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The many metrics of cardiac chronotropy: A pragmatic primer and a brief comparison of metrics

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Abstract

This paper focuses on pragmatic issues in obtaining measures of cardiac vagal control, and overviews a set of freely available software tools for obtaining several widely used metrics that putatively reflect sympathetic and/or parasympathetic contributions to cardiac chronotropy. After an overview of those metrics, and a discussion of potential confounds and extraneous influences, an empirical examination of the relationships amongst these metrics is provided. This study examined 10 metrics in 96 unselected college students under conditions of resting baseline and serial paced arithmetic. Intercorrelations between metrics were very high. Factor analyses were conducted on the metrics reflecting variability in cardiac rate, once at baseline and again during mental arithmetic. Factor structure was highly stable across tasks, and included a factor that had high loadings of all variables except Toichi's ''cardiac sympathetic index'' (CSI), and a second factor that was defined predominantly by the CSI. Although generally highly correlated, the various metrics responded differently under challenge.

Keywords: Cardiac vagal control; Cardiac vagal tone; Heart rate variability; Measurement; Methods; Respiratory sinus arrhythmia

1. Introduction

Measures of heart rate variability (HRV) and respiratory sinus arrhythmia (RSA) in particular have been used to index autonomic function and reactivity in relation to diverse phenomena, such as diabetes (Ewing et al., 1981), hypertension and other cardiovascular disease (Masi et al., 2007; Thayer and Lane, 2007), attentional capacity (Porges, 1992), emotion regulation (Calkins and Johnson, 1998), coronary artery disease (Carney et al., 1988), daily stressors (Fabes and Eisenberg, 1997), major depression (Chambers and Allen, 2002; Rottenberg, 2007), anxiety (Friedman, 2007), and children's levels of empathy (Eisenberg et al., 1996), among many others. Numerous metrics have been used to summarize variability in cardiac chronotropy in the literature, but the selection of particular metrics is highly variable across studies, and metrics are often used interchangeably, or with little justification. Rarely have the various metrics been compared to assess the degree to which they assess similar constructs. This article will provide a pragmatic overview of obtaining measures of heart rate variability, followed by an empirical comparison of some of the more popular and easily obtained metrics of cardiac chronotropy. Finally, a suite of freely available software tools is introduced for converting EKG signals to metrics that may be used as indices of cardiac vagal control, in the hope that more researchers may incorporate such indices into their experimental protocols.

1.1. The physiological basis of heart rate variability

Heart rate variability results from a dynamic relationship between sympathetic and parasympathetic nervous system influences. HRV can occur from a co-activation, coinhibition, or activation of one with an inhibition of the other division of the autonomic nervous system (Bernston et al., 1991). Studies suggest that the vagus nerve is responsible for heart rate variability within the respiratory frequency band, as pharmacological blockades of vagal synapses at the sino-atrial node of the heart nearly abolish this coupling of heart rate and respiration, whereas interruption of the cardiac sympathetic inputs via beta-adrenergic blockade do not (Japundzic et al., 1990; McCabe et al., 1985; Pagani et al., 1986), although there

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is some evidence that high levels of sympathetic activity may attenuate RSA by inhibiting phasic vagal driving (see Berntson et al., 1993). This association of respiratory-linked heart rate variability, often termed respiratory sinus arrhythmia (RSA), with vagal influence as its putative mechanism, has led to the use of RSA as an approximation of vagal efferent activity to the sino-atrial node, since no direct noninvasive assessment of vagal cardiac efferent activity exists (e.g. Grossman and Taylor, 2007). The present manuscript will focus on how to obtain a variety of metrics of respiratory-linked variability, presenting a pragmatic overview and a description of tools available for use by researchers wishing to assess cardiac vagal control.

1.2. From the electrocardiogram to metrics

1.2.1. The EKG

Assessing cardiac vagal control begins with a simple digitized time series of the electrocardiogram (EKG), typically recorded from bipolar recordings between pairs of limbs according to Einthoven's triangle (Einthoven et al., 1913), although any placement that permits a reliable identification of the R-spike in the EKG is acceptable. The EKG reflects voltage changes associated with phases of the cardiac cycle, with peaks and valleys in the waveform associated with the timing of atrial and ventricular depolarization and repolarization. Accurate identification of the QRS complex of the EKG, associated with ventricular depolarization, provides an easy-to-identify and reliable index of cardiac timing. Data should be digitized at a rate of 500 Hz or faster (Bernston et al., 1991), and the distance in milliseconds between each R-spike (the most prominent feature of the EKG waveform) will form the basis of an interbeat interval (IBI) series (see Fig. 1). This IBI series will form the input data for most algorithms that compute metrics of heart rate variability.

1.2.2. Artifacts in the detection of R–R intervals

Instead of the laborious task of detecting each IBI by hand, beat detection algorithms can automate this process, but such algorithms are seldom perfect, either skipping R-spikes (indicated by an unusually large IBI value, e.g. 1800 ms) or detecting a spurious beat (indicated by an unusually small IBI value, e.g. 150 ms). Even one artifact can result in an invalid index of HRV or RSA, regardless of whether the metric was derived from spectral analysis or time series analysis (Berntson and Stowell, 1998). It is thus important that the IBI series created from the beat detection program be hand-corrected for artifacts, a process that is facilitated by one of the two software tools (QRSTool) discussed in Appendix A.

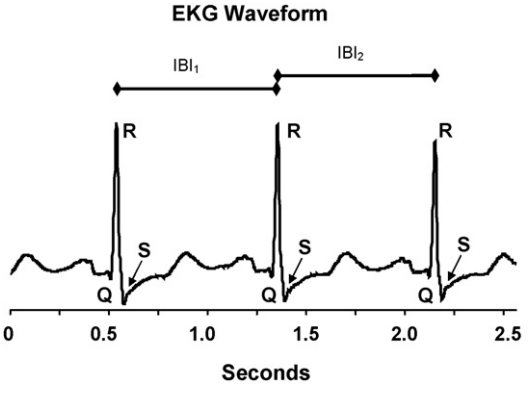

Fig. 1. Illustration of interbeat intervals (IBIs) and the electrocardiogram (EKG) waveform.

1.3. The many metrics of HRV and RSA

1.3.1. Time domain metrics of variability

Time–domain metrics are plentiful and easily obtained (see Stein and Kleiger, 1999, for an accessible review). Several metrics summarize overall variability, but are not necessarily specific to respiration-linked changes in heart rate. Such metrics provide crude estimates of HRV and as such they are more appropriate for clinical trials than for use in most psychophysiological studies (Berntson et al., 1997). Other metrics are better indices of respiratory linked changes, and thus may serve as better indices of cardiac vagal control.

Measures of overall variability include the variance of the IBI series, with greater beat-to-beat variability reflected in greater variance. This metric is often log-transformed to make it more suitable to parametric statistical analyses. Similarly, the standard deviation of the interbeat intervals (SDNN; Murray et al., 1975) has been recommended as a measure for overall variability (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Because the variance and SDNN measures will potentially be larger as recording length increases, as slow changes in overall heart rate will influence these measures in addition to the beat-to-beat changes (Ewing et al., 1981), the Task Force (1996) recommends standardizing the recording length to 5 min to aid comparisons across studies.

Several other time–domain measures may more closely reflect respiratory-linked changes in heart rate, and thus provide better indices of the parasympathetic nervous system's contribution to heart rate variability (respiratory sinus arrhythmia). These include: the percentage of the absolute differences between consecutive IBIs that are greater than 50 ms (pnn50; Ewing et al., 1984); the mean of the absolute value of the difference between successive interbeat intervals (MSD); the mean square successive difference (MSSD); the square root of the mean of squared successive differences between interbeat intervals (root mean square of successive differences, or RMSSD; Von Neumann et al., 1941); the peak to valley method (Grossman and Svebak, 1987; Grossman et al., 1990; Katona and Jih, 1975); the Porges adaptive polynomial filter method (also known as V_{HAT} and V_{NA}), a method that is closely approximated using one of the software tools (CMetX) described in Appendix A; Toichi's cardiac vagal index (Toichi et al., 1997).

MSD and MSSD provide nearly identical values (Ewing et al., 1981). These metrics primarily index parasympathetic influences to HRV, because slow changes to HR that are not due to respiration produce little change from one successive beat to

the next, and thus do not appreciably influence the metrics (Berntson et al., 2005; Porges and Bohrer, 1990). Friedman et al. (2002) compared RSA (derived from impedance pneumography, Ernst et al., 1999) and MSD during tasks that would elicit distinct cardiac responses (e.g. hand grip for sympathetic response). MSD and RSA slightly diverged during a mixed sympathovagal task (i.e. playing a video game while wearing a cold facial mask), such that MSD reflected more sympathetic influence while RSA reflected parasympathetic influences. In primarily parasympathetic and primarily sympathetically mediated tasks, RSA and MSD responded similarly (across tasks, except for mixed sympathovagal task, $r = .89$), suggesting MSD captures much but not all of the respiratory-linked variability in cardiac rate during most tasks.

The peak-to-valley or ''peak-to-trough'' method involves averaging the differences between the shortest IBI during inspiration and the longest IBI during exhalation across respiration cycles, thus, requiring a separate measure of respiration. Although this metric has been criticized for not accounting for slow periodic and aperiodic variations in heart period that are unrelated to respiration (Porges, 1986), Grossman (1992) notes that such variations produce very small effects on the peak to valley estimate of RSA (although for more detail see Weber et al., 1992a,b). Moreover, the peak to valley method correlates highly with both the Porges method (see below) and spectral analysis (Grossman et al., 1990), with within-subject correlations typically greater than .9, and between-subject correlations of .93 during rest and .94 during task conditions. Such convergence would be expected under conditions in which heart rate levels are relatively stable, but under periods of metabolic demands or recovery, slow trends may confound the peak-valley measure but not measures based on a limited frequency band (such as the Porges method or spectral analysis).

The Porges method removes complex baseline low-frequency nonrespiratory trends by using the patented Porges–Bohrer algorithm with a moving polynomial that filters out nonrespiratory frequencies and remove non-stationarities, but with some amplification of signals close to the low frequency cutoff due to a broad ripple in the frequency response of this filtering method (Litvack et al., 1995) The method converts the IBI series to a time series (see also Appendix A for further description with CMetX), applies the filter, and then computes the log-transformed variance of the remaining data (in the respiration range .12–.40 Hz, after loss of data points for the filter; Bohrer and Porges, 1982). The use of an average respiration range for all individuals and populations (e.g. anxious population; Kollai and Kollai, 1992) might result in an attenuated estimate of RSA, as the breathing rate may occur outside .12–.40 Hz (Grossman et al., 1990); however, the Porges method allows specification of the respiration bands based on the respiration rate.

Toichi et al. (1997) developed an alterative assessment of cardiac vagal activity, derived from the logic of a Lorenz plot of each IBI plotted against the subsequent IBI (Fig. 2). The length of the transverse axis (T) to the line $IBI_n = IBI_{n+1}$reflects beat-to-beat variability and large deviations along this axis

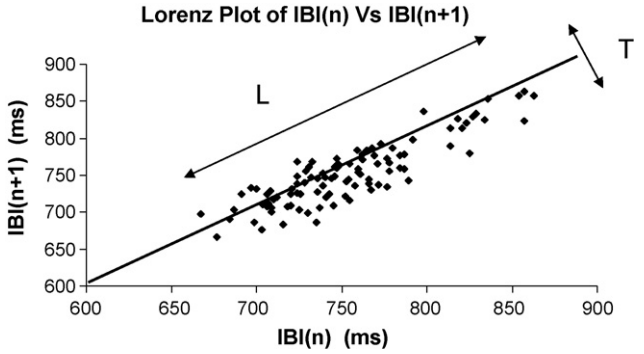

Fig. 2. A Lorenz plot of each IBI (x-axis) plotted against the subsequent IBI (y-axis) for a single participant. Deviations in the direction transverse to the line $IBI_n = IBI_{n+1}$reflect high beat-to-beat variability and putatively reflect predominately parasympathetic influences.

putatively reflect predominately parasympathetic influences while the length of the longitudinal axis (L) reflects the overall range of IBIs, resulting from both sympathetic and parasympathetic influences. Two indices are calculated from L and T: cardiac vagal index (CVI) = $\log_{10}(L \times T)$; cardiac sympathetic index (CSI) = L/T.

Toichi et al. (1997) examined changes in these metrics during sympathetic and parasympathetic blockades during supine resting, mental arithmetic, sitting, and standing. Although the T metric was responsive to some extent to both sympathetic and parasympathetic manipulations, the CVI was unaffected during sympathetic blockade with propranolol and significantly decreased under parasympathetic blockade with atropine, especially during the postural change from standing to supine. The CSI, by contrast, was significantly lower during sympathetic blockade, except for in the supine resting condition.

1.3.2. Frequency–domain metrics of variability

A variety of frequency domain techniques are available for examining the extent to which heart rate varies within specific frequency ranges, such as those associated with typical respiration. Fourier methods involve taking a time–domain representation of the IBI series and converting it to a frequency–domain representation, usually in the form of a power spectrum, and then summarizing activity in the frequency band of interest (e.g. .12–.40 Hz). An assumption underlying the Fourier transform is that a signal has at least weak stationarity (i.e. has a similar mean and variance across time) and periodicity (i.e. it repeats, and does so at uniformly spaced intervals of time). Although strictly speaking IBI series signals are not stationary nor periodic, as heart rate can shift over time and changes due to respiration and other sources do not recur at uniform intervals, these violations are not likely to be sufficient to invalidate the method (Friedman et al., 2002; Houtveen and Molenaar, 2001). Wavelet methods, an alternative method for frequency decomposition that do not assume stationarity, provide an other approach that produces results that are highly similar to those provided with conventional Fourier methods (Houtveen and Molenaar, 2001).

Autoregressive techniques are based on lagged correlations and also produce an index of periodic fluctuations in a time

series, such as those due to respiration. Autoregressive and fast Fourier transform methods are highly related (r = .96; Hayano et al., 1991).

An extension of the Fourier-based frequency–domain method involves comparing the IBI series with a measure of respiration to assess the degree to which there is covariation between the IBI series and respiration. These two signals may be compared using cross-spectral analysis (e.g., Porges and Bohrer, 1990) to derive an estimate of the coherence (ranging from 0 to 1) between the signals at a given frequency, or the weighted coherence (see Porges et al., 1980) for a range of frequencies such as those that are within the respiratory range. A variant of this method calculates the transfer magnitude (e.g., Freeman et al., 1995), which expresses the gain between heart rate (in beats per minute) per liter of lung volume, with a large gain indicating that relatively small changes in lung volume result in sizable changes in heart rate.

Although this lengthy list of metrics is not exhaustive, it covers the majority of those that have been used in the attempt to estimate vagal influence on cardiac chronotropy. In the present study, a subset of these measures are examined that are widely used and easy to obtain using only an EKG signal, and that can be derived using the freely-available software tools described in Appendix A.

1.4. Comparisons of measures

A few studies have compared the many metrics of cardiac chronotropy, typically comparing a subset of the measures to each other (Fahrenberg and Foerster, 1991; Friedman et al., 2002; Grossman et al., 1990; Hayano et al., 1991; Kleiger et al., 1991). In an examination of time and frequency domain measures derived from 24 h ambulatory Holter monitors, Kleiger et al. (1991) reported high correlations between SDNN, pnn50, RMSSD, and spectral analysis, with moderate correlations between SDNN and the other metrics (.68–.78), and strong correlations amongst pnn50, RMSSD, and spectral analysis (r's = .92–.98). Similarly high correlations (mean r = .85) between RMSSD and spectral high frequency power were found by Berntson et al. (2005).

Despite suggestions that the peak-valley estimation is vulnerable to artifacts associated with low frequencies (Byrne and Porges, 1993; Porges, 1985; Porges and Bohrer, 1990) and concerns that spectral analysis and the Porges–Bohrer method do not individually tailor the respiration range and instead use a mean or modal range of respiration (typically somewhere in the range of 6–30 breaths per minute; e.g. Grossman and Wientjes, 1986), a comparison suggests near equivalency of the peak-valley estimation, the Porges method, and spectral analysis during a 5 min period (Grossman et al., 1990). Moderate relations between MSD and the peak-valley method and spectral analysis also exist (r's .58–.9; Fahrenberg and Foerster, 1991; Hayano et al., 1991).

Despite the comparison of methods, no single measure has been hailed as the "gold standard." The Society for Psychophysiological Research's Task Force committee report concluded "A number of approaches are currently available for analyzing periodic components of heart rate variability . . . and direct comparisons have revealed generally comparable results. Each of these approaches has advantages and disadvantages, and no general consensus has emerged on a single optimal analytic method" (Berntson et al., 1997, p. 641). There may, however, be specific research questions and research designs when one measure is preferable over the other (see pp. 151–154; Grossman, 1992), and some measures are more closely tied to respiratory-linked variability and may thus be preferred over global measures (HRV, SDNN) of variability.

1.5. Methodological concerns

1.5.1. Participant characteristics and behaviors

There are numerous issues that should be considered when deriving heart rate metrics as summarized by the Committee Report of Berntson et al. (1997). Beyond the issues that are summarized in the committee report, it is worth noting that there are a number of study participant behaviors and demographics that can influence indices of heart rate variability and, specifically, respiratory-linked variability. These factors may be considered when designing a study or assessed in the context of the study, and include: use of caffeine or alcohol, exercise, smoking, age, gender, and obesity. Table 1 provides a tabular summary of the impact of these various factors.[1]

1.5.2. The thorny issue of respiration

1.5.2.1. Might RSA be an imperfect index of vagal control?.

A concern that has inspired considerable debate is the question of controlling for respiration. The principal issue is whether RSA is primarily a reflection of cardiac vagal control or whether RSA can under certain circumstances also reflect changes in respiration that are independent of central vagal effects. Investigators want to determine whether there are legitimate changes in vagal control, and not merely the influence of changes in respiration on the imperfect measure, RSA. This problem arises when respiration rates differ between groups, or between conditions.

Grossman et al. (1991) demonstrated that when there is large variability in respiration rates within individuals, rapid breathing will reduce RSA and slow breathing will increase RSA, independent of changes in tonic vagal modulation of heart rate, in a study designed to eliminate sympathetic contributions by administering 10 mg propranolol to block sympathetic influences. Houtveen et al. (2002) concur that RSA does not reflect solely tonic vagal modulation of heart rate during activities that affect the central respiratory drive (e.g. exercise), but they conclude, however, that during activities when respiration is not expected to greatly vary (i.e. during resting conditions and most clinical mental stress tasks), RSA uncorrected for respiration accurately reflects vagal modulation of heart rate.

Some studies pace participants' breathing at a fixed rate or rates (e.g. Grossman et al., 1991) in order to avoid the

[1] Articles were included in this synopsis if they included healthy adult samples.

Table 1
Individual difference variables and participant characteristics that can impact measures of cardiac vagal control

Variable	Specific comparisons	Description of sample	Study design	Cardiac measures	Effect on HRV and RSA	Effect size (Cohen's *d*)	Citation
Smoking							
Chronic smoking	Resting RSA of non-smokers vs. moderate smokers (1–24 cigarettes per day) who are less than 30 years of age	28 males: aged 19–30 years; 15 non-smokers and 13 moderate smokers; refrained from smoking or drinking caffeine or alcohol 8 h before the study and consumed a light breakfast	5 min resting recording in the supine position	RSA calculated by power spectral density of RR interval variability	No difference in resting levels of RSA	Not significant	Hayano et al. (1990)
	Resting RSA of non-smokers vs. heavy smokers (>25 cigarettes per day) who are less than 31 years of age	26 males: aged 19–30 years; 15 non-smokers and 11 heavy smokers; refrained from smoking or drinking caffeine or alcohol 8 h before the study and consumed a light breakfast	5 min resting recording in the supine position	RSA calculated by power spectral density of RR interval variability	Reduced levels of RSA (power spectral density) in heavy smokers >31 years of age	D_{RSA} = .88	Hayano et al. (1990)
	Resting RSA of non-smokers vs. moderate and heavy smokers greater than 31 years of age	26 males: aged 30–52 years; 10 non-smokers, 18 moderate smokers, and 14 heavy smokers; refrained from smoking or drinking caffeine or alcohol 8 h before the study and consumed a light breakfast	5 min resting recording in the supine position	RSA calculated by power spectral density of RR interval variability	No differences between groups	Not significant	Hayano et al. (1990)
Acute effects of smoking	Acute effects of smoking in regular smokers	Nine males: aged 24–30 years who were regular cigarette smokers (mean 27 ± 8 cigarettes per day) refrained from smoking or drinking caffeine or alcohol 8 h before the study and consumed a light breakfast	5-min pre-smoking recording and post-smoking resting recording 3, 10, 17, and 24 min after start of smoking in the supine position. Participants smoke 4 cm of 1 cigarette containing 1.0 mg of nicotine during 2 min in the supine position	HRV calculated by standard deviation o f the R–R interval; RSA calculated by power spectral density of RR interval variability	No changes in HRV. Reduced RSA at 3 min post-smoking and returned to control level by 10 min	d_{HRV} = not significant; d_{RSA} = 1.01	Hayano et al. (1990)
	Change during smoking in regular smokers (mean = 25.75 ± 11.9 cigarettes)	16 participants (8 female) aged 18–75 who smoked regularly (mean 25.75 ± 11.9); refrained from smoking and from drinking alcoholic or caffeinated beverages for 12 h before study	10-min baseline and the last 10 min of each 30 min cigarette smoking period resting recording in a seated position. Ten fixed doses of 1.0 mg of nicotine were administered in a standardized manner for three separate cigarettes spaced 30 min apart	Autoregressive spectral analysis from the IBI series in the full spectrum for HRV and in the respiratory frequency range for RSA	Reduced HRV and RSA	d_{HRV} = 1.86; d_{RSA} = .52 (effect sizes reported for only the effects of the first cigarette as cardiac measures were not significantly different for number of cigarettes)	Nabors-Oberg et al. (2002)

Table 1 (*Continued*)

Variable	Specific comparisons	Description of sample	Study design	Cardiac measures	Effect on HRV and RSA	Effect size (Cohen's *d*)	Citation
Exercise							
Pre- to post-training changes in resting recordings of heart rate after exercise program	Pre- to post-training changes in HRV and RSA in those in an exercise training program for 16 weeks vs. those not exercising	Nineteen healthy males ages 45–68: 11 in exercise group and 8 in control group. Exercise group underwent a prolonged program of physical training (30 ± 1 week, range 25–36). During first 14 weeks of training, participants walked/jogged 3 days/week for 33 min/day. Level of exercise was progressively increased so that during final 16 weeks of training participants walked/jogged 3.5 days for 43 min per day	5 min in supine position while breathing at a rate of 10 breaths per minute	SDNN for HRV and peak to trough times series analysis for RSA	HRV increased; no change in RSA	d_{HRV} = .76 (estimated from graphs)	Seals and Chase (1989)
	Pre- to post-comparison of RSA after exercise training three times per week for 3 months	17 healthy males: 10 "young group" ages 19–29; "middle-aged group" ages 50–59. Refrained from smoking, caffeine, and alcohol on the day before EKG	1 h resting condition in supine position for 60 min	Standard deviation of the R–R interval for HRV and spectral analysis in the respiratory frequency for RSA	No differences between exercisers and controls and no differences between pre- to post-resting HRV or RSA in exercisers	Not significant	Catai et al. (2002)
Comparison of regular exercisers to non-exercisers	Between group differences in RSA	30 healthy participants (4 women) ages 22–44. Participants who did not exercise regularly and participants who exercised less than 60 min, three times a week, for more than 6 months and intermediately were those who fell in between the two groups; participants were nonsmokers and refrained from caffeine and alcohol and refrained from moderate, heavy, or sustained exercise on the morning prior to the EKG	24 h Holter and participants avoided moderate, heavy, or sustained exercise	Spectral analysis in the respiratory frequency range; time domain measures of root mean square of successive differences; and the proportion of successive differences greater than 50 ms for measures of RSA	Greater levels of resting HRV and RSA in exercisers compared to non-exercisers	Not available	Goldsmith et al. (1997)
	Between group differences in RSA	12 college aged males (6 aerobically trained). Aerobically trained participants were members of the men's cross-country team for at least 1 year and had a minimum of 3 years of competitive experience in cross-country events	3 min resting condition	Porges method	No difference in RSA in aerobically trained men	Not significant	Hatfield et al. (1998)

Chronic effects of exercise	Resting HRV and RSA levels between senior sedentary controls, moderate exercisers, and high exercisers	80 women ages 60–70 years old; 14 low activity, 13 moderate activity (from adult gymnastics group for >4 years); 14 in high-activity from cycling tour club for >4 years	10 min resting with and without paced breathing; participants refrained from exercise, alcohol, and caffeine within last 24 h	For HRV, standard deviation of the RR series; for RSA, square root of the mean squared differences and spectral analysis in the respiratory frequency	HRV and RSA were higher in the high activity group in comparison to the low activity group	d_{SDNN} = .49; d_{rMMSD} = 3.75; d_{RSA} = 2.86	Reland et al. (1994)
	Resting HRV and RSA levels between senior sedentary controls and senior competitive athletes (vigorous exercise for at least 45 min, four times a day)	29 healthy seniors (>60 years old): 14 sedentary persons and 15 athletes (45 min, 4 times per week), who refrained from alcohol and caffeine for 12 h before and during ambulatory recording, and 24–36 h after last bout of exercise	24 h Holter and participants refrained from exercise during Holter	SDNN for HRV and rMMSD and HRV in the respiratory frequency range for RSA derived from time series analysis of the IBI series	Senior competitive athletes had greater HRV and RSA in comparison to sedentary controls	d_{SDNN} = 1.05; d_{rMMSD} = 1.19; d_{RSA} = 1.16	Yataco et al. (1997)
Age and gender							
Age					HRV and RSA decrease with age		See De Meersman and Stein (2007) for summary
Gender					Mixed Findings		See De Meersman and Stein (2007) for summary
Caffeine							
Acute effects of caffeine	Resting levels of RSA from pre- to post-consumption of caffeine	10 healthy participants (1 female) aged 21–25 years old. Participants abstained from food, drink, and exercise for the previous 3 h	Breathed 1 breath every 4 s during 5 min resting baseline and 20 min after the beverage was consumed. Each participant had the caffeinated beverage one day and the uncaffeinated beverage the next day	Spectral analysis of the IBI series in the respiratory frequency for a measure of RSA	RSA increased and reached the maximum about 20–30 min after consumption of caffeine compared to placebo	Not available	Hibino et al. (1997)
	Resting levels of RSA from pre- to post-consumption of caffeine	20 healthy participants who regularly consumed 350–700 ml of coffee per day (approximately 180–360 mg caffeine). Participants fasted and abstained from caffeine from 10 p.m. the night before each examination	5 min baseline recording pre- and 1 h post-consumption of either 300 mg caffeine pill or matching placebo capsule on two consecutive days	Spectral analysis in the respiratory frequency for a measure of RSA	No change in RSA from pre- to post-consumption of caffeine	Not significant	Waring et al. (2003)

Table 1 (*Continued*)

Variable	Specific comparisons	Description of sample	Study design	Cardiac measures	Effect on HRV and RSA	Effect size (Cohen's *d*)	Citation
Acute effects of caffeine in non-habitual caffeine users	Compared resting levels of RSA between placebo, and 100 and 200 mg of caffeine	10 participants (4 women) ages 23–32 randomized to receive 100 or 200 mg of caffeine dissolved in honey or a placebo in a crossover design over 3 days. Refrained from caffeine for 4 days	At baseline, 60 and 90 min after ingestion	Time domain for HRV was standard deviation of IBIs and the root mean-squared difference in successive IBI intervals, the percentage of successive RR intervals > 50 ms, and the respiratory frequency all served as measures for RSA	RSA and HRV decreased after 90 after caffeine consumption	100 mg: d_{SDNN} = 3.41; d_{rMMSD} = .84; d_{pnn50} = n.s.; d_{RSA} = 1.34; 200 mg: d_{SDNN} = 3.76; d_{rMMSD} = .89; d_{pnn50} = 1.06; d_{RSA} = 1.15	Sondermeijer et al. (2002)
Long-term effects of caffeine	Compared resting levels of HRV and RSA after 2 weeks of no caffeine in comparison to 2 weeks of caffeine	10 healthy (5 female) mean age 41.4(±10.8). All maintained a low-caffeine diet (<50 mg/day)	48 h Holter pre- and post-2 weeks. Randomized cross-over design for 2 weeks ingested 2 250 mg (comparable to two to three cups of drip coffee) caffeine per day or matched placebo	Time domain measures of counts of RR intervals > 50 ms (sNN50) and spectral analysis in the respiratory frequency for RSA	Pre- to post-changes after 2 weeks of daily caffeine increased HRV but no changes in RSA	d_{HRV} = .80 d_{RSA} = not significant	Richardson et al. (2004)
Alcohol							
Chronic effects of alcohol	Pre- to post-changes in resting levels of RSA after 1 week of daily consumption of 24 g of vodka in comparison to 1 week of no alcohol	21 healthy participants (7 female) aged 21–41 years	30 min supine rest followed by 5 min EKG with controlled breathing	Spectral analysis in respiratory frequency	Increased RSA after 1 week of daily alcohol compared to after 1 week of no alcohol	d_{RSA} = .93	Flanagan et al. (2002)
Acute effects of alcohol	Pre- to post-changes in HRV and RSA with consumption of alcohol	18 healthy males (mean age 28.5 ± 4.3 years old) who were infrequent drinkers consumed .3 g of alcohol/kg of body weight	10 min resting recording before and after (at 15, 30, 45, and 60 min post) at 9 a.m.; participants were told to fast overnight	RSA measured by spectral analysis in the respiration frequency band	Decrease in RSA from 15 to 60 min after consumption of alcohol	d_{RSA15} = 1.83; d_{RSA30} = 1.90; d_{RSA15} = 1.44; d_{RSA15} = 1.77	Gonzalez Gonzalez et al. (1992)
	Within-subject comparison of baseline levels of RSA after alcohol or fruit juice consumption	12 healthy males (mean age 23.8 ± 1.5 years old) whose weekly alcohol consumption averaged 77 g (range 20–150 g); refrained from alcohol for 48 h and fasted and refrained from coffee and cigarettes for 4 h	Pre- to post-changes after drinking 1 g/kg of body weight in juice or in alcohol one week apart. Thirty min resting heart rate before and after ingestion every hour for 3 h during controlled breathing	RSA measured by root mean square of R–R intervals and spectral analysis	RSA decreased after alcohol consumption up to 3 h post consumption	Not available	Koskinen et al. (1994)
Obesity							
Effects of obesity	Comparison of HRV and RSA in normal, overweight, and obese participants	653 healthy participants (361 women) mean ages of 40 ± 12 years	24 h Holter	HRV measured by standard deviation of RR intervals; RSA by root mean square of successive RR interval differences, percentage of successive normal sinus RR intervals >50 ms, and spectral analysis in the high frequency range	No differences	Not significant	Antelmi et al. (2004)
	Comparison of RSA and body mass index	282 healthy males ages 21–59 years old who refrained from consuming food or beverages 2 h prior	3 min resting heart rate	RSA measured by autoregressive spectral analysis in the respiratory frequency band	Higher levels of RSA with lower body mass index	Not available	Kageyama et al. (1997)

	Comparison of HRV before and after propranolol	56 males aged 25–36 with stable weight within the last 4 months with body mass index ranging from 2 to 46.3 (mean 24 ± 1.3). Participants consumed a weight-maintaining liquid diet for 4 days preceding heart rate monitoring. Participants refrained from tobacco and caffeine on the day of the recording	6 min heart rate during fixed breathing rate (5 breaths per minute) before and after propranolol	Standard deviation of R–R intervals for HRV and standard deviation of R–R intervals after propranolol during paced breathing for RSA	No relationship between body mass index and HRV; lower RSA with higher body mass index	Not available	Peterson et al. (1998)
	Comparison of RSA in obese and lean participants	84 (39 women) aged 39–60: 46 obese and 26 lean matched controls	24 h Holter	HRV measured by standard deviation of all normal RR intervals; RSA measured by spectral analysis in the respiratory frequency band during 24 h, daytime and evening	Less HRV and RSA in obese patients	d_{HRV} = .86; d_{RSA_T} = .60	Karason et al. (1999)
	Between group comparison of HRV and RSA in obese and lean women	20 obese women aged years old, weight between kg and 18 women aged 22–39 years old of normal weight	15 min heart rate recording in the supine position	Standard deviation of the R–R interval for HRV and spectral analysis in the respiratory frequency range for RSA	HRV and RSA less in obese women	d_{HRV} = .73; d_{RSA} = .66	Zahorska-Markiewicz et al. (1993)
	Between group comparison of HRV and RSA in obese and lean participants	120 participants: 42 obese patients (24 females) ages 15–55 whose body mass index was greater than 30 and 78 lean healthy participants (30 females) aged 15–69. Participants refrained from caffeine on the day of the study	4-min resting heart rate while breathing deeply at a rate of 6 breaths per minute	The mean of the differences between the maximum and minimum heart rate during three successive breathing cycles measured HRV and cross-correlation function analyses (correlates spectral analysis of the interbeat interval series within the respiratory frequency range with the respiratory signal indexed RSA)	HRV and RSA less in obese participants	d_{HRV} = 1.33; d_{RSA} = 1.28	Rossi et al. (1989)
	Between group comparison of HRV and RSA in three groups of varying body mass indices	23 patients (16 females) ages in three groups: 17 in 27–32 kg/m^2 group, 13 in 33–39 kg/m^2 group, and 12 in above 40 kg/m^2 group	5 min resting heart rate	Spectral analysis in the respiratory frequency range for RSA	No differences in resting RSA between groups and no relationship between RSA and body mass index	d_{RSA} = not significant	Laederach-Hofmann et al. (2000)
Circadian rhythm							
Circadian rhythm					HRV and RSA decrease during the daytime and increases during the nighttime		See Guo and Stein (2002)

Effect sizes are provided when possible.

possibility that changes in respiration, unrelated to central vagal efferent activity, can produce changes in the metric used to assess vagal control, RSA. An unresolved issue, however, is whether paced intentional breathing alters the very system the investigator wishes to assess. Under spontaneous conditions, breathing is a probe that allows investigators to observe the direct effects of the vagal system on cardiac rate, assessing the degree to which rate is responsive to each breath. Changing the probe itself, by pacing breathing for example, could render the assessment invalid for a variety of reasons, among them the fact that one would no longer be observing the spontaneous activity of the system, but the activity under artificial conditions. Whether the behavior of the system under paced conditions is an adequate model for its behavior under spontaneous conditions remains an open question. Moreover, evidence that manipulating the depth and frequency of breathing can impact subjective emotion (Philippot et al., 2003) suggests that pacing breathing may additionally affect behaviors of central interest to researchers utilizing measures of vagal control. The debate over whether to control for respiration is discussed in depth by several authors in this volume (Denver et al., 2007; Grossman and Taylor, 2007; Porges, 2007) and elsewhere (Berntson et al., 1997; Grossman et al., 1991).

1.5.2.2. Statistical "control" for respiration. In addition to attempting to experimentally control respiration by pacing breathing, for example, authors have attempted to statistically control for respiration frequency through the use of covariate analysis (e.g. Hughes and Stoney, 2000) or residualizing data by first accounting for variance that overlaps with respiration frequency. The use of an analysis of covariance (ANCOVA) procedure has both valid and invalid uses, as detailed below, but adjusting estimates of RSA using respiratory frequency as a covariate will not "solve" the problem when experimental conditions or groups of participants differ in terms of respiratory frequency. Such an analysis can address whether variance between groups of participants or between conditions in respiratory frequency may account for any differences in RSA observed between those groups of participants or between conditions. But this analysis does not in any way "fix" the underlying fact that groups or conditions differ in terms of respiration, nor does it allow one to proceed with covariate adjusted RSA data as if one had "corrected for" or "statistically controlled for" the impact of respiration.

This issue has received statistical and conceptual discussions (e.g. Chapman and Chapman, 1973; Miller and Chapman, 2001; Siddle and Turpin, 1980, among others), but the fundamental issue is that covariance analysis is designed to remove variance due to a factor that is *statistically independent of* (i.e. uncorrelated with) the effect of interest, as in the case of removing variance associated with an individual difference such as body mass index when participants are randomly assigned to conditions. When a dependent variable (RSA) and a covariate (respiration frequency) are correlated, however, removing the effect of the covariate on the dependent variable can remove relevant variance in RSA that is due to the group difference or experimental manipulation, thus, misleading a researcher to conclude that no meaningful differences exist between subjects or conditions in terms of RSA. Vexingly, in other cases, such an ANCOVA can in fact create spurious group or condition effects (e.g. see Wainer's, 1991 discussion of Lord's Paradox). In short, this use of covariance may remove too much of the effect of interest, or conversely create spurious effects (Elashoff, 1969; Miller and Chapman, 2001).

Among the recommendations of the Society for Psychophysiological Research Committee Report (Berntson et al., 1997) is "to use respiratory frequency and possibly depth as covariates in statistical analysis or to remove possible contributions by regression prior to analysis" (p. 639). Although this at first glance appears to be a recommendation to use ANCOVA in the problematic manner outlined above, they note one paragraph later that "these correction procedures ... may remove actual experimental effects that correlate with respiratory changes" (p. 639), thus reinforcing the message that ANCOVA does not solve the underlying confounding of respiratory parameters and the group or condition differences. The Committee's recommendation to use ANCOVA makes sense when the variance to be removed is understood to be noise or error variance, rather than variance systematically associated with the independent variable.

Despite such problematic use of ANCOVA with respiration and RSA, there remain some valid uses that can rule out some important alternative hypotheses. Instead of asking whether effects of group or condition on RSA remain after "accounting for" or "controlling for" variance due to respiration, one can ask the question of whether respiration can account for these effects of interest. If an investigator finds significant group or condition effects on RSA in a simple ANOVA, and subsequent results using respiration as a covariate in an ANCOVA leave the effects of interest intact, then it is safe to conclude that respiration cannot account for the effects of interest, and the investigator can then interpret the RSA effects due to group or condition. If, however, including the covariate changes the statistical outcome, one is left not knowing whether the effects of group or condition on vagal control are legitimate, or an artifact of respiration differences between group or condition.

1.5.2.3. Simple recommendations regarding respiration. The Society for Psychophysiological Research's Task Force committee report recommends the consideration of respiration when interpreting RSA as a measure of cardiac vagal control, specifically to ensure that the frequency band used to define RSA (e.g. .12–.40 Hz) actually encompasses the respiratory frequency of participants included in the analysis (Berntson et al., 1997). Common lower cutoffs for the respiratory band are .12 Hz (one breath every 8.3 s) and .15 Hz (one breath every 6.7 s), but these cutoffs will yield an inaccurate estimate of RSA for participants who breath more slowly than the lower cutoff (and a similar problem would exist in the less likely case that participants breath more rapidly than the upper cutoff). Moreover, the impact of breathing at frequencies near the cutoff will be attenuated to some extent due to the transition band on

most filters (see Fig. A.2 in Appendix A for examples of such transition bands).

Assuming that participants are breathing in the range defined to be captured by a given metric of RSA, there remain several desiderata for experimental design and analysis with respect to respiration:

(1) Plan the experiment to increase the likelihood that respiration rates will not differ between conditions (e.g. keep activity levels similar across conditions).
(2) Monitor to ensure respiratory rate does not differ between conditions and occurs in a range that will be captured by the metric of RSA. This can be done by monitoring respiration, or also by looking at the peak in the spectral representation of the time-sampled IBI series.
(3) If rates differ, use covariate analysis not to adjust means, but to assess whether effects from a standard ANOVA survive after accounting for variance due to respiration. If they do, then respiration rate effects did not account for the effects of interest and interpretations based on RSA can proceed.
(4) If after covariate analysis the effects of interest disappear, then one is left with an interpretive enigma with respect to whether the observed differences in the measure of RSA may accurately reflect differences in vagal control per se.

1.6. The present study

Empirical data are presented to illustrate the discriminant and convergent validity of easy to derive time domain measures of heart rate variability that can be calculated using the suite of programs described in Appendix A. These are metrics that do not require respiration for their calculation, but are based on normative ranges of respiratory frequency. As these results were obtained under relatively inactive laboratory conditions, they may or may not apply to vastly different settings where respiration may vary substantially.

2. Method

2.1. Participants

Participants were undergraduate students recruited by telephone for an experiment on ‘‘personality characteristics,’’ for which they would receive credits toward a course in introductory psychology. These participants were also included in Movius and Allen (2005), but only the single RSA metric and its relationship to personality measures was reported in that paper. Participants who were currently taking cardiovascular medications or those with a history of cardiac disease were ruled ineligible for the study. A total of 116 of the participants contacted by phone agreed to participate in the study. Due to electrode failure (16), resting RSA more than three standard deviations below the mean (2), and too many abnormal beats (2), data from a total of 96 participants (51 females) were available.

2.2. Procedures

After providing informed consent, three Ag/AgCl electrodes were attached to each participant in a Lead-II formation (Einthoven et al., 1913) plus a right forearm ground, with impedances reduced to less than 20 kΩ on all electrodes. EKG signals were amplified 1000 times with a bandpass of .05–100 Hz, then digitized at 500 Hz.

2.2.1. Tasks for recording

Participants were seated in a sound-dampened chamber and briefly oriented to their surroundings. Participants sat quietly for five minutes to obtain baseline values. For a measure of heart rate reactivity, participants were then asked to perform serial paced mental arithmetic by counting backward in varying intervals, starting with a four-digit number for five 1 min periods. Following the paced arithmetic stressor task, participants sat quietly for another 5 min period.

2.3. Data reduction

2.3.1. EKG signal reduction

Raw digitized EKG signals were analyzed off-line. IBI series were hand corrected for artifacts and then processed by CMetX (see Appendix A) for measures of heart rate and heart rate variability. CMetX converted the IBI series to a time-series sampled at 10 Hz, filtered the series using a 241-point optimal finite impulse response digital filter designed using FWTGEN V3.8 (Cook and Miller, 1992) with half-amplitude frequencies of .12 and .40 Hz, and then took the natural log of the variance of the filtered waveform to be as the estimate of RSA. CMetX also derived several other measures of heart rate variability: the proportion of consecutive interbeat intervals differing by more than 50 ms (pnn50); the mean of the absolute value of the difference between consecutive IBIs (MSD); the cardiac vagal index (CVI; Toichi et al., 1997); the standard deviation of the interbeat intervals (SDNN); the root mean square of successive differences between interbeat intervals (RMSSD); and natural-log-transformed variance of the unfiltered IBI times series, across the entire frequency range (HRV). Finally, CMetX calculated the cardiac sympathetic index (CSI; Toichi et al., 1997), average heart rate, and average heart period. All metrics were calculated for resting baseline and during the stressor task.

3. Results

3.1. Transfer functions of various classes of metric

Two metrics of variability (HRV: natural log of the variance of the IBI series; SDNN: standard deviation of IBI series) are based on the raw IBI series and summarize cardiac variability across all frequency ranges, and thus do not reflect solely vagal contributions to cardiac chronotropy. Other metrics transform the IBI series via various ‘‘filters,’’ and thus may attenuate non-respiratory contributions to heart rate variability. The mean successive difference (MSD) metric is based on successive differences, which have been noted to attenuate lower frequencies, and the root mean square of successive differences between IBIs (RMSSD) might be expected to have a similar effect. The pnn50 is essentially a course successive difference filter, dichotomizing the successive differences as zero or one. And the estimate of RSA (natural log of .12–.40 Hz band-limited time-sampled IBI series) will specifically eliminate those frequencies outside of the typical respiration band.

To derive the transfer functions of these various transformations, for each IBI series (across all participants at baseline and task conditions) a successive-difference (SD) series was created, as well as a dichotomized SD series (to correspond to the pnn50 metric) with values greater than 50 ms receiving a value of one, and those $\leq$50 receiving a value of 0. Time series with sampling rates of 10 Hz were created for each of these series using cubic-spline interpolation. To compare the frequency response of these series with the method used to estimate RSA (CMetX), an additional series was created by applying a .12–.40 Hz filter to a 10 Hz sampled time series interpolation of the original IBI series.

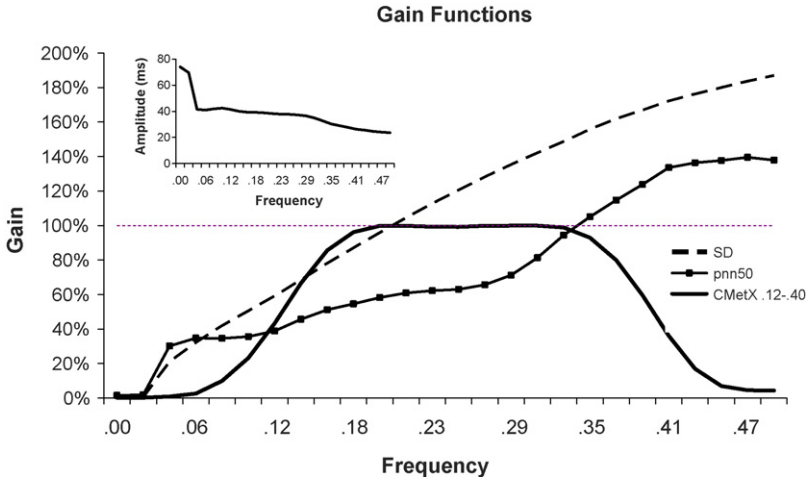

Fig. 3. Gain functions for various "filters" including mean successive difference (SD), proportion of consecutive interbeat interval (IBI) differences differing by more than 50 ms (pnn50), and .12–.40 Hz bandpass filter (CMetX). Inset shows average amplitude spectrum of the non-transformed time-sampled IBI series across all participants and tasks. Note that the gain functions for the SD and .12–.40 Hz filters are based on a comparison of spectra using values in ms as input, and thus provide transfer functions that will produce meaningful gain estimates compared to the ms units for the raw IBI series. The pnn50 filter, by contrast, assigns dichotomous values of zero or one, and thus is not calibrated in milliseconds as the other metrics. The pnn50 gain function has thus been multiplied by 100 for display purposes, leaving the shape of the transfer function unchanged, and displaying the relative weighting of various frequencies with this transformation.

Power spectral density estimates were obtained for each series by average FFT (successive windows of length 5.12 s, overlapping by 3.84 s, with a 50% Hamming window). These spectral densities were used to calculate the gain function for each derived series, taken as the ratio between amplitudes of the derived and original IBI at each frequency. Average gain functions were obtained for each derived series (SD, dichotomized SD [pnn50] and .12–.40 Hz filter) as the grand mean of the respective transfer functions across all original IBI series (all participants and both resting and stress task).

The gain functions for each metric, representing the average across all participants and task conditions, are presented in Fig. 3, with the inset of Fig. 3 showing the amplitude spectrum from the raw IBI series. As expected, the empirical transfer function for the .12–.40 Hz filtered series closely approximates the transfer function of that filter, and substantially attenuates frequencies outside of that bandwidth. The SD, and pnn50 transfer functions, by contrast, are characterized by: (1) broader transition bands at the low frequency end, thus, allowing some non-respiratory linked low-frequency variance to be included; (2) uneven transfer functions, especially for pnn50, with large changes in gain from one frequency to the next; (3) accentuation of higher frequencies, such that frequencies beyond the normal breathing range are most heavily weighted in the calculation of the metric. This empirical derivation is in general agreement with the transfer ratio modeled by Berntson et al. (2005), who estimated a similar shaped transfer function, based only on successive differences in simulated data based on sinusoidal respiration functions at individual frequencies.

3.2. Convergent validity

As shown in Table 2, measures of heart rate and heart rate variability were highly correlated with one another during both rest (median magnitude of Pearson $r = .75$) and during paced arithmetic stressor (median magnitude of $r = .73$). Metrics putatively measuring parasympathetic nervous system activity were also highly intercorrelated during rest (median $r = .89$) and during paced arithmetic stressor (median $r = .87$).

3.3. Discriminant validity

Although many measures of heart rate and heart rate variability were moderately to highly correlated both during rest and stressor, the putative sympathetic metric, Toichi's CSI, was significantly negatively correlated to all other measures of heart rate variability both at rest and under stress.

3.4. Exploratory factor analysis

To examine the degree to which the measures were indexing similar constructs, exploratory factor analysis using principal component extraction and varimax rotation was utilized, extracting two factors. Measures of variability were entered into the analysis (HRV, SDNN, RMSSD, CSI, MSD, pnn50, RSA, and CVI), with a separate factor analysis conducted at rest and during stressor. Two factors accounted for 94% and 95% of the variance during rest and stressor, respectively. As shown in Table 3, Factor 1 had high loadings for every metric except CSI, whereas Factor 2 had a very high loading for CSI, and smaller and inverse loadings for most but not all other metrics. The factor loadings were descriptively quite similar at rest and during stressor.

These results suggested that across participants and across tasks, the inter-relationships of the various metrics were highly stable, and that measures putatively reflecting total variability and parasympathetically mediated variability loaded on a single

Table 2
Intercorrelations between metrics at rest and during paced arithmetic

	IBI	HR	HRV	SDNN	RMSSD	CSI	MSD	pnn50	RSA
Rest									
HR	−.98								
HRV	.62	−.62							
SDNN	.59	−.56	.95						
RMSSD	.65	−.61	.84	.93					
CSI	−.58	.59	−.33	−.39	−.63				
MSD	.65	−.61	.80	.88	.98	−.65			
pnn50	.73	−.72	.75	.77	.88	−.75	.91		
RSA	.62	−.61	.90	.90	.91	−.60	.89	.87	
CVI	.72	−.72	.96	.94	.92	−.58	.88	.88	.95
Paced arithmetic									
HR	−.97								
HRV	.49	−.45							
SDNN	.53	−.47	.97						
RMSSD	.74	−.67	.74	.83					
CSI	−.67	.75	−.31	−.31	−.61				
MSD	.78	−.71	.71	.80	.99	−.61			
pnn50	.79	−.72	.71	.77	.97	−.61	.97		
RSA	.68	−.68	.84	.83	.88	−.71	.87	.87	
CVI	.69	−.68	.92	.91	.89	−.63	.87	.87	.97
Change from rest to paced arithmetic									
HR	−.84								
HRV	.14	−.05							
SDNN	.11	−.03	.92						
RMSSD	.45	−.26	.62	.75					
CSI	−.48	.72	.08	.11	−.15				
MSD	.45	−.26	.53	.66	.96	−.14			
pnn50	.39	−.14	.40	.47	.78	−.11	.84		
RSA	.30	−.35	.76	.71	.64	−.45	.57	.45	
CVI	.35	−.30	.92	.87	.75	−.27	.65	.54	.89

Note: $N = 96$; correlations greater in magnitude than .199 are significant at the $P < .05$ level. Each panel has measures of rate (IBI = mean interbeat interval; HR = mean heart rate), measures of total variability (HRV = natural log of variance of IBI time series; SDNN = standard deviation of IBIs; RMSSD = root mean square of differences between IBIs), an estimate of sympathetic-related variability (CSI = Toichi cardiac sympathetic index), and estimates of parasympathetically controlled variability (MSD = mean of absolute value of consecutive IBI differences; pnn50 = proportion of consecutive IBI differences greater than 50 ms; RSA = natural log of variance of filtered (.12–.40 Hz) IBI time series; CVI = Toichi cardiac vagal index).

factor. These analyses addressed the extent to which these various measures shared variance across individuals, but did not address the extent to which they were similarly sensitive to change within individuals. The change in scores from baseline to stressor was therefore examined.

3.4.1. Change scores in measures

To reflect reactivity due to the stress task, change scores (stressor task minus rest) were computed. Intercorrelations amongst change scores for these metrics of heart rate and heart rate variability (Table 2) were moderate to high (median

Table 3
Factor loadings for metrics at rest and during paced arithmetic

	Rest		Paced arithmetic (PA)		Change from rest to PA	
	Factor 1	Factor 2	Factor 1	Factor 2	Factor 1	Factor 2
HRV	.97	–	.96	–	.89	–
SDNN	.96	–	.97	–	.93	–
RMSSD	.82	−.52	.73	−.63	.90	–
CSI	–	.97	–	.95	–	.97
MSD	.78	−.57	.70	−.65	.84	–
pnn50	.67	−.69	.69	−.65	.71	–
RSA	.85	−.46	.73	−.63	.81	−.37
CVI	.89	−.41	.83	−.52	.93	–

Note: $N = 96$; loadings greater in magnitude than .3 are shown. Factor analysis included measures of total variability (HRV = natural log of variance of IBI time series; SDNN = standard deviation of IBIs; RMSSD = root mean square of differences between IBIs), an estimate of sympathetic-related variability (CSI = Toichi cardiac sympathetic index), and estimates of parasympathetically controlled variability (MSD = mean of absolute value of consecutive IBI differences; pnn50 = proportion of consecutive IBI differences greater than 50 ms; RSA = natural log of variance of filtered (.12–.40 Hz) IBI time series; CVI = Toichi cardiac vagal index).

Table 4
Ability of metrics to discriminate between tasks

	Mean (S.E.)		F	P	η^2
	Rest	Paced arithmetic			
IBI	840.2 (12.71)	724.8 (12.60)	325.6	<.001	.77
HR	73.4 (1.09)	86.0 (1.54)	201.2	<.001	.68
HRV	7.9 (.08)	8.3 (.07)	28.4	<.001	.23
SDNN	61.3 (2.90)	68.6 (2.45)	9.0	<.003	.09
RMSSD	56.2 (3.70)	43.5 (2.60)	27.7	<.001	.23
CSI	2.2 (.07)	3.5 (.17)	91.9	<.001	.49
MSD	44.4 (2.97)	32.0 (1.95)	39.7	<.001	.30
pnn50	29.4 (2.32)	18.6 (1.62)	66.3	<.001	.41
RSA	6.7 (.10)	6.5 (.10)	7.1	<.01	.07
CVI	4.6 (.04)	4.6 (.04)	.39	n.s.	.004

Note: $N = 96$; analysis included measures of rate (IBI = mean interbeat interval; HR = mean heart rate), measures of total variability (HRV = natural log of variance of IBI time series; SDNN = standard deviation of IBIs; RMSSD = root mean square of differences between IBIs), an estimate of sympathetic-related variability (CSI = Toichi cardiac sympathetic index;), and estimates of parasympathetically controlled variability (MSD = mean of absolute value of consecutive IBI differences; pnn50 = proportion of consecutive IBI differences greater than 50 ms; RSA = natural log of variance of filtered (.12–.40 Hz) IBI time series; CVI = Toichi cardiac vagal index).

magnitude $r = .45$) and metrics of parasympathetically influenced variability were more strongly intercorrelated (median $r = .61$). Exploratory factor analysis extracted two factors that were highly similar to those described above, jointly accounting for 81% of the variance (Table 3).

3.5. Sensitivity to experimental manipulations

To determine whether metrics were able to discriminate between tasks, separate analyses were conducted using repeated measures general linear model for each metric. All metrics except Toichi's CVI (Table 4) discriminated robustly between the rest and stressor task, although effect sizes varied quite substantially.

4. Discussion

Although several metrics converged as expected, the overall pattern of results suggests that metrics putatively tapping vagally mediated cardiac variability correlate highly with metrics summarizing total variability. This may reflect that, at rest, a majority of variability in cardiac chronotropy is due to parasympathetic influences. Fig. 3 supports this interpretation, as most of the power in the raw spectrum is in ranges passed by the various transformations of the IBI series that form the basis of the various metrics. The present results apply only to relatively sedentary laboratory conditions, and generalization beyond these conditions is not warranted.

Evidence from the analysis of metrics during rest and arithmetic stressor suggests that measures are differentially sensitive to the stressor manipulations, with all measures except for Toichi's CVI significantly discriminating rest from stressor task but with markedly different effect sizes. Given the relatively high correlation of CVI with other measures of vagally influenced variability, it is surprising that this measure was not sensitive to the task manipulation, but suggests it may not be used interchangeably with the more standard measure of RSA based on respiratory band-limited variance.

Given the present results, it may be tempting to assume the interchangeability of many of the metrics, but given the variability between the transfer functions of the various metrics, a band-limited filtered IBI series (e.g. .12–.40 Hz in the present study), or power from the same frequency range using spectral analysis, may be preferred as they adequately summarize cardiac variability in the respiratory frequency range, and attenuate slower frequencies with a steep roll-off. Other metrics such as the Toichi metrics are included as they appear to hold some promise, but are largely unexplored. Computationally simple metrics with broad and irregular transfer functions were included merely to assess comparability across studies. Although at rest all metrics perform somewhat similarly, it is worth noting that they have widely different effect sizes in terms of discriminating rest from the arithmetic stress task, and if researchers are interested in solely vagal contributions, they would be well advised to use measures that are most likely to reflect respiratory-linked vagally mediated control of cardiac chronotropy.

A freely available suite of software tools for obtaining the IBI series from EKG data and computing many metrics of cardiac chronotropy is described in Appendix A. Given the increasing interest in using measures of cardiac vagal control in research on health, emotion, development, and psychopathology, it is hoped that these tools will stimulate further interest in the field, and encourage researchers new to this field to begin using measures of cardiac vagal control in their research.

Acknowledgments

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Appendix A. QRSTool and CMetX: a software suite to obtain metrics of cardiac vagal control

A suite of tools for transforming EKG data to metrics of cardiac variability is freely available from http://www.psychofizz.org, under the appropriate link. These tools run under various versions of the Microsoft Windows operating system. QRSTool provides a graphical user interface that will allow for the extraction of the IBI series from EKG data, whereas CMetX is a command-line based utility that will calculate several metrics of cardiac chronotropy given a simple IBI series as input. The tools are integrated such that users who choose to extract the IBI series with QRSTool can have metrics calculated directly by CMetX. The programs also can be used independently: CMetX can derive metrics given any IBI series

Table A.1
Common QRS detection problems and solutions in QRSTool

Problem	Possible cause	Common solution	QRSTool solution
Time-varying amplitude of PQRST complexes	Amplifier drift	Adaptive threshold techniques	*Average minimum/maximum* beat detection algorithm
	Skin potential artifact		*Peri-beat filter* correction algorithm (following manual insertion of beats)
	Changes in sensor impedance Participant movement		
Atypical PQRST morphology	Sensor placement	Accentuate higher frequency components, e.g.	Derivative transform
T-waves larger than R-spikes	Participant physiology	Derivative based algorithms	Length-transform beat detection algorithm
Large Q- or S-spikes		Filtering	*Peri-beat filter* correction algorithm (following manual insertion of beats)
Baseline drift of EKG signal/dc noise (dc-coupled amplifiers)	dc-coupled amplifier	High-pass filter	Filtering (externally created filters)
	Skin potential artifact		*Peri-beat filter* correction algorithm (following manual insertion of beats)

as input, and QRSTool can extract the IBI series to then analyze using other programs or algorithms. Each program is described below.

A.1. QRSTool: software for deriving a reliable IBI series from EKG data

There are a number of software packages designed to detect beats in raw EKG data. Typically, such software does not allow online modification of the beat series, and as such, the output must be visually inspected for missed or extra beats. Errors in beat series must be modified manually, e.g. deletion of extra beats, or interpolation of beat times for skipped beats. This process can require multiple passes of correction and visualization before a beat series is acceptable.

QRSTool is an EKG beat detection software program that allows for modification of beat assignments while simultaneously viewing the resulting IBI series. Missed and extra beats can be identified by visual inspection of the IBI series, and corrected by manual addition or removal of individual beats. The EKG and IBI series are displayed in separate vertically stacked time-locked windows, with identified beats marked on the EKG series.

There are a number of EKG transform functions (e.g. filtering) that create additional, separate EKG series, each of which can be selected as the currently visible EKG series. Beat markers are placed directly on the visible EKG series, allowing for comparison of beat placement between different series. This function can be useful in assessing the extent to which features in the transformed data are shifted with respect to the same features in the raw series (e.g. filtering; see below).

QRSTool also includes automated beat detection algorithms. The simplest of these is a threshold function, which assigns beats to all maxima or minima in the EKG time series that exceed a set threshold (typically, the maxima corresponding to the R-wave). Like many of the functions in QRSTool, the threshold detection tool can be applied to the entire series or to manually selected portions. Limiting beat detection to selected portions of the EKG series can be useful in cases where the series varies with respect to signal or noise amplitude.

QRSTool also includes a limited filtering function. Externally generated filters may be imported into QRSTool (as text coefficients), and then applied to the EKG series. It is important to note that, since the PQRST complex is not symmetrical, portions of the filtered complex (e.g. the R-spike) may be phase-shifted with respect to the original series. The degree to which this occurs depends on both the filter parameters and possibly time-varying characteristics of the original EKG signal (e.g. PQRST complex amplitude). As such, if threshold detection is applied to a filtered series, it is often useful to compare beat locations in both the original and filtered series (by changing which is visible in the EKG window).[2]

QRSTool is equipped to handle those cases where threshold beat detection is not possible. Table A.1 lists common QRS detection problems and the solutions offered in QRSTool. EKG recorded with dc-coupled amplifiers may include baseline drift, making any given threshold valid for only a few beats. The *peri-beat filter* correction algorithm (described below) may be useful for such data, since the algorithm uses only local signal characteristics. In cases where T-wave amplitudes are similar to or exceed R-spike amplitude, a first-order derivative transform is available (essentially amplifying higher-frequency components). A somewhat more sophisticated function uses the *length-transform* (Gritzali, 1988) to accentuate R-spikes in the process of beat detection (however, as of this writing, it is not clear that this algorithm is more robust than filtering or derivative transforms).

QRSTool also includes beat correction algorithms, which are applicable to series where at least some beats have already been

[2] It is not clear how much the phase shift that result from filtering affects measures of heart-rate variability. Unless PQRST morphology varies throughout the ECG signal, the phase-offset for any given filter should be relatively constant.

identified. One of these is a function which locks beats to local maxima or minima in the visible series; this algorithm may be applied, for example, to move beats detected by phase-shifted peaks in a filtered series to the actual maxima or minima in the original series. Another function that has proven useful for both beat detection and correction is the *peri-beat filter*. This algorithm uses existing beats to create an "average" beat, which is in turn applied as a filter to the EKG time series. Since the center of the filter corresponds to existing beat locations, the newly created series will have maxima at points where the original series is similar to the "average" beat. This algorithm is useful in cases where the EKG signal is not globally similar, e.g. baseline drift, or time-varying changes in PQRST amplitude.

QRSTool also allows hand placement of peaks for those cases where visual inspection is required to identify the beat. Also, in case of ectopic beats due to premature ventricular contraction that are then followed by a lengthy compensatory delay before the subsequent beat, Porges (2007) recommends that a beat be placed midway between beats on either side of the ectopic beat, a feature that is easily implemented with QRSTool. Porges (2007) notes that these ectopic ventricular complexes will artificially inflate the estimate of RSA by adding ventricular-related variance that is independent of the vagal modulation of the sino-atrial node.

Once an acceptable IBI series has been created, the series may be exported for further analysis (as of this writing, QRSTool does not incorporate any functions for the analysis of heart-rate variability). QRSTool was specifically developed to work in conjunction with CMetX, and as such, automates both the export of the IBI series and execution of CMetX on the resulting file. However, QRSTool can export IBI series in a number of different formats for use with other analysis packages. Manual selection, or selection based on events, may be used to export portions of the IBI series. This functionality may be useful for experiments where one EKG record exists for a number of different conditions. Currently, QRSTool allows both manually inserted events, as well as events imported from Neuroscan CNT files.

QRSTool has, in addition to the GUI menu and button driven methods of processing data, some scripting functionality that can automate parts of the process such as exporting the artifact free series, opening files, applying certain types of filters, etc. Commands stored in ASCII text files can be opened and executed within QRSTool.

A.2. CMetX: software for calculating many metrics of cardiac chronotropy

CMetX is a command-line based program that calculates many metrics of cardiac chronotropy, given an IBI series as input. The IBI series is contained within an ASCII file, with each IBI in ms on a separate line. The resultant output provides metrics summarized in Table A.2.

A.2.1. Filtering the IBI series, following transformation to a time series

An IBI series in not, strictly speaking, a time series, as the data occur at uneven intervals, provided that there is variability in cardiac chronotropy, the very phenomenon of interest! An IBI series can be converted to a time series by interpolating data points at a fixed sampling rate. CMetX program implements a 10 Hz sampling rate with linear interpolation, as illustrated in Fig. A.1.

Whereas Porges' MXEdit program uses a moving polynomial filter, CMetX uses an optimal finite impulse response digital filter designed using FWTGEN V3.8 from Cook and Miller (1992). The default filter is a 241-point FIR filter with a .12–.40 Hz bandpass, constructed using a Hamming windowing option. It is applied to a time-series representation of the IBI series, at a sample rate of 10 Hz. The transfer function of the filter is shown in Fig. A.2. In the process of convolving a filter over a time series, data points at the beginning and end of the series will not be filtered, and are thus "lost" (see Fig. A.3). CMetX also includes three other filters that can be selected instead of the default .12–.40 Hz filter, including .15–.40 (alternate for adult, in line with the recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996), .24–1.04 (for infant), and .3–1.3 (for newborn). Additionally, with version 2.6 and later, CMetX users can specify filter parameters ranging from 0 to 5 Hz, and CMetX will design and apply an optimal finite impulse response 241-point digital bandpass filter using the algorithm specified in Cook and Miller (1992) with a hamming window. Finally, users

Table A.2
Metrics output by CMetX, with notes concerning computation

Metrics of rate, which are influenced by both parasympathetic (PNS) and sympathetic (SNS) influences
Mean interbeat interval (IBI), calculated as simple average of IBIs
Mean heart rate (HR), calculated as the average of the rate-transformed IBIs, not as the rate-transformation of the average IBI
Metrics summarizing total heart rate variability, which are influenced by both SNS and PNS
Heart rate variability (HRV), operationalized as the natural log of the variance of the IBI time series
Standard deviation of IBI series (SDNN); NN in the acronym SDNN is the abbreviation for "normal-to-normal intervals," which is the artifact-free IBI series
Root mean square of successive differences between IBIs (RMSSD)
Putative sympathetic metric
A cardiac sympathetic index (CSI; Toichi et al. (1997), see Fig. 1)[a]
Putative parasympathetic metrics
Mean absolute successive IBI difference (MSD)
Proportion of consecutive IBI differences >50 ms (pnn50)
Respiratory sinus arrhythmia (RSA), defined as natural log of band-limited (.12–.40 Hz) variance of IBI time series
A cardiac vagal index (CVI; Toichi et al. (1997), see Fig. 1)[a]

Note: IBI series refers to the series of IBI values in ms; IBI time series refers to the 10 Hz sampled interpolation of the IBI series to create a time series.

[a] Toichi parameters are based on the Lorenz plot, in which deviations in the direction transverse to the line $IBI_n = IBI_{n+1}$ reflect high beat-to-beat variability and putatively reflect predominately parasympathetic influences. For calculations, axes were rotated -45° so that L was now parallel to the X-axis (the authors wish to thank Scott Vrana for this helpful suggestion with the rotation of the axes). After rotation L and T were estimated as four times the standard deviation of points along the respective axes (4 S.D.s reflecting a truncated normal distribution). Finally, two indices were calculated as described by Toichi et al. (1997)—cardiac vagal index: $CVI = \log(L \times T)$; and, cardiac sympathetic index: $CSI = L/T$.

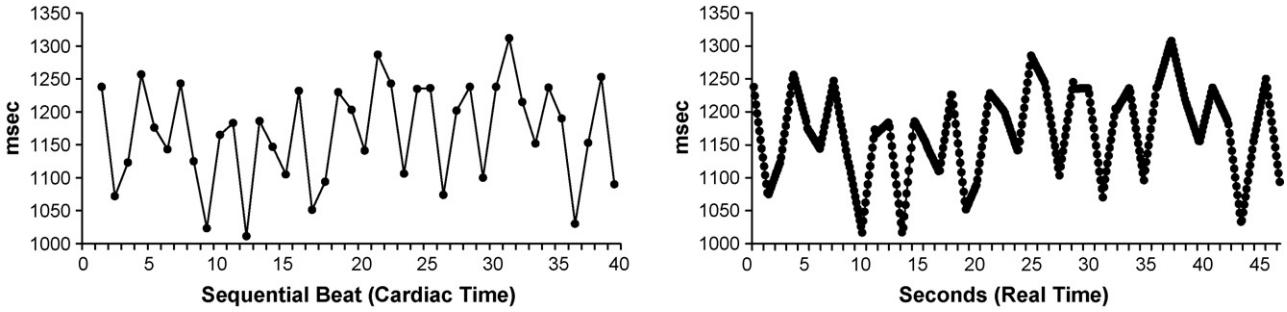

Fig. A.1. The first 40 IBIs for a sample participant in cardiac time (left) and real time sampled at 10 Hz (right).

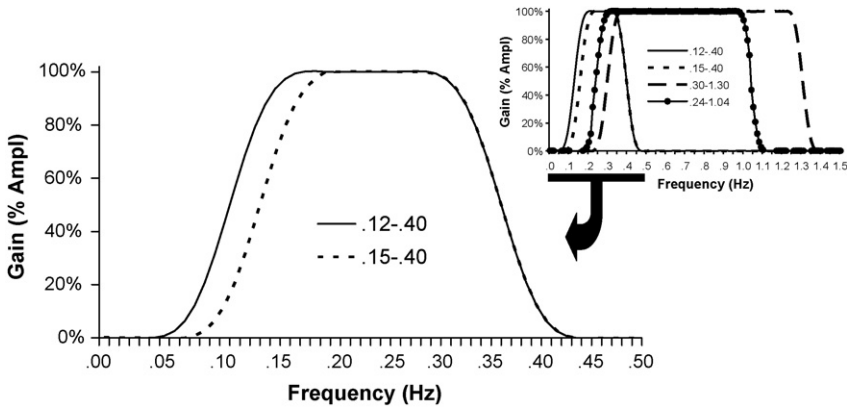

Fig. A.2. Transfer function of filters used by CMetX. Larger panel shows the default .12–.40 Hz bandpass filter and the alternate .15–.40 Hz filter for adults, whereas inset shows all four available filters. All filters are 241-point FIR filters.

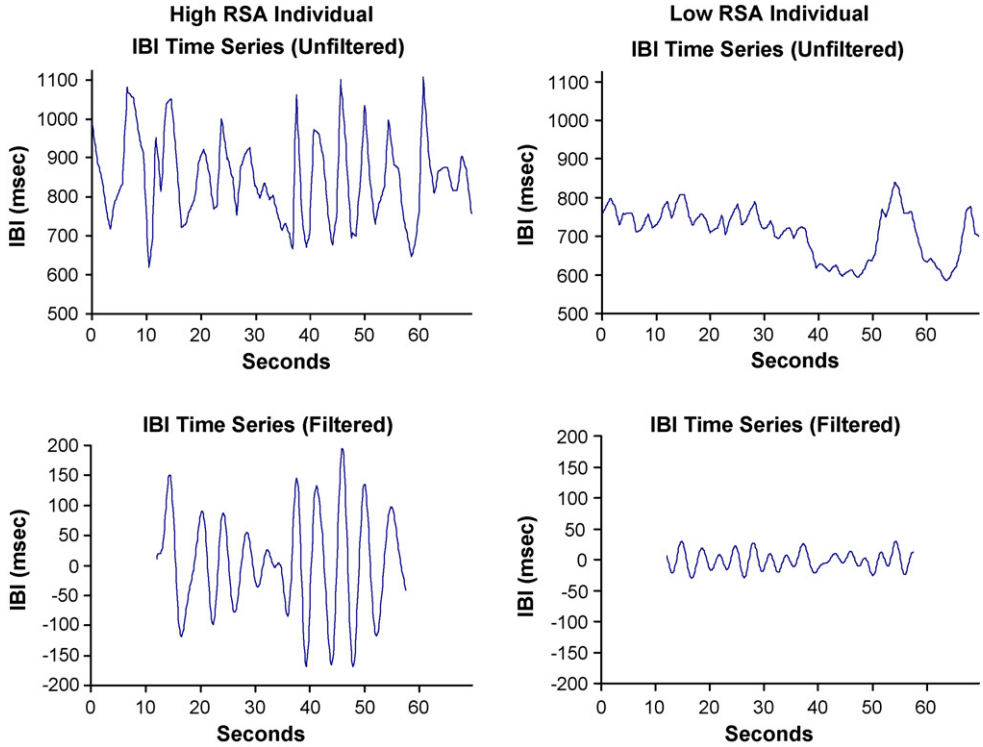

Fig. A.3. Time-series representation of IBI series for two participants (top panels), and the filtered versions of those series (lower panels), resulting in a loss of 12 s of data at both ends of the series. The variability in the lower panel represents that potion of the total variability within the respiratory frequency band, and that putatively reflects vagal influence.

Table A.3
Correlations between metrics obtained with CMetX and with Porges' MXEdit program

Metric	Baseline	Arithmetic
HRV	.995	.997
RSA	.992	.995
Mean IBI	1.000	1.000
Mean HR	1.000	1.000
# IBIs	1.000	1.000

familiar with filter design can create and import their own coefficients as well.

A.2.2. Computation of metrics, and comparison to Porges' V_{Hat}

All metrics are calculated for only the IBIs that correspond to the sampled timepoints that are retained after the filter is applied to band-limit the signal to calculate RSA. The filter results in a loss of 12 s of data at the beginning and 12 s at the end of the file. All metrics are therefore based on the same subset of the data, but users may wish to include data 12 s prior to and 12 s following the time window of interest to accommodate this data loss.

Many metrics are calculated on the raw IBI values, as they involve the standard deviation, the variance, or a root mean square of the raw IBI values (or difference between successive values). Others, such as RSA – which involves the extraction of specific frequencies of variability – require series in real time.

Correlations were obtained between the metric of RSA from CMetX and that from MXEdit V2.21 from Porges with a sample of 96 college students (described in Section 2), at rest and paced arithmetic. Correlations were near unity, as presented in Table A.3. Thus, CMetX produces data that appear highly comparable to those obtained using the Bohrer and Porges method that is part of the more user-intensive MXEdit program. Moreover, CMetX can be called directly from QRSTool, obviating the need for a separate user session to derive the metrics of cardiac chronotropy.

Finally, comparisons of the estimate of RSA from CMetX were compared to spectral power from the FFT of the IBI series for these same 96 subjects. Power spectral density estimates were obtained for the 10 Hz sampled time series representation of the IBI series, averaging successive windows of length 5.12 s, overlapping by 3.84 s, with a 50% Hamming window. Natural log-transformed spectral power and natural log-transformed spectral amplitude were extracted from the .12–.40 Hz range. At rest, RSA from CMetX correlated .986 with spectral power, and .984 with spectral amplitude; during the stressor, correlations were .992 for spectral power, and .997 for spectral amplitude. Thus although the filter used for CMetX has a transition band at the low and high cutoff, the resultant time–domain metric (natural log of band-limited variance) provides a result that would be virtually indistinguishable from that derived in the frequency domain (natural log of .12–.40 Hz spectral power or amplitude).

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