Heart rate complexity: A novel approach to assessing cardiac stress reactivity

Ryan C. Brindlea\*, Annie T. Gintyb, Anna C. Phillipsa, James P. Fishera, David McIntyrea, Douglas Carrolla

aSchool of Sport, Exercise, and Rehabilitation Sciences, University of Birmingham, Birmingham, B15 2TT, UK

bDepartment of Psychiatry, School of Medicine, University of Pittsburgh, USA

RUNNING HEAD: Cardiac Stress Reactivity and Heart Rate Complexity

Please Address Correspondence to: Ryan C. Brindle, School of Sport, Exercise, and Rehabilitation Sciences, University of Birmingham, Birmingham, B15 2TT, UK: Email Address: RCB236@bham.ac.uk: Telephone: +44 121 414 8745

**Abstract**

 Correlation dimension (D2), a measure of heart rate complexity, has been shown to decrease in response to acute mental stress and relate to adverse cardiovascular health. However, the relationship between stress-induced change in D2 and HR has yet to be established. The present studies aimed to assess this relationship systematically while controlling for changes in respiration and autonomic activity. In study 1 (N = 25) D2 decreased during stress and predicted heart rate reactivity even after adjusting for changes in respiration rate, and cardiac vagal tone. This result was replicated in experiment 2 (N = 162) and extended by including a measure of cardiac sympathetic activity; correlation dimension remained an independent predictor of HR reactivity in a hierarchical linear model containing measures of cardiac parasympathetic and sympathetic activity and their interaction. These results suggest that correlation dimension may provide additional information regarding cardiac stress reactivity above that provided by traditional measures of cardiac autonomic function.

Keywords: Correlation dimension; stress; heart rate; pre-ejection period; heart rate variability

**Introduction**

It is now a widely accepted that individuals differ markedly in the magnitude of their cardiovascular reactions to acute psychological stress and that such individual differences have implications for both health and behavior (Chida & Steptoe, 2010; Phillips, Ginty, & Hughes, 2013; Treiber et al., 2003). Understanding the physiological determinants of cardiovascular stress reactions thus becomes an important area of inquiry. Although a recent large-scale meta-analysis implicated both the parasympathetic and sympathetic nervous systems in cardiovascular responses to mental stress (Brindle, Ginty, Phillips, & Carroll, 2014), characterizing individual differences in autonomic activity is difficult as the activity of the cardiac autonomic nerves must be assessed indirectly in humans, and due to their inaccessibility it is not plausible to make intra-neural recordings. Hence, researchers have had to rely on indirect measures of autonomic activity.

Measures of HR variability, namely the spectral analysis variable of high-frequency variability and its time domain analogue root mean square of successive differences (RMSSD), have allowed researchers to gauge changes in cardiac vagal activity induced by acute mental stress. On the whole, acute withdrawal of cardiac vagal influence has been observed during stress leading to increased HR and augmented cardiac output (). However, beta-adrenergic sympathetic activation has also been directly implicated in cardiac output augmentation. Using pre-ejection period, a measure of myocardial contractility that can be accurately captured using impedance cardiography or Doppler ultrasonography (Cacioppo, Uchino, Berntson, 1994), studies have shown that cardiac reactivity to psychological stress is largely driven via beta-adrenergic activation. Focusing on adaptation to recurrent stressors Kelsey and colleagues (2001, 2004) showed when evaluation observation disrupted the adaptation process the rebound in HR reactivity was accompanied by a resurgence of beta-adrenergic activation. In addition, in a series of studies examining challenge and threat appraisals to mental stress it was found that challenge appraisals were often characterized by augmented heart rate and pre-ejection (PEP) reactions, further implicating the beta-adrenergic sympathetic nervous system in cardiovascular reactivity (Tomaka, Blascovich, Kelsey, & Leitten, 1993; Tomaka, Blascovich, Kibler, & Ernst, 1997).

However, a notable amount of inter-individual variability in cardiovascular reactivity remains unaccounted for; variability that could potentially lend insight into stress-disease associations. Emerging evidence suggests that cardiac pacemaker cells in the sino-atrial node can operate in a seemingly non-linear or chaotic manner due to the convergence of autonomic, hormonal, and hemodynamic influences (Hagerman, Berglund, Lorin, Nowak, & Sylvén, 1996; Lipsitz, 1995; Wagner, & Persson, 1998) and this has prompted some researchers to examine the effect of acute stress on measures of HR complexity. This class of cardiac measures, based on nonlinear dynamics and chaos theory, characterize the dynamic properties of the HR generating system (for reviews please refer to Acharya, Joseph, Kannathal, Lim, & Suri, 2006; Goldberger et al., 2002a; Goldberger, Peng, & Lipsitz, 2002b, Hagerman et al., 1996; Wagner & Persson, 1998).

Although various HR complexity measures have been shown to be perturbed by acute stress (Anishchenko, Igosheva, Yakusheva, Glushkovskaya-Semyachkina, & Khoklova, 2001; Brisinda, Fioravanti, Sorbo, Venuti, & Fenici, 2015; Melillo, Bracale, & Pecchia, 2011), the correlation dimension (D2) appears to be particularly relevant in the context of mental stress. First, studies have shown D2 to decrease during acute mental stress episodes. A study by Schubert and colleagues (2009) observed a significant decrease in D2 during an acute socially evaluative speech task and reported a negative association between baseline D2 and a self-reported measure of chronic stress. In the field, D2 derived from ambulatory ECG traces was significantly lower during a university exam compared to a holiday period (Melillo, et al., 2011). Taken together, these data support the notion that D2 is sensitive to mental stress and to some extent generalizes outside the laboratory. Second, clinical research has shown D2 to be particularly relevant in the context of myocardial ischemia and ventricular fibrillation, conditions also associated with increased mental stress and mental stress induced sudden cardiac death (Kamarck & Jennings, 1991; Lampert, Joska, Burg, Batsford, McPherson, & Jain, 2002; Lane et al., 2005). D2 has been reported to significantly decrease in response myocardial ischemia and hours to minutes before ventricular fibrillation in animals (Skinner, Carpeggiani, Landisman, & Fulton, 1991) and humans (Skinner, Pratt, & Vybiral, 1993) as well as predict the onset of ventricular fibrillation in prefibrillation heart rate data of arrhythmia patients (Kroll & Fulton, 1991).

Although D2 has been shown to decrease under mental stress, no study has addressed the relationship between stress-induced changes in HR and D2. Two studies are reported that address this relationship. The aims of the studies were two-fold. First, they were concerned with determining if D2 reactivity related to stress-induced changes in HR and whether controlling for changes in RMSSD and respiration rate influenced the relationship. Second, the studies attempted to determine whether changes in D2 withstood further adjustment for changes in PEP and the interaction between RMSSD and PEP.

**Study 1**

**Methods**

*Participants and Experimental Design*

 Twenty five healthy individuals (Mean age (SD) = 19.77 (3.86); 50% female) were recruited for the present study. Potential participants were excluded if they reported having a history of cardiovascular or metabolic disease or had previously participated in the psychological stress protocol. All participants were required to refrain from alcohol and vigorous exercise 12 h, caffeine 2 h, and food and drinks other than water 1 h before testing. They received participation credit and the study was approved by the University of Birmingham Ethics Committee.

 Upon arrival to the laboratory anthropometric measures were completed and participants were sat quietly while being instrumented. Participants then completed a standardized 8-min adaptation period before data was obtained during a formal 8-min baseline and an 8-min Paced Auditory Serial Addition Test (PASAT; Gronwall, 1977). During the baseline period, participants were asked to remain seated and rest quietly, while during the PASAT participants were required to consecutively add single digit numbers, presented via CD recording, while remembering the most recent number so that it may then be added to the next number presented. Participants answered via a keypad and numbers were presented at increasing speeds beginning with an inter-number interval of 4.0 seconds that decreased by 0.5 seconds every two mins. The PASAT was chosen in the present study as it has been shown to reliably perturb cardiac activity (Mathias, Stanford, & Houston, 2004; Ring, Burns, & Carroll, 2002), through both vagal withdrawal (Brindle et al., 2014; Mezzacappa, Kelsey, Katikin, & Sloan, 2001) and beta-adrenergic sympathetic activation (Kelsey et al., 2000; Rousselle et al., 1995; Winzer, Ring, Carroll, Willemsen, Drayson, & Kendall, 1999), and demonstrate acceptable test-retest reliability (Ginty, Gianaros, Derbyshire, Phillips, & Carroll, 2013; Willemsen et al., 1998). Participants completed a brief questionnaire (Knafner et al., 2011) immediately upon completion of the task, which required them to rate task stressfulness on a standard Likert scale ranging from 1-10 anchored by “not at all stressful” to “extremely stressful.” PASAT score, an index of task performance, was calculated by subtracting 5 points for every wrong or unanswered question from a starting value of 1000.

*Physiological Measurements*

 Heart rate and respiration rate were measured continuously during all task phases via three-lead ECG and a leak-free respiratory mask and gas analyzer (ML206; ADInstruments, Bella Vista, NSW, Australia), respectively. All data were continuously sampled at 200 Hz using an analogue-digital converter (PowerLab, ADInstruments) and analyzed offline using LabChart software (Version 7, ADInstruments). Continuous data from each phase (i.e., baseline, stress) was used to calculate phase means for HR, respiration rate, and HR variability and complexity measures. Reactivity was defined as the difference between stress and baseline phase means.

*Cardiac Data Processing*

 Raw ECG data was visually inspected for artifacts. Following removal of artifacts, RR interval data was subjected to analysis using Kubios HRV 2.0, a software suite specifically designed for the analysis of human HRV and complexity (Tarvainen & Niskanen, 2008). RMSSD of the RR intervals was used as an index of cardiac parasympathetic activity as it has been shown to capture high frequency changes in HR variability which positively relate to cardiac parasympathetic control (Kleiger et al., 1991; Task Force of the ESC and NAPE, 1996). D2 was calculated using the algorithms outlined by Tarvainen & Niskanen (2008).

*Statistical Analysis*

 Repeated-measures (baseline, stress) ANOVAs were used to confirm that the acute stress exposure significantly perturbed HR and other HR measures. Individual simple linear regressions were used to assess the associations between D2 and RMSSD change score and HR reactivity. Next, individual hierarchical regressions were undertaken to control for respiration rate, BMI, PASAT score and baseline HR as these measures have been shown to influence HR reactivity (Carroll et al., 2000; Carroll, Phillips, & Der, 2008; Ginty, Phillips, Roseboom, Carroll, & de Rooij, 2012b). HR reactivity was again the outcome variable, changes in respiration rate and other control variables were entered at step 1 and D2 or RMSSD were then entered at step 2. Finally, all remaining significant cardiac autonomic predictor variables were then entered into a single regression model to assess their independent contributions to HR reactivity. Differences in participant number as noted in the tables reflect occasional missing data.

**Results**

*Participants*

 The mean (SD) BMI was 22.46 (3.20) kg/m2. Participants achieved a mean score of 878.15 (86.98) on the PASAT and reported an average task stressfulness of 5.08 (1.98).

*Cardiovascular, Autonomic, and Respiratory Reactions to Acute Stress*

Repeated measures (baseline, stress) ANOVA revealed a significant increase in HR, F(1,25) = 27.08, *p* < .001, η2 = .520, respiration rate, F(1,25) = 37.09, *p* < .001, η2 = .597, and ApEn, F(1,24) = 24.00, *p* = .006, η2 = .277, and a significant decrease in D2, F(1,24) = 9.06, *p* = .006 .001, η2 = .274 and RMSSD, F(1,24) = 8.77, *p* = .007 .001, η2 = .268 (Table 1).

[Insert Table 1 about here]

*Autonomic Correlates of Heart Rate Reactivity*

Both RMSSD, *r* = -.688, *p* <.001, and D2, *r* = -.563, *p* =.003, significantly correlated with HR reactivity. Changes in respiration rate did not significantly relate to HR reactivity (*p* =.64)

*Autonomic Predictors of Heart Rate Reactivity*

Unadjusted regression models revealed that D2, β = -.56, *p* =.003, ΔR2 = .317 and RMSSD, β = -.69, *p* < .001, ΔR2 = .473, were significant predictors of HR reactivity (Table 2). In all cases, decreases in each cardiac autonomic variable were associated with increased HR reactivity. In models that were adjusted for changes in respiration rate and other confounders, D2 and RMSSD both continued to predict HR reactivity. When both autonomic predictors were included in a single model, with changes in respiration rate, both measures independently predicted HR reactivity (Table 2).

[Insert Table 2 about here]

*Sensitivity Analysis: Other HR Complexity Measures*

 As several other HR complexity measures have been shown, in some circumstances, to be perturbed by acute mental stress ((Anishchenko et al., 2001; Brisinda et al., 2015; Melillo et al., 2011) stress-induced change scores were also derived for SD1, SD2, DFα1, DFα2, and ApEn using previously outlined algorithms (Tarvainen & Niskanen, 2008). Pearson’s correlations were undertaken to assess the relationship between stress-induced changes in these measure and HR reactivity. DFα1 (r = 0.599, *p* = .002), SD1 (r = -0.688, *p* <.001), and SD2 (r = -0.414, *p* = .039), were all related to HR reactivity; SD2 and ApEn were not (all *p* >.05).

**Summary**

The PASAT significantly increased HR and decreased RMSSD and D2. RMSSD and D2 were predictors of HR reactivity when various control variables, including changes in respiration rate, were accounted for and both independently predicted HR reactivity when entered into a single linear model.

**Study 2**

**Methods**

*Participants*

Adolescent participants (N = 185) were recruited from local high schools proximal to the University. Data from 9 participants were unusable due to signal acquisition problems resulting in a final sample of 176 participants (age, M = 18.04, SD = 0.43; 82% female). Exclusion criteria included history of cardiovascular or metabolic disease and participants were required to refrain from alcohol and vigorous exercise 12h, caffeine 2h, and food and drinks other than water 1h before testing. All participants and legal guardians, if participants were under 18, gave informed consent. Participants received £10 for study participation. The study was approved by the University Ethics Committee.

*Procedure*

Height and weight were measured on arrival at the laboratory and body mass index (BMI) calculated. Participants were then asked to recline in a lateral decubitus position while ECG electrodes were attached and the Doppler probe positioned. After a 10-min adaptation period, participants were asked to rest quietly for a 10-min formal baseline period, followed by a 10-min stress phase, in which participants undertook the PASAT. Afterwards, participants completed a brief questionnaire regarding task stressfulness. Participants responded on a standard Likert scale ranging from 0-6 anchored by “not at all” and “extremely” (Khanfer, Carroll, Lord, & Phillips, 2012).

*Acute Psychological Stress Task*

A similar PASAT was used to that of Study 1 with some modifications to enhance task stressfulness. The PASAT was 10 min in length and was presented at a faster speed beginning at 2.5 sec intervals and shortening .5s every two minutes. In addition, an experimenter overtly recorded every answer, and participants were informed that in response to wrong answers or hesitation, they would hear brief bursts of loud aversive noise. Participants were required to watch themselves in a mirror and were videotaped so that “body language experts” could assess anxiety levels. In reality, participants were not videotaped and were given a standardized number of noise bursts which were administered every 10 numbers, coinciding with wrong answers or hesitation where possible (Ginty, Phillips, Higgs, Heaney, & Carroll, 2012a).

*Cardiovascular Measures*

Continuous ECG data, digitized at 1000Hz was collected during the same task phases as Study 1 using Grass amplifier (Grass P511, Grass Instruments, USA), CED Power1401 and Spike 2 software (Cambridge Electronic Design, Cambridge, UK). Using the same protocol as Study 1 HR, D2, and RMSSD were derived from the ECG trace. PEP was measured using a Philips Sonos 7500 ultrasound machine with a S3 two dimensional transducer (1-3MHz). Digital images of spectral waveforms were recorded for offline processing. For each measurement point averages were obtained from 3 or more recorded waveforms; phase means were derived from the measurement point averages in each phase. An apical five-chamber view was used to identify blood flow through the aortic valve during systole. The velocity profile of aortic flow was captured immediately below the orifice of the aortic valve (2.0mm sample volume) using pulsed-wave spectral mode at a screen sweep speed of 100mm/s. PEP was quantified using the velocity time integral and overlaid ECG trace to measure the elapsed time between the onset of ventricular depolarization (beginning of QRS complex) and ventricular ejection (beginning of velocity profile). Relatively high correlations have been reported between measures of PEP derived from echo- and impedance cardiography (Carvalho et al., 2010; Cybulski, Michalak, Koźluk, Piątkowska, & Niewiadomski, 2004). Finally, to account for interactions between the parasympathetic and sympathetic branches of the autonomic nervous system an interaction term (INT) was created using the mean-centered change scores of RMSSD and PEP.

*Statistical Analysis*

The same statistical analyses were carried out as in Study 1.

**Results**

*Participants*

The mean (SD) BMI was 23.40 (4.51) kg/m2. Participants achieved a mean score of 600.20 (177.65) on the PASAT and reported an average task stressfulness of 4.42 (1.12).

*Cardiovascular and Autonomic Reactions to Acute Stress*

Repeated measures (baseline, stress) ANOVA revealed that the PASAT significantly increased HR, F(1,160) = 427.66, *p* < .001, η2 = .728. Significant decreases in D2, F(1,160) = 55.26, *p* < .001, η2 = .257, RMSSD, F(1,160) = 122.56, *p* < .001, η2 = .434, and PEP, F(1,160) = 223.15, *p* < .001, η2 = .582, were also observed in response to stress (for summary statistics see Table 3). No main effect of stress was observed for the INT, F(1,160) = 0.01, *p = .92*, η2 = < .001.

[Insert Table 3 about here]

*Autonomic Predictors of Heart Rate Reactivity*

Unadjusted regression models revealed that D2, β = -.40, *p* < .001, ΔR2 = .163, RMSSD, β = -.36, *p* < .001, ΔR2 = .126, PEP, β = -.63, *p* < .001, ΔR2 = .392, and INT, β = -.17, *p* = .03, ΔR2 = .028, were all significant predictors of HR reactivity (Table 4). All measures were negatively related to HR reactivity. In models that were adjusted for HR baseline, BMI, PASAT score, and gender, D2, RMSSD, PEP, and INT all continued to predict HR reactivity. When all autonomic predictors were included in a full single model only RMSSD, PEP, and D2 independently predicted HR reactivity (Table 5).

[Insert Table 4 about here]

[Insert Table 5 about here]

*Sensitivity Analysis: Other HR Complexity Measures*

 As in Study 1, DFα1(r = 0.369, *p* < .001), SD1(r = -0.358, *p* < .001), and SD2 (r = -0.246, *p* = .001) were related to HR reactivity. In contrast to Study 1, DFα2 (r = 0.509, *p* < .001), and ApEn (r = -0.639, *p* < .001), were also related to HR reactivity.

S**ummary**

Results from experiment 2 are consistent with experiment 1. A significant decrease in D2 was again observed in response to acute mental stress and D2 independently predicted HR reactivity even after controlling for several cardiac and non-cardiac confounders. The results also extend the findings of Study 1 by showing that D2 remained an independent predictor of HR reactivity to mental stress when both cardiac parasympathetic and sympathetic activity and the interaction between the autonomic branches were taken into account.

 **Discussion**

 With the present study we sought to examine whether D2, a measure of HR complexity, accounted for a significant portion of the variance in HR reactivity to acute psychological stress when measures of cardiac sympathetic and parasympathetic activity, indexed by PEP and RMSSD, respectively, were accounted for.In the Study 1 it was demonstrated that D2 predicted HR reactivity above and beyond RMSSD, even when changes in respiration rate were taken into account. This finding is consistent with those of Schubert and colleagues (2009) who reported that stress-induced changes in D2 withstood adjustment for respiratory activity. Study 2 included a measure of cardiac beta-adrenergic activity and results showed that D2 still accounted for a unique portion of the variation in HR reactivity when RMSSD, PEP, and their interaction term were included in a single regression model.

The finding of reduced HR complexity under stress accords with others who have reported decreased HR complexity in response to acute mental (Melillo et al., 2011; Schubert et al., 2009), performance (Williamon et al., 2013), and physical (Butler, Yamamoto, & Highson, 1994; Hagerman et al., 1996; Osaka, Saitoh, Atarashi, & Hayakawa, 1993) stress. Presently, the basic physiological meaning of transient stress-induced reductions in HR complexity remains unclear. However, the clinical significance of D2 is better established and may provide some insight into the physiological underpinnings of D2. For example, D2 appears to be related to the electrical stability of the heart. Both environmental and acute stresses have been shown in animals and humans to modulate the electrical stability of the heart. In rats, a social defeat model of stress produced a significant increase in isolated ventricular premature beats (Sgoifo et al., 1999), whereas in dogs, a restraint model of stress significantly reduced the stimulation threshold for repetitive extrasystole (Verrier & Lown, 1984). In humans, mental stress (for review see Taggert, Boyett, Logantha, & Lambiase, 2011) has been reported to significantly increase the presence T-wave alternans (Kop et al., 2004, Lampert et al.,2005), a marker of cardiac electrical instability, increase ventricular premature beats (Lown & DeSilva, 1978), and cause myocardial ischemia (Strike & Steptoe, 2003), which can compromise cardiac electrical stability through changes in ion concentration and tissue conduction (Opie, 1985; James, Taggert, McNally, Newman, Sproton, & Hardman, 2000). Also, a substantial body of literature exists showing an association between stress and sudden cardiac death from ventricular arrhythmia, a clinical condition with etiological roots in the electrical stability of the heart (Kamarck & Jennings, 1991; Lampert, Joska, Burg, Batsford, McPherson, & Jain, 2002; Lane et al., 2005). Consistent with the notion of D2 relating to cardiac electrical stability D2 has been reported to decrease significantly in response to myocardial ischemia and before ventricular fibrillation in animals (Skinner, Carpeggiani, Landisman, & Fulton, 1991) and humans (Skinner, Pratt, & Vybiral, 1993) as well as predict the onset of ventricular fibrillation in prefibrillation heart rate data of arrhythmia patients (Kroll & Fulton, 1991).

Efforts have been made to determine the physiological underpinning of HR generation under stress and there are currently two prominent models of HR stress reactivity. Based on data collected using single and double pharmacological blockades Stemmler and colleagues (1991) advanced a theoretical 6-variable quantitative model of HR reactivity that accounted for the influence of basal HR, α- and β-adrenergic activity, cholinergic influence, as well as interactive effects, and residual effects which they defined as local tissue influences (i.e., metabolic effects). However, when employed to analyze blockade data, a restrictive model was employed that omitted interaction and residual terms. This model was expanded upon by Berntson and colleagues (1991, 1993, 1994) who developed the concept of autonomic space to account for the origins of cardiac reactions to acute psychological stress. This model positioned sympathetic and parasympathetic cardiac control in a 2-dimensional space where changes in HR reactivity could result from a number of autonomic changes ranging from independent changes in a single autonomic branch to co-activation, or reciprocal activation of the autonomic branches. Although this model represented an advance in that it formally acknowledged and accounted for the possibility that the sympathetic and parasympathetic nervous systems may interact, it took no account of the possibility of non-linear influences on HR generation during acute stress exposure. In the present study, D2 continued to predict HR reactivity even when RMSSD, PEP, and INT were accounted for suggesting that a fuller account of the origins of HR stress reactivity should also include measures of complexity such as D2.

A few examples of non-linear physiological mechanisms, that have been shown to be relevant in the context of behavioral stress, include accentuated antagonism, baroreflex function, and central processing. Accentuated antagonism is the autonomic interaction by which increases in sympathetic activity have a lesser chronotropic influence at higher levels of parasympathetic activity and increases in parasympathetic activity have greater chronotropic influence at high levels of sympathetic activity (Levy, & Zieske, 1969; Warner, & Russell, 1969). This interaction was shown in a small study to be relevant in the context of behavioral stress as a significant interaction between left ventricular ejection time and respiratory sinus arrhythmia, indicators of sympathetic and parasympathetic function, respectively, was found to contribute, alongside sympathetic and parasympathetic influences, to the chronotropic response to a battery of behavioral stressors (Uijtdehaage, & Thayer, 2000). D2, in the current study, continued to predict HR reactivity even when this autonomic interaction, modelled using INT, was taken into account. Similarly, the baroreflex, which modulates HR in response to acute changes in blood pressure (Chapleau, Cunnigham, Sullivan, Wachtel, & Abboud, 1995; Gianaros, Onyewuenyi, Sheu, Christie, & Critchley, 2012; Head, & McCarty, 1987), has been implicated in cardiovascular reactions to acute psychological stress (Forsman, & Lindblad, 1983; Sleight, Fox, Lopez, & Brooks, 1978; Stephenson, 1984; Steptoe, & Sawada, 1989). Finally, cortical and subcortical brain regions have been directly implicated in the modulation of HR in response to stress with some regions exerting positive and others negative chronotropic influence (Wager, et al., 2009a; Wager, et al., 2009b). It should be noted that these are just a few of the possible non-linear cardiac influences and that other modulators (e.g., receptor physiology; Marsland et al., 1995; Mills & Dimsdale, 1991; Mills, Dimsdale, Ziegler, Berry, & Bain, 1990; Pacák et al., 1989) may impact HR under behavioral stress but remain to be more fully characterized in this context.

Our overall study is not without limitations. First, respiratory parameters were not measured in Study 2 and, accordingly, the influence of changes in respiration rate during stress on the D2 response could not be assessed. However, previous studies of D2 and respiration have produced mixed results (Kanters, et al., 1997; Mangin, et al., 2008; Schubert et al., 2009), and in Study 1 we found that D2 predicted changes in HR even when changes in respiration rate were controlled for. Second, no clinical endpoints were included in these studies, as they focused on young healthy participants. Accordingly, the relationship between changes in D2 and clinical outcomes could not be assessed. As decreases in D2 have been shown to precipitate adverse cardiac events and stress was found in both studies to decrease D2, further research using large epidemiological datasets should be undertaken to assess the relationship between stress-induced changes in D2 and clinical outcomes.

The advent of HR complexity measures has made possible further progress in characterizing HR generation during acute stress exposure by facilitating quantification of cardiac influences not captured by traditionally used measures (e.g., RMSSD, PEP, RMSSD x PEP interaction). D2 quantifies the number of underlying functional components responsible for a HR time series and may, accordingly, provide insight into stress-induced cardiac change above that gained by measures of cardiac sympathetic and parasympathetic activation. However, current models of cardiac reactivity have largely neglected HR complexity variables. As stress-induced decreases in D2 significantly predicted HR reactivity and remained an independent predictor when measures of cardiac sympathetic and parasympathetic activity and their interaction were controlled for, this study provides evidence to support the inclusion of such measures in models of cardiac stress reactivity. In addition, since decreases in D2 have been shown to predict adverse cardiac events linked to altered electrical stability of the heart future research should aim to evaluate if stress-induced changes in D2 hold clinical value.

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**Table 1. Cardiac descriptive statistics Experiment 1**

|  |  |
| --- | --- |
|  | Mean (SD) |
|  | Baseline | Stress |
|  HR (beats/min) | 73.19 (10.99) | 80.40 (12.90) |
|  RMSSD (ms) | 63.43 (36.66) | 49.58 (45.79) |
|  Correlation Dimension (ms) | 3.41 (1.11) | 2.65 (1.56) |
|  Respiration Rate (breaths/min) | 16.46 (2.22) | 20.78 (4.06) |

**Table 2. Predictors of heart rate reactivity**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | β | *t* | *p* | ΔR2 |
| Individual Models |  |  |  |  |
| RMSSD (n = 25) |  |  |  |  |
|  Unadjusted | -.69 | -4.54 | <.001 | .473 |
|  Adjusted | -.69 | -4.00 | =.001 | .424 |
| Correlation dimension (n = 25) |  |  |  |  |
|  Unadjusted | -.56 | -3.27 | =.003 | .317 |
|  Adjusted | -.66 | -3.65 | =.002 | .382 |
| Full Model (n=25) |  |  |  |  |
| RMSSD | -.57 | -4.04 | =.001 |  |
| Correlation Dimension | -.41 | -2.78 | =.011 | .615 |

**Table 3. Cardiac descriptive statistics Experiment 2**

|  |  |
| --- | --- |
|  | Mean (SD) |
|  | Baseline | Stress |
|  HR (beats/min) | 74.52 (12.09) | 90.70 (14.78) |
|  RMSSD (ms) | 64.31 (42.96) | 34.60 (26.51) |
|  PEP (ms) | 0.091 (0.012) | 0.077 (0.015) |
|  INT | 0.07 (0.49) | 0.09 (1.79) |
|  Correlation dimension  | 3.09 (1.38) | 2.05 (1.53) |

**Table 4. Individual predictors of heart rate reactivity**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | β | *t* | *p* | ΔR2  |
| RMSSD (n = 176) |  |  |  |  |
|  Unadjusted | -.36 | -4.79 | <.001 | .126 |
|  Adjusted | -.34 | -4.56 | <.001 | .099 |
| PEP (n = 162) |  |  |  |  |
|  Unadjusted | -.63 | -10.12 | <.001 | .392 |
|  Adjusted | -.63 | -10.04 | <.001 | .350 |
| INT (n = 162) |  |  |  |  |
|  Unadjusted | -.17 | -2.16 | .03 | .028 |
|  Adjusted | -.28 | -2.97 | .003 | .048 |
| Correlation dimension (n = 176) |  |  |  |  |
|  Unadjusted | -.40 | -5.57 | <.001 | .163 |
|  Adjusted | -.41 | -5.78 | <.001 | .149 |

**Table 5. Full regression model of heart rate reactivity (n = 162)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | β | *t* | *p* | ΔR2 |
|  RMSSD | -.27 | -4.93 | <.001 |  |
|  PEP | -.51 | -8.89 | <.001 |  |
|  INT | -.11 | -1.78 | .076 |  |
|  Correlation dimension | -.28 | -4.91 | <.001 | .539 |