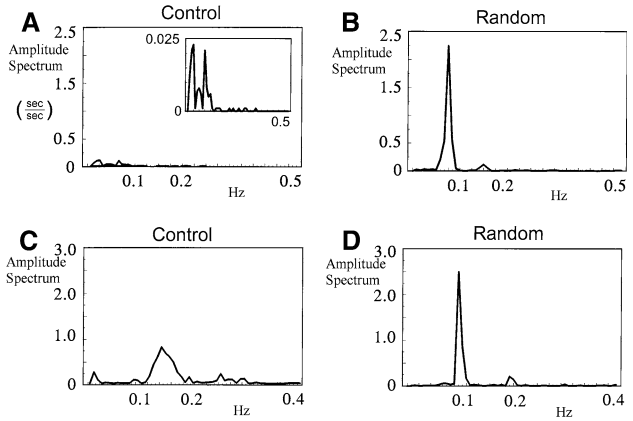

Fig. 6 Heart period variability spectra corresponding to the heart period records shown in Fig. 5. Note the very large increase in area of the spectrum at the respiratory frequency (approximately 0.09 Hz) from **a** to **b** in subject 4, while the area over the band at respiratory frequency for subject 3 (from **c** to **d**) increases only moderately (Fig. 8). Control and Random conditions are shown at the same amplitude scale, with the scale magnified in the upper right corner of **a** to give resolution to this amplitude spectra

software-assisted editing. Short-term recordings from the young healthy subjects in this study were free of ectopy and missing data. The mean HP was subtracted from the data to reduce error in the spectral determination, and the data were then divided by the mean to facilitate inter-subject comparisons. These normalized time-interval data records were subjected to a Blackman window and the power spectra determined, during CONTROL and RANDOM conditions in each subject for 128 point (R–R interval) segments (Fig. 6). A more detailed description of the signal processing methods is given in Messenheimer et al. (1989).

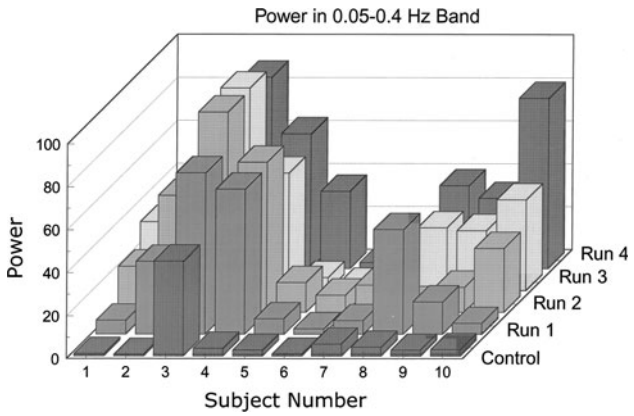

Fig. 7 The power in the broad 0.05–0.4 Hz band of the heart period data is shown for all ten subjects for the control condition, followed by each of the four runs with random input. Note that only subject 3 shows only a moderate increase in power (factor of 2 max) to the random input in all four runs

From the power spectra of the heart period data, the power was summed in three bands (0.05–0.15, 0.15–0.3, and 0.05–0.4 Hz). This provides a measure of the variance of the portion of the time domain data corresponding to each frequency band. This summed power for each band was determined for the CONTROL and each RANDOM condition. Data for the 0.05–0.4 Hz band are presented in Fig. 7.

The pNN50 was determined for the CONTROL and four RANDOM conditions in each subject. For each calculation, the pNN50 was determined as the percent of sequential beat-to-beat R–R interval variations of 50 ms or greater from 256 sequential normal beats.

Analysis of variance (Model I ANOVA) was used to test the null hypothesis that the mean heart rate was unchanged between CONTROL and RANDOM conditions (Sokal and Rohlf 1969). Due to the sample size (four replications in ten subjects) and because the sampled population was not controlled for physical conditioning, the null hypothesis for equal means was also tested using a non-parametric (Sokal and Rohlf 1969) repeated measures comparisons (Friedman's test). The Student's *t* test was used to test the null hypothesis that the mean power in each of several frequency bands (0.05–0.15, 0.05–0.40, 0.15–0.30 Hz) was unchanged from CONTROL by the RANDOM input. Again, a non-parametric test (Wilcoxin Matched Pairs) was also used to test the null hypothesis (the median difference between the groups was zero; Sokal and Rohlf 1969). Similarly, the Student's *t* test for equal means and the Wilcoxin test for equal medians were used to test the null hypothesis that the input did not change the pNN50 measure.

Results

Respiratory rate during random ventilatory input

A short segment (approximately 20 s) of the pressure wave generated at the mouthpiece is shown in Fig. 3a. This is a combination of spontaneous respiration and the speaker-driven random pressure wave. The corresponding power spectrum, determined from five segments of 2,048 points each, is shown in Fig. 3b. The spontaneous respiratory input, apparent as the low frequency component in Fig. 3a, corresponds with the low frequency peak at approximately 0.1 Hz in the power spectrum (Fig. 3b). The power of this pressure wave falls to nearly −20 dB by 1.5 Hz. The bandwidth for the random respiratory input, without respiration, is represented in Fig. 2. From Fig. 3b, with increasing frequency, the contribution of this random input is observed to build from ambient noise at approximately 2 Hz and is significantly above ambient noise at approximately 3 Hz. It is important to note that the spontaneous respiratory frequency in these subjects varied between 0.06 and 0.23 Hz (see Table 1; Fig. 8). Spontaneous respiration was generally lower than normal when the subjects breathed through the mouthpiece, particularly with the presence of forced oscillations.

Table 1 The peak respiratory and peak heart period frequency (Hz)

Showing all four runs (*f*1–*f*4) for all ten subjects, with forced oscillation imposed on spontaneous respiration

* No discernable peak was apparent

Subj. no.	f_1 (resp)	f_1 (HP)	f_2 (resp)	f_2 (HP)	f_3 (resp)	f_3 (HP)	f_4 (resp)	f_4 (HP)
1	0.156	0.163	0.14	0.13	0.15	0.12	0.12	0.112
2	0.146	0.137	0. 098	0. 098	0.735	0.714	0.098	0.096
3	0.098	0.097	0.11	0.113	0.13	0.13	0.098	0.096
4	0.073	0.07	0.075	0.076	0.1	0.11	0.075	0.081
5	0.244	*	0.23	0.176	0.09	0.08	0.13	0.12
6	0.171	0.165	0.17	0.17	0.317	*	0.205	0.21
7	0.22	0.217	0.22	0.22	0.22	0.21	0.24	0.23
8	0.12	0.127	0.12	0.11	0.22	0.228	0.11	0.11
9	0.122	0.128	0.125	0.145	0.14	0.135	0.135	0.115
10	0.22	0.209	0.22	0.21	0.146	0.135	0.12	0.124

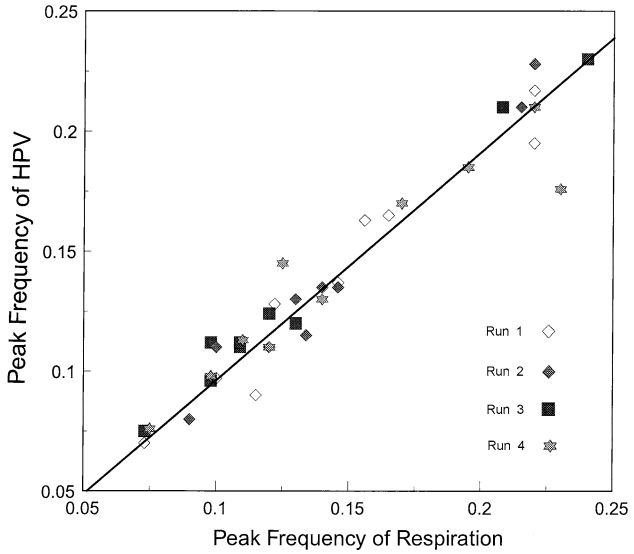

Fig. 8 The peak frequency of the respiratory spectrum is plotted against the peak frequency of the heart period spectrum (HPV) for all four random input trials in all ten subjects. This graph illustrates that the peak frequency of the HPV corresponds to the respiratory frequency in all subjects. These data are from Table 1. Note that the "peak frequency" is approximate in many of the spectra, as a single frequency at which the peak occurred was not always definitive

Table 2 The mean heart period (s) for the control (C_HP) and all four forced oscillation test conditions (R1_HP–R4_HP) for all ten subjects

Subj. no.	C_HP	R1_HP	R2_HP	R3_HP	R4_HP
1	0.701	0.813	0.807	0.788	0.77
2	0.918	0.739	0.714	0.742	0.735
3	1.119	1.052	1.07	1.071	1.053
4	1.084	1.01	0.973	0.97	0.963
5	0.896	0.891	0.842	0.836	0.797
6	0.847	0.806	0.827	0.828	0.855
7	0.85	0.829	0.823	0.852	0.789
8	0.996	0.944	0.986	0.955	0.937
9	0.755	0.758	0.772	0.76	0.755
10	1.018	0.917	0.854	0.811	0.883
HPav (s)	0.9184	0.8759	0.8668	0.8613	0.8537
HRav (bpm)	65.3	68.5	69.2	69.7	70.3

The outcomes for parametric and non-parametric tests are given

ANOVA (equal mean) $F_{0.05[4,45]} = 2.58 >> F_S = 0.511$ (not significant)

Friedman (equal mean) $p = 0.053$ (not significant at $p = 0.05$ level)

Mean HP

Resting mean heart rates ranged from 54 to 95 beats/min (average 67 beats/min). Across all subjects, mean HP increased slightly in one subject, remained unchanged in one subject, and decreased slightly from CONTROL to RANDOM conditions in each RANDOM trial for eight of the ten subjects (Table 2). However, from a single classification ANOVA on the effect of Random Input on mean HP (Table 2), the observed variance ratio $:F_s = 0.5112 \ll F_{0.05[4,45]} = 2.55$. Therefore, from the parametric test, we accept the null hypothesis that the random input does not have a significant treatment effect on mean HP, and we conclude that the random input had no significant effect on mean heart rate. The non-parametric Friedman test also indicated that there was no significant change in the median HR at the 0.05 level of significance ($p = 0.053$). However, this test, which is based on ranks of the data rather than their values, was more sensitive to the observed trend for HR to increase slightly due to the RANDOM input in eight of the ten subjects.

HPV steady-state response

All subjects reached a steady-state response within 30 s of the application of the random ventilator input. The pNN50 results are presented in Table 3. Although three subjects showed almost no change in the pNN50 measure from CONTROL through all four RANDOM tests, the change in over all subjects was significant for each trial condition at the 0.05 level using the Student's t test. The Wilcoxin Signed-Rank test gave similar results (Table 3), and in both the parametric and non-parametric tests the null hypothesis was rejected.

The R–R interval graphs, for two subjects (subjects 3 and 4), are presented in Fig. 5 and their corresponding auto spectra are shown in Fig. 6. These two subjects represented two extremes in the ten subjects tested. For all subjects except subject 3, the control HP time record had variability similar to the record of Fig. 5a. In all of these (excepting subject 3), there was a pronounced increase in variability in the heart period in at least one of the four RANDOM runs, as illustrated in Fig. 5b. This increased variance in the heart period was further analyzed in the frequency domain. The increased variance in R–R interval from Fig. 5a to b is observed to result in a dramatic increase in power in the band between 0.06 and 0.095 Hz (Fig. 6a to b). The spectrum of Fig. 6b very nearly represents that of a single sinusoid.

In subject 3 the high amplitude, narrow band variation in the R–R interval plot for the CONTROL condition (respiratory frequency in Fig. 6c, centered at 0.13 Hz) was very typical of the RANDOM condition appearance of the time records for most subjects (compare Fig. 5b, c), and atypical for any of the other CONTROL conditions (compare Fig. 5a, c). In fact, there was very little overall increased spectral power from the CONTROL to RANDOM conditions in this subject, but rather a narrowing and increased

Table 3 The pNN50 (percent of 50 ms or greater beat-to-beat changes in HP) for the control and four forced oscillation test conditions for all ten subjects

Subj. no.	Control pNN50	R1 pNN50	R2 pNN50	R3 pNN50	R4 pNN50
1	1.4	30.1	29.7	31.3	32.4
2	20.3	36.3	33.2	42.2	36.3
3	77.0	69.1	69.1	70.7	68.8
4	41.8	41.8	44.9	45.7	41.8
5	23.0	50.0	36.7	29.3	36.7
6	0.4	13.3	16.0	9.0	7.4
7	28.5	36.7	39.5	46.5	26.2
8	23.4	54.7	59.4	56.3	52.0
9	5.9	16.0	14.8	25.4	22.7
10	57.4	73.0	56.6	48.8	62.1
pNN50av (%)	27.9	42.1	40.0	40.5	38.7
Student *t* statistic (two-tail) Control vs. Rx (*p* value)		0.00585	0.0159	0.0203	0.0274
Wilcoxin Signed-Rank (two-tail) Control vs. Rx (*p* value)		0.010	0.0178	0.0394	0.05

The outcomes for parametric and non-parametric tests are given

amplitude of the predominant band as seen in Fig. 6c and d. From inspection of Fig. 5c, the broader band at respiratory frequency in Fig. 6c is more likely due more to a single sinusoid modulated `frequency, rather than the inclusion of a broad spectrum of sinusoids in the time series. This suggests that the white-noise input, in this case, entrained the subject's respiratory response to a single frequency without changing the overall sinoatrial effect on HP.

The summed power for frequency bands 0.05–0.15, 0.15–0.30, and 0.05–0.04 Hz of the autospectra of the normalized HP data was compared (Table 4) between the CONTROL data and the RANDOM data for all ten subjects collected under the steady-state circumstances of random pressure modulated respiratory input. These results for the 0.05–0.4 Hz bands (which contained the respiratory band for all subjects) are shown in Fig. 7. While the responses to the random input were generally very narrow band, the peak frequency of this response varied between subjects from 0.07 to 0.28 Hz; and the RANDOM response was generally at the same or slightly lower frequency than the predominant peak in the CONTROL spectra. It was, therefore, necessary to use a broad band in comparing the power change between CONTROL and RANDOM conditions. The total summed power in the autospectra is presented in Fig. 7, excluding power attributed to activity of the renin–angiotensin system below 0.05 Hz (Akselrod et al. 1981). The high frequency was limited to 0.4 Hz because in all subjects there was no significant power beyond 0.4 Hz. For all subjects, except subject 3, there was a marked increase in total power from CONTROL to RANDOM conditions. For the RANDOM condition, the peak frequency of HPV very closely matched the peak frequency of respiration, as demonstrated in Fig. 8. These data indicate that the large amplitude response of HPV corresponds in frequency with the spontaneous respiratory input, which has been entrained to a very regular rhythm by the random input. This response is greatly amplified beyond that of the spontaneous respiratory input due, apparently, to the presence of the random signal.

Discussion

The wide application of heart period and HRV is restricted by its limited diagnostic capability for any disease involving autonomic dysfunction (excepting severe heart disease). In part this difficulty is related to the multiple factors which influence the variability. One method for improving the diagnostic capabilities of analysis is to evaluate a response of the system to a set stimulus. Perturbation of the autonomic nervous system with a known input offers a quantifiable measure to analyze this dynamic system (Berger et al. 1989). We have found that the superposition of a random pressure wave onto the spontaneous respiration of relaxed subjects dramatically increased the normal variability of heart period at the respiratory frequency. This increase is illustrated by the significant increase in the pNN50 time domain measure (Table 3) as well as a dramatic increase in the power spectrum of the R–R interval. Significance is observed for the pNN50 measure in all four RANDOM tests, from the CONTROL, even without a notable change in three of the ten subjects (significance for both the Student's *t* test, and the Wilcoxin Signed-Rank test; Table 3). Highly significant increases in the HRV power spectrum are shown in Table 4 for the 0.05–0.15 and the 0.05–0.40 frequency bands, but not in the 0.15–0.3 band. Significance

Table 4 Outcomes for tests for significance (parametric and non-parametric) between the control and random test conditions for summed power in three frequency bands (0.05–0.15, 0.05–0.40, and 0.15–0.30) are given for the ten subjects

Test and frequency band	C vs. R1	C vs. R1	C vs. R1	C vs. R2	C vs. R2	C vs. R2
	0.05–0.15	0.05–0.4	0.15–0.3	0.05–0.15	0.05–0.4	0.15–0.3
t Statistic (two-tail) Control vs. Rx	0.0187*	0.00261**	0.212	0.0346*	0.0169*	0.161
Wilcoxin Signed-Rank (two-tail) Control vs. Rx	0.0054**	0.0054**	0.0784	0.0178*	0.0074**	0.134
Test and frequency band	C vs R3	C vs R3	C vs R3	C vs R4	C vs R4	C vs R4
	0.05–0.15	0.05–0.4	0.15–0.3	0.05–0.15	0.05–0.4	0.15–0.3
t Statistic (two-tail) Control vs. Rx	0.00289**	0.00119**	0.996	0.000978**	0.00122**	0.430
Wilcoxin Signed-Rank (two-tail) Control vs. Rx	0.0135*	0.0054**	0.741	0.0135*	0.0054**	0.298

* Significant at $p = 0.05$

** Significant at $p = 0.01$

was greater in the broader band (for both the Student's *t* test and the Wilcoxin Signed-Rank test; Table 4), as spontaneous respiration for three of the ten subjects was at a frequency higher than the 0.05–0.15 band for one or more of the RANDOM tests. Controlled frequency of breathing was not used in this study, as this would have been a confounding input to that used in the forced oscillations method. The observed increase in spectral power was characterized by a sharp narrowing and an increase in power at respiratory frequency, with a slight decrease in the frequency of the respiratory band from the control condition often observed. The maximum overall increase in spectral power for each subject (except subject 3) ranged from one to two orders of magnitude (Fig. 7), with no significant change in mean heart rate. There was an initial transient response at the onset of the random input, lasting 20–30 s (Fig. 4), and a tendency to accommodate to the input after 6–12 min. All subjects reported no discomfort, although the presence of the input was very apparent by the subject's perception of a pulsation in the chest.

Our subjects did show a non-significant trend of an increase in mean heart rate with the dramatic increase in HRV (eight of ten subjects; Table 2). Although we typically think of average autonomic effect changing in parallel with a change in cardiac output, our subjects showed that the change in HRV could be distinct from mean heart rate. This type of change was reported by Brown et al. 1993 who reported no significant change in mean HR with large changes in HRV power at respiratory frequency during controlled breathing. However, their data indicate a trend of increased HR with increased respiratory frequency (Brown et al. 1993; Fig. 2). Friedman's (non-parametric) test, which is based on rank rather than the value of the data (and indicates a mean change in HR which is close to significance), is consistent to the observed trend for HR to increase slightly due to the RANDOM input in eight of the ten subjects (Table 2). Increases in mean HR are typically due to change in demand for cardiac output such as related to posture or metabolic rate. Our subjects did not have a posture or exercise change between the CONTROL and RANDOM conditions. Although we did not measure metabolism, there is no reason to believe that our "RANDOM" condition altered the metabolic rate, increasing demand for cardiac output. Alternatively, mental stress may also cause an increase in heart rate, although our subjects remained relaxed during the study.

We compared the peak frequency of the HPV and respiration and found a direct correspondence (Fig. 8). This is consistent with the observations of others that the high frequency HRV peak is related to respiratory rate (Angelone and Coulter 1964; Akselrod et al. 1981; Ahmed et al. 1982). The modulation of HPV seen with the input of random ventilatory noise may have effects mediated via direct mechanical stimulation of baroreceptors and esophageal receptors as well as a direct mechanical effect on the heart. Both of these mechanical receptors have an influence over HPV. Respiration is known to have significant reflex as well as direct mechanical effects on blood pressure (Peters et al. 1988; Saul et al. 1991; Novak et al. 1993; Parati et al. 1995; Eckberg 2009; Karemaker 2009). Esophageal receptors may also play a role in influencing HPV. Both electrical and mechanical stimulations of the esophagus have been demonstrated to increase the power in the high frequency band (Bajwa et al. 1997; Tougas et al. 1997), mediated via vagal esophageal afferents. However, this effect may be small under normal physiologic conditions (Koh et al. 1998). The direct mechanical effect of respiration on HRV has also been well documented. Following heart transplant or after pharmacological blockade of both arms of the autonomic nervous system, a minor component remained which is attributed to the direct mechanical effect of respiration on the heart (Peters et al. 1988; Bernardi et al.

1989; Saul et al. 1991; Parati et al. 1995). In our protocol there were two sources of direct mechanical input to the heart: the random component and that of spontaneous respiration. We have not identified the single pathway through which this device influences HRV, and further investigation would be required to determine the involvement for each of these mechanisms.

Exercise is noted to have a dramatic influence over the characteristics of sedentary HRV (Perini et al. 1990; Dixon et al. 1992; Goldsmith et al. 1992; Puig et al. 1993; Al-Ani et al. 1996). A large increase in HRV in endurance trained athletes over sedentary subjects has been described (Dixon et al. 1992; Goldsmith et al. 1992). While the subjects in our study were neither selected nor tested for physical condition, the largest variability in HP was observed in the only subject (subject 3) who was engaged in vigorous daily physical training. This subject also demonstrated the highest baseline power in the respiratory band (Figs. 4c, 5c) and had the least change in the HPV with the respiratory stimulation (Figs. 4c, 5c to 4d, 5d). Dixon (Dixon et al. 1992) found that subjects who trained vigorously had less change in HPV with moderate exercise, which may explain the absence the dramatic increase in the HPV in this subject (twofold maximum increase) during the RANDOM input, contrasted with the other subjects (10- to 100-fold maximum increase).

Angelone and Coulter (1964) first described the relationship between heart rate and respiration as an input/output system, giving the frequency response of heart rate due to respiration (1–40 breaths/min input) both in amplitude and phase. Ahmed et al. (1982) found, using spectral and coherence estimates, a linear interaction between respiration and heart rate in the frequency band between 0.05 and 0.40 Hz, but not outside this range. Cohen's group has confirmed that respiration modulated HRV at the frequency of respiration in both the parasympathetic and sympathetic bands. Berger and Saul used a metronome to pace respiration at random intervals from 1 to 15 s (Berger et al. 1989; Saul et al. 1991), and the coherence function to evaluate the linear interaction between respiration and HR (Saul et al. 1991).

Our study demonstrates that when the subject is presented with a forced oscillation input, outside of the respiratory frequency, the system responds in a non-linear fashion. We superimposed a low-level random pressure wave, with spectral content entirely outside the HR spectral band, onto spontaneous respiration. This input delivered no significant energy at the frequency of spontaneous respiration. For linear systems this random input should produce no effect at the observed respiratory frequency. Alternatively, the profound effect (as much as 2 orders of magnitude) observed at the respiratory frequency from the superposition of the random input can only be attributed to significant non-linear effects. A fundamental distinction between linear and non-linear systems is that linear systems are not input dependent. We conclude that the system for respiratory modulation of heart rate is strongly non-linear (Novak et al. 1993), but has been shown to approximate linear system behavior with carefully controlled inputs (Ahmed et al. 1982; Saul et al. 1991).

Certainly, autonomic output, as observed through the autonomic control of heart rate (HRV), is limited to a much narrower frequency band that characterizes autonomic activity. Our random input frequency band extended well beyond respiratory rate and below the 40–150 Hz firing rate of sensory input to the ANS (Pack et al. 1986). Pulmonary Type I and II stretch receptors as well as chest proprioception both play a role in sensory feedback of respiratory control, and are responsive within the entire bandwidth of the random input presented to our subjects. Our random respiratory input may augment the vagus cardiac output by increasing the activity of these two sensory feedback mechanisms. If this is correct, these sensory mechanisms may have major influences on respiratory-related cardiac control well beyond the bandwidth of physiologic respiration, including impulse input to the respiratory system (impacts and jolts to the diaphragm and chest). Some of these pulmonary stretch receptors have static and dynamic roles. Clues to the static component may be gained through the application of constant positive airway pressure (CPAP) and examining HRV. Török et al. 1997–1998 found that CPAP applied to healthy subjects during patterned breathing increases the low frequency HRV by approximately 30%. These findings are impressive but are not of the same magnitude found in our test group; therefore, more dynamic forces may be contributing to the influence (Török et al. 1997–1998). Further investigation in lung transplant patients and patients with spinal cord transaction may delineate the roles of these inputs. Other mechanisms involved with modulation of HRV involving respiration include HR modulation from medullary respiratory neurons, reflex arterial baroreceptor or atrial stretch receptor HR modulation in response to blood pressure changes (Hirsch and Bishop 1981), or direct mechanical effect on HR through variation of venous blood flow and thus cardiac preload due to modulation of interthoracic pressure. Irrespective of the mechanism, the forced oscillation method has profound effect on HRV. The direct mechanical effect through cardiac preload is unlikely to be the mechanism responsible for the forced oscillation effect. This high frequency, mean-zero pressure input would not be expected to cause a non-zero thoracic pressure change over one respiratory cycle. The other mechanisms all involve autonomic-mediated HRV effects.

In this study the random ventilatory signal was generated by forced oscillation, which has been used to estimate pulmonary impedance (DuBois et al. 1956; Michaelson et al. 1975; Pimmel et al. 1977; Eyles et al. 1982; Landser et al.

1982; Hantos et al. 1986; Oosstveen et al. 2003; LaPrad and Lutchen 2008; Ionescu et al. 2010). For estimates of impedance in these studies, it is presumed that the system remains unchanged by the forced oscillatory input. Our findings suggest that a significant change in autonomic output is induced by the forced oscillation method for estimating pulmonary impedance. The concomitant alteration in autonomic outflow may have implications on the normalcy of parameters of compliance, resistance, and inertance estimated using this method of pulmonary testing. While this pronounced effect on the modulation of heart rate was observed using forced random ventilation, it is not known if the single sinusoidal method of estimating respiratory impedance has a similar effect (DuBois et al. 1956; Veiga et al. 2009) or how the response may be dependent on the frequency of the sinusoid. To our knowledge, this profound effect of forced oscillation on HRV has not been previously described.

In future studies, it would be of interest to include graded levels of amplitude as well as subsets of the broad band input used in this study; including a range of single sinusoidal inputs above the bandwidth of the heart period spectrum, as well as non-integer multiple sinusoidal inputs. Other protocol of interest would include controlled breathing with and without CPAP. Also, investigations relating physical fitness with the response to the forced oscillation method may be of interest.

Conclusion

The procedure described in this study is non-invasive and does not require patient cooperation. We have found that broad spectrum ventilation, outside the heart period bandwidth, produces a pronounced effect on HPV in the spectral region where heart rate has been demonstrated to be under autonomic control. However, further investigation is needed to determine if this technique of determining the responsiveness of the pulmonary-autonomic mechanisms to a random oscillatory input may be clinically useful. Certainly, the profound effect of forced oscillation input to respiration on HRV, autonomically mediated at respiratory frequency, may have implications on estimates of respiratory impedance using the method of forced oscillation.

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