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Biological Psychology 74 (2007) 263–285
BIOLOGICAL PSYCHOLOGY
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Toward understanding respiratory sinus arrhythmia: Relations to cardiac vagal tone, evolution and biobehavioral functions

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Accepted 16 November 2005
Available online 1 November 2006

Abstract

Respiratory sinus arrhythmia (RSA, or high-frequency heart-rate variability) is frequently employed as an index of cardiac vagal tone or even believed to be a direct measure of vagal tone. However, there are many significant caveats regarding vagal tone interpretation:

1. Respiratory parameters can confound relations between RSA and cardiac vagal tone.
2. Although intraindividual relations between RSA and cardiac vagal control are often strong, interindividual associations may be modest.
3. RSA measurement is profoundly influenced by concurrent levels of momentary physical activity, which can bias estimation of individual differences in vagal tone.
4. RSA magnitude is affected by beta-adrenergic tone.
5. RSA and cardiac vagal tone can dissociate under certain circumstances.
6. The polyvagal theory contains evolution-based speculations that relate RSA, vagal tone and behavioral phenomena. We present evidence that the polyvagal theory does not accurately depict evolution of vagal control of heart-rate variability, and that it ignores the phenomenon of cardiac aliasing and disregards the evolution of a functional role for vagal control of the heart, from cardiorespiratory synchrony in fish to RSA in mammals.

Unawareness of these issues can lead to misinterpretation of cardiovascular autonomic mechanisms. On the other hand, RSA has been shown to often provide a reasonable reflection of cardiac vagal tone when the above-mentioned complexities are considered. Finally, a recent hypothesis is expanded upon, in which RSA plays a primary role in regulation of energy exchange by means of synchronizing respiratory and cardiovascular processes during metabolic and behavioral change.
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Keywords: Cardiac vagal control; Cardiac vagal tone; Respiratory sinus arrhythmia; Heart-rate variability; Parasympathetic; Vagus; Autonomic; Cardiovascular; Evolution; Respiration; Polyvagal theory

1. Introduction

Respiratory sinus arrhythmia (RSA) is a cardiorespiratory phenomenon characterized in mammals by heart rate (HR) or R-R interval (RRI) fluctuations that are in phase with inhalation and exhalation. Typically, HR accelerates during inspiration and slows down during expiration, but the exact phase relationship between respiratory and HR oscillations is dependent upon the prevailing respiration rate (Eckberg, 1983). Furthermore, even when autonomic tone remains stable, the amplitude of these rhythmic HR fluctuations (i.e. the magnitude of RSA) is greatly dependent upon both respiratory frequency and depth of ventilation (i.e. tidal volume; Hirsch and Bishop, 1981). The central, neural, humoral and mechanical feedback mechanisms that together generate RSA are a complex of integrated respiratory and cardiovascular responses (Grossman, 1983; Jordan and Spyer, 1987; Spyer, 1990). Therefore, RSA must be conceptualized as a phenomenon that directly results from the interaction between the cardiovascular and respiratory systems.

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0301-0511/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.biopsycho.2005.11.014

RSA has been shown to importantly reflect rhythmic waxing and waning of cardiac vagal efferent effects upon the sinoatrial node and, therefore, HR (Eckberg, 2003; Hedman et al., 1995b). The relationship between RSA and vagal control of HR has generated great interest among scientists who wish to explore and exploit noninvasive estimates of cardiovascular autonomic control. Along a related line, evidence that RSA magnitude in humans is sometimes predictive of both physiologic and psychological morbidity has also engendered research focused upon using RSA more as a marker of risk than as an index of discrete parasympathetic cardiac control (e.g. Bigger et al., 1992; Hayano et al., 1990; Janszky et al., 2004; Kluge et al., 1988; Nishimura et al., 2004).

Whatever the basic motivation for investigation, a firm understanding of what RSA is – and what RSA is not – would seem essential. All too commonly, a thorough grasp of RSA appears missing in the literature, and this has led to contradiction, confusion, misinterpretation and misattribution with respect to research findings and appropriate methods of measurement and analysis. The major purposes of this article are (1) to clarify the nature of RSA, (2) to elucidate certain misconceptions regarding RSA, and (3) to elaborate upon a new theoretical model that integrates RSA and biobehavioral functioning. In this model, RSA plays a significant role in coordinating physical energy requirements and continuously changing behavioral activities.

RSA – in addition to being simply employed as a cardiac vagal index – has become embedded in a theoretical framework of evolutionary, biological and psychobiological adaptation. Therefore, we will address both proximal concerns about its accuracy as a parasympathetic measure and broader aspects of its functional roles and its evolutionary origins in non-mammalian vertebrates. Specifically, we will discuss the biological function that RSA is likely to serve in coordinating and maintaining interplay between the respiratory and cardiovascular systems, which are together responsible for meeting metabolic demands over a range of highly variable internal and external conditions. Both respiratory and cardiovascular processes are responsive not only to gross metabolic demands but also to levels of alertness and, in humans at least, different types of emotion, mental activity and arousal (and the latter may only minimally or not at all change metabolism). Coupling of respiratory and cardiovascular systems, consequently, are likely to be pertinent to psychological and behavioral variations, as well as physiological state.

Our arguments fall under two themes: (1) clarification of the relationships between RSA and vagal tone in mammals and particularly humans, and (2) the evolution of central, vagal control of cardiorespiratory interactions in vertebrates. With respect to evolutionary issues, we will also critique the polyvagal theory (Porges, 1995), a currently popular view based upon assumptions about the evolution of the autonomic nervous system. The polyvagal theory attempts to introduce an evolutionary perspective into relations between parasympathetic activity and behavior and to explain situations in which changes in RSA clearly do not correspond to alterations in vagally mediated HR (i.e. cardiac vagal tone; Porges, 1995, 2001, 2003b). The theory maintains that RSA is generated in functionally distinct vagal systems that first evolved in the brainstem of mammals (Porges, 1995, 2003b). In recent years, this theory has been expanded to encompass a wide range of postulates regarding physical, psychophysiological and even social functioning in humans (e.g. Porges, 2003b; Sahar et al., 2001).

The following six points will guide the structure of our presentation:

1. Respiratory parameters of rate and volume can confound relations between RSA and cardiac vagal tone.
2. Although within-subject relations between RSA and cardiac vagal control are often strong (when properly measured), between-subject associations may be relatively weak.
3. RSA measurement is strongly influenced by concurrent levels of momentary physical activity, which can bias estimation of individual differences in vagal tone.
4. RSA amplitude is affected by beta-adrenergic tone and may not be a pure vagal measure.
5. RSA and cardiac vagal tone may dissociate under certain circumstances.
6. Basic assumptions of the polyvagal theory regarding RSA are at odds with current knowledge of the neuroanatomical and functional evolution of cardiac vagal control.

RSA can be quantified in a number of different ways, most commonly including spectral analysis, time-domain peak-valley analysis or application of a band-pass filter. Units of measurement can also consequently vary. For time-domain measures, RSA is typically estimated in ms (e.g. the inspiratory–expiratory difference in RRI). With spectral analysis and other frequency-domain approaches, the variation of RRI occurring within the range of the respiratory frequency is estimated; thus ms^2 is frequently employed, consistent with usual statistical units of variance. Often RSA measures are logarithmically transformed to normalize distribution, but this is not always the case. Because different methods are almost perfectly correlated with each other when properly employed (Grossman et al., 1990b), we will not detail quantification methods when reviewing the literature, except when it might be pertinent to a specific topic. A fuller treatment of measurement issues is beyond the scope of this article.

2. Respiratory confounds in RSA estimation of cardiac vagal tone

Numerous studies have documented the effects upon RSA of voluntary and spontaneous changes in respiration rate and tidal volume under steady-state conditions and during mental tasks. Steady state, in this context, connotes conditions during which metabolic activity and autonomic tone remain largely constant. A sample of these studies include the following: Ahmed et al. (1982, 1986), Althaus et al. (1998), Angelone and Coulter (1964), Badra et al. (2001), Ben Lamine et al. (2004), Brown et al. (1993), Clynes (1960), Cooke et al. (1998), Eckberg (1983, 2003), Eckberg et al. (1984), Grossman (1992),

Grossman et al. (1991, 2004), Grossman and Kollai (1993), Hayano et al. (1994), Hedman et al. (1995a), Hirsch and Bishop (1981), Patwardhan et al. (1995), Penttila et al. (2001), Poyhonen et al. (2004), Ritz et al. (2001), Saul et al. (1989), Schipke et al. (1999), Scott et al. (2004), Selman et al. (1982), Stark et al. (2000), Strauss-Blasche et al. (2000), Taylor et al. (2001b), and Wilhelm et al. (2004).

Results across studies are highly consistent and very similar, no matter how RSA is quantified (e.g. including the most common methods of spectral analysis, peak-valley analysis or digitally filtered time-domain procedures). RSA magnitude under steady-state conditions is inversely related to respiration rate and directly related to tidal volume. Hence, rapid shallow breathing will greatly attenuate RSA, and slow deep breathing will produce maximal RSA levels. Respiration rate and tidal volume produce independent and interactive effects (e.g. Hirsch and Bishop, 1981). For example, increases of tidal volume at slower respiration rates will cause larger RSA elevations than the same tidal volume increases at more rapid respiration rates, although natural changes in respiration and tidal volume are usually reciprocal at stable levels of metabolic activity. Furthermore, these respiratory influences are sizeable within the normal physiological range of respiration rates and tidal volumes that characterize states of rest and mental or emotional activation (e.g. Althaus et al., 1998; Bernardi et al., 2000; Grossman et al., 1991). Therefore, an increase of only 3–4 breaths/min from rest to mental task may attenuate RSA magnitude substantially. Consequently, RSA magnitude under conditions of mental activation or emotion can be significantly altered solely as a function of those respiratory changes.

Measurements of RSA magnitude are commonly taken to indicate the level of cardiac vagal tone. This, as defined and understood in cardiovascular physiology, is the mean vagal efferent effect upon HR or RRI (Grossman and Kollai, 1993; Hayano et al., 1991; Kollai et al., 1994). Cardiac vagal tone is not constant for an individual but changes greatly as a consequence of age, posture, metabolic activity and other factors (e.g. Craft and Schwartz, 1995; Robinson et al., 1966). A number of investigations have shown that pharmacologically induced changes in cardiac vagal tone can be tracked by RSA amplitude: thus, stepwise decreases in cardiac vagal tone produced by the cumulative action of increasing doses of the muscarinic cholinergic receptor antagonist atropine, or other vagolytic (i.e. vagally blocking) agents, are accompanied by a dose-related reduction in RSA magnitude (Dellinger et al., 1987; Medigue et al., 2001; Pyetan et al., 2003; Raczkowska et al., 1983; Scheinin et al., 1999). Atropine does not produce a systematic effect upon respiratory parameters; therefore, alterations in cardiac vagal tone and RSA during atropine administration are not accompanied by changes in respiration rate or tidal volume (Elstad et al., 2001; Grossman and Kollai, 1993; Rauniar et al., 1998).

Respiratory influences upon RSA amplitude become a problem for assessment of cardiac vagal tone (1) whenever respiratory rate and/or tidal volume substantially differ between groups or conditions, and (2) whenever RSA, respiratory parameters and cardiac vagal tone all do *not* systematically covary with each other. We have examined this issue (Grossman et al., 1991) by adapting the same kind of pharmacological autonomic blockade paradigm that has long been employed in physiological studies to characterize autonomic control during specific challenges such as dynamic or isometric exercise. To evaluate the degree of covariation of RSA, respiration and vagal tone, we expanded the task domain to include mental tasks (a cognitive reaction-time task) and ventilatory tests (e.g. voluntary hyperventilation and CO_2

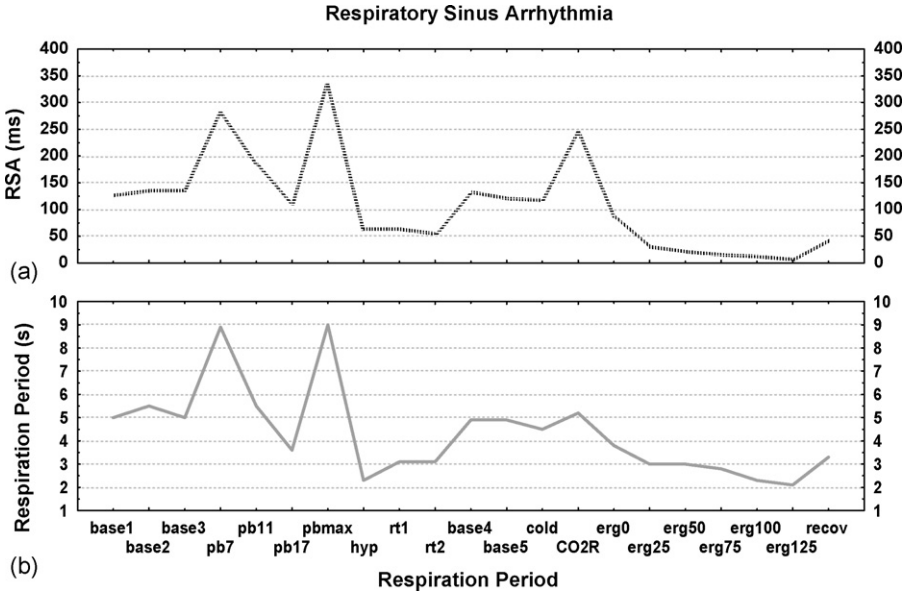

Fig. 1. Mean respiratory sinus arrhythmia (RSA; a) and respiration period (b) across different behavioral task; data from Grossman et al. (1991): five baseline phases (base), four paced-breathing phases (pb; 7, 11 and 17 cpm; max = 7 cpm at large tidal volume), hyperventilation (hyp), two cognitive reaction-time phases (rt), cold pressor (cold), bicycle ergometry at 0, 25, 50, 75, 100 and 125 W (erg), and recovery (recov). Note the similar patterning of respiratory period and RSA over the range of tasks, suggesting the very close within-individual relationship that was found.

rebreathing): healthy subjects were intravenously administered a dosage of propranolol sufficient to effectively block sympathetic, beta-adrenergic influences upon HR. Because variations in normal HR almost exclusively derive from sympathetic beta-adrenergic and vagal effects, this meant that HR changes under pharmacological beta-blockade could be ascribed solely to alterations in vagal tone. We were able, therefore, to answer the question of whether changes in RSA necessarily reflect alterations in cardiac vagal tone when respiratory rate and tidal volume vary.

Our findings clearly indicated that behaviorally induced fluctuations in cardiac vagal tone were not discernibly tracked by changes in RSA magnitude under variable respiration. RSA magnitude was much more closely related to changes in respiratory parameters – particularly respiration rate – than to any changes in cardiac vagal tone (Fig. 1a and b). It was only when respiratory variables were statistically controlled (using a within-individual regression approach) that there was a clearly improved but still imperfect association between RSA and vagal tone (Fig. 2a–c).

A second autonomic blockade study (Grossman and Kollai, 1993) that manipulated respiratory rate and volume under individual and dual beta-adrenergic and vagal blockade provided additional evidence that respiratory variation confounds RSA indices of within-subject changes in cardiac vagal tone. Alterations in respiration rate and tidal volume had profound effects upon RSA magnitude that were unrelated to directly determined levels of cardiac vagal tone.

We recently addressed the question of whether, under relatively constant levels of cardiac autonomic tone, normal respiratory variations to psychological tasks would significantly alter RSA independently of cardiac vagal tone (Grossman and Stemmler, in preparation). Within-subject relations between RSA and respiratory parameters were examined during performance of a variety of mental tasks during which autonomic tone appeared to be relatively constant. Tasks included completion of written psychological inventories, two rest phases, guided meditation, nonstressful speaking, quiet reading, attention to auditory stimuli (five levels), a memory-comparison reaction-time task (three levels), a computerized multiple-choice math task, and a post-task recovery period. Forty healthy adults (24–40 years) were tested. In order to create the least stressful environment possible, tasks were administered in a comfortable, nonclinical setting by an female assistant chosen and instructed to be very friendly and relaxed with subjects. We wanted to examine the physiological effects of discrete mental tasks, themselves, upon cardiorespiratory variables, relatively unconfounded by emotional stress.

No task-related HR changes were found. Self-reported negative affect (anxiety and anger/irritation complaints) also barely changed on a 10-point scale (from a mean low of 0.4 during meditation to a high of 0.9 during stress tasks). Therefore, results indicated that cardiac autonomic tone and mood were not significantly altered as a function of task.

Respiratory parameters, however, did reliably vary across tasks. Both respiration rate and tidal volume showed large condition effects, as did peak-valley RSA (see Fig. 3a and b). Using multiple regression with RSA as criterion measure and respiration rate and tidal volume as predictors, the average *within-subject* R was .76, indicating that almost 60% of the RSA variance could be accounted for by respiratory parameters. In the complete absence of parallel HR changes (i.e. no suggestion of cardiac autonomic change), these findings underscore the powerful confounding effects of respiratory parameters on RSA under conditions of psychological activation.

A recent paper has suggested that respiratory rate and tidal volume need not be considered in RSA studies of cardiac vagal control during non-exercise behavioral variations (Houtveen et al., 2002). However, the single mental stress task employed in

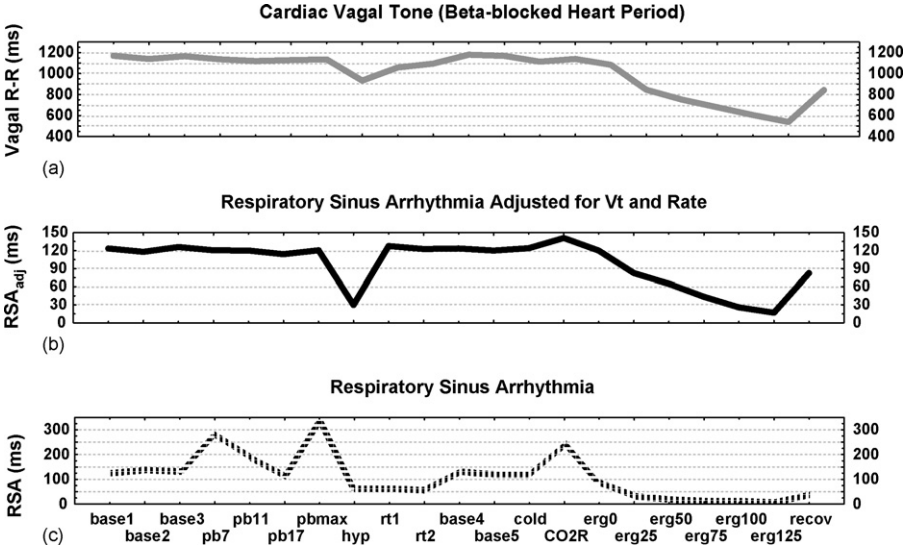

Fig. 2. Cardiac vagal tone (a), respiratory sinus arrhythmia adjusted within individual for respiratory variables (b) and raw unadjusted RSA (c) across conditions from (Grossman et al., 1991). Cardiac vagal tone (beta-blocked HR) and unadjusted RSA are obviously not strongly associated. However, RSA, corrected for respiratory parameters, shows a clear covariation with changes in cardiac vagal tone.

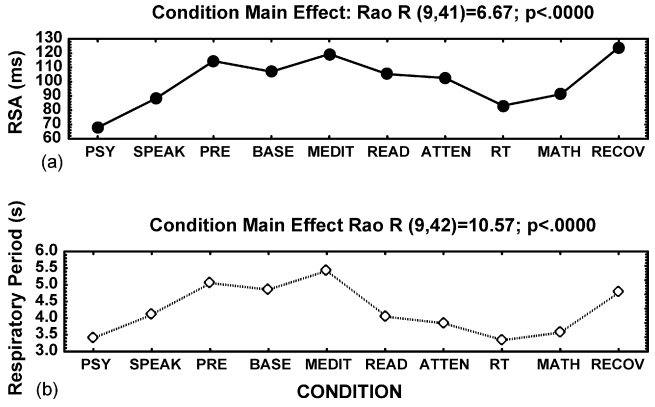

Fig. 3. RSA and respiration period across a number of mental tasks (Grossman and Stemmler, in preparation, $n = 40$ healthy subjects): completing written psychological inventories (PSY), conversational speaking (SPEAK), pre-baseline (PRE), baseline (BASE), guided meditation (MEDIT), reading (READ), auditory attention task (ATTEN), cognitive reaction-time task (RT), computerized, nonverbal math task (MATH), and recovery (RECOV); five levels of ATTEN and three levels of RT averaged. RSA and respiratory period show similar patterns of change, and RSA was not related to HR across conditions (not shown); HR only varied as a function of time (decreasing across the fixed order of tasks).

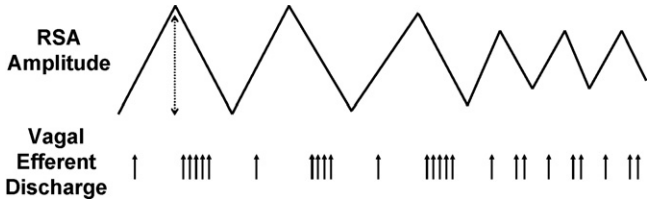

Fig. 4. A simulated model of relations between RSA (top) and bursts of vagal efferent traffic to the heart (bottom) as a function of respiration period (vertical arrow indicates RSA magnitude). The first three RSA cycles occur at a respiration period that is twice as long as the last three cycles. Note that the same quantity of vagal bursts occur per time unit (i.e. six bursts during one slow breath; six breaths during two rapid breaths), indicating the same mean level of vagal discharge per minute, but a different pattern of vagal discharge and impact on RSA at different respiration rates.

that study exerted an unusually small effect upon respiration rate (<2 breaths/min) compared to what others and we typically find (e.g. Figs. 1 and 3). It may be expected that smaller respiratory changes will have lesser effects on RSA. Additionally, determination of cardiac autonomic control was made purely on the basis of indirect sympathetic and vagal indices, and they ignored potentially important sympathetic–parasympathetic interactions (see later discussion). Nevertheless, this study should remind us that not all conditions will exert significant effects upon respiratory parameters (e.g. Gianaros and Quigley, 2001; Gianaros et al., 2001; Grossman et al., 2001). In the absence of respiratory differences between groups or tasks, the respiratory confound is obviously not a problem. Nevertheless, as indicated in the preceding discussion, respiration is often variable and may significantly affect RSA magnitude, independently of changes in cardiac vagal tone. The only way to ascertain whether RSA differences are related to respiratory parameters in a particular study is to measure both respiration and RSA, as well as to examine relations between the two.

2.1. Why does RSA, unrelated to vagal tone, vary with respiration?

In order to understand the autonomic contributions to the phasic waxing and waning of HR that define RSA, consider the distinction between *phasic* and *tonic* influences. Assuming that RSA is primarily vagally mediated, the most likely explanation is that those RSA variations that are due to changes of respiration rate and volume, represent merely differences in *phasic* patterning of vagal effects upon HR, without necessarily reflecting differences in mean vagal influence upon the heart. Clear evidence for this effect has been shown in dogs (Hedman et al., 1995a). The simulated data in Fig. 4 illustrates a case in which the same mean vagal discharge (i.e. cardiac vagal tone) occurs at two different respiration rates, with the faster rate twice the respiratory period length as the slower rate. In agreement with current knowledge, vagal efferent discharge to the heart is largely gated (blocked) during inspiration and active during expiration.

The figure depicts an equal number of vagal bursts during two shorter breaths as during one longer breath (i.e. six bursts), indicating the same mean vagal discharge per minute (i.e. cardiac vagal tone) for faster and slower breathing. The close grouping of five bursts during the expiration of slow breathing is certain to produce greater expiratory reduction of HR than the mere two bursts during the expiration when breathing is twice as fast. Hence, the average cardiac vagal tone remains the same, although RSA magnitude is very different during rapid *versus* slow breathing. The varying pattern and magnitude of RSA would tell us little about *tonic* vagal mechanisms but does indicate that breathing frequency has changed the *phasic* pattern.

2.2. Practical solutions to exclude or control for respiratory variables when measuring RSA

Based on the preceding discussion, it should be obvious that respiratory parameters may confound the relation between RSA and cardiac vagal tone. There are, nevertheless, some strategies that can be employed to eliminate or at least reduce the problem (Grossman et al., 1991, 1990a; Wilhelm et al., 2004). One approach, sometimes possible, is to have subjects pace their breathing at a certain rate during study tasks. We have found an auditory signal that patterns inspiration and expiration times to be most effective, in terms of comfort, ease, and quickness to learn (Grossman et al., 2001; Wilhelm et al., 2004). This procedure is feasible when other behavioral demands are absent or minimal (resting baseline, or an easy attention task). It does, nevertheless, require pre-task training to ensure that subjects are comfortable with the pacing, can perform it rather automatically and can find the right depth of breathing, so that they do not hyperventilate (i.e. that they do not breathe too deeply). With sufficient training there is evidence that respiratory pacing even under more complex psychological demands does not notably distort normal cardiovascular

responses (Grossman et al., 1990a,b), although this approach may not be easy to achieve, especially with mentally more demanding tasks.

Objections that paced breathing affects RSA differently than spontaneous breathing are largely unsupported: the vast majority of carefully performed investigations support the idea that relations between RSA and respiratory measures remain the same under spontaneous and voluntarily paced respiratory conditions (e.g. Ben Lamine et al., 2004; Bloomfield et al., 2001; Eckberg et al., 1976; Grossman et al., 1991; Hirsch and Bishop, 1981; Patwardhan et al., 2001, 1995). This makes sense, because respiration during awake states is importantly under the regulation of higher brain centers that control voluntary behavior (Longobardo et al., 2002). Therefore, covariation between RSA magnitude and respiratory parameters during alert states inherently reflects the interaction of cardiovascular control mechanisms and higher central nervous system (CNS) behavioral control of breathing.

Another strategy is statistical control for respiratory parameters, using covariance procedures. This is a frequently employed approach (e.g. Burleson et al., 2003; Hughes and Stoney, 2000), but is problematic when a simple between-group analysis of covariance is performed, with respiratory measures as covariates: relations between RSA and respiratory measures are systemic, that is to say that these relationships are typically very strong within individuals but not between individuals (e.g. Ben Lamine et al., 2004). This is because (a) the magnitude of RSA is highly variable between subjects, and (b) the slope of the regression between RSA and respiratory variables can also vary greatly from subject to subject, independent of individual differences in RSA magnitude, even at very similar levels of within-individual correlation. A normal analysis of covariance approach pools between- and within-subject variance and can lead to erroneous conclusions about the contribution of respiratory parameters to RSA fluctuation, due to violations of assumptions concerning homogeneous variances/covariances per cell (Browne and Shapiro, 1991). Simulated data in Fig. 5a–d illustrate this point, by displaying that even with a large difference between groups in respiratory period response from baseline to task, analysis of covariance effects for RSA controlled for respiratory period are still significant; each of the simulated subjects showed a covariation between RSA and respiratory period, but the slopes of the within-subject relations were highly variable within and between cells.

A more appropriate procedure may be a *within-subject* approach in which RSA is residualized against respiratory variables (Grossman et al., 1991). This is performed when respiration rate or tidal volume changes as a consequence of experimental conditions or time. Several condition or measurement epochs are needed to compute individual regressions. Condition levels of RSA are regressed against respiration variables using a multiple regression analysis, and the residuals are used as an index of vagal tone (RSA variation disproportional to respiratory change; positive residuals index increase in cardiac vagal tone, and negative values reflect vagal withdrawal; see Fig. 2b). However, this procedure may yield a somewhat conservative estimate of cardiac vagal control under circumstances in which respiratory parameters partially covary with vagal control.

Yet another approach is to pace subjects across the normal physiological range of respiration rates during a baseline period. Once again, care must be taken in training subjects to ensure that subjects remain eucapnic (i.e. do not hyperventilate) and are not so uncomfortable or distressed by the pacing that autonomic tone changes. We have found that with clear instruction, most subjects can ordinarily learn within 10 min to comfortably pace their breathing at a range of rates, at the same time maintaining baseline HRs and avoiding hyperventilation. Also stable estimates of RSA can be acquired with pacings of about 1–1.5 min per respiratory rate. Therefore, even pacing across five frequencies can be accomplished without excessively prolonging a protocol. The regression line of RSA on respiratory parameters can then be used to estimate task-related RSA changes that systematically exceed or are lower than expected values (i.e. reflecting cardiac vagal augmentation or decline, respectively).

Tidal volume is often considered a less important respiratory variable to control in RSA research (Berntson et al., 1997). Nevertheless, tidal volume can have marked independent effects upon RSA magnitude (Hirsch and Bishop, 1981), and attention to tidal volume may be warranted whenever it changes substantially and these changes are not tightly reciprocally related to changes in respiration rate (e.g. Grossman et al., 1991, 2004; Ritz et al., 2001). Tidal volume can be nonintrusively assessed rather easily and inexpensively using air bellows strain gauges (see Morel et al., 1983).

A common approach that adjusts for the influence of tidal volume upon RSA is to calculate the transfer function (Berger et al., 1989; Grossman et al., 1991, 2004; Wilhelm et al., 2004). This procedure derives the gain of RSA related to tidal volume (ms RSA per liter tidal volume). The measure is simply RSA divided by tidal volume when time domain-measures are used, or the transfer function from cross-spectral analysis of the RRI and respiratory time series when spectral analysis is employed. In both cases, it characterizes the amount of RSA amplitude change per liter tidal volume. Due to the reciprocal relation between rate and tidal volume, this adjustment may dampen or even eliminate the RSA dependency upon respiration rate: in an ambulatory study of alert, active subjects (Grossman et al., 2004), the transfer function was more closely associated with cardiac vagal tone than raw, unadjusted RSA; additionally statistical control for respiration rate did not improve the degree of correlation between the adjusted measure and cardiac vagal tone. On the other hand, the transfer function measure appears to remain importantly related to respiration rate under laboratory conditions (where physical activity does not exert a large impact on cardiac vagal tone; Berger et al., 1989; Grossman et al., 1991; Wilhelm et al., 2004). Therefore, transfer function analysis can be used as an adjustment to variations in tidal volume, and, perhaps, requires no additional control for respiration rate when the major focus is upon metabolically associated changes in cardiac vagal tone (as in most ambulatory studies). However, this parameter alone does not appear adequate for evaluating tonic vagal changes under

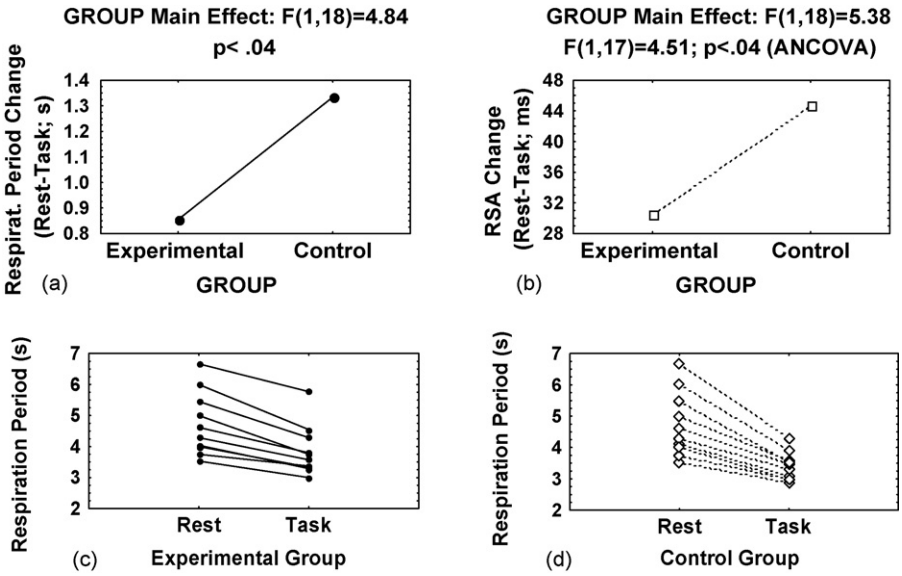

Fig. 5. Simulated respiration period and RSA data of two groups (experimental *vs.* control) during rest and task. RSA and respiration period decreased from rest to task; the control group showed larger changes (mean changes in a and b). (c) and (d), both individual data, indicate that the control group consistently manifested larger decreases in respiration period, although the main effect for RSA change was still significant after a group analysis of covariance (ANCOVA) adjustment (see b). In all cases, RSA decrease was accompanied by respiration period reduction, although, corresponding to real findings, within-individual slopes of the relation were highly variable.

the typically sedentary circumstances of most psychophysiological or clinical investigations.

The above-described approaches all have methodological and practical limitations. Furthermore, there is no systematic evidence about which method is superior for estimation of cardiac vagal tone. Hopefully enhanced awareness of the problem of respiratory confounding of vagal tone estimation will lead to additional research regarding this matter.

3. Individual differences in vagal tone may not always be reflected by variations of RSA

Studies validating RSA as a *within-individual* index of cardiac vagal tone have shown RSA to decrease proportionately to levels of atropine-induced cardiac vagal withdrawal. Along a different line, *individual differences* in cardiac vagal tone among humans are defined as the decrease in mean RRI (ms), produced by complete vagal blockade using atropine or some other vagolytic drug under basal conditions. In other words, cardiac vagal tone is operationalized as the difference between the average RRI before atropine infusion and the mean RRI after maximal dose of atropine (at which HR change is maximal). Several studies have examined the correlation between RSA and this pharmacological measure of cardiac vagal tone, considered the gold standard of estimation in human cardiovascular physiology (Cacioppo et al., 1994; Fouad et al., 1984; Grossman and Kollai, 1993; Hayano et al., 1991; Kollai and Mizsei, 1990; Maciel et al., 1985). All but two studies (Cacioppo et al., 1994; Maciel et al., 1985) found a significant correlation between cardiac vagal tone and RSA. However, the extent of the association varied widely in those investigations that did find a relationship, with r's ranging from 0.5 to 0.9. In the two studies with the highest level of association (both $r = 0.9$), methodological issues cast doubt over the actual strength of the relation between RSA and vagal tone in a homogeneous population: in one study (Fouad et al., 1984), hypertensives and normotensives were combined in the data set. It is well known that hypertension reduces RSA and vagal tone, and this would serve to exaggerate both the range of normal variation of measures and the correlation coefficient. A similar effect could be expected from the other investigation (Hayano et al., 1991) that pooled half sedentary and half physically active subjects in the analyzed sample (physically active individuals often show higher levels of RSA, e.g. Dixon et al., 1992; Shin et al., 1997). The other larger and homogeneous sample of young healthy subjects (Grossman and Kollai, 1993; Kollai and Mizsei, 1990) reported much more modest correlations between 0.5 and 0.6, indicating that only 1/4 to 1/3 of the individual variation in cardiac vagal tone could be explained by RSA alone, even when respiratory parameters were controlled. However, the inclusion of HR as an additional predictor increased explained variance to 76% ($r = 0.9$).

Taken together, these studies indicate that caution is warranted when employing RSA as an index of individual differences in cardiac vagal tone. There is apparently a relationship, but it has not been demonstrated to be close enough to assume RSA to be more than roughly associated with individual variations in cardiac vagal tone. In fact, in one set of studies, resting HR, in comparison to RSA, was much more highly correlated to cardiac vagal tone (Grossman and Kollai, 1993; Kollai and Mizsei, 1990); a later report of a small sample of female students yielded similar results (Cacioppo et al., 1994). These findings suggest the utility of including both variables whenever predicting individual differences in cardiac vagal tone. They also suggest that baseline HR may be as good or better an index of individual differences in

cardiac vagal tone than RSA. This evidence has long been neglected in the literature and may have implications for our understanding of RSA and autonomic control. Exactly what individual differences in RSA represent, thus, remains an intriguing question.

4. Concurrent physical activity alters cardiac vagal tone and RSA

Recent evidence (Bernardi et al., 1996; Grossman et al., 2004) shows that accurate estimation of RSA is seriously biased by variations in concurrent physical activity. Heart rate during mild-to-moderate change in physical activity, characteristic of normal variations of daily activity, is predominantly under parasympathetic control (Boushel et al., 2001; Ekblom et al., 1972; Epstein et al., 1965; Grossman et al., 1991; Hopkins et al., 2003; Janicki et al., 1996; Maciel et al., 1986; O'Leary and Seamans, 1993; Robinson et al., 1966, 1953; Rowell and O'Leary, 1990; Vatner and Pagani, 1976). Thus, disease-specific, temperamental or other psychological effects upon range, frequency and duration of daily activity may interact with and confound the assessment of individual differences in autonomic regulation and RSA during ambulatory monitoring. Markers of cardiac vagal activity may reflect not only individual differences in constitutional parasympathetic control but also variations in daily activity pattern. Fig. 6 illustrates the extent of effects of concurrent activity upon RSA magnitude among young, healthy adults (Grossman et al., 2004), displayed as a function of quintiles of physical activity during awake hours. There is a systematic decrease in RSA as activity increases.

Although it seems clear that certain clinical groups may differ in activity from healthy individuals, it is less obvious that even small differences of activity in the laboratory or field may also have significant effects upon RSA magnitude: we found in the same study that the distinction in activity level in the lowest two quintiles was extremely subtle (see Fig. 7). Nevertheless, RSA differed markedly and very reliably between these lowest quintiles of activity (see Fig. 6). Therefore, it is plausible that even small laboratory differences in movement during baseline measurement (i.e. frequent postural shifts, tapping or even subtle limb or body motion) may produce effects upon RSA that could be wrongly inferred as evidence of constitutional differences in autonomic control. Simultaneous monitoring of activity in field, experimental and clinical settings may, therefore, be required when examining group differences in RSA, especially when group effects, as so often the case, are significant but relatively small in magnitude.

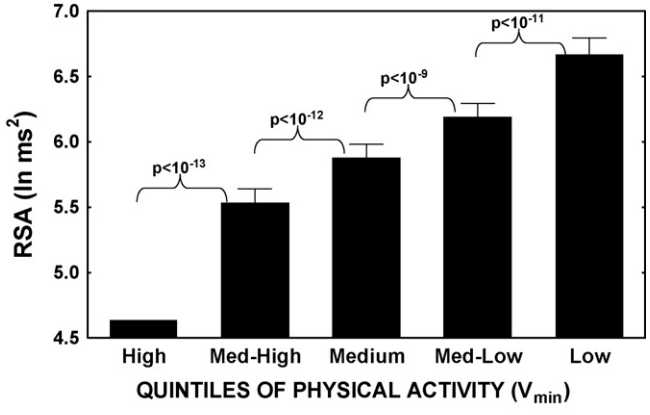

Fig. 6. RSA magnitude (ln spectral power) as a function of quintile of physical activity (based on minute ventilation, V_{min}) during awake ambulatory recording over 12 h in 40 healthy subjects (from Grossman et al., 2004). Quintiles were determined by evenly distributing activity levels over time (i.e. the highest quintile being equivalent to mean RSA for that 20% of the day during which V_{min} was highest; this analysis was made for each subject, then averaged over all subjects). RSA varied lawfully across all levels of activity, as indicated by the p values.

5. RSA is affected by sympathetic tone and may not be a 'pure' vagal index

As previously mentioned, within-individual validation studies of RSA are based upon evidence of changes in RSA during progressive pharmacological blockade of cardiac parasympathetic control (Ali-Melkkila et al., 1991; Coker et al., 1984; Dellinger et al., 1987; Hayano et al., 1991; Julu and Hondo, 1992; Medigue et al., 2001; Pyetan et al., 2003; Raczkowska et al., 1983; Scheinin et al., 1999). In almost all studies, an exponential reduction of RSA (quantified in different ways) is found as HR rises. In other words, HR increases as a function of progressive attenuation of vagal tone, and RSA tracks that change (exponentially) and is, consequently, assumed to be a noninvasive marker of the changing mean level of cardiac vagal tone.

HR fluctuations, as previously noted, are under the joint control of sympathetic beta-adrenergic and parasympathetic branches of the autonomic nervous system. In some of these validation investigations, pretreatment with a beta-adrenergic blocking drug was performed to prevent cardiac sympathetic influences from affecting the findings (e.g. Coker et al., 1984; Hayano et al., 1991; Medigue et al., 2001). Sympathetic blockade made little difference, in terms, of the *relative* relations found between RRI and RSA during progressive vagal

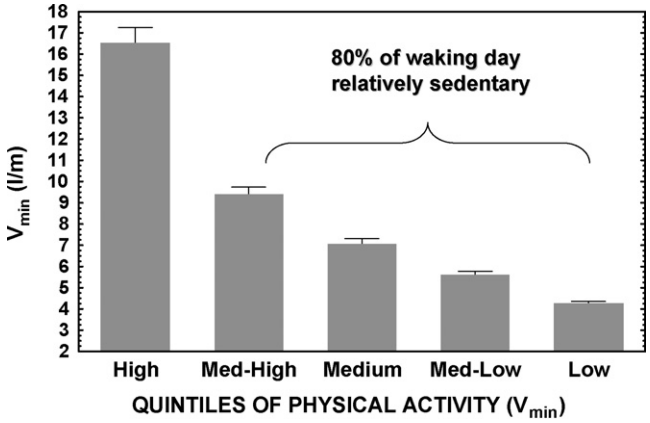

Fig. 7. Physical activity levels (V_{min}) across the quintiles (Grossman et al., 2004), but not reported there); quintile calculation described in Fig. 6. Note that physical activity is relatively sedentary during normal awake functioning, especially at the lowest two quintiles.

withdrawal, attesting to the parasympathetic effects of the vagolytic drugs (e.g. Grossman and Kollai, 1993). However, an important finding in a large number of dual cardiac blockade investigations (vagal and sympathetic) was that beta-adrenergic blockade augmented RSA under baseline conditions (e.g. Eckberg et al., 1976; Coker et al., 1984; Grossman and Kollai, 1993; Pitzalis et al., 1998; Taylor et al., 2001b), unrelated to respiratory alteration. This effect was often relatively large (RSA increases typically $\geq$30%) and was independent of whether the beta-blocker was cardiac-specific (i.e. affected only the periphery) or had central effects because the drug crossed the blood–brain barrier (Pitzalis et al., 1998). Even in small-sample studies in which this effect was not significant, mean RSA tended to increase under beta-blockade, e.g. Cacioppo et al. (1994). For example, beta-blockade during baseline sitting in the latter investigation induced a mean RSA increase of 65% when reported natural log units were transformed to ms^2 spectral power (28% if converted to time domain measures).

Beta-adrenergic effects upon RSA magnitude are relevant for physiological and psychophysiological studies, because the change in cardiac sympathetic tone (often about 150 ms RRI or 10 bpm HR; e.g. Cacioppo et al., 1994) is within the range of what can be expected in laboratory situations or among different clinical samples. The finding of sympathetic effects upon RSA magnitude points to a serious problem of intra-individual validation studies. Namely, throughout the validation literature, the covarying RSA and RRI decline during vagal withdrawal has always been examined within the context of stable levels of cardiac sympathetic tone (either during or without beta-blockade). These investigations have provided necessary *but insufficient* evidence for the general utility of RSA as a cardiac vagal index: they showed that RSA was sensitive to variations in cardiac vagal tone when cardiac sympathetic tone was absent, or was relatively low and stable. However, they failed to confirm the parasympathetic specificity of RSA, i.e. that no matter how cardiac sympathetic activity changes, RSA always specifically reflects cardiac vagal control. Beta-adrenergic effects upon RSA challenge the vagal specificity of RSA.

There are several possible interpretations of these findings: (a) cardiac vagal tone is directly altered by varying levels of sympathetic effects upon HR; (b) RSA, but not cardiac vagal tone, is influenced by changes in cardiac sympathetic tone; or (c) vagal-sympathetic interactions can occur that alter the relation between RSA and cardiac vagal tone in predictable or unpredictable ways. Whichever in the end proves correct, any of these possibilities, at the very least, clearly indicates that concurrent levels of cardiac sympathetic activity can alter respiratory modulation of HR (i.e. RSA).

One invasive dog study (Hedman et al., 1995b), indeed, may confirm that RSA, but not mean level of cardiac vagal efferent activity (vagal outflow from the brain to the heart), is altered by beta-adrenergic blockade: sympathetic and vagal nerves to the heart were electrically stimulated after their pathways from the brain were severed. Vagal nerve impulses were rhythmically stimulated so as to simulate RSA patterns at a frequency of 12 cpm, and this patterning of impulses was presented both in the presence and in the absence of mild-to-moderate sympathetic stimulation. In comparison to a background of no sympathetic activity, even mild sympathetic stimulation drastically attenuated RSA, despite the fact that mean levels of efferent vagal traffic to the heart remained constant.

What seems clear from this and other investigations is that even mild levels of sympathetic activity can substantially depress RSA, as a function of either sympathetic–vagal interactions or a direct suppressive effect upon RSA magnitude.

In human studies, there are indications that the influence of beta-blockade on RSA can be removed or greatly attenuated by a simple procedure that normalizes RSA for mean RRI (Hayano et al., 1990):

$$\text{RSAnorm} = \sqrt{\frac{(\text{spectral power of high-frequency RRI power } (\text{ms}^2))}{\text{mean RRI } (\text{ms})}}$$

In the case of time-domain peak-valley amplitude measures (which are linearly related to the square root of the spectral power; Laude et al., 1995), the peak-valley measure is divided by RRI.

This normalized index has been found to be more sensitive to incremental vagal blockade than more commonly used indices (e.g. the spectral power uncorrected) in one sophisticated and well-documented pharmacological investigation (Scheinin et al., 1999). Therefore, we explored whether this RSA measure, which corrects for changes in mean RRI, would be less sensitive to beta-adrenergic effects than uncorrected measures of RSA.

We found one study that reported beta-blockade to have no effect upon this measure (Schachinger et al., 2001). Additionally, we calculated this normalized index from mean group data in three studies that had reported clear effects of beta-blockade upon RSA (Grossman and Kollai, 1993; Pitzalis et al., 1998; Taylor et al., 2001b). Beta-blocker effects were almost completely removed (all differences between beta-blockade and no blockade $<$10%). This was also confirmed in data recalculated from our own study (Grossman and Kollai, 1993), which found a 30% increase in peak-valley RSA with beta-blockade before normalizing, and a 5% difference after normalizing for mean RRI.

These findings suggest that RRI correction of RSA can reduce or eliminate the influence of basal levels of cardiac sympathetic tone (resting HR with *versus* without the normally mild level of sympathetic activity). In the autonomic stimulation study described above (Hedman et al., 1995b), normalized RSA also substantially lowered the impact of sympathetic stimulation: with a constant level of vagal efferent discharge, normalized RSA was reduced 50% during sympathetic stimulation, compared to no sympathetic stimulation; without correction, RSA was reduced by a factor of 12.

5.1. RSA and vagal–sympathetic interactions

In light of preceding conclusions, it may be useful to consider more precisely the meaning of the term cardiac vagal

tone. Many scientists assume that cardiac vagal tone reflects the extent of central vagal discharge to the heart, but the term is actually operationalized as the mean level of cardiac vagal effects upon HR over a defined interval of time. This definition focuses upon the *final vagal effects upon HR*. Vagally mediated HR changes depend, in turn, upon a number of prior processes and events.

Limiting discussion to the efferent side of vagal neural transmission, the vagus nerve must first transmit efferent activity in the direction of the sinoatrial node, located in the posterior wall of the heart's right atrium. In healthy individuals, the sinoatrial node initiates each beat of the heart, and vagal effects on HR are contingent upon the release of acetylcholine from the parasympathetic nerve endings at junctures to the sinoatrial node. Any process that interferes with acetylcholine transmission can alter the relationship between central vagal efferent traffic and its effect on HR. For example, as dosage rises, atropine appears to progressively increase average central vagal efferent traffic to the heart, while, at the same time, it blocks the action of acetylcholine at the periphery, i.e. at the sinoatrial node (Katona et al., 1977). The net result at higher doses of atropine is complete vagal blockade – elimination of cardiac vagal tone – despite elevated, mean levels of central vagal efferent activity.

Findings from the previously described experimental study (Hedman et al., 1995b) showed that mild-to-moderate stimulation of cardiac sympathetic activity greatly attenuated beat-to-beat modulation of RRI (simulated RSA) in dogs, although average level of vagal efferent traffic to the heart remained constant during both the absence and presence of sympathetic stimulation. Perhaps related to the effects of the latter study, numerous investigations have demonstrated that neuropeptide Y, an important neurotransmitter, inhibits the release of acetylcholine at parasympathetic effector junctions *of the heart, itself* (including the sinoatrial node), and thereby reduces effectiveness of vagal action on the heart (e.g. Moriarty et al., 1993a,b; Serone and Angus, 1999; Smith-White et al., 1999, 2002; Warner and Levy, 1989).

In mammals, it has long been recognized that the cardiac response to neural activity in one autonomic division depends on levels of activity in the other division (Levy, 1984). Complex peripheral interactions between sympathetic and parasympathetic nerve supplies to the heart are of importance in modulating control of cardiac function. Many of the terminal fibers of the two subdivisions of the autonomic nervous system lie close to each other on the mammalian heart (Jacobowitz, 1967). Consequently, transmitters released from the nerve endings of one division can readily diffuse to the nerve terminals of the other, as well as to the cells of the cardiac ganglion and the myocardium (Revington and McCloskey, 1990).

These and many others studies illustrate that what we measure with any HR index of vagal tone are *only the final functional vagal effects* on cardiac activity. Attenuation of cardiac vagal tone may be caused by reduced central vagal efferent activity, blocking of preganglionic or postganglionic neuronal actions of acetylcholine, impaired vagus nerve conduction, or combined mechanisms. A pronounced decrease in RSA magnitude may therefore signify (1) true reduction of vagal outflow from brain to heart, (2) a primary increase in sympathetic tone that leads to an interaction with vagal activity in the periphery, or (3) both. Such considerations underline the difficulties in inferring CNS mechanisms whenever there is a reduction in RSA, even when respiratory parameters are controlled and when RSA does reflect some aspect of parasympathetic modulation.

These interactions, nevertheless, may not always be apparent in pharmacological blockade studies (e.g. Berntson et al., 1994; Cacioppo et al., 1994). However, autonomic blockade studies, such as the latter, in which sympathetic and vagal influences are eliminated one at a time or jointly, may not be informative about interactions between the two autonomic branches when both are active to varying degrees. Interactions also depend upon the extent of activation of both autonomic branches (Levy, 1984). For example, in the previously described canine study (Hedman et al., 1995b), substantial RSA attenuation was found at mild to moderate levels of cardiac sympathetic stimulation. On the other hand, under conditions during which sympathetic stimulation is consistently very modest (e.g. estimated sympathetic effects upon RRI $\leq$ 30 ms, in Berntson et al., 1994), autonomic interactions may play an insignificant role.

To further complicate the matter, there are numerous ways in which autonomic blocking drugs may potentially influence estimations of cardiac autonomic tone: drugs like atropine and different beta-blockers may sometimes have central effects, as well as induce indirect, secondary effects due to distressing symptoms. However, the use of double blockade studies would seem to eliminate such sources of bias when only two influences can be responsible (e.g. vagal and sympathetic effects upon HR). Moreover, sequential studies of dual vagal and beta-sympathetic blockade do not indicate that central or indirect concomitants of autonomic blockade significantly bias estimates of cardiac autonomic tone (e.g. Kollai et al., 1994). Nevertheless, further research may still be useful to examine such possible influences upon pharmacological determination of cardiac parasympathetic and sympathetic tone.

Whatever the explanation for occasionally disparate findings, the repeated evidence of beta-sympathetic influence upon RSA and peripheral sympathetic–parasympathetic interactions should be of concern for both physiological and psychophysiological investigations of RSA and cardiac vagal control. This does not mean we should cease employing RSA as a marker of *final* vagal effects upon the heart. However, it does imply that variations in RSA magnitude currently provide an unreliable index of *central* vagal outflow or tone. The implications for interpretation of past and present RSA research are far-reaching.

6. RSA and cardiac vagal tone can dissociate under certain circumstances

There is evidence that RSA magnitude can sometimes dissociate from cardiac vagal tone, even under conditions of no

apparent sympathetic–vagal interaction and when respiration is controlled. One example replicated in several studies is when extreme levels of cardiac vagal tone are provoked by pharmacological enhancement of the cardiac baroreflex (Goldberger et al., 1994, 1996, 2001). The baroreflex is a cardiovascular feedback system with a primary function to stabilize blood pressure within a certain range. An important component of the baroreflex is a vagally mediated slowing of HR when blood pressure rises above a set point (slowing the heart will reduce levels of arterial blood flow and consequently blood pressure). Therefore, phenylephrine, a drug that raises blood pressure produces increased cardiac vagal tone in a dose–response manner, i.e. HR progressively slows as a result of enhanced vagal tone. The relation between RSA and vagal tone (vagal HR change) is relatively proportional and linear until HR slows down to very low levels. As HR further decreases, so does magnitude of RSA (e.g. Goldberger et al., 2001). The relation between RSA and vagal tone is quadratic across the entire range of vagally mediated HR change (see Fig. 8). Why this happens is unclear but may have something to do with saturation of the vagal effects across the respiratory cycle, loss of phasic respiratory changes in vagal nerve discharge, or a simple floor effect in which minimal HR has no room left to oscillate.

Another instance of dissociation between cardiac vagal tone and RSA appears to occur during stimulation of the carbon dioxide chemoreceptors (involved in the control of respiration; Sasano et al., 2002; Yasuma and Hayano, 2001; Yasuma et al., 2001). When high levels of inspired CO_2 stimulate these receptors, RSA increases disproportionately to changes in cardiac vagal tone (or respiratory variables; also see the CO_2 rebreathing condition of Fig. 2a and b; Grossman et al., 1991). On the basis of this and other evidence, it has been suggested that the primary adaptive function of this RSA augmentation during elevated levels of inspired and arterial CO_2 is to expel excess levels of CO_2 from pulmonary circulation, thus enhancing pulmonary gas exchange (Hayano and Yasuma, 2003).

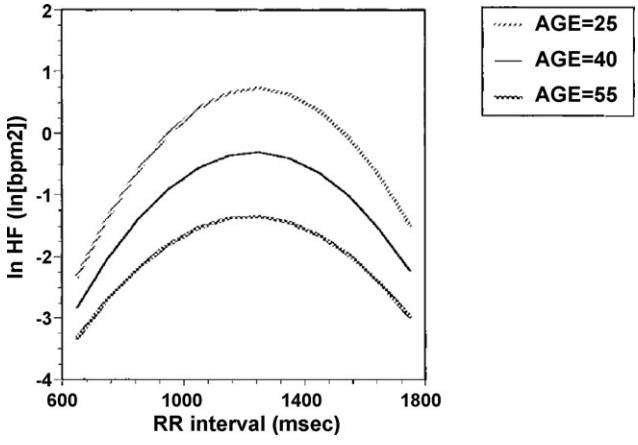

Fig. 8. Dissociation between RSA and cardiac vagal tone during baroreflex stimulation (from Goldberger et al., 2001). RR-interval alteration reflects pharmacologically induced (phenylephrine) change in cardiac vagal tone as a consequence of baroreflex-mediated parasympathetic responses; RSA (y axis) is the natural logarithm of high-frequency HR variability (ln HF) in bpm^2. There is a quadratic relation between RSA and cardiac vagal tone that flattens somewhat with increasing age (to be reprinted with permission).

These and other investigations (e.g. infant RSA during attentional responses; Richards and Casey, 1991) point to specific conditions during which RSA is not an accurate index of cardiac vagal tone. It should be noted, however, that these instances often seem to be at the outer range of normal physiological functioning (see Fig. 8). In the baroreflex studies described above, the association between RSA and vagally induced HR change was directly proportional until HR fell to approximately 40 bpm, clearly at the bottom range of HR seen during normal physiological states. Likewise, the elevated levels of CO_2 in the second set of experiments could only be expected during respiratory distress approaching asphyxiation. Therefore, these studies, by themselves, do not severely compromise RSA as an index of cardiac vagal tone within a wide spectrum of normal physiological functioning. However, the findings once again underline the fact that RSA is not synonymous with vagal tone and point to the risks in assuming that RSA variations necessarily reflect cardiac vagal tone in every circumstance.

The preceding discussion highlighted a number of issues that should be considered when employing RSA as an index of cardiac vagal tone. A substantial body of literature documents the value of RSA, although it is often replete with contrasting findings. Our preceding review should not be taken to negate the utility of RSA as a marker of vagal control. To the contrary, we hope it many be used as a guide to improve understanding and measurement of RSA and cardiac vagal activity. With these aims in mind, Table 1 provides a list of possible solutions to the various concerns and caveats we have here raised.

7. A critique and an alternative to the polyvagal theory

As stated earlier, the polyvagal theory was first proposed in 1995 (Porges, 1995, 2001, 2003b) as an attempt to (a) introduce an evolutionary perspective into relations between parasympathetic activity and behavior (possibly on the basis of a phylogenetic overview of vagal control of the heart in vertebrates reviewed by Taylor, 1994, as suggested by Medigue et al., 2001), and (b) to explain situations in which changes in RSA clearly do not correspond to alterations in vagally mediated HR (i.e. cardiac vagal tone). In recent years, this theory has been expanded to encompass a wide range of postulates regarding physical, psychophysiological and even social functioning, including speculations regarding various disorders including asthma, autism, and post-traumatic stress disorder (e.g. Porges, 2003b; Sahar et al., 2001).

At the core of the polyvagal theory lie several assumptions regarding two separate populations of vagal nuclei that reside in the brainstem – the ventrally located nucleus ambiguus (nA) and the dorsal motor nucleus (DMN) – and their role in vertebrate evolution. Fig. 9 provides a cartoon of the two brainstem nuclei, their cardiac projections, and the functional respiratory gating of central vagal outflow that characterizes RSA. The polyvagal theory maintains that these areas not only represent functionally distinct vagal systems in the mammal but also that they can be clearly characterized in terms of their evolution (Porges, 1995, 2003b): the DMN is proposed as the

Table 1
Proposed methods for addressing caveats of RSA estimation of cardiac vagal control

Problems with RSA as index of cardiac vagal tone	Possible methods to improve estimation
Respiratory confounds of within-individual RSA change	1. Pace breathing during experimental measurement at standard respiration rate(s) 2. Examine deviations between RSA and respiration during experimental conditions in relation to RSA at different paced respiratory rates during basal conditions 3. Perform within-subject residual analysis of RSA upon respiration rate and tidal volume 4. Normalize RSA for tidal volume (probably valid for ambulatory but not for laboratory conditions)
RSA as index of individual differences in cardiac vagal tone	1. Always examine and report mean heart-rate levels 2. Employ both RSA and heart rate as joint predictors of cardiac vagal tone 3. Assume that RSA is an approximate *marker* of individual differences in vagal tone, and not vagal tone, itself
Beta-adrenergic confounding of RSA as a within-subject vagal index	1. Possibly normalize RSA for heart rate 2. When RSA is large (under controlled respiratory conditions), assume high levels of central vagal discharge and high levels of cardiac vagal tone 3. When RSA is small (under controlled respiratory conditions), assume that cardiac vagal tone is low, but level of central vagal traffic to heart is unknown (due to possible sympathetic-vagal interactions at heart): RSA = final vagal effects upon sinus node 4. When RSA changes (under controlled respiratory conditions) but indices of cardiac sympathetic tone remain constant (e.g. pre-ejection period), assume cardiac vagal tone changes
Physical activity confounding of RSA and vagal tone	1. Measure and control for degree of activity and posture, even under sedentary conditions 2. Recognize the potent change of RSA in response to metabolic changes
Dissociation of RSA and cardiac vagal tone	1. Always examine and report heart-rate levels 2. Suspect dissociation under circumstances whenever both heart rate and RSA become extremely low 3. Make sure that heart rate is at least twice that of respiration rate to prevent cardiac aliasing (see Section 7.1) (a potential problem with very young infants, certain clinical disorders, and other species)

sole vagal system in reptiles (and by implication all non-mammalian vertebrates) and therefore phylogenetically more primitive than the nA; the primary function of the DMN is seen as innervation of the ''subdiaphragmatic visceral organs including the digestive tract (Porges, 2003b).'' On the other hand, mammals are seen to possess an additional vagal system mediated by the nA that primarily is tied to neural control of the heart and bronchia, is essential for regulation of facial and head muscles, and is crucially implicated in social behavior and communication.

This analysis seems to be based on the premise that precise vagal control of the heart first evolved in mammals, for which there is much evidence to the contrary (Taylor, 1994; Taylor et al., 1999, 2001a). The review by Porges (2003b) also claims that modern texts have consistently ignored the afferent functions of the vagus, which is not true, as a typical student text describes the important roles of lung stretch receptor and aortic receptor afferents in generating reflex control of the respiratory and cardiovascular systems, in, for example, the diving response (Randall et al., 1997).

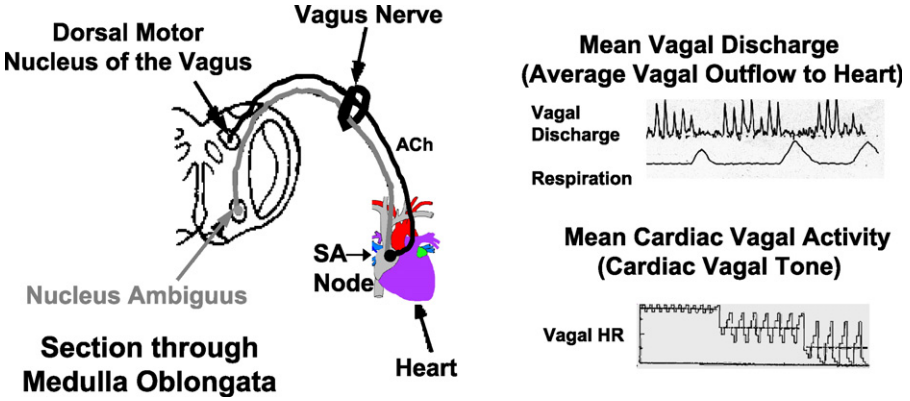

Fig. 9. Depiction of the nucleus ambiguus and the dorsal motor nucleus as they innervate the heart (left); both project to the sinoatrial node, SA node (also the atrioventricular node but not shown). Right upper graphs, a simulated cartoon of vagal efferent discharge as gated by respiratory phase. Note predominant silence during inspiration, suggesting the inherently phasic nature of RSA. Right lower graph, simulated graph of changes in vagal tone (vagal HR) when related to RSA, indicated by the systematically increasing amplitude of the rapid HR oscillations as vagal tone increases (i.e. HR decreases); this would then be a case during which RSA and vagal tone covary.

An additional and very essential implicit premise of the polyvagal theory is that RSA *always* reflects tonic vagal efferent discharge originating in the nA, and that dissociation between vagally mediated HR changes and RSA can be explained by divergent activities of the nA and the DMN. The theory assumes that nA activity is at all times associated with beat-to-beat changes in HR, i.e. RSA, whereas the DMN produces flat changes in HR that show no respiratory modulation or any other type of beat-to-beat variation. Therefore, according to this theory, vagally mediated HR reductions that are not accompanied by RSA increase are supposed to originate from the DMN; changes in RSA magnitude, on the other hand, from the nA. Hence the accuracy of RSA as a measure of mean levels of nA-generated vagal efferent discharge is critical to the theory, because RSA is *the* vagal measure used in studies to evaluate premises and postulates of the theory.

The polyvagal theory has been very broadly stated in terms of premises, extrapolations and speculations. Yet the above-mentioned major assumptions provide a basis by which we may evaluate the very fundament of the theory. There are a number of lines of evidence that challenge this foundation of the polyvagal theory. Several of these arguments relate to points already discussed. Others concern questionable inferences made in the polyvagal theory regarding the evolution of the vertebrate parasympathetic nervous system. Each of these points is addressed below.

7.1. Evolution of vagal control of cardiorespiratory interactions in vertebrates

7.1.1. Evolution of the nA and two sites for cardiac vagal preganglionic neurons

The polyvagal theory first proposed that the nA regulation of cardiac vagal tone is only to be found in mammals (Porges, 1995): ‘‘Reptiles, unlike mammals, have only the older vagal system.’’ Later this was qualified (Porges, 2003b): ‘‘In general, phylogenetic development results in increased neural control of the heart via the myelinated mammalian vagal system [i.e. nucleus ambiguus].’’ This assumption, in either form, appears to be unfounded.

It seems that a dual location for vagal preganglionic neurons (VPN) has important functional correlates in all vertebrates (Taylor, 1994; Taylor et al., 1999, 2001a). This may be particularly the case with the central vagal control exerted over the heart by cardiac vagal preganglionic neurons (CVPN). About 30% of VPN but up to 70% CVPN are in the nA of mammals where inhibitory inputs from neighboring inspiratory neurons are the primary central mechanism generating RSA (Jordan and Spyer, 1987). There is a similar proportional representation of VPN between the major vagal nuclei in amphibians and turtles. When stimulated to metamorphose by injection of thyroxine, the neotenous axolotl shows a doubling of numbers of VPN and relocation of 15% into a ventrolateral group outside the DMN that may constitute a primitive nA (Taylor et al., 2001a,b) This change is accompanied by an increased HRV (Taylor and Choudhury, unpublished observations). In fish and crocodilians the proportion of VPN in the nA is closer to 10% and in some lizards and birds it is 2–5%. However, the CVPN are distributed unequally between these nuclei so that 45% of CVPN are located in the nA of the dogfish; and about 30% in *Xenopus* and the duck (Taylor et al., 2001a; see Fig. 10). This topographical separation of CVPN seems to be of importance in the central control of the heart. Cells in one location may show respiration-related activity (e.g. those in the DMN of dogfish and in the nA of mammals, where they are in close proximity to respiratory neurons), while cells in the other location are sporadically active. Their different activities and separate functions will be determined by their different afferent inputs from the periphery or elsewhere in the CNS, which in turn will relate to their central topography.

What emerges from this brief overview of the vertebrates is that separation of VPN and in particular CVPN into two major nuclei seems virtually ubiquitous and has a long evolutionary history (Taylor et al., 2001a). Its functional significance is the subject of debate, with elasmobranch fish providing a viable model for studying basic mechanisms of cardiac vagal control (Taylor, 1989).

7.1.2. Relationships between ventilatory and heart rates

The polyvagal theory has suggested that the beat-to-beat control of HR that generates RSA is restricted to mammals, which have evolved myelinated vagal pathways that originate in the nA (Porges, 1995, 2003a,b). This idea is not supported by existing research.

In fish there is a close matching of the rates of respiratory water flow and cardiac output, according to their relative

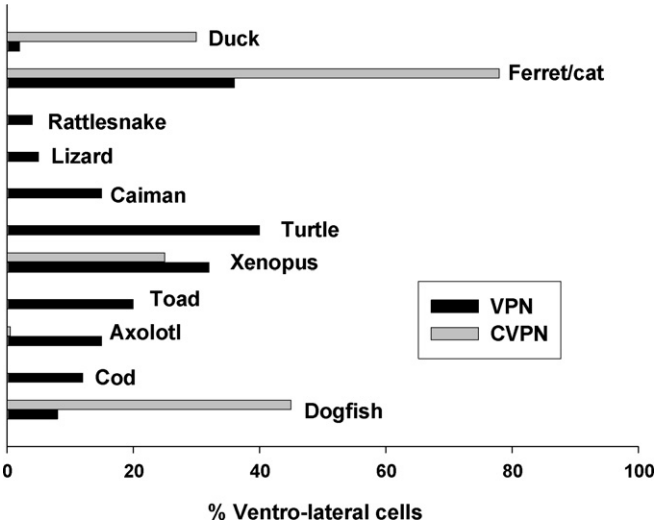

Fig. 10. The proportion of VPN located in ventro-lateral locations outside the DMN in a range of vertebrates. In mammals this location is chiefly the nA with a few cells scattered in the reticular formation. In ‘‘lower’’ vertebrates a discrete area similar to the nA exists in birds and some amphibians and reptiles while in fish and other reptiles the ventro-lateral cells have a more scattered distribution across the medulla. The neotenous axolotl has no cells outside the DMN but 15% following induced metamorphosis (see text). The location of CVPN is known for a small number of species. The proportion of CVPN located outside the DMN is similar to or much larger than the proportion of VPN in this location. This implies that two locations for CVPN in the brainstem have early origins in vertebrate phylogeny, linked to a significant functional role that we postulate is the generation of cardio-respiratory coupling.

capacities for oxygen (the ventilation/perfusion ratio), that is thought to optimize respiratory gas exchange over the functional counter-current at the gills (Hughes and Shelton, 1962; Piiper and Scheid, 1977; Taylor, 1992). As both water and blood flow have been shown to be pulsatile over the gills (e.g. Jones et al., 1974), close beat-to-beat temporal relationships between heart beat and ventilation, or cardiorespiratory synchrony (CRS), has long been hypothesized for fish (Satchell, 1960). CRS has been reported in both resting dogfish (Taylor, 1992) and hypoxic trout (Randall and Smith, 1967). Cardiac vagotomy or injection of atropine abolished CRS in the dogfish (Taylor, 1992) and HR variability in the unanaesthetized trout (Le Mevel et al., 2002). In the sculpin, *Myoxocephalus scorpius*, injection of atropine raised mean HR in normoxia and abolished a hypoxic bradycardia (Taylor et al., 2006), and cardiac vagotomy abolished HR variability (Campbell et al., 2004). These observations confirm the dependence of beat-to-beat variability of HR on tonic vagal control in these non-mammalian vertebrate species.

Peripheral, phasic electrical stimulation of a cardiac vagus in the dogfish (an elasmobranch) can recruit the heart, so that it beats at a frequency determined by the rate of the phasic bursts of stimuli (Taylor et al., 2006). This frequency can be either higher or lower than the intrinsic HR (Young et al., 1993). Current work on pacu (a Brazilian teleost) has shown that the heart can be paced by peripheral stimulation of the cardiac vagus and by central stimulation of respiratory branches of the vagus (Taylor et al., unpublished observations). Thus, in both of these two major groups of fish, the generation of CRS is likely to depend on a combination of central, feed-forward and reflex, receptor-based control (Taylor et al., 1999).

Recordings from the central cut ends of cardiac nerves in decerebrate and paralyzed dogfish showed two different forms of activity, comprising smaller units that fired sporadically or continuously but without any clear pattern and larger units showing respiration related, bursting activity (Barrett and Taylor, 1985b,c; Taylor, 1992; Taylor and Butler, 1982). These different units were shown to originate from CVPN having separate locations in the medulla (Barrett and Taylor, 1985a,c). Central recordings revealed that the sporadically active units arose from a clearly distinguishable lateral group of VPN that contribute axons solely to the cardiac branch of the vagus (Barrett and Taylor, 1985c). These CVPN comprise 8% of the total population of VPN but supply 45% of vagal preganglionic output to the heart and cardiac ganglion in the dogfish. About 9% of VPN are in a scattered ventrolateral location in the ray, *Raja clavata*, and they are also solely CVPN, innervating the heart via a single pair of cardiac vagi (J.J. Levings and E.W. Taylor, unpublished).

Thus in elasmobranch fishes, a very ancient group (dating back some 400 million years), CVPN are in two locations; in the DMN, where they are contiguous with and possibly directly influenced by respiratory vagal motor-neurons (RVM), generating respiration-related activity, and in a ventrolateral location, outside the DMN, possibly homologous to and even the evolutionary antecedent of the mammalian nA, where they are less likely to be influenced by the respiration-related activity in RVM. Stimuli that caused a marked bradycardia, such as hypoxia or capsaicin (the latter an airway irritant, which stimulates J-receptors innervated by pulmonary C-fibers in mammals), induced increased activity in the sporadically active units, thus increasing cardiac vagal tone (Taylor, 1992). When vagal tone is relatively low, in settled normoxic or hyperoxic fish (Taylor, 1992), firing rates in the sporadically active units were reduced while the respiration related activity in the larger units continued. This bursting activity may then recruit HR, generating CRS. This implies that reflex control of HR in response to environmental change is influenced by the sporadically active units while cardiorespiratory synchrony can be generated in the dogfish by central, feed-forward control from units showing respiration-related activity, arising from the central respiratory pattern generator (CRPG). The dogfish heart lacks sympathetic innervation; however, vagal tone on the heart is influenced by levels of circulating catecholamines, with normal resting levels augmenting vagal tone and elevated levels associated with hypoxia or disturbance antagonizing vagal tone (Agnisola et al., 2003). Thus, similar complex interactions between cholinergic (i.e. parasympathetic) and adrenergic (i.e. sympathetic) control of the heart as have been reported in mammals already exist in this primitive group of vertebrates.

In trout, CRS developed during progressive hypoxia, as HR slowed to match ventilation, and could be generated by pulsatile forced ventilation, independently of central respiratory rhythmicity, presumably due to reflex control, arising from stimulation of branchial mechanoreceptors (Randall and Smith, 1967). Thus, fundamentally different mechanisms may underlie the generation of CRS in dogfish and trout (Taylor et al., 1999). However, both species have CVPN located both in the dorsal motor nucleus of the vagus (DMN) and in a ventrolateral location outside the DMN that may constitute a primitive nA (Taylor, 1992).

7.1.3. Myelinated versus unmyelinated vagal efferent fibers

In contra-distinction to mammals, the interaction between respiratory neurons and CVPN occurs in the DMN of the dogfish rather than in the equivalent of the nA of the mammal. This difference probably reflects the migration of respiratory neurons into dorsal and ventral groups during the evolution of the lung and aspiratory air-breathing. In mammals, CVPN in the DMN have unmyelinated fibers while those in the nA have myelinated fibers. It is these latter neurons that are thought to generate RSA, possibly dependent on their faster conduction velocities. This is the root of the polyvagal theory, which postulates that the nA and the associated myelinated fibers are a late innovation, coming with evolution of the mammals. However, all fibers in the branchial cardiac nerves of the dogfish are myelinated (Short et al., 1977) with conduction velocities varying between 7 and 35 m/s^{-1} (Barrett and Taylor, 1985c). These velocities fall within the range of mammalian B fibers (e.g. Katona et al., 1970) and definitely above the range for unmyelinated C fibers, reported by Bennett et al. (1985).

The faster conduction velocity of mammalian fibers may relate in part to the high body temperature and to faster HRs of these animals. However, beat-by-beat modulation of HR may

be possible in fish, despite the somewhat slower conduction velocities of the vagal fibers innervating the heart, because of their relatively slower HRs. Additionally, there is also evidence, even in mammals, that the DMN is capable of generating beat-to-beat modulation of HR: dorsal vagal neurons in the rabbit apparently manifest conduction properties that are indistinguishable from nA vagal neurons, including expiratory-related firing patterns (Jordan et al., 1982).

7.1.4. Mistaken inferences when heart rate is not at least double the respiration rate—aliasing and the Nyquist frequency

One-to-one CRS is not the only possible temporal interaction between HR and ventilation rate. For many years researchers seeking piscine CRS reported complex and often drifting phase relationships between HR and ventilatory frequency (Satchell, 1960; Taylor and Butler, 1971). These relationships were often subject to relatively complex analysis (e.g. Hughes, 1972), but the techniques of power spectral analysis were not available to fish physiologists of that generation. Interest in HR variability (HRV) research increased once it became clear that in both the time and frequency domains, oscillations in cardiac interval generated by sympathetic, parasympathetic and circadian inputs could be detected. Power spectral statistics has, of course, become well established for research and clinical application in mammals and especially in humans.

The few power spectral studies undertaken on non-mammalian vertebrates have shown interspecific differences, with lizards and some fish having dual spectral peaks (Campbell et al., 2004; De Vera and Priede, 1991; Gonzalez Gonzalez and De Vera Porcell, 1988; Le Mevel et al., 2002) whilst other fish have a single main component (Altimiras et al., 1995, 1996; Armstrong and Priede, 1988). These components have been characterized as of a relatively low frequency, well below that of the ventilation rate. For example, spectral analysis of instantaneous ECG in the sculpin produced dual spectral peaks at 0.018 and 0.05 Hz in the frequency domain, equivalent to periodicities of 55 and 20 s, respectively, in the time domain. As ventilation occurred every 3.3 ± 0.2 s (0.30 Hz), it was about six times faster than the highest-frequency component (0.05 Hz). It was concluded that this component probably did not represent centrally generated respiration-related activity, analogous to the RSA described in recordings of HRV in mammals, and consequently that the sculpin did not show CRS (Campbell et al., 2004).

However, one very important point that has gone overlooked in the vertebrate literature is that RSA is only possible when HR is at least twice as fast as the ventilation (i.e. respiration rate see Fig. 11). Two cardiac intervals (RRIs) are required for each breath in order to have a characteristic waxing and waning of HR with each respiratory cycle. In other words, at the very minimum, there must be a longer RRI associated with expiratory phase and a shorter one associated with inspiratory phase. This minimum two-to-one relationship is known as the Nyquist frequency. Therefore, if ventilation rate is greater than one-half the HR, another phenomenon occurs that is termed

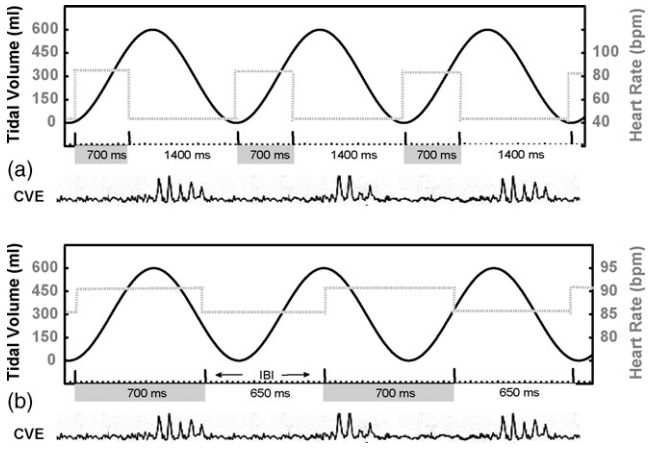

Fig. 11. Simulated examples of RSA (a) and cardiac aliasing (b). Solid line, respiration; dotted line, HR; CVE, simulated cardiac vagal efferent discharge. Note the clear phase relation in (a) between respiratory phase and HR; this example represents the minimal condition in which there are just two beats per respiratory cycle. In (b), aliasing occurs because HR approximates respiration rate; there is little more than a single beat during each respiratory cycle making it impossible for RSA to occur. Also note the changing (and drifting) phase relation between signals in (b). This can happen even when vagal efferent discharge is phasically related to respiration (see CVE).

aliasing: it is no longer possible to meaningfully calculate RSA because there are less than two beats per breath, and any possible synchrony between respiration period and RRI must be evaluated over a longer duration than the single breath. If one knows exactly what the cardiac frequency and the ventilation frequency are for an animal, then the exact aliasing frequency can be determined as the difference between the sampled signal (HR) and the sampling frequency (respiration rate; Mintchev et al., 2000).

Application of the Nyquist criterion and an anti-aliasing digital filter to the data from Campbell et al. (2004) revealed that HRV in the sculpin included a component that appeared to be synchronous with the respiratory rhythm (Taylor et al., 2006), so that, contrary to previous analysis of these data, a respiratory coupling may indeed contribute to HRV in fish and perhaps other lower vertebrates (see Fig. 12). Campbell et al. (2004) noted that as rates of oxygen uptake (MO_2) in the sculpin fell during recovery from surgery, a time-domain index of HRV (standard deviation of successive RRIs) mirrored this change more closely than the progressive reduction in HR. Reexamination of their data, using anti-aliasing techniques has led them to conclude that HRV was an important component of the mechanisms optimizing respiratory gas exchange (Taylor et al., 2006).

This problem of cardiac aliasing has also been thoroughly documented and discussed in the human infant HRV literature for over a decade (Rother et al., 1989; Witte et al., 1988; Zwiener et al., 1994, 1995, 1990) but has been completely ignored in the discussion of the polyvagal theory. As we shall see, aliasing may lead to important misinterpretations regarding the polyvagal theory.

In a study comparing two types of lizards (Porges et al., 2003), the authors concluded on the basis of RSA findings that vagal tone deriving from the nA is tied to the evolution of

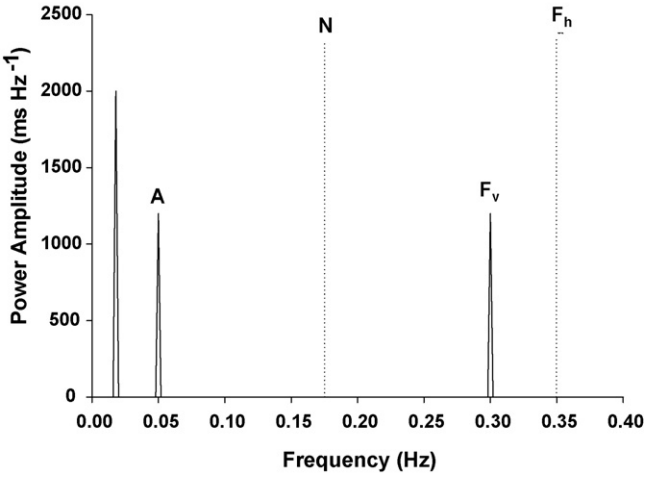

Fig. 12. Power spectra calculated from IBÍs of a resting sculpin *Myocephalus scorpious*; only the amplitude and frequency of major peaks displayed. Peaks of HR variability are apparent in the calculated spectra at 0.018 and 0.05 Hz. The Nyquist criterion (*N*) shows limit of spectral window set by the mean HR frequency (f_h). The actual frequency component set by ventilation rate (f_v) would be folded back as the aliased component (A). In *M. scorpious* (Campbell et al., 2004), the recorded mean RRI was 2.85s thus producing an actual sampling frequency (f_a) of 0.35 Hz, and consequently a Nyquist limit (*N*) of 0.175 Hz. The fish was observed to ventilate its gills every 3.33 ± 0.2 s ($f_s = 0.3$ Hz). Therefore, any spectral component at the frequency of ventilation would appear above the Nyquist criterion and outside the limits of the spectral bandwidth. The consequence of this is a true component (0.018 Hz) close to the aliased, folded-back component (0.05 Hz). When an anti-aliasing filter (a low pass bandwidth filter at the limit of Nyquist) was applied to the spectra calculated for *M. scorpious* this peak at 0.05 Hz was abolished confirming that it was aliased, but the low frequency peak at 0.018 Hz was unaffected, confirming that it is a true component in the spectra and perhaps corresponds to adrenergic effects as described by De Vera and Priede (1991).

certain aspects of social behavior among reptiles. However, data presented in the tables and figures of the article clearly indicate that aliasing occurred during measurement. Consequently, RSA could not have possibly been identified in the presumably 'less vagally and socially evolved' species of lizard because the respiration rate approximated the HR (greater than 0.5 the cardiac frequency). Nevertheless, examination of the spectral analysis data suggests that beat-to-beat cardiorespiratory synchrony did, in fact, occur. Thus, due to the aliasing, there is no basis to believe that the mere numerical relationship between respiration rate and HR should be meaningful in terms of the evolution of cardiac vagal control.

Both reptiles and amphibians regularly show marked changes in HR associated with periodic breathing. Modulation of HR by ventilation can be clearly seen in species that show prolonged periods of regular breathing, particularly when this is at a markedly slower rate than HR (e.g. Wang et al., 2001). In a recent study on the rattlesnake, *Crotalus durissus*, long-term measurements of HR, using data-loggers in undisturbed and resting snakes, clearly documented an oscillatory component in the HRV signal at the frequency of ventilation (Campbell et al., 2006). These oscillations resemble the rise in HR during inspiration in mammals, and are very likely an example of reptilian RSA. RSA in rattlesnakes was most pronounced at low HRs and was diminished when HR was increased due to spontaneous activity, handling stress or recovery from instrumentation. Cholinergic tone is highest at low HRs in reptiles (Wang et al., 2001), and as in mammals, the incidence of RSA seems to require a relatively high vagal tonus on the heart. This interpretation is also consistent with our observation that the cholinergic antagonist atropine abolished RSA in the rattlesnakes.

Thus evidence of cardiorespiratory coupling and sometimes apparent RSA is found throughout the vertebrate literature, among not only fish but amphibians (Shelton, 1985; Wang et al., 1999a,b, 2004; West and Burggren, 1983); reptiles (Birchard and Reiber, 1996; Burggren, 1975; Wang and Hicks, 1996; Campbell et al., 2006), and birds (Butler, 1982; Butler and Jones, 1997; Butler and Taylor, 1983), as well as, of course, mammals. These findings do not provide support for the polyvagal theory. To the contrary, they suggest a clear primacy of cardiorespiratory coupling in the evolution of cardiac vagal control. It may well be neglect of the respiratory system within the polyvagal theory that has led to a series of unsupported assumptions.

7.1.5. Mammalian evidence of DMN contribution to cardiac vagal tone

We were unable to discover any publications that document DMN effects upon mammalian vagally mediated HR during any conditions other than severe experimental irritation of the airways, extreme hypoxia, or lung congestion (Jones et al., 1998; Jordan et al., 1982; Wang et al., 2000). DMN generation of bradycardia has only been suggested as a response to stimulation of pulmonary C fibers (also called J receptors), which do not have a clear role in normal breathing. Existing research, therefore, suggests that tonic DMN HR alterations are restricted to physiological circumstances in which ventilation may be critically compromised by toxic airborne agents, lung edema or asphyxia (Paintal, 1983). These conditions in no way correspond to any hypotheses in the polyvagal theory concerning when DMN influence upon HR may be active, e.g. during behavioral immobilization. Hence, there appears to be little or no evidence of DMN effects upon vagal HR under circumstances that reflect behavioral variation and not physiological, respiratory emergency.

7.1.6. Accuracy of RSA as a measure of mean level of nA-generated vagal efferent tone

A clear and fundamental assumption of the polyvagal theory is that RSA is a precise measure of vagal efferent discharge to the sinoatrial node emanating from the nA. Several lines of evidence already reviewed appear to contradict this assumption: (a) Some fish studies mentioned above indicate that a phenomenon similar or identical to RSA can be produced via vagal efferent traffic originating in the DMN. (b) Changes in respiratory pattern can confound any relationship between RSA magnitude and cardiac vagal tone. (c) RSA magnitude can be influenced by concurrent changes in sympathetic activity at constant rates of vagal efferent discharge. (d) Peripheral blocking of vagal activity at the sinoatrial node can mask

increased central vagal activity, indicating that, at best, RSA reflects the final vagal effects upon HR and may not necessarily be correlated with the amount of central vagal efferent traffic. (e) RSA may even sometimes dissociate from mean vagal effects upon HR, in a manner that cannot be explained by the polyvagal theory (e.g. a quadratic relation over the entire range of normal vagal heart variation). (f) Beat-to-beat vagal cardiorespiratory coupling can occur in the absence of RSA when respiration rate is above the Nyquist frequency. Therefore, RSA cannot be assumed to be a direct measure of vagal tone or nA-generated vagal efferent discharge as suggested by the polyvagal theory, nor is it always a relatively accurate index of central efferent vagal traffic, or vagal tone, in general, or cardiac vagal tone, specifically.

The extent to which cardiac vagal tone and RSA are differentially affected by sympathetic mechanisms remains unclear, because it unknown whether sympathetic effects upon RSA reflect true autonomic interactions or only affect the amplitude of RSA. RSA, however, does not provide a measure of nA vagal activity. For all these reasons, the pivotal role of RSA in the polyvagal theory is seriously challenged. Without an adequate metric to accurately measure some aspect of tonic vagal control, or nA efferent discharge specifically, the polyvagal theory would appear to lack basic supporting evidence for several of its fundamental assumptions.

7.2. RSA and energy regulation during behavioral and metabolic demands

The evolutionary literature on cardiac vagal control emphasizes the biological advantage of tight coupling between ventilation and cardiovascular function in order to achieve optimal gas exchange and to meet changing metabolic and behavioral requirements (Taylor et al., 1999; Taylor, 1994; Barrett and Taylor, 1985b). It is plainly the case that the respiratory and cardiovascular systems are intimately related structurally, neurally and functionally, with a common goal of coordinating and modulating energy expenditure and uptake in all vertebrates. Within this theoretical framework, it makes sense to consider that RSA has evolved as a mechanism that contributes to functional cardiorespiratory interactions (Hayano and Yasuma, 2003; Hayano et al., 1996; Yasuma and Hayano, 2004). Because the heart is largely under vagal control during the normal range of metabolic activity, it is also logical to assume that a covariation of cardiorespiratory coupling, e.g. RSA, CRS and vagal tone, may often occur. This has, indeed, been found to be the case even among ''lower'' non-mammalian vertebrates, such as the dogfish and the sculpin (Campbell et al., 2004; Taylor and Barrett, 1985; Taylor et al., 2006). The precise mechanisms underlying the covariation of cardiorespiratory synchrony and vagal control may vary from species to species of vertebrates (Taylor et al., 2001a). At present we can only speculate on how the dramatic ventilatory adaptations involved in the switch from aquatic to terrestrial breathing may have influenced the evolution of mechanisms concerned with cardiorespiratory coupling, but it seems likely that these ventilatory modifications played a significant role.

A number of experimental studies have recently begun to provide support for the hypothesis that RSA serves an active biological function, in that it enhances the efficiency of pulmonary gas exchange by matching blood perfusion to air flow in the lung throughout each breathing cycle, particularly during rest (Hayano and Yasuma, 2003; Hayano et al., 1996; Sasano et al., 2002; Yasuma and Hayano, 2001, 2004; Yasuma et al., 2001). It is proposed that in basal states, cardiovascular and respiratory systems save energy by reducing unnecessary heartbeats and, therefore, pulmonary blood flow during expiration when alveolar gas volume is reduced. The extent of resting RSA is seen as reflecting a functional energy reserve capacity from which the organism can draw during more active states. Evidence for this theory comes primarily from invasive dog investigations in which ventilation and cardiac activity were experimentally manipulated. However, human studies, employing paced breathing, also provide preliminary support (Giardino et al., 2003, 2004). Hence, the theory appears to be gathering direct and indirect confirmation and is a plausible alternative to the polyvagal theory.

This gas exchange hypothesis of RSA alludes to but does not elaborate upon behavioral and psychophysiological mechanisms that may be involved (Hayano and Yasuma, 2003). Nor does it consider the diversity of respiratory patterns that modify RSA amplitude and that mediate both behavioral and metabolic demands during changing levels of activity. The human breathing pattern varies systematically and often precisely to degree of alertness, behavioral task, emotional state and motivation factors (Boiten, 1998; Boiten et al., 1994; Grossman and Wientjes, 2001; Longobardo et al., 2002; Wientjes and Grossman, 1994; Wientjes et al., 1998; e.g. see Fig. 13). Variations of breathing are obviously essential during behavioral acts such as speaking, expression of emotions like laughing and crying, eating, drinking and defecation (Boiten, 1998; Overeem et al., 2004; Phillipson et al., 1978; Reilly and Moore, 2003). Breathing also changes more covertly during quiet performance of different mental tasks (e.g. see Section 2; and Grossman and Wientjes, 2001) and may subserve clear

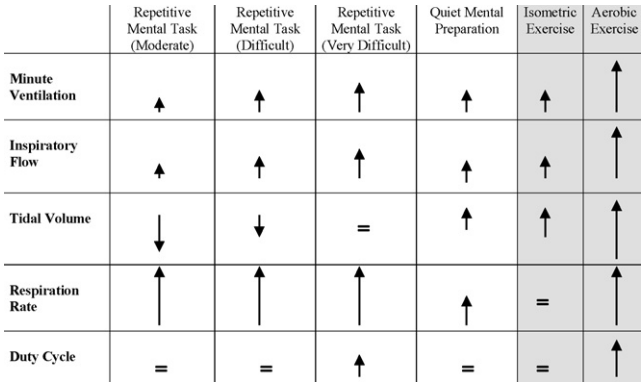

Fig. 13. Respiratory responses from resting baseline to varying mental physical tasks (from Grossman and Wientjes, 2001). Arrow up, increase; arrow down decrease; =, unchanged from baseline; length of arrow proportional to amount of change. Note that each condition is associated with a distinctive pattern of respiratory adjustment. Also note changes both respiration rate and tidal volume even during moderate mental demand.

social communication functions, e.g. modulation of utterances and facial expressions that occur with anger, disgust, sadness, excitement or joy. Emotional experience and expression occur within a context of physical activity, whether this means changes in muscle tone or in animation of head, trunk and/or limbs. Such alterations are inherently tied to metabolic change, to which ventilation must also respond. Thus, breathing necessarily adapts to both behavioral and metabolic requirements.

Vagal control of HR often dominates the rapid metabolic cardiac adaptations required by a range of behavioral activities in humans (e.g. Grossman et al., 2004). Furthermore, according to arguments above, concomitant variations in breathing must be integrated into an efficient pattern of cardiorespiratory synchrony in order to achieve optimal gas exchange for a given level of physical or mental activity. Therefore, it is unsurprising that RSA, vagal mechanisms and behavioral function have evolved in a manner in which coordination, and covariation, often occur. The cranial nerves (including the Xth, the vagus) are implicated in ventilation and in head, neck and upper torso muscle activity in mammals. In fact, this has been the case all along the evolutionary path of vertebrates with respect to corresponding anatomical regions (Taylor et al., 1999): in fish, cranial nerves V, VII, IX and X innervate the respiratory muscles, as well as other musculature in the head, and in the case of X (i.e. the vagus), the heart and other viscera are innervated. Fish recruit the feeding muscles, innervated by the hypobranchial nerves, into the respiratory rhythm when the level of respiratory drive is high (Taylor et al., 2006). It is these same muscles that are responsible for buccal (approximately, the floor of the mouth) ventilation in amphibians and reptiles. The hypobranchial nerve of fish is replaced in four-limbed vertebrates by its equivalent the hypoglossal, which innervates the tongue. It is interesting that the muscles of the tongue and jaws are used in suckling by infant mammals, or even during an expressive sigh, in humans.

The very rapid beat-to-beat modulation of HR that occurs with RSA can only be realized by means of parasympathetic influences upon HR: vagal neural effects upon HR are almost instantaneous (i.e. within milliseconds), whereas sympathetic effects are delayed for a few seconds and then take several more seconds to achieve a maximum response (e.g. Berger et al., 1989). Therefore, *phasic* vagal modulation, but not necessarily *tonic* vagal activity, must be implicated in cardiac changes occurring within a single breath.

RSA magnitude, cardiac vagal tone and behavioral adjustment, in fact, need not always covary, if the primary biological role of RSA is to enhance cardiopulmonary efficiency. The rapid, relatively shallow breathing characteristic of continuous mental processing (e.g. a cognitive reaction-time task) may require a different kind of coupling between ventilation and perfusion (reflected by reduced RSA) than that occurring during quiet relaxation, when breathing is slower and deeper (Boiten, 1998; Boiten et al., 1994; Grossman and Wientjes, 2001; Wientjes and Grossman, 1994; Wientjes et al., 1998). Cardiac vagal tone may, at times, be hardly altered between these two conditions (Grossman and Stemmler, in preparation; see Section 2), but the pattern of cardiorespiratory synchrony will be different, solely as a function of the respiratory change.

On the other hand, RSA amplitude locked to variations in vagal tone would mean that vagal tone was invariably bound to respiration rate and depth (i.e. every respiratory adaptation would be accompanied by a HR change). Not only has this idea been disproved (see Section 2), but it would also imply that behaviorally mediated changes in breathing pattern would necessarily trigger changes in cardiac vagal tone, even in the absence of metabolic alterations. Such interdependency between respiration and cardiac autonomic control is not likely to confer any biologically adaptive advantage: behavioral control of respiration, which prevails during alert states, can greatly alter time and volume parameters of ventilation independently of metabolic activity (Boiten, 1998; Boiten et al., 1994; Grossman and Wientjes, 2001; Wientjes and Grossman, 1994; Wientjes et al., 1998; also Section 2).

Within a context of behavioral and metabolic demands, this hypothesis linking RSA, energy efficiency and behavior is compatible with the concept of allostatic regulation, which posits that in a world of ever-occurring exogenous and endogenous perturbations, the organism maintains stability by means of active change (Sterling and Eyer, 1988). The notion of allostatic regulation, indeed, originated to describe how the cardiovascular system adjusts from resting to active states by means of autonomic and other mechanisms. We propose that RSA may help match the behavioral and metabolic requirements of respiratory and circulatory systems by adjusting the relationship of ventilation and pulmonary perfusion to immediate demands. Thus, the almost instantaneous changes in RSA accommodate the diversity of often rapid and interactive metabolic, behavioral and psychological alterations that characterize our waking (and even much of our sleeping) hours (e.g. scratching an itch, simple posture changes, experience and expression of emotion, or verbal and nonverbal communication). The range of RSA from rest to maximal exertion would then indicate the reserve energy capacity available to an individual under physical or mental load.

In conclusion, we have presented evidence that clearly indicates that RSA is not synonymous with cardiac vagal tone but may sometimes reflect it. Our theoretical formulation, derived from evolutionary biology, cardiovascular physiology and respiratory psychophysiology, posits that vagally modulated RSA may facilitate enhanced gas exchange and closely interact with behavioral, respiratory and both phasic and tonic cardiac parasympathetic mechanisms. Thus it seems likely that coordination of breathing and blood flow through pulmonary capillaries (in land-dwelling vertebrates) would contribute to the efficiency of oxygen uptake and carbon dioxide release in response to a variety of tasks and settings.

Consistent with the concept of allostasis, this hypothesis suggests that dynamic changes in RSA may reflect broad plasticity of function across a wide variety of physiological, behavioral and psychological conditions. As such, it supports the concept, not so much of autonomic flexibility (Friedman and Thayer, 1998), as of the extent of capacity to integrate

behavioral and metabolic demands by means of efficient energy exchange. Aberrant RSA levels may signal breakdown of a range of processes, including impaired ventilatory, cardiovascular or autonomic function, psychological or behavioral disorder, or disturbed integration of behavioral and metabolic function. We propose this model in the hope it may offer a new, testable and more inclusive perspective upon RSA.

Acknowledgements

Preparation of this article was partially supported by a grant from HRCA Research and Training Institute, Boston MA, U.S.A. We would like to thank Dr. Frank H. Wilhelm for discussion and comments, as well as all reviewers for their remarks.

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