IMPROVING ESTIMATION OF CARDIAC VAGAL TONE DURING SPONTANEOUS BREATHING USING A PACED BREATHING CALIBRATION

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ABSTRACT

Respiratory sinus arrhythmia (RSA) is a commonly employed non-invasive measure of cardiac vagal control. It has been demonstrated that respiratory parameters such as tidal volume and respiratory frequency can change RSA without altering tonic vagal activity. Thus, within-individual comparisons of cardiac vagal control across different behavioral tasks might benefit from an adjustment for respiratory confounds. We tested an adjustment method using transfer function analysis and paced breathing at 3 different respiratory frequencies as the basis for regressing out respiratory related RSA changes in a task where breathing was not controlled. Electrocardiogram and calibrated respiration were recorded with the LifeShirt system from 15 young adult participants. Time series of RR intervals and lung volume change were computed and the respiration-to-RR-interval transfer-function magnitude (RSA-TF, in ms/liter) estimated. Mean (SD) of RSA-TF was 142 (68) at 9 breaths/min, 78 (52) at 13.5 breaths/min, 57 (43) at 18 breaths/min, and 121 (56) during baseline, with a respiratory frequency of 12.5 (3.8) breaths/min. At baseline, measured and predicted RSA-TF values (mean 94+/-82) differed significantly and correlated only moderately (r=0.67). Factors contributing to a less than perfect correlation included slightly elevated subjective anxiety levels and hyperventilation during paced breathing, both of which may have affected cardiac vagal tone. This study demonstrates a novel procedure for computing a respiratory unrelated RSA index. Results provide some support for the utility of this adjustment method for improving the estimation of cardiac vagal tone from RSA, but also indicate that the paced breathing procedure may need to be further refined.

Keywords: Heart Rate Variability, Respiration, Ambulatory Monitoring, Autonomic Nervous System, Transducers, Psychophysiology

INTRODUCTION

Activation of the parasympathetic branch of the autonomic nervous system can be estimated noninvasively from the variations in heart period occurring synchronous with respiratory phase, termed respiratory sinus arrhythmia (RSA). During inspiration the RR interval is shorter (heart rate is higher) than during expiration, an effect mediated primarily by the vagus nerve [1]. Several methods have been used to quantify this phenomenon, with spectral analysis (high-frequency heart rate variability) being the method of choice [2]. The validity of RSA as a measure of parasympathetic tone has been established pharmacologically and by behavioral manipulations [e.g., 3].

Methodological concerns have been raised about respiratory confounds in the measurement of within-individual changes in vagal tone by RSA. Changes in respiratory frequency (f_b) that commonly occur

during varying behavioral tasks (e.g., mental arithmetic, bicycle ergometry, exposure to anxiety inducing stimuli) can greatly affect RSA levels without underlying change in tonic vagal activation [e.g., 4]. It has been shown that RSA decreases nonlinearly with increasing f_b [5], which is related to attenuation of the transmission of vagal nerve impulses at the cardiac sino-atrial node acting similarly to a low-pass filter [6].

Standardizing f_b in each behavioral task by requiring paced breathing can solve this problem, but paced breathing cannot always be implemented. It may also interfere with certain tasks (e.g., speaking) and by requiring a dual-task performance may change the autonomic characteristics of the assessed behavior. Thus, adjustment for confounding within-individual changes in f_b is sometimes attempted using analysis of covariance. However, this statistical adjustment is weak since it uses a regression relating RSA to f_b derived from group statistics, which cannot take into account the large variability between individuals in slopes and intercepts for these regressions [e.g., 7]. Theoretically, a more precise adjustment would take into account idiosyncratic characteristics of the f_b-RSA regression to derive a purer index of cardiac vagal control for each individual under conditions associated with varying f_b.

Another respiratory parameter that can vary with behavioral tasks is the volume, or depth, of breathing [8]. It has been demonstrated that respiratory depth linearly modulates RSA amplitude without an underlying change in vagal tone: with increasing tidal volume (V_t), RSA increases [5]. A relatively straightforward adjustment for this confound can be achieved by computing the V_t-to-RR-interval transfer-function magnitude (RSA_{TF}) using spectral analysis. This computation effectively adjusts for the confounding effect of respiratory depth in the assessment of RSA as an index of parasympathetic activation [9], and provides a measure of the amount of RSA per liter air breathed.

Here we present a procedure that attempts an improved measurement of within-individual changes in vagal tone by adjusting conventional RSA for respiratory depth and frequency. To establish individual f_b-RSA relationships, subjects are asked to breathe at 3 frequencies within a normal physiological range. To concurrently adjust for V_t variations, we calculate the transfer function relating RR interval oscillations to lung volume change at these frequencies. We will explore characteristics of this calibration procedure and test its performance by relating adjusted RSA values to measured RSA values during a period where breathing was not controlled.

METHODS

Participants

Fifteen female students participated in the study. Their mean (SD) age was 21.3 (2.4). All participants were physically healthy, were not currently smoking cigarettes, and had no history of respiratory or cardiac disease.

Procedures

Subjects arrived in the laboratory in the morning. After the procedures were fully explained, all participants signed an informed consent form. Subjects were then asked to put on the LifeShirt garment with embedded sensors (Vivometrics Inc., Ventura, CA). The experimenter assisted by attaching the electrocardiogram electrodes and the cable connector to the multi-channel ambulatory monitor. Then the monitor was started and subjects followed the instructions on the screen to calibrate the respiratory sensors of the device by breathing in and out of an 800 ml bag 7 times, filling and emptying it completely. This procedure was conducted in sitting and standing posture. Then subjects were asked to sit quietly with eyes open (baseline, 5 min) and then pace their breathing at 3 respiratory frequencies (6 min).

During this task, subjects heard a tape recording of tones with increasing and decreasing pitch. They received the following instructions:

> *"The speed of breathing has an impact on the heart rate. To assess this, we will have you breathe at 3 different speeds. For the next 6 minutes you will hear tones with rising and falling pitch. Please breathe in with the rising pitch, out with the falling pitch, and pause between breaths when there is no tone. During the first two minutes the tones will be rather slow, then they will switch to a normal breathing speed, and after another two minutes, to a fast speed."*

The tones had a pleasant volume and pitch. Their timing was modeled after a normal relaxed breathing cycle, with a duty cycle of 0.4, and included a pause after expiration. Paced breathing started at 9 breaths/min and was switched to 13.5 and 18 breaths/min without interruption. Each pace had a 2-min duration. (Electronic Wave-files with paced breathing tones are available upon request from the first author). After baseline and paced breathing participants rated their level of anxiety on a 0 (not at all) to 10 (extremely) Likert-type scale.

Data acquisition

Physiological signals were continuously registered via a multi-channel ambulatory monitor (LifeShirt System; Vivometrics Inc., Ventura, CA) and stored on compact flashcards. Placement of electrodes and sensors and data recording followed established conventions. Electrocardiogram lead-II was measured from the thorax using 3 spot electrodes. Respiratory pattern was measured using thoracic and abdominal inductance plethysmography bands integrated in the LifeShirt garment. Data was stored on a flash memory card inserted in the LifeShirt recorder.

Respiratory and autonomic data reduction

The data stored on the memory card was downloaded to a personal computer and loaded into the VivoLogic analysis and display software. The calibration periods marked on the recordings were automatically analyzed to derive multiplication factors for the thoracic and abdominal inductance plethysmography sensors [10]. These were then automatically applied to the signals to compute the calibrated respiratory lung volume curve (in liters). The VivoLogic software then computed a variety of parameters for each breath across the entire recording. The experimenter marked the segments to be analyzed (baseline, paced breathing at 9, 11.5 and 18 breaths/min). Average respiratory frequency (f_b) and tidal volume (V_t), and their product, minute ventilation, were computed for each segment.

The electrocardiogram was analyzed with a program that detects R-waves and calculates consecutive RR intervals [11]. Beat-by-beat values were edited for outliers due to artifacts or ectopic myocardial activity by computer algorithm and visual inspection. Lung volume curve and RR interval were resampled into time series with 0.25-sec temporal resolution using weighted-average interpolation. We quantified RSA_{TF} (in ms/liter) using fast Fourier transform and Welch's averaged periodogram method [12] as the magnitude of the transfer function relating RR interval oscillations to lung volume oscillations at the peak f_b [13]. This method effectively adjusts for the confounding effect of respiratory depth in the assessment of RSA as an index of tonic parasympathetic activation [5] and measures variance in the RR interval signal that is accounted for by respiration.

RR interval and lung volume time series, both resampled at 4 Hz, were first partitioned into 60-sec segments, overlapping by 30 sec. Each segment was then linearly detrended, Hanning windowed, and zero padded to 64 sec (128 data points). The segments were subjected to fast Fourier transform for frequency decomposition. The resulting power spectral density functions were adjusted to account for attenuation

produced by the Hanning window. For each segment, the cross-spectral density function was calculated as the product of respective power spectral density functions for RR interval and lung volume signals. Successive power spectral density and cross-spectral density functions were then averaged across segments to obtain reliable estimates. The transfer function (in ms/liter) was calculated as the averaged spectral density function of the lung volume signal divided by the averaged cross-spectral density function.

The peak f_b was automatically detected as the greatest local maximum in the 0.08-0.50 Hz lung volume power spectral density function. Spectral coherence between lung volume and RR interval at this frequency was required to be at least 0.5 for the transfer function RSA estimate to be valid (which was the case for all measurement points in this study; less coherence would have indicated sources for RR interval variation other than respiration).

Statistical data analysis

To characterize relationships between parameters, we calculated means, paired t-tests (two-sided), Pearson product-moment correlations, and regression equations. The principal comparisons were made between the spontaneous-breathing baseline and parameters derived from the paced breathing procedure. P-levels at or below 0.05 were considered statistically significant.

RESULTS

All participants were able to follow the instructions to pace their breathing at the required respiratory frequencies. Figure 1 shows the mean RSA_{TF} values at the 3 paces of breathing for the 15 participants. As has been demonstrated before, RSA_{TF} values declined non-linearly with increasing f_b.

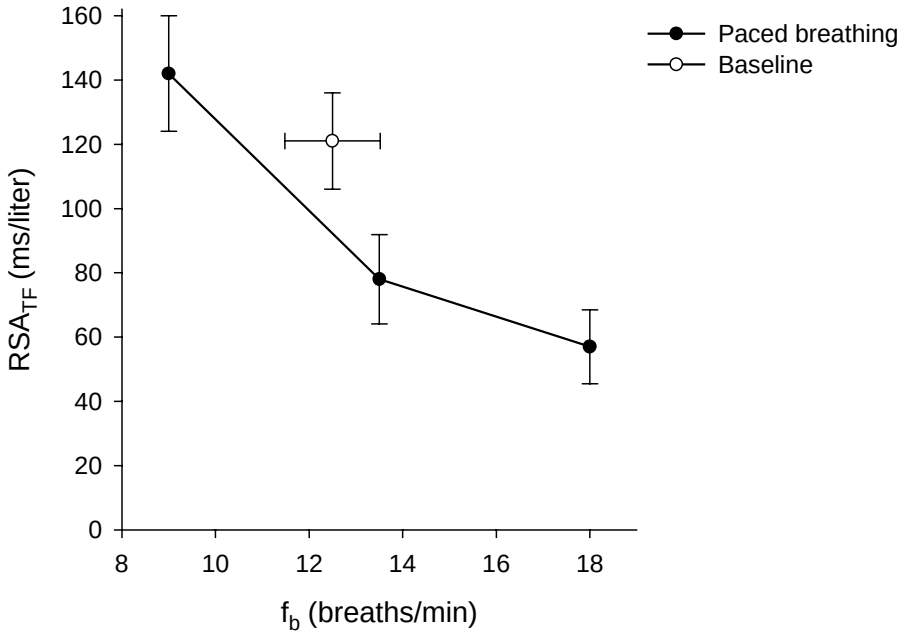

Figure 1. RSA_{TF} as a function of respiratory frequency (f_b) during the paced breathing calibration and at baseline (means and standard error bars).

In the same figure, the mean (SEM, y-axis) baseline RSA_{TF} value is plotted at the mean (SEM, x-axis) baseline f_b. Baseline f_b had a range of 5.6-20.3 breaths/min, with 28% of subjects below 9 breaths/min (0.15 Hz). As is apparent from the figure, the baseline RSA_{TF} was above the RSA_{TF} level to be expected for the corresponding f_b on the basis of the paced breathing procedure.

For each individual, we computed a linear regression equation in a least squares sense to best represent the 3 RSA_{TF} values measured during paced breathing by their corresponding f_b values:

$$RSA_{TF} = b \cdot f_b + a$$

Using this formula, after slope b and intercept a were established for each individual, the expected RSA_{TF} values (based on paced breathing values) could be estimated for each baseline f_b. Mean (SD) of linear regression parameters derived from the paced breathing calibration were for slope b = -9.4 (5.9) and for intercept a = 218 (109). R^2, an index of the goodness of fit of the linear regression, was 0.82 (.29).

Based on the regression equation of each individual, we computed the RSA_{TF} value that was to be predicted for the individual's specific f_b at baseline. At this f_b, measured RSA_{TF} (121 +/- 56) and predicted RSA_{TF} (94 +/- 82) differed significantly ($t(14)=2.25$, $p<0.05$). The values correlated moderately, with an r of 0.67. Nonlinear regression did not improve predictive accuracy: the average correlation coefficient for logarithmic regression fit was r=.44 and for hyperbolic (1/x) regression fit was r=.65.

Similarly, we calculated linear regression equations for each individual to predict V_m at the baseline f_b from the 3 V_m values measured during the paced breathing procedure. Measured V_m (5.4 +/- 1.2) and predicted V_m (5.8 +/- 1.8) differed significantly ($t(14)=2.31$, $p<0.05$), indicating that V_m was elevated from baseline values during the paced breathing procedure. A parallel analysis was performed for heart rate. Measured heart rate (74.3 +/- 8.2) and predicted heart rate (76.8 +/- 9.5) differed significantly ($t(14)=2.46$, $p<0.05$), indicating that heart rate was slightly, but reliably, elevated from baseline values during the paced breathing procedure. Self-reported anxiety during baseline was 0.78 (0.51) and during paced breathing 1.37 (0.74), which was significantly different ($p<.05$).

DISCUSSION

We have presented a procedure that – on theoretical grounds – should enhance the accuracy of RSA for indexing within-individual changes in vagal tone by removal of two important confounds. Adjustment for V_t variations was achieved by spectral transfer function analysis relating the RR interval time series to the concurrently measured calibrated lung volume curve, resulting in RSA_{TF}. Adjustment for f_b variations was achieved by establishing the f_b-RSA_{TF} regression for each individual using a 3-step paced breathing procedure, and then applying this regression to estimate RSA_{TF} for a period where breathing was not controlled. Change in vagal control free of V_t and f_b confounds then can be calculated as the difference between the estimated and measured RSA_{TF}, which could be termed "respiratory unrelated RSA."

The transfer function magnitude relating cardiac RR interval to lung volume change decreased with increasing f_b as expected, allowing computation of individual f_b-RSA regressions. The 0.82 goodness of fit for the regressions was acceptable. Surprisingly, RSA_{TF} was significantly higher during baseline than was extrapolated from the paced breathing regressions. This was also the case for heart rate, a parameter that correlates highly with vagal tone under many conditions. This suggests that the paced breathing procedure was not performed in a steady physiological baseline state. The reduced vagal tone observed

during the paced breathing procedure is undesirable since it partially counteracts its calibration function. On the other hand, only small differences in both vagal tone and RSA_{TF} can be expected from baseline to paced breathing in comparison to the full range of normal vagal variation [see 14]. This may mean that accuracy of estimation of vagal control when using respiratory unrelated RSA measures may be somewhat compromised when only modest alterations of cardiac vagal tone occur. Nevertheless, our data still suggest a reasonably accurate depiction of even such minimal changes of cardiac vagal tone

We examined several factors that might have contributed to this. Apparently, the paced breathing procedure induced significant state anxiety, which is known to reduce vagal tone [e.g., 15]. Additionally, an increase in minute ventilation indicated significant hyperventilation during the paced breathing procedure. Hyperventilation has been demonstrated to be associated with a reduction in vagal tone [16]. The two factors might also have interacted since anxiety can cause hyperventilation, and vice versa [17].

In this study, we have not applied our paced breathing adjustment procedure to a range of mental stress tasks inducing variations in f_b and V_t. Adjustment for these confounds would be its ultimate important function. However, our preliminary analysis indicates that the paced breathing procedure first needs to be improved. We think that a reduction in the undesirable stress inducing quality of the procedure can be achieved by 1) emphasizing in the instructions that paced breathing should be effortless and performed in a relaxed way, 2) first training participants in the task of following tones of varying pitch with their breathing, which should also reduce any first-time effects, 3) shortening the task to half its duration (3 x 1 min instead of 3 x 6 min). From observing paced breathing data we get the impression that during paced breathing peak-valley RSA amplitude is relatively stable and can be measured reliably even from a small sample of breaths. Stress reduction during the procedure might also reduce the tendency of participants to hyperventilate. Additionally, it might be advantageous to have the instruction include an anti-hyperventilation statement; e.g., “Please breathe fairly shallowly, especially at the end when tones are presented more quickly.” Concurrent capnometry could provide immediate feedback to the experimenter about any hyperventilation tendency of participants and allow assisting subjects in regular breathing.

Even with these improvements in the paced breathing procedure the predictive accuracy of respiratory unrelated RSA values will not be perfect. However, applying some calibration for the effect of within-individual f_b variation on RSA is essential. Our data show that even a moderate increase in f_b by 4.5 breaths/min (from 9 to 13.5 breaths/min) induces a spurious decrease in RSA_{TF} by 45%. Furthermore, a behavioral task that increases f_b will often be characterized by decreased V_t to counteract hyperventilation. This makes the problem of misestimating vagal tone worse for any metric that does not take V_t variation into account.

It is important to note that baseline f_b had a range of 5.6-20.3 breaths/min, with a significant proportion of subjects breathing below 9 breaths/min (0.15 Hz). A frequency of 0.15 Hz is typically used as the cutoff defining the spectral band for estimation of conventional RSA (high-frequency heart rate variability) that is solely based on RR interval measurement, neglecting respiratory confounds. In the present study, high-frequency heart rate variability would be spuriously low for these subjects, not well representing respiration-related variance in their heart rate variability. This additionally underlines the importance of controlling for f_b in the assessment of RSA.

Monitoring of 24-hr heart rate variability and quantification of autonomic function in clinical populations has tried to elucidate potential pathways of their increased cardiac mortality [e.g., 18]. However, the commonly used index of vagal activity, high-frequency heart rate variability, is extremely suscepti-

ble to both f_b and V_t confoundation. Adjustment for respiratory confounds is especially important in ambulatory studies because respiratory parameters vary profoundly during day and night [19]. The LifeShirt system to our knowledge is the first ambulatory recording system that allows reliable breath-by-breath quantification of the calibrated respiratory waveform necessary for computing more precise parameters of vagal activity [20]. A recent ambulatory study by Grossman, Wilhelm and Spoerle [14] used this technology and has shown that adjustment for V_t substantially improves relations between RSA and vagally mediated heart rate. RSA_{TF} was approximated by dividing RSA oscillation amplitude (peak-valley RSA) by V_t, rather than by the computationally demanding cross-spectral analysis, since this is more practical for very large measurement periods.

CONCLUSIONS

In summary, our study demonstrates a novel procedure that allows computing a respiratory unrelated RSA measure to improve prediction of vagal tone under conditions of varying respiratory activity. The study provides some support for the utility of the paced breathing calibration adjustment method for improving estimation of cardiac vagal tone from RSA, but also indicates that the paced breathing procedure can be refined further by making it less stressful. Additional validation of this method over a range of tasks inducing large respiratory alterations is desirable.

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