

Aberrant Parasympathetic Stress Responsivity in Pure and Co-Occurring Major Depressive Disorder and Generalized Anxiety Disorder

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Abstract Major Depressive Disorder (MDD) and Generalized Anxiety Disorder (GAD) are highly comorbid; we know little, however, about the shared physiological features of these disorders. In the present study, we examined whether aberrant parasympathetic stress responsivity represents a transdiagnostic process in MDD, GAD, and co-occurring MDD-GAD. Adult women diagnosed with MDD only, GAD only, and co-occurring MDD-GAD and never-disordered controls (CTLs) completed a standardized laboratory task that involved anticipating, confronting, and recovering from a social stressor. Participants' levels of respiratory sinus arrhythmia (RSA) were measured to index parasympathetic responses. The three clinical groups combined (participants with MDD only, GAD only, and co-occurring MDD-GAD) exhibited a similar pattern of RSA responsivity that differed significantly from that of the CTL group. Specifically, whereas CTL participants exhibited a sharp decrease in RSA when confronting the stressor and a sharp increase in RSA when recovering immediately following the stressor, the clinical participants exhibited a blunted response pattern that involved weaker fluctuations in RSA when confronting and recovering from the stressor. There were no significant differences among the three clinical groups in RSA responses. Interestingly, clinical and CTL participants did not differ in self-reported fluctuations in

negative emotional arousal. Finally, for clinical participants patterns of RSA reactivity to the acute stressor were associated differentially with trait rumination and worry as maladaptive forms of emotion regulation. These findings support the formulation that aberrant parasympathetic stress responsivity is a shared feature of MDD, GAD, and co-occurring MDD-GAD that is characterized by diminished reactivity to and recovery from stress.

Keywords Respiratory sinus arrhythmia (RSA) · Stress · Major depressive disorder · Generalized anxiety disorder · Comorbidity · Transdiagnostic

Mood and anxiety disorders are the two most prevalent classes of mental disorder (Kessler et al. 2005a); in particular, Major Depressive Disorder (MDD) and Generalized Anxiety Disorder (GAD) confer significant personal and societal costs. MDD is defined as having one or more clinically significant depressive episodes involving dysphoria, anhedonia, and at least five total symptoms for two or more weeks. GAD is defined by at least 6 months of excessive and pervasive worry that is difficult to control and is accompanied by physical and cognitive symptoms (American Psychiatric Association 2013). MDD and GAD are each frequently characterized by significant impairment across multiple life roles (Kessler et al. 1999) and a recurrent or chronic lifetime course (Ballenger et al. 2001; Kessler et al. 2003). In addition to having substantial consequences as independent disorders, MDD and GAD are the most commonly comorbid mood and anxiety disorder (Kessler et al. 2005b); their rates of co-occurrence are especially high in clinical samples (e.g., Brown et al. 2001). Notwithstanding areas of symptom overlap in current nosology, these associations between MDD and GAD have raised questions about whether the two disorders represent different

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manifestations of similar liability constructs (Krueger and Markon 2006). For example, theorists have proposed that MDD and GAD share high levels of negative affectivity and distress (e.g., Clark and Watson 1991), genetics, and pharmacotherapy response (e.g., Gorwood 2004). In contrast to this established theoretical literature, however, empirical research directly comparing psychobiological processes across MDD, GAD, and co-occurring MDD-GAD is limited.

In the present study, we examined parasympathetic stress responsivity as one potential shared, or transdiagnostic, psychobiological process that transects MDD, GAD, and co-occurring MDD-GAD. Specifically, we assessed parasympathetic regulation of heart rate (HR) during the anticipation of, confrontation with, and recovery from a laboratory social stressor. This form of parasympathetic regulation is subserved by the vagus (10th cranial) nerve, a pathway through which the brain stem exerts control over visceral organs by slowing HR and inhibiting sympathetic nervous system (SNS) influences to the heart. Vagal control of HR varies with respiration, such that vagal activity decreases and HR accelerates during inhalation, and vagal activity increases and HR decelerates during exhalation. Thus, vagal input to the heart can be quantified using the rhythmic HR fluctuations associated with breathing frequencies (0.15–0.40 Hz; i.e., high-frequency heart rate variability [HF-HRV]), which is referred to as respiratory sinus arrhythmia (RSA; Berntson et al. 1997). Importantly, in response to acute stressors, the temporary withdrawal of vagal control or decrease in RSA level that supports SNS activation is viewed as adaptive, indexing flexible psychophysiological responsivity to environmental demands (Porges 1995, 1997). Indeed, both relatively high RSA levels at rest and relatively greater RSA *decreases*, or temporary withdrawals in response to stressors, have been found to predict better mental and physical health outcomes (e.g., Porges et al. 1996; Salomon 2005).

With respect to RSA in MDD, two meta-analyses have shown that individuals diagnosed with MDD exhibit lower resting levels of cardiac vagal control than do individuals with no psychiatric disorder. While findings have been somewhat variable across studies, the overall effect size for this group difference is small to medium (Kemp et al. 2010; Rottenberg 2007). In addition, a handful of studies have examined patterns of RSA responsivity to stressors in MDD. In an initial investigation, Rottenberg and colleagues (2003) examined RSA levels in depressed and nondepressed participants during and following a film clip that was designed to elicit tearful crying. Whereas in nondepressed participants crying in response to the film clip was associated with subsequent RSA increases or rebound, indicative of a regulatory parasympathetic response to strong emotion, in depressed participants this RSA rebound was blunted. These findings were extended in an investigation of psychophysiological responses to two different types of laboratory stressors, a speech task and a mirror-tracing task (Rottenberg et al. 2007): whereas healthy

control participants showed an adaptive response profile involving decreases in RSA from baseline to both of the tasks, participants diagnosed with MDD failed to exhibit a significant decrease in RSA in response to either task. Neither of these studies examined the potential influence of comorbid anxiety on RSA in depressed individuals. Finally, Bylsma and colleagues (2014) recently examined RSA fluctuations throughout a laboratory social stress task in participants with current MDD, participants with remitted MDD, and never-disordered control participants. Interestingly, the current MDD participants showed weaker RSA fluctuations than did the control participants throughout all phases of the task, including during preparation for, confrontation with, and recovery from the stressor. The remitted MDD group did not differ from control participants, suggesting that aberrant RSA responsivity characterizes active depressive symptoms. Moreover, RSA levels were significantly associated with depressive symptom severity, but not with anxiety symptom severity.

A separate body of research has examined parasympathetic functioning in GAD, both at rest and in response to various experimental conditions. Similar to the literature on MDD, several investigators have demonstrated lower resting levels of cardiac vagal control in persons diagnosed with GAD compared to persons with no psychiatric disorder (e.g., Lyonfields et al. 1995; Pittig et al. 2013; Thayer et al. 1996). Not all studies have shown this effect, however (e.g., Aldao and Mennin 2012; Llera and Newman 2010), although it is important to note that GAD is generally not as well researched or understood as are other anxiety disorders (Dugas et al. 2010). In one relevant investigation, Lyonfields and colleagues (1995) assessed participants' fluctuations in HR variability (mean successive differences in interbeat intervals [MSD]) as they shifted from a resting baseline to two emotionally evocative laboratory tasks: an aversive imagery task followed by a worrisome thinking task. Paralleling the findings for MDD described above, whereas nondisordered control participants exhibited significant decreases in MSD from baseline to the imagery task and from the imagery task to the worry task, participants diagnosed with GAD did not exhibit any significant fluctuations in MSD in response to these changing conditions. More recent studies have produced equivocal results. For example, Pittig et al. (2013) reported that participants with GAD did not differ from controls in their degree of RSA change from baseline to a hyperventilation task, and Llera and Newman (2010) found greater RSA fluctuations in participants with GAD than in controls as a function of induced worry versus relaxation. To date, no studies have examined RSA responsivity to social stressors in GAD, which is critical given the significant interpersonal concerns, sensitivity, and problems that characterize this disorder (reviewed in Newman et al. 2013). In the present study, we hypothesized that a blunted pattern of RSA stress responsivity would characterize the diagnoses of both MDD and GAD.

Evidence for aberrant parasympathetic functioning as a transdiagnostic process in MDD and GAD comes from three investigations of individuals with co-occurring MDD-GAD. First, Hofmann et al. (2010) assessed participants with GAD only and participants with co-occurring MDD-GAD as they completed a resting baseline followed by instructed worry and relaxation tasks. Participants with GAD only exhibited lower RSA during each of the tasks than did participants with co-occurring MDD-GAD; the two groups did not differ, however, in their degree of RSA fluctuation from one task to another. Unfortunately, Hofmann et al. did not include a control group to which to compare these findings. Second, Kemp and colleagues (2012) assessed resting cardiac vagal control in participants with MDD only, participants with co-occurring MDD and anxiety disorders (including a subgroup with co-occurring MDD-GAD), and non-disordered control participants. Resting RSA levels were lower in both the MDD only and the co-occurring MDD-GAD groups relative to the control group; in fact, RSA was even lower in the MDD-GAD group than in the MDD only group. Finally, in the single study that has included participants from all four diagnostic groups relevant to the present investigation, Chang et al. (2013) assessed resting RSA in participants with MDD only, GAD only, co-occurring MDD-GAD, and never-disordered controls. The investigators found that resting RSA was reduced both in GAD and in MDD-GAD relative to controls and was lowest in MDD-GAD. Thus, taken together, two of these three studies documented lower resting levels of RSA in MDD-GAD than in either disorder alone (Chang et al. 2013; Kemp et al. 2012). The single study that assessed fluctuations in RSA in GAD and MDD-GAD, however, did not find any group differences (Hofmann et al. 2010), which suggests that MDD-GAD is characterized by a level of RSA stress responsivity similar to that of the single disorders.

Finally, researchers have now begun to examine the relations of RSA to other core processes in MDD and GAD, with a particular focus on rumination and worry as maladaptive forms of emotion regulation. In brief, the construct of rumination refers to perseverative negative thinking about one's feelings and problems; it is a significant risk factor for and associated feature of MDD (Nolen-Hoeksema et al. 2008). Similarly, worry refers to perseverative negative thinking about multiple sources of potential threat in one's life and is the cardinal symptom of GAD (American Psychiatric Association 2013). Interestingly, although no study has examined these constructs in relation to RSA stress responsivity, higher levels of rumination (Woody et al. 2014) and worry (Llera and Newman 2010) have been associated with lower RSA levels in the laboratory. Moreover, there is increasing evidence that rumination and worry are transdiagnostic processes that cut across MDD and GAD (reviewed in Ehring and Watkins 2008), which may ultimately feature prominently in dimensional research approaches to psychopathology (e.g., the

National Institute of Mental Health [NIMH] Research Domain Criteria [RDoC]). These emerging findings highlight the importance of integrating the examination of RSA stress responsivity with the assessment of rumination and worry across MDD, GAD, and co-occurring MDD-GAD.

In the present study, we examined RSA stress responsivity in participants diagnosed with MDD only, GAD only, and co-occurring MDD-GAD and never-disordered controls (CTL). All participants completed a standardized laboratory task that involved anticipating a social stressor, confronting the stressor, and recovering after the stressor. We utilized a social stressor based on its documented ability to produce a strong physiological stress response (e.g., Dickerson and Kemeny 2004) and because social stressors are ecologically relevant to both MDD and GAD (e.g., Conway et al. 2012; Kendler et al. 2003). Fluctuations in participants' RSA levels were measured in order to index parasympathetic responses. We hypothesized that, compared with the CTL group, the three clinical groups combined (i.e., participants with MDD only, GAD only, and co-occurring MDD-GAD) would exhibit a similar pattern of blunted RSA responsivity, including a smaller decrease in RSA when confronting the stressor and a smaller increase in RSA when recovering from the stressor. In addition, we examined participants' subjective levels of negative emotional arousal throughout the task. Finally, we assessed participants' self-reported trait levels of rumination and worry and explored the relations of these variables to fluctuations in RSA across the MDD, GAD, and co-occurring MDD-GAD groups.

Method

Participants

Sixty-seven women (14 MDD, 15 GAD, 20 MDD-GAD, and 18 CTL) between the ages of 18 and 50 years completed the study. We restricted our sample to women both to strengthen statistical power and because MDD, GAD, and their co-occurrence are approximately twice as prevalent in adult women as in men (Kessler et al., 2005ab). Recruitment was conducted through local psychiatric clinics and online advertisements; however, none of the potential participants recruited through clinics met inclusion/exclusion criteria for this study. Participants were initially screened for inclusion and exclusion criteria through a telephone interview. Exclusion criteria were: not fluent in English; learning disabilities; history of severe head trauma; psychotic symptoms; bipolar disorder; *Diagnostic and Statistical Manual of Mental Disorders* (4th ed.; *DSM-IV-TR*; American Psychiatric Association 2000)-defined alcohol or substance abuse in the past 6 months; and electrocardiogram (ECG) confounds (e.g., diagnosis of cardiovascular disorder, cardiac pacemaker).

Clinical Assessment

Diagnostic Interview Participants who were identified as likely to meet inclusion criteria were invited to participate in a laboratory diagnostic evaluation based on *DSM-IV-TR* criteria using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I; First et al. 1996), administered by a trained interviewer. Participants in the MDD group met diagnostic criteria for current MDD, with no diagnosis of GAD concurrently or within the past 24 months. Participants in the GAD group met diagnostic criteria for current GAD, with no diagnosis of MDD concurrently or within the past 24 months. Previous research on the comorbidity of MDD and GAD has documented that individuals with 12-month comorbidity and individuals with current co-occurrence have similar clinical correlates (e.g., clinical severity, impairment; Kessler et al. 1999; Wittchen et al. 2000). Therefore, in order to increase both the homogeneity of the ‘pure’ MDD and GAD groups and our statistical power to detect differences between these groups, we used a more conservative window of 24 months. Participants in the MDD-GAD group met criteria for current MDD and current GAD. *DSM-IV-TR* contains a hierarchical exclusion criterion in which GAD caseness cannot be met when the syndrome occurs only in the context of MDD; however, because the epidemiological and experimental literature and a *DSM-5* work group have indicated that there is little utility and potentially significant problems associated with this exclusion criterion (Andrews et al. 2010; Lawrence et al. 2009; Zimmerman and Chelminski 2003), we did not impose it so that we could fully capture this important and understudied co-occurring MDD-GAD group.¹ Finally, participants in the CTL group did not meet criteria for any current or lifetime Axis I disorder. Diagnostic interviews were audio recorded and a randomly-selected 25 % of audiotapes across the four groups were used to re-rate current diagnoses of MDD and GAD by a different interviewer who was blind to the original diagnoses. Inter-rater reliability was excellent for classifying the presence/absence both of MDD ($k=1.00$) and of GAD ($k=0.87$).

¹ Indeed, within our MDD-GAD group, in 40% of cases GAD preceded MDD; in 30% of cases MDD preceded GAD; in 10% of cases MDD and GAD started at the same time; and in 20% of cases participants stated that they were unable to report with precision their relative timing of onset. Consistent with our multilevel model across the three clinical groups, we ran the multilevel model of RSA stress responsivity in the MDD-GAD group only in order to examine whether there were any differences between participants for whom GAD preceded MDD and for whom GAD occurred in the context of MDD (using a Level 2 variable dummy-coded as 0 or 1). There were no significant differences between these two subgroups in any of the RSA measures, all $ps > .523$.

Self-Report Questionnaires We administered the Beck Depression Inventory (BDI-II; Beck et al. 1996) and the Generalized Anxiety Disorder Questionnaire-IV (GAD-Q-IV; Newman et al. 2002) to assess symptom severity of MDD and GAD, respectively. These measures have shown strong psychometric properties in previous studies (e.g., BDI-II $\alpha=.91$, Dozois et al. 1998; GAD-Q-IV $\alpha=.83$, Rodebaugh et al. 2008). In the current sample, there was high internal consistency reliability for both the BDI-II ($\alpha=.96$) and GAD-Q-IV ($\alpha=.83$), with the dichotomous items summed to analyze them as a continuous item (Rodebaugh et al. 2008). To assess habitual depressive rumination, participants completed the five-item Brooding subscale of the Ruminative Response Styles (RRS) scale (Nolen-Hoeksema and Morrow 1991). Brooding entails maladaptive and passive repetitive thinking about one’s feelings and problems, and scores on this subscale of the RRS have been associated most reliably with negative outcomes (Nolen-Hoeksema et al. 2008). To assess habitual worry, participants completed the well-validated Penn State Worry Questionnaire (Meyer et al. 1990). These measures have also shown excellent psychometric properties in previous studies (e.g., RRS Brooding subscale $\alpha=.77$, Treynor et al. 2003; PSWQ $\alpha=.86-.95$, Brown et al. 1992). In the current sample, internal consistency reliability was high for both the RRS Brooding Subscale ($\alpha=.87$) and PSWQ ($\alpha=.94$).

Participants completed questionnaires concerning their demographic information, height and weight, current use of psychotropic medication, and current engagement in outpatient psychosocial treatment. Importantly, some investigators have suggested that the use of psychotropic medications, including tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRIs), contributes to the suppression of RSA in depression and anxiety disorders (e.g., Licht et al. 2008, 2009). We included participants who were taking medication in order to increase the generalizability of our sample, and we recorded current use of all psychotropic medications so that we could examine the effects of medication on RSA stress responsivity. We assessed height and weight in order to calculate body mass index (BMI), which has been found to be associated with RSA. Finally, on the day of the laboratory session, participants reported on their subjective quality of sleep during the previous night and whether they engaged in exercise, smoked, or drank a caffeinated beverage earlier that day, as additional variables that have been found to be associated with RSA (e.g., Bylsma et al. 2014; Pittig et al. 2013).

Participant Characteristics

Demographic and clinical characteristics for the MDD, GAD, MDD-GAD, and CTL groups are presented in Table 1. There were no group differences in age, $F(3, 66)=1.64, p=.189$,

Table 1 Demographic and clinical characteristics of the MDD, GAD, MDD-GAD, and CTL groups

Variable	MDD <i>M (SD)</i> or %	GAD <i>M (SD)</i> or %	MDD-GAD <i>M (SD)</i> or %	CTL <i>M (SD)</i> or %
Age	30.29 (9.97)	30.13 (6.92)	35.50 (10.10)	35.17 (9.93)
% college educated	64.29 %	60.00 %	65.00 %	66.67 %
Race/ethnicity*				
Non-Hispanic White	50.00 %	67.67 %	60.00 %	61.11 %
Hispanic	0.00 %	20.00 %	10.00 %	0.00 %
African-American	0.00 %	0.00 %	5.00 %	11.11 %
Asian-American	21.43 %	13.33 %	15.00 %	5.56 %
Native American	0.00 %	0.00 %	0.00 %	5.56 %
Mixed race	28.57 %	20.00 %	10.00 %	11.11 %
Psychiatric comorbidities				
Specific phobia	0.00 % ^a	26.67 % ^b	15.00 % ^a	0.00 % ^a
Social anxiety disorder	0.00 % ^a	13.33 % ^a	20.00 % ^b	0.00 % ^a
Panic disorder/agoraphobia	7.14 %	13.33 %	0.00 %	0.00 %
Obsessive-compulsive disorder	0.00 %	6.67 %	0.00 %	0.00 %
Posttraumatic stress disorder	0.00 % ^a	0.00 % ^a	20.00 % ^b	0.00 % ^a
Eating disorder	0.00 %	0.00 %	5.00 %	0.00 %
% taking psychotropic medication	35.71 % ^b	13.33 % ^b	25.00 % ^b	0.00 % ^a
% receiving psychosocial treatment	14.29 %	13.33 %	15.00 %	0.00 %
BDI-II score	28.26 (9.16) ^c	14.40 (9.72) ^b	30.70 (10.63) ^c	1.56 (2.68) ^a
GAD-Q-IV score	7.73 (4.25) ^b	11.06 (1.09) ^c	10.38 (2.62) ^c	2.01 (2.23) ^a
RRS Brooding score	13.43 (2.68) ^b	11.80 (3.95) ^b	15.50 (2.65) ^c	6.44 (1.20) ^a
PSWQ score	57.50 (13.79) ^b	68.47 (6.31) ^c	61.00 (13.26) ^{b,c}	41.72 (12.15) ^a

BDI-II Beck Depression Inventory-II, *CTL* no past or current psychiatric disorder, *GAD* current generalized anxiety disorder, *GAD-Q-IV* Generalized Anxiety Disorder Questionnaire-IV, *MDD* current major depressive disorder, *PSWQ* Penn State Worry Questionnaire, *RRS* Ruminative Response Styles Questionnaire. *Race and ethnicity data were missing for one CTL participant. ^{a,b,c} Significant pairwise comparisons, $p < .05$

proportion of college-educated participants, $\chi^2(3, N=67)=0.17, p=.983$, proportion of non-Hispanic White participants, $\chi^2(3, N=66)=1.01, p=.798$, or proportion of participants receiving psychosocial treatment, $\chi^2(3, N=67)=2.90, p=.408$. The groups differed in current use of psychotropic medication, $\chi^2(3, N=67)=7.84, p=.049$: MDD, GAD, and MDD-GAD participants were more likely to be taking medication than were CTL participants. Significant pairwise comparisons on rates of other specific psychiatric comorbidities and on the BDI-II, GAD-Q-IV, RRS Brooding subscale, and PSWQ are denoted in Table 1.

Psychophysiological Assessment

Data Acquisition

Physiological activity was recorded continuously at a sampling rate of 1 kHz using the Biopac MP150 system and AcqKnowledge software package (Biopac Systems, Goleta, CA). Specifically, we recorded participants' cardiovascular activity using the ECG amplifier module and three disposable electrodes positioned in a modified lead II configuration.

Signal Processing

Physiological data were scored in 300-second intervals using ANSLAB (Wilhelm et al. 1999), a customizable physiological software package that has been used in previous studies of RSA responsivity and emotion regulation in clinical samples (e.g., Butler et al. 2006; Hopp et al. 2013). Using ANSLAB, we inspected the ECG signal for artifacts and missing R-peaks (based on improbable interbeat intervals). For each 60 s, if one R-peak was missing, an R-peak was inserted at a time point halfway between the two adjacent R-peaks. If more than one R-peak was missing, that 60-second period was not scored (Berntson et al. 1997). After correcting for artifacts and missing R-peaks, we used fast Fourier transformation to derive average spectral power values and integrated the power values in the 0.15–0.40 Hz spectral bandwidth as our index of RSA level for each 300-second interval.

Subjective Assessment

Participants' subjective experience was assessed using two visual analog scales: negative affect (left pole=*not at all*

negative; right pole=*extremely negative*) and arousal (left pole=*not at all aroused*; right pole=*extremely aroused*). These scales were administered using a computer and participants' responses along the scales were tagged with a numerical value ranging from 0 (left pole) to 1000 (right pole). Data were missing for four participants due to an error in computerized administration.

Procedure

This study was part of a larger research project approved by the Stanford University Institutional Review Board. Informed consent was obtained from participants prior to the start of the diagnostic evaluation. Immediately after the diagnostic session, participants completed a laboratory session that involved several computer tasks, which was followed by a 1-week experience sampling protocol. Participants received monetary compensation for each component of the overall research project.

Laboratory Session Entry and Baseline

Approximately 10 days after participants completed the diagnostic evaluation, participants arrived at the laboratory session. Participants were instructed to refrain from eating and drinking (except water) 1 h before the session. Upon their arrival, the experimenter familiarized participants with the psychophysiological recording equipment and attached the ECG sensors as well as a respiration band and skin conductance sensors (data not presented here). Participants were seated for a 10-minute habituation period, followed by a 10-minute baseline recording during which resting RSA level was assessed. Levels of negative affect and arousal were assessed immediately after baseline recording.

Modified Trier Social Stress Task

Participants completed a modified version of the Trier Social Stress Task (TSST; Kirschbaum et al. 1993). The TSST is a dual-task stressor entailing a public speaking task and mental arithmetic task, and a recent meta-analysis found the TSST to be most effective in eliciting physiological responsivity to an acute laboratory stressor (Dickerson and Kemeny 2004). As adapted for the present study, the TSST consisted of four periods: anticipation (10 min); confrontation (15 min); immediate recovery (15 min); and final recovery (15 min). At the start of the anticipation period, the experimenter obtained informed consent for the speech task, informed participants that they would soon be preparing and delivering a speech in the presence of an evaluator, and instructed participants to sit quietly for the next 10 min. The experimenter did not state the topic of the speech, in an attempt to elicit subjective uncertainty and anxiety. The experimenter next re-entered the room and

instructed participants to prepare a 5-minute speech promoting their candidacy for a job. Following 5 min of solitary preparation, the acute stressor proceeded with the participant in a standing position throughout. A trained confederate dressed in a laboratory coat entered the room and set up a video camera to purportedly record participants; the confederate was trained to observe participants stoically, silently take notes on the participants' behaviors, and respond in a standardized manner to any lapses in speaking. After 5 min, the confederate instructed participants to complete a second unexpected task involving subtracting 13 serially from 1022 for 5 min. If participants made an error, the confederate instructed them to stop and start over. The confederate then left the room. At the start of the immediate recovery period, the experimenter seated participants and asked them to sit quietly for 15 min. Finally, the experimenter re-entered the room and instructed participants to wait for an additional 15 min, which corresponded to the final recovery period. We allotted 30 min total for recovery as the minimum amount of recovery time that was used in the canonical TSST (Kirschbaum et al. 1993). Levels of negative affect and arousal were assessed immediately after each period of the task. At the end of the session, participants were debriefed and compensated for their participation.

Data Reduction and Statistical Analyses

For each participant, RSA data were scored in 300-second (5-minute) intervals, resulting in a total of 13 successive RSA values: baseline (10 min; 2 successive values), anticipation (10 min; 2 successive values), confrontation (15 min; 3 successive values), immediate recovery (15 min; 3 successive values), and final recovery (15 min; 3 successive values). To analyze these data, we conducted multilevel modeling using HLM software, Version 6.01 (Raudenbush et al. 2004). This statistical approach enabled us to model the repeated measurements of RSA within persons as a function of the TSST 'conditions,' or periods. Specifically, we were able to simultaneously model each participant's baseline RSA level and temporal slopes of RSA in response to all of the different TSST periods. Our Level 1 model quantified within-person baseline RSA level and fluctuations in RSA, and our Level 2 model quantified individual differences in baseline RSA level and fluctuations in RSA.

With respect to subjective responsivity, for each participant, we rotated scores on the negative affect and arousal scales by 45° ($NA = \text{negative affect}/\sqrt{2} + \text{arousal}/\sqrt{2}$; adapted from Knutson et al. 2005) to derive the dimension of negative arousal (NA) both as most theoretically relevant to RSA stress responsivity and to reduce multiple testing. We computed NA separately for each period of the task: baseline, anticipation, confrontation, immediate recovery, and final recovery. As described below, we used multilevel modeling to analyze the NA data in a parallel manner to the RSA data.

Results

Differences in RSA Between Clinical and Control Participants

Raw RSA values scored at 5-minute intervals at baseline and over the course of the TSST are presented in Fig. 1. In examining our primary research question, we used a four-rate piecewise linear growth model (Raudenbush and Bryk 2002). A piecewise model separates repeated measurements into discrete periods of time; because we expected that the different periods of the TSST would exert varying influences on RSA, a piecewise model was well suited to test our hypotheses. We simultaneously estimated levels of RSA during the baseline period and slopes of RSA in response to the anticipation, confrontation, and two recovery periods. We specified the following Level 1 model:

$$\begin{aligned} \text{RSA} = & \pi_{0j} + \pi_{1j}(\text{anticipation}) + \pi_{2j}(\text{confrontation}) \\ & + \pi_{3j}(\text{immediaterecovery}) + \pi_{4j}(\text{finalrecovery}) \\ & + e_{ij} \end{aligned}$$

in which π_{0j} corresponds to baseline RSA level for participant j , π_{1j} corresponds to the slope of RSA as a function of stressor anticipation for participant j , π_{2j} corresponds to the slope of RSA as a function of stressor confrontation for participant j , π_{3j} corresponds to the slope of RSA as a function of

immediate recovery for participant j , and π_{4j} corresponds to the slope of RSA as a function of final recovery for participant j (see Fig. 1 for the RSA values that were used to compute baseline level and slope terms). e_{ij} denotes the within-person random effect. Three participants (1 MDD, 1 GAD, and 1 CTL) declined to complete the speech task in the TSST; therefore, only data corresponding to baseline and anticipation were collected and analyzed for these participants.

At Level 2 we evaluated differences between the CTL and clinical participants in baseline RSA level and fluctuations in RSA, with this variable dummy-coded as 0 (CTL group) or 1 (clinical group; i.e., participants with MDD, GAD, and MDD-GAD combined). Prior to conducting our full Level 2 model, we used one Level 2 model to test the associations of all potential Level 2 covariates to the prediction of RSA baseline and slope terms specified in the Level 1 model, with the exception of psychotropic medication and psychosocial treatment, which were necessarily confounded with membership in the clinical group. Age (centered at grand mean) and engagement in exercise on the day of the session (dummy-coded) each emerged as a significant predictor of several the baseline and slope terms for RSA. Therefore, we included these variables in all five Level 2 equations in the full model in order to be most consistent in partialing their effects from that of the clinical group. We specified the intercept and slope effects as random, given that previous studies of RSA stress responsivity indicate sizeable within-group variability (reviewed in Rottenberg et al. 2007) and maximal random-effects structure is recommended for

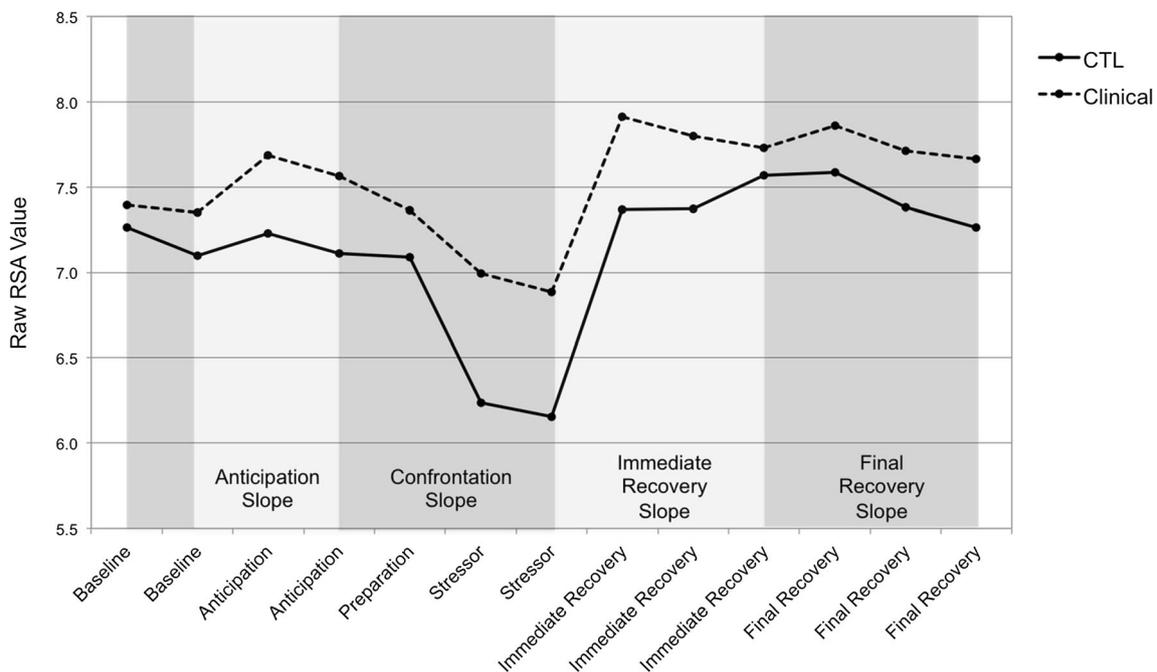


Fig. 1 Raw RSA values scored at 5-minute intervals at baseline and over the course of the TSST for the control and clinical groups. Note. CTL= never-disordered control participants; Clinical=participants with current

major depressive disorder (MDD) only, current generalized anxiety disorder (GAD) only, or current co-occurring MDD-GAD

hypothesis testing when model convergence can be achieved (Barr et al. 2013). Indeed, all of the models for RSA stress responsivity converged. Therefore, we specified the following Level 2 model, which included five equations:

$$\text{Baseline} : \pi_{0j} = \beta_{00} + \beta_{01}(\text{clinical group}) + \beta_{02}(\text{age}) \\ + \beta_{03}(\text{exercise}) + r_0$$

$$\text{Anticipation slope} : \pi_{1j} = \beta_{10} + \beta_{11}(\text{clinical group}) \\ + \beta_{12}(\text{age}) + \beta_{13}(\text{exercise}) \\ + r_1$$

$$\text{Confrontation slope} : \pi_{2j} = \beta_{20} + \beta_{21}(\text{clinical group}) \\ + \beta_{22}(\text{age}) + \beta_{23}(\text{exercise}) \\ + r_2$$

$$\text{Immediate recovery slope} : \pi_{3j} = \beta_{30} + \beta_{31}(\text{clinical group}) \\ + \beta_{32}(\text{age}) + \beta_{33}(\text{exercise}) \\ + r_3$$

$$\text{Final recovery slope} : \pi_{4j} = \beta_{40} + \beta_{41}(\text{clinical group}) \\ + \beta_{42}(\text{age}) + \beta_{43}(\text{exercise}) \\ + r_4$$

In the first Level 2 equation above, β_{00} denotes the mean baseline RSA level in the CTL group, β_{01} denote the difference between the CTL group and the clinical group in mean baseline RSA, and r_0 denotes the between-persons random effect. In the second Level 2 equation, β_{10} denotes the mean slope of RSA for the anticipation period in the CTL group, β_{11} denotes the difference between the CTL group and the clinical group in mean slope of RSA for the anticipation period, and r_1 denotes the between-persons random effect. Following this same system of denotation, we tested all potential interactions between Level 1 and Level 2 predictors in all of our models.

Coefficient estimates and significance tests are presented in Table 2. As shown in Table 2, three Level 1 RSA slope terms were significant, indicating that the CTL participants exhibited a significant decrease in RSA when confronting the stressor, $p < .001$, a significant increase in RSA when recovering immediately after the stressor, $p < .001$, and, unexpectedly, a significant decrease in RSA during final recovery, $p < .001$. At

Table 2 Hierarchical linear model of RSA stress responsivity as a function of CTL and clinical groups

Predictor	Coefficient	SE	t	p
Baseline (intercept)	7.31	0.32	22.89	< .001
Clinical group	0.08	0.35	0.23	.816
Age	-0.05	0.02	-2.89	.006
Exercise	-0.07	0.41	-0.16	.874
Anticipation (linear)	-0.08	0.07	-1.20	.235
Clinical group	0.16	0.08	2.12	.038
Age	0.01	0.00	2.33	.023
Exercise	0.10	0.07	1.40	.165
Confrontation (linear)	-0.32	0.06	-5.67	< .001
Clinical group	0.15	0.06	2.59	.012
Age	0.01	0.00	1.85	.068
Exercise	0.18	0.07	2.74	.008
Immediate recovery (linear)	0.54	0.08	7.16	< .001
Clinical group	-0.24	0.13	-1.95	.055
Age	0.00	0.01	0.11	.915
Exercise	-0.28	0.13	-2.15	.035
Final recovery (linear)	-0.22	0.05	-4.51	< .001
Clinical group	0.15	0.06	2.72	.009
Age	-0.00	0.00	-0.24	.809
Exercise	0.18	0.09	2.09	.040

Results reflect one model that includes one Level 1 equation and five Level 2 equations. Predictors listed are at Level 2. Never-disordered control group is reference group. Clinical group=participants with current major depressive disorder (MDD) only, current generalized anxiety disorder (GAD) only, or current co-occurring MDD-GAD, as compared to control group

Level 2, clinical group was not a significant predictor of baseline RSA level, $p = .816$, but it was a significant predictor of the RSA slope terms. Surprisingly, compared to the CTL participants, the clinical participants exhibited a relative increase in RSA when anticipating the stressor, $p = .038$. As predicted, the clinical participants exhibited a smaller decrease in RSA when confronting the stressor, $p = .012$, and a marginally smaller increase in RSA when recovering immediately after the stressor, $p = .055$, than did CTL participants. Finally, the clinical participants exhibited a smaller decrease in RSA during final recovery, $p = .009$, than did CTL participants.

In addition to testing this transdiagnostic model of RSA stress responsivity, we conducted a series of follow-up HLM analyses testing for differences among the MDD, GAD, and co-occurring MDD-GAD groups in baseline RSA level and fluctuations in RSA. The Level 1 model was identical to the

Level 1 model reported above. At Level 2 we evaluated differences among the diagnostic groups, with these variables dummy-coded as 0 (referent group; e.g., MDD only group) or 1 (comparison group; e.g., GAD only group). Consistent with the transdiagnostic model, prior to conducting our full Level 2 model, we tested the associations of all potential Level 2 covariates, including the current use of psychotropic medication and receipt of psychosocial treatment, to the prediction of RSA baseline and slope terms specified in the Level 1 model. Only age (centered at grand mean) emerged as a significant predictor of a subset of the terms for RSA, and we included this variable in all Level 2 equations in the full models. The results of all of these models demonstrated that the three clinical groups did not differ significantly from one another in their baseline RSA level or RSA fluctuations when anticipating, confronting, or recovering from the stressor. In the one marginally significant effect ($p < .10$), the MDD group exhibited a greater increase in RSA during final stressor recovery than did the GAD group, $p = .077$.²

Differences in Subjective Responsivity Between Clinical and Control Participants

We used a corresponding four-rate piecewise linear growth model to simultaneously evaluate baseline NA and fluctuations in NA as a function of anticipating, confronting, and recovering from the stressor. We specified the following Level 1 model:

$$\text{NA} = \pi_{0j} + \pi_{1j}(\text{anticipation}) + \pi_{2j}(\text{confrontation}) \\ + \pi_{3j}(\text{immediate recovery}) + \pi_{4j}(\text{final recovery}) + e_{ij}$$

in which, consistent with the Level 1 model for RSA, the different denotations represent baseline NA and fluctuations in NA, and only data corresponding to baseline and anticipation were analyzed for the three participants who declined to complete the speech task. At Level 2 we evaluated differences between the CTL and clinical participants in baseline NA and fluctuations in NA. Prior to conducting our Level 2 model, we tested the Level 2 covariates used in the model for RSA, age and

engagement in exercise, and found that neither variable was a significant predictor of the baseline or terms for NA.³ Therefore, we specified the following Level 2 model, which included five equations:

$$\text{Baseline} : \pi_{0j} = \beta_{00} + \beta_{01}(\text{clinical group}) + r_0$$

$$\text{Anticipation slope} : \pi_{1j} = \beta_{10} + \beta_{11}(\text{clinical group}) + r_1$$

$$\text{Confrontation slope} : \pi_{2j} = \beta_{20} + \beta_{21}(\text{clinical group}) \\ + r_2$$

$$\text{Immediate recovery slope} : \pi_{3j} = \beta_{30} + \beta_{31}(\text{clinical group}) \\ + r_3$$

$$\text{Final recovery slope} : \pi_{4j} = \beta_{40} + \beta_{41}(\text{clinical group})^4$$

Coefficient estimates and significance tests are presented in Table 3. As shown in Table 3, two Level 1 NA slope terms were significant, indicating that the CTL participants reported a significant increase in NA when confronting the stressor, $p = .011$, and a significant decrease in NA when recovering immediately after the stressor, $p = .022$. At Level 2, clinical group was a significant predictor of baseline NA, $p < .001$, indicating that the clinical participants reported a significantly higher baseline level of NA than did CTL participants. However, clinical group was not a significant predictor of any of the NA slope terms, all $ps > .219$, indicating that the clinical participants did not differ from CTL participants in reported NA stress reactivity or recovery.

We conducted a series of follow-up HLM analyses testing for differences among the MDD, GAD, and co-occurring MDD-GAD groups in baseline NA and fluctuations in NA. As with the transdiagnostic model of NA, we tested the Level 2 covariate used in the model for RSA, age, and found that it did not significantly predict any of the baseline or slope terms for NA.⁴ The results of all of these models demonstrated that

² We re-ran our analyses comparing RSA stress responsivity across the clinical groups while controlling for the presence of other DSM-IV anxiety disorders (Specific Phobia, Social Anxiety Disorder, Panic Disorder/Agoraphobia, Obsessive-Compulsive Disorder, and Posttraumatic Stress Disorder). The presence of other anxiety disorders was not significantly associated with any of the RSA measures, all $ps > .484$, and no new significant differences emerged among the clinical groups in any of the RSA measures, all $ps > .102$.

³ There were no new significant results of these models when we included the covariates used in the models of RSA stress responsivity.

⁴ The multilevel models for NA, both across all four groups and across the three clinical groups only, did not converge when all of the Level 2 equations included random effect terms and required removal of one of these random effects in order to converge. We elected to remove the random effect term for the final recovery period, as across the NA models this period was associated with the lowest proportion of total variance of explained.

Table 3 Hierarchical linear model of subjective negative arousal responsiveness as a function of CTL and clinical groups

Predictor	Coefficient	SE	<i>t</i>	<i>p</i>
Baseline (intercept)	305.82	32.98	9.27	< .001
Clinical group	214.53	42.17	5.09	< .001
Anticipation (linear)	1.28	26.39	0.05	.962
Clinical group	47.13	37.89	1.24	.219
Confrontation (linear)	134.31	50.83	2.64	.011
Clinical group	14.08	63.17	0.22	.824
Immediate recovery (linear)	-108.54	46.12	-2.35	.022
Clinical group	-32.53	54.06	-0.60	.549
Final recovery (linear)	-47.99	36.57	-1.31	.191
Clinical group	-25.36	43.09	-0.59	.556

Results reflect one model that includes one Level 1 equation and five Level 2 equations. Predictors listed are at Level 2. Never-disordered control group is reference group. Clinical group=participants with current major depressive disorder (MDD) only, current generalized anxiety disorder (GAD) only, or current co-occurring MDD-GAD, as compared to control group

the three clinical groups did not differ significantly from one another in their baseline level of NA or fluctuations in NA when anticipating or confronting the stressor. There was one marginally significant effect in which the MDD group reported greater baseline NA than did the MDD-GAD group, $p=.063$. There was also one significant effect during the final recovery period, in which the GAD group reported a greater decrease in NA than did the MDD group, $p=.006$.

Associations of RSA Stress Responsivity to Subjective Stress Responsivity

In supplementary analyses, we explored the associations between patterns of RSA stress responsivity and NA responsiveness across groups. For each participant, we computed a change score for negative arousal (NA) from each period of the task to the next (e.g., anticipation change score=NA level for anticipation period – NA level for baseline period). We then centered and used these change scores and their interaction with clinical group as additional Level 2 predictors in the transdiagnostic model of RSA stress responsivity. Therefore, we specified the following Level 2 model:

$$\begin{aligned} \text{Baseline} : \pi_{0j} = & \beta_{00} + \beta_{01}(\text{clinical group}) + \beta_{02}(\text{baseline NA}) \\ & + \beta_{03}(\text{baseline NA} \times \text{clinical group}) \\ & + \beta_{04}(\text{age}) + \beta_{05}(\text{exercise}) + r_0 \end{aligned}$$

$$\begin{aligned} \text{Anticipation slope} : \pi_{1j} = & \beta_{10} + \beta_{11}(\text{clinical group}) \\ & + \beta_{12}(\text{anticipation NA change score}) \\ & + \beta_{13}(\text{anticipation NA change score} \\ & \times \text{clinical group}) \\ & + \beta_{14}(\text{age}) + \beta_{15}(\text{exercise}) + r_1 \end{aligned}$$

$$\begin{aligned} \text{Confrontation slope} : \pi_{2j} = & \beta_{20} + \beta_{21}(\text{clinical group}) \\ & + \beta_{22}(\text{confrontation NA change score}) \\ & + \beta_{23}(\text{confrontation NA change score} \\ & \times \text{clinical group}) \\ & + \beta_{24}(\text{age}) + \beta_{25}(\text{exercise}) + r_2 \end{aligned}$$

$$\begin{aligned} \text{Immediate recovery slope} : \pi_{3j} = & \beta_{30} + \beta_{31}(\text{clinical group}) \\ & + \beta_{32}(\text{immediate recovery NA change score}) \\ & + \beta_{33}(\text{immediate recovery NA change score} \\ & \times \text{clinical group}) \\ & + \beta_{34}(\text{age}) + \beta_{35}(\text{exercise}) + r_3 \end{aligned}$$

$$\begin{aligned} \text{Final recovery slope} : \pi_{4j} = & \beta_{40} + \beta_{41}(\text{clinical group}) \\ & + \beta_{42}(\text{final recovery NA change score}) \\ & + \beta_{43}(\text{final recovery NA change score} \\ & \times \text{clinical group}) + \beta_{44}(\text{age}) \\ & + \beta_{45}(\text{exercise}) + r_4 \end{aligned}$$

There was one marginally significant interaction between confrontation NA change score and clinical group (coefficient=0.0004, $SE=0.0002$, $t=1.90$, $p=.063$); whereas CTL participants exhibited a more negative coupling between RSA and NA when confronting the stressor (coefficient=-0.0003, $SE=0.0002$, $t=-1.63$, $p=0.109$; i.e., greater NA increases were associated with greater RSA decreases), the clinical participants exhibited an opposing pattern of more positive coupling (coefficient=0.0001, $SE=0.0001$, $t=0.95$, $p=0.348$; i.e., greater NA increases were associated with lesser RSA decreases). As a negative coupling would be expected between RSA and NA, these results further support an aberrant pattern of stress responsivity in MDD, GAD, and MDD-GAD.

In addition to testing this transdiagnostic model of RSA stress responsivity, we conducted a series of follow-up HLM analyses testing for differences among the MDD, GAD, and co-occurring MDD-GAD groups in the associations between RSA stress responsivity and NA responsiveness. We used the NA change scores and their interaction with the diagnostic groups as additional Level 2 predictors in the group-specific model of RSA stress responsivity. There was one significant and several marginally significant interactions between NA

change scores and the various diagnostic groups. With respect to baseline, there were marginally significant interactions between baseline NA and MDD-GAD group (relative to MDD group: coefficient=0.0038, *SE*=0.0020, *t*=1.90, *p*=.064; relative to GAD group: coefficient=0.0035, *SE*=0.0019, *t*=1.86, *p*=.071): whereas MDD participants and GAD participants exhibited a more negative coupling between baseline RSA and baseline NA (MDD group: coefficient=-0.0019, *SE*=0.0013, *t*=-1.51, *p*=.140; GAD group: coefficient=-0.0016, *SE*=0.0010, *t*=-1.58, *p*=.122), the MDD-GAD participants exhibited an opposing pattern of more positive coupling (coefficient=0.0019, *SE*=0.0016, *t*=1.20, *p*=0.240). With respect to final recovery, there was a significant and a marginally significant interaction between NA final recovery change score and MDD-GAD group (relative to MDD group: coefficient=-0.0006, *SE*=0.003, *t*=-1.76, *p*=.086; relative to GAD group: coefficient=-0.0006, *SE*=0.0002, *t*=-2.72, *p*=.010): whereas MDD participants and GAD participants exhibited a more positive coupling between RSA recovery slope and NA final recovery change score (MDD group: coefficient=0.0004, *SE*=0.0003, *t*=1.26, *p*=.216; GAD group: coefficient=0.0004, *SE*=0.0002, *t*=2.13, *p*=.040), the MDD-GAD participants exhibited an opposing pattern of more negative coupling (coefficient=-0.0002, *SE*=0.0001, *t*=-2.02, *p*=0.051).

Associations of RSA Stress Responsivity to Rumination and Worry

Finally, to evaluate the associations of RSA stress responsivity to trait maladaptive emotion regulation, we conducted an HLM analysis in the clinical participants only. The Level 1 model was identical to the Level 1 model for RSA reported above. At Level 2 we evaluated individual differences in baseline RSA level and RSA stress responsivity as a function of RRS Brooding and PSWQ scores (both centered at grand mean). We included age (centered at grand mean). We specified the following Level 2 model, which included five equations:

Baseline : $\pi_{0j} = \beta_{00} + \beta_{01}(\text{RRS Brooding score}) + \beta_{02}(\text{PSWQ score}) + \beta_{03}(\text{age}) + r_0$

Anticipation slope : $\pi_{1j} = \beta_{10} + \beta_{11}(\text{RRS Brooding score}) + \beta_{12}(\text{PSWQ score}) + \beta_{13}(\text{age}) + r_1$

Confrontation slope : $\pi_{2j} = \beta_{20} + \beta_{21}(\text{RRS Brooding score}) + \beta_{22}(\text{PSWQ score}) + \beta_{23}(\text{age}) + r_2$

Immediate recovery slope : $\pi_{3j} = \beta_{30} + \beta_{31}(\text{RRS Brooding score}) + \beta_{32}(\text{PSWQ score}) + \beta_{33}(\text{age}) + r_3$

Final recovery slope : $\pi_{4j} = \beta_{40} + \beta_{41}(\text{RRS Brooding score}) + \beta_{42}(\text{PSWQ score}) + \beta_{43}(\text{age}) + r_4$

Interestingly, higher RRS Brooding score predicted a smaller decrease in RSA when confronting the stressor, $\beta=0.01$, *SE*=0.00, *t*=2.52, *p*=.016, whereas higher PSWQ score predicted a greater decrease in RSA when confronting the stressor, $\beta=-0.01$, *SE*=0.00, *t*=-2.35, *p*=.023. No other associations of RSA stress responsivity with rumination or worry were significant.

Discussion

The aim of the present study was to investigate aberrant parasympathetic stress responsivity as a transdiagnostic psychobiological process that cuts across MDD, GAD, and co-occurring MDD-GAD. Using RSA as a well-established index of parasympathetic regulation, we found that, indeed, these three diagnostic groups collectively exhibited a pattern of RSA stress responsivity that differed significantly from that of CTL participants. As hypothesized, the clinical participants exhibited a blunted pattern of responsivity that was characterized by weaker fluctuations in RSA when confronting and recovering immediately after the stressor. Contrary to previous literature, however, the clinical participants did not differ from CTL participants in resting RSA level, and they also displayed a relatively greater increase in RSA from rest to the stressor anticipation period.

Although the clinical participants reported a higher baseline level of NA than did CTL participants, there were no significant differences between the clinical and CTL groups in degree of NA stress responsivity. Importantly, this suggests that the group differences in RSA stress responsivity were not driven by group differences in subjective stress. However, CTL participants exhibited a marginally more negative coupling between RSA and NA responses to the acute stressor than did clinical participants, which suggests that MDD,

GAD, and MDD-GAD may be characterized by weaker coherence between psychophysiological and subjective stress response systems. Finally, within the clinical sample, trait rumination and worry were differentially associated with patterns of RSA reactivity to the acute stressor. All of these findings may be fruitful to explore further in research examining psychophysiology, subjective emotion, and emotion regulation in psychopathology. Below we discuss in greater detail these study findings, implications, and directions for future research.

As evidenced by the strong fluctuations of both RSA and NA in CTL participants when they confronted and recovered immediately following the stressor, the current results generally support the perspective that the flexible withdrawal of vagal control in response environmental demands is adaptive and reflective of healthy psychiatric functioning (Porges 1995, 1997). There was one unexpected effect for CTL participants in which they exhibited a relatively large decrease in RSA during final recovery. In interpreting this finding, it is possible that the final recovery period was experienced as a mild stressor, to which the CTL participants responded more strongly than did the clinical participants. Specifically, when the experimenter re-entered the room after immediate recovery, participants might have expected to be released from the experiment, but instead were instructed to wait silently for another 15 min. This extended waiting might have been experienced as mildly aversive in itself or generated anxiety about when the experiment would be finished. However, CTL participants reported no significant changes in subjective NA from immediate to final recovery.

In contrast, for individuals diagnosed with MDD, GAD, and co-occurring MDD-GAD, a blunted or more rigid profile of parasympathetic stress reactivity may be associated with decreased ability to cope effectively with stressors, potentially contributing to an impaired stress recovery response. Interestingly, while we found support for blunted RSA stress responsivity as a transdiagnostic process that cuts across these disorders, we found no evidence for the aberrant RSA at rest in any of these disorders that has been reported in several studies (Chang et al. 2013; Hofmann et al. 2010; Kemp et al. 2012). One possible explanation for this lack of significant findings is that participants were familiarized with the laboratory setting, given that they had completed a diagnostic interview and unrelated experimental tasks prior to attending the current session. A more novel laboratory or clinical context might elicit greater anxiety in the clinical groups, serving to decrease RSA levels during baseline recording. Thus, the lower resting levels of RSA that have been found in MDD, GAD, and MDD-GAD in previous studies may reflect heightened responsivity to more novel environments rather than stable differences across all types of situations.

In addition, the clinical participants exhibited an unexpected increase in RSA during stressor anticipation; it is possible

that the intended elicitation of an anxious anticipation state as relevant to GAD was not sufficient to reduce RSA levels, as would be predicted on the basis of previous associations between worry and RSA (e.g., Aldao et al. 2013; Llera and Newman 2010). Given that the clinical participants exhibited more of a decrease in RSA immediately following anticipation, in response to the preparation period, it is possible that the imminence of the stressor or the effort involved in coping with the stressor during the preparation period was more effective in suppressing vagal control. Finally, while not fully captured by the RSA slope terms used in our statistical analyses, it is noteworthy that the clinical participants exhibited a more delayed trajectory of RSA levels during recovery than did the CTL participants. Future investigations that use stress responsivity paradigms should continue to include sustained recovery periods in order to further assess possible delayed effects of stressors on parasympathetic functioning in clinical groups.

Importantly, patterns of acute RSA responsivity to the stressor in MDD, GAD, and MDD-GAD were differentially associated with habitual rumination and worry, two maladaptive emotion regulation processes that are increasingly considered to be transdiagnostic (Ehring and Watkins 2008; McEvoy et al. 2013). In particular, whereas more blunted vagal withdrawal when confronting the stressor was associated with higher self-reported rumination, more pronounced vagal withdrawal when confronting the stressor was associated with higher self-reported worry. It is important to note that in our clinical participants, levels of rumination and worry were uncorrelated ($r=.07$, $p=.621$); thus, it is unlikely that these effects were due to statistical artifacts arising from shared variance among the measures. The effect for trait worry is particularly interesting in light of Aldao et al.'s (2013) finding that state worry, but not state rumination, was associated with lower levels of HRV in response to emotional film clips. Building on Aldao et al.'s cogent arguments, it is possible that the construct of worry is more closely tied to threat states than is rumination, and that the association of worry with heightened vagal withdrawal in the present study reflects a more imminent general threat stance toward stressors. In reconciling the notion that rumination and worry are conceptually related and seem to be transdiagnostic, with these divergent findings for the two variables, it may be that the relative ratio of engagement in these forms of emotion regulation is critical. For example, given the current findings, persons with MDD, GAD, or MDD-GAD who engage in relatively more worry than rumination may exhibit more pronounced RSA reactivity to stressors. Furthermore, the fact that such individuals respond physiologically more strongly to stressors may reciprocally reinforce their tendency to worry, given recent theory that worry serves to avoid negative emotional contrasts in individuals who are especially sensitive to their own negative state (Newman and Llera 2011). More broadly, the unique relations

of rumination and worry to divergent patterns of RSA responsiveness in this study contrast with the lack of significant differences among the clinical groups. This provides support for emerging research frameworks (i.e., the NIMH RDoC) that aim to characterize relations of psychophysiological measures to core dimensionally-assessed processes rather than to categorical entities. Clearly, it will be important to replicate and extend these findings concerning rumination, worry, and RSA.

With respect to implications for clinical procedures, most traditional models and methods of treatment focus on single disorders. Increasingly, both research (Insel 2013) and treatment (e.g., Barlow et al. 2004) efforts are adopting an alternative construct-based perspective. The present findings highlight anomalous RSA stress responsivity as one cross-cutting construct that can be relatively easily recorded and quantified across the clinical syndromes of MDD, GAD, and co-occurring MDD-GAD. Critically, we found RSA to be distinct from subjective stress responsivity in its ability to distinguish between psychiatric and non-psychiatric groups. In clinical settings, therefore, RSA may provide a more objective and sensitive index of aberrant stress responsivity than does patient self report. In addition, given our documented association of worry with reductions in parasympathetic regulation, along with similar prior findings (Aldao et al. 2013; Llera and Newman 2010), interventions that aim to target worry as a cognitive process might utilize RSA as an objective measure of treatment progress or outcome. Furthermore, it may be fruitful to use biofeedback procedures to directly target aberrant RSA stress responses in treatment as one method to help patients to cope more effectively with social stressors in their daily lives.

Four limitations of the current study warrant discussion. First, in constructing our groups for this study, we prioritized particular diagnostic inclusion/exclusion criteria (i.e., current and 24-month diagnoses of MDD and GAD), which made it challenging to precisely balance the groups on all other variables while still recruiting a sufficient sample size. Thus, we recruited a smaller sample than in some previous studies of MDD and of GAD. In addition to using relatively stringent diagnostic criteria, we attempted to enhance statistical power to detect group differences by recruiting only women and including multiple measurement points both within participants and within the periods of the TSST. However, there is no clear rule for determining power in complex multilevel modeling contexts (Snijders 2005). Future studies should consider recruiting both female and male participants on the basis of dimensionally-assessed constructs rather than diagnostic syndromes, which may increase both statistical power relative to categorical models and the generalizability of findings to other forms of psychopathology. Indeed, while aberrant RSA stress responsivity may be common to these two disorders and their co-occurrence, this feature may extend to other disorders as well.

Second, the activities that comprised the confrontation period - speaking and standing - both produce shifts in RSA that make it difficult to directly compare this period to the other periods of the TSST. While this does not preclude examination of group differences during this type of stressor, future studies might extend our findings by utilizing a stressor that does not involve speaking or standing. Third, we implemented a highly standardized laboratory stressor in order to increase the internal validity of our study. Much less is known about individuals' naturalistic responses to stressors; we are currently pursuing this research question in MDD, GAD, and MDD-GAD using experience sampling methodology. Fourth, we focused on parasympathetic stress responsivity as a potential transdiagnostic process, and we also found evidence for common patterns of NA stress responsivity across these diagnoses. Future studies should examine potential disorder-specific processes using expanded assessments of shared and unique dimensions in depression and anxiety (Clark and Watson 1991).

In sum, these findings support the formulation that aberrant parasympathetic stress responsivity is a shared feature of MDD, GAD, and co-occurring MDD-GAD, which is characterized by diminished reactivity to and recovery from stress and is associated with core maladaptive emotion regulation processes. In contrast to traditional research paradigms that examine single diagnoses, incorporating multiple clinical groups, constructs, and measures in a single study will increase our understanding of these disorders and their common and unique processes.

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Conflict of Interest Katharina Kircanski, Christian E. Waugh, M. Catalina Camacho, and Ian H. Gotlib report no conflicts of interest.

Experimental Participants All procedures performed in studies involving human participants were in accordance with the ethical standards of institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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