Relative burst amplitude of muscle sympathetic nerve activity is an indicator of altered sympathetic outflow in chronic anxiety

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Abstract

Relative burst amplitude of muscle sympathetic nerve activity (MSNA) is an indicator of augmented sympathetic outflow and contributes to greater vasoconstrictor responses. Evidence suggests anxiety-induced augmentation of relative MSNA burst amplitude in patients with panic disorder, thus we hypothesized that acute stress would result in augmented relative MSNA burst amplitude and vasoconstriction in individuals with chronic anxiety. Eighteen participants with chronic anxiety (ANX, 8 men/10 women, 32±2 years) and 18 healthy controls with low/no anxiety (CON, 8 men/10 women, 39±3 years) were studied. Baseline MSNA and 24-hour blood pressure were similar between ANX and CON ($P>0.05$); however, nocturnal systolic blood pressure % dipping was blunted among ANX ($P=0.02$). Relative MSNA burst amplitude was significantly greater among ANX compared with CON immediately preceding (anticipation) and during physiological stress (2-min cold pressor test, ANX: 73±5% vs. CON: 59±3% AU, $P=0.03$) and mental stress (4-min mental arithmetic, ANX: 65±3% vs. CON: 54±3% AU, $P=0.02$). Increases in MSNA burst frequency, incidence, and total activity in response to stress were not augmented among ANX compared with CON ($P>0.05$), and reduction in brachial artery conductance during cold stress was similar between ANX and CON ($P=0.92$). Relative MSNA burst amplitude during mental stress was strongly correlated with state ($P<0.01$) and trait anxiety ($P=0.01$) (State-Trait Anxiety Inventory), independent of age, sex and BMI. Thus, in response to acute stress, both mental and physiological, individuals with chronic anxiety demonstrate selective augmentation in relative MSNA burst amplitude, indicating enhanced sympathetic drive in a population with higher risk for cardiovascular disease.

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47 **New & Noteworthy**

48 Relative burst amplitude of muscle sympathetic nerve activity in response to acute mental and
49 physiological stress is selectively augmented in individuals with chronic anxiety, which is a
50 prevalent condition that predicts the development of cardiovascular disease. Augmented
51 sympathetic burst amplitude occurs with chronic anxiety in the absence of common
52 comorbidities. These findings provide important insight to the relation between anxiety, acute
53 stress, and sympathetic activation.

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Introduction

Anxiety is the most common mental health problem in the United States, occurring in about 18% of adults per year (30). Anxiety is also predictive of later incidence of hypertension (27) and coronary heart disease (29, 48). The idea that comorbidities such as hypertension can arise from prolonged stress and anxiety has been supported by a significant number of epidemiological and clinical studies (15, 49). Alteration in the autonomic nervous system resulting in sympathetic overactivity is characteristic of hypertension and stressor-induced cardiac events (5, 42). Therefore, exaggerated sympathetic responses to stress may be an important link between anxiety and development of cardiovascular disease.

Key regions of the brain that become dysregulated with anxiety, such as the amygdala, send projections to areas of the brainstem essential in regulating sympathetic outflow (12, 47, 65). Brain areas involved with stress responses such as the periaqueductal gray are also associated with changes in sympathetic nerve activity (56). Electrical stimulation of the dorsolateral periaqueductal gray in humans leads to increased muscle sympathetic nerve activity (MSNA) burst amplitude but not burst frequency (56). Burst amplitude and frequency of multi-unit MSNA are important characteristics of sympathetic drive because they reflect the firing pattern of single-unit sympathetic neurons. An increase in firing probability of single-unit neurons, or recruitment of latent single-unit neurons, may increase burst frequency, but they may also fire (typically once) in synchrony to increase burst amplitude. Additionally, active single-unit neurons can increase firing rate within a burst to increase burst amplitude, such as during intense physiological stimuli (34, 39, 43) [For supplementary description of sympathetic discharge patterns in humans, the reader is directed to recent expert reviews: (40, 53)]. Interestingly, patients with intense forms of anxiety such as panic disorder exhibit augmented

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MSNA burst amplitude but not burst frequency during panic attacks (67). Augmented MSNA burst amplitude in panic disorder has been associated with an increase in firing rate of individual sympathetic nerve fibers from once to up to 3-4 times during a single burst of MSNA (34, 36). This is important because previous studies indicate that MSNA can shift toward higher burst amplitude before an observed increase in burst frequency in patients with heart failure (57, 58), suggesting that MSNA burst amplitude is a sensitive and unique indicator of pathological increases in sympathetic activity. In addition, larger MSNA burst amplitude is associated with greater vasoconstrictor responses in healthy humans (17, 18). Moreover, studies by Lambert and colleagues (2010) demonstrated a correlation between anxiety and greater firing rate of individual sympathetic fibers during bursts of MSNA, which would theoretically constitute greater MSNA burst amplitude (35). However, it remains unclear whether augmented MSNA burst amplitude is in fact a unique characteristic of sympathetic outflow in chronic anxiety.

In the present study, we examined multi-unit MSNA in individuals with moderate/high chronic anxiety and controls with low/no anxiety at rest and during sympathoexcitatory stress. Given that previous data suggests anxiety-induced augmentation of MSNA burst amplitude in patients with panic disorder (67), we hypothesized that individuals with chronic anxiety would exhibit augmented MSNA burst amplitude responses to a sympathoexcitatory stimuli (cold stress) compared with controls with low/no anxiety, and that augmented MSNA burst amplitude responses would lead to greater sympathetic vasoconstriction. Also, to determine whether augmented MSNA burst amplitude in anxiety manifests in response to psychological stress, MSNA was examined during a mental stress task (mental arithmetic) in a subset of the study participants.

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Methods

All experimental procedures and protocols conformed to the Declaration of Helsinki and were approved by the University of Iowa Institutional Review Board (Project#: 201409782). Each subject received a verbal and written explanation of the study objectives, measurement techniques, and risks and benefits associated with the investigation prior to providing written informed consent.

Subjects: A total of 36 participants were studied (age range: 25-63 years). Eighteen healthy participants with moderate/high anxiety (ANX, 8 men/10 women) based on anxiety assessments (see Anxiety Assessments) and 18 controls with low/no anxiety (CON, 8 men/10 women) who were nonsmokers and free of cardiovascular, metabolic, or neurological disease were recruited through the University of Iowa. Timing of study visits for women were not controlled for menstrual cycle phase because previous studies demonstrate that there is no effect of menstrual cycle phase on MSNA and BP responses to acute mental stress (10). A urine pregnancy test confirmed the exclusion criteria of pregnancy. All participants visited the lab for screening and written informed consent prior to the experimental day.

Experimental Measurements

Anxiety assessments: Generalized Anxiety Disorder 7-item (GAD-7), a valid and reliable self-report scale for screening generalized anxiety disorder (55), was used in part for study entry criteria. A GAD-7 score ≥10 was used for eligibility for ANX participants, and a score ≤5 was used for eligibility for CON participants. Participants met individually with a psychiatrist (J.G.F.) who performed a structured diagnostic interview (M.I.N.I-International Neuropsychiatric

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Interview) before participation in the study. Anxiety was also assessed using the State-Trait Anxiety Inventories (STAI) surveys among all participants (54). The STAI is a self-report survey that assesses "state" anxiety, which reflects how the person feels right at a specific moment in time, and "trait" anxiety, which is longer-term tendency to be anxious. Total scores for the STAI are the sum of all responses and range from 20-80. Anxiety and depressive symptoms were also assessed using the Beck Anxiety Inventory (BAI) and Beck Depressive Inventory (BDI-II) surveys (3, 4).

Muscle sympathetic nerve activity: Multiunit postganglionic MSNA was recorded using the standard microneurographic technique, as previously described (24, 25, 59). Briefly, a tungsten microelectrode was placed into the peroneal nerve near the left fibular head. Signals were amplified, filtered (bandwidth 0.7-2.0 kHz), rectified and integrated (0.1 s time constant) to obtain mean voltage neurograms (Nerve Traffic Analyzer; University of Iowa Bioengineering, Iowa City, IA). MSNA was identified by the presence of spontaneous bursts with characteristic pulse synchronicity and morphology, and by its responsiveness to end-expiratory breath holds (apnea), but not to arousal or skin stimulation. MSNA data was acquired at a frequency of 1,000 Hz using a Powerlab data acquisition system (ADInstruments) and analyzed using LabChart version 8.1.5 (ADInstruments).

Brachial artery conductance: Brachial artery blood velocity and diameter were measured longitudinally in the distal third of the upper arm with a high-resolution ultrasound system (Logiq7, GE). Diameter and blood velocity were measured continuously (beat-to-beat) using a 12-MHz linear-array Doppler probe in pulsed-wave mode with an insonation angle of 60

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degrees. Blood flow was calculated as the product of mean blood velocity (cm/s) and cross-sectional area (cm^2) and multiplied by 60 (milliliters per minute). Mean blood flow was divided by beat-to-beat mean blood pressure (BP) (conductance), and was expressed as percent change from the 2-min baseline immediately preceding the stimulus. The vasoconstrictor response to elevations in MSNA was assessed during cold stress, but not mental stress, because data demonstrate that changes in forearm blood flow during mental arithmetic are not associated with changes in MSNA (6, 9).

Cardiorespiratory measures: Heart rate was determined from lead II of the 3-lead ECG. Beat-to-beat BP was estimated using finger photoplethysmography (Nexfin), and arm cuff BP was estimated using electrosphygmomanometry over the brachial artery. Respiratory movements were monitored using a strain-gauge pneumobelt placed around the abdomen (Pneumotrace, UFI). Respiration rate was calculated as the number of inspiratory peaks per minute. The amplitude of the respiratory movements, used as an estimate of tidal volume, was measured in arbitrary units and calculated for each respiratory cycle as the range from the inspiratory peak to the next expiratory nadir of the tracing. Respiratory rate and amplitude were used to estimate minute-ventilation (V_E). Estimated minute ventilation was not quantified during mental stress because participants were verbally communicating with the researcher administering the mental stress task.

Ambulatory 24-hour BP: Noninvasive ambulatory 24-hour BP was obtained using oscillometric SpaceLabs 90207 monitors (SpaceLabs Inc) (45). SpaceLabs monitors were programmed to obtain BP readings at intervals of 30 minutes during the day from 0600-2300 hours and

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nighttime every 60 min from 2300-0600 hours. Participants were instructed to record their activities and sleep periods for the 24-hour monitoring period. At least 10 daytime readings and 5 nighttime readings and at least 80% successful readings of planned measurements over the 24 hours were required (38). Average values for systolic, diastolic, and mean BP, and BP variability (standard deviation) were determined from individual 24-hour recordings. Daytime (awake) and nocturnal (sleeping) BP was adjusted to the nearest hour based on each participant's written record of their activities and sleep periods for the 24-hour monitoring period. The percent of nocturnal systolic BP dipping was calculated as: [(nocturnal systolic BP – daytime systolic BP) / daytime systolic BP] × 100. Two ANX participants elected to not wear the 24-hour BP cuff.

Experimental protocol: On the experimental day, participants arrived at the laboratory in the Institute for Clinical and Translational Science Clinical Research Unit between 7:00am and 9:00am following an overnight fast. Participants were instructed to refrain from medication use the morning of the study (one ANX participant was taking a SSRI, and another ANX participant was taking a tricyclic antidepressant). Participants were also requested to abstain from caffeinated beverages the morning of the study and strenuous physical activity and alcohol for at least 24 h before experimental sessions. All experiments were performed in a dimly-lit room at an ambient room temperature of 22-24ºC. Upon arrival, a venous catheter was inserted into the antecubital or a hand vein of the right arm for blood sampling of norepinephrine and a metabolic panel. The venous catheter was not able to be placed in 3 ANX and 4 CON participants, therefore only a metabolic panel via butterfly needle was obtained in these participants. Next, while supine, subjects were instrumented for HR, BP, and MSNA. Once all signals were acquired, data were collected for at least a 10-min baseline period to determine resting values.

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MSNA and vascular conductance responses to cold stress were primary measures, therefore the cold stress protocol (ANX: n=18, CON: n=18) preceded the mental stress protocol (ANX: n=13, CON: n=15) for all participants (separated by 15 min) and order was not randomized. At the end of the study visit, participants were fitted for a 24-hour ambulatory BP monitor.

Physiological (cold) stress: A cold pressor test was used to determine MSNA, HR, brachial artery conductance and BP responses to a physiological sympathoexcitatory stimulus (60). The left hand was placed in ice water for 2 min. All variables were recorded during a 2-min baseline period, during cold stress, and during a 2-min recovery. Participants were then asked to rank the pain/discomfort of the cold stress on a scale of 0-10. Study investigators did not speak to participants during the 2-min baseline period prior to the cold stress, but did give a verbal countdown before beginning the 2-min baseline period ("beginning cold stressor 2-min baseline, 3, 2, 1, start"). Order of events was explained to each participant before beginning, therefore participants understood approximately when the cold stress would begin.

Mental stress: Mental arithmetic was used to determine MSNA, HR and BP responses to a mental stress task. Following a 2-min baseline period, participants were subjected to 4 min of verbal arithmetic that involved the continuous subtraction of a one- or two-digit number (randomly chosen) from a 3- or 4-digit number (e.g., 1547 minus 13), and a new number from which to begin subtracting was given every 20-30 s (1, 2). Participants were pressed to answer verbally as quickly and accurately as possible. All participants reported the task to be frustrating and demonstrated obvious relief at the end of the task. MSNA, HR and BP were continuously recorded at baseline, during mental arithmetic, and during a 2-min recovery. Study investigators

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did not speak to participants during the 2-min baseline period prior to mental stress, but did give a verbal countdown before beginning the 2-min baseline period.

Data Analysis. Resting neural and cardiovascular variables were calculated as mean values over the initial 10-min baseline period. MSNA was quantified as burst frequency (bursts/min), burst incidence (bursts/100heartbeats), and total activity (burst frequency multiplied by mean burst amplitude, AU/min). If no MSNA burst was detected for a particular cardiac cycle, a value of zero was assigned to that cardiac cycle and not included in MSNA total activity. Absolute MSNA burst amplitude cannot be compared between individuals because maximum height of a burst is determined by how close the tip of the microelectrode is to the sympathetic axons, which cannot be exactly replicated (40). Therefore, relative MSNA burst amplitude was used, and was calculated by attributing the value of 100 to the maximum burst height during the baseline recording, which was determined from the average of the 3 largest bursts, and expressing all other burst amplitudes as a percentage of the maximum burst height as previously described by our lab and others (17, 24, 25, 36, 63). Therefore, relative MSNA burst amplitude for each condition was an average of normalized burst amplitudes based on the maximum burst height of the preceding baseline. Relative MSNA burst amplitude included only cardiac cycles with identified bursts of MSNA. The change in MSNA, HR and BP in response to stress was relative to the 2-min baseline immediately preceding the stimulus.

Statistical Analysis: All data are reported as mean ± SEM. Statistical comparisons of baseline variables between ANX and CON were made using t-tests and analysis of covariance

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(ANCOVA) to adjust for age, sex and BMI where indicated. Statistical analyses of physiological responses to stress were made using two-way repeated measures ANOVA (2-min baseline, cold or mental stress, 2-min recovery). Bivariate correlational analyses between measures of anxiety and physiological responses to stress were adjusted for age, sex and BMI using partial correlation. Data were analyzed using SigmaPlot 13 (Systat Software Inc.) and statistical significance was set at $P < 0.05$.

Results

Subject characteristics: As expected, measures of anxiety and depression were significantly higher in ANX compared to CON (Table 1). No significant differences were observed in plasma triglycerides (P=0.86) and HDL (P=0.36) between ANX and CON, but fasting glucose tended to be higher in CON (P=0.07), and fasting insulin was unexpectedly higher in CON (P=0.03). No differences were observed between ANX and CON in resting HR (P=0.25) and mean BP (P=0.26). 24-hour ambulatory BP and BP variability were also similar between ANX and CON (all P>0.05) (Table 1). However, ANX had significantly blunted nocturnal systolic BP % dipping compared with CON (P=0.02). Indeed, systolic BP % dipping during sleep was significantly and inversely correlated with trait anxiety score (R=-0.36, P=0.039) (i.e., the higher the anxiety score the lesser the systolic BP % dipping). No difference was observed between ANX and CON in self-reported physical activity during work and leisure time (Total aerobic min/week: ANX 265 ± 44 vs. CON 277 ± 40, P=0.83) [12-month avg. based on Modifiable Activity Questionnaire (32, 33, 64) and ACSM physical activity guidelines (22)].

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Resting sympathetic activity: Examples of individual resting MSNA recordings from 5 ANX participants and 5 CON participants of same sex and comparable age are presented in Figure 1. There were no significant differences between ANX and CON in resting MSNA burst frequency (ANX: 18 ± 3 vs. CON: 24 ± 2 bursts/min, P=0.07), burst incidence (ANX: 30 ± 5 vs. CON: 40 ± 4 bursts/100hb, P=0.14), relative burst amplitude (ANX: 48 ± 1 vs. CON: 47 ± 1 AU, P=0.46), and total activity (ANX: 862 ± 139 vs. CON: 1155 ± 122 AU/min, P=0.12). Means adjusted for age, sex, and BMI (ANCOVA) were also not significantly different between ANX and CON: MSNA burst frequency (ANX: 18 ± 3 vs. CON: 24 ± 3 bursts/min, P=0.13), burst incidence (ANX: 33 ± 4 vs. CON: 36 ± 4 bursts/100hb, P=0.55), relative burst amplitude (ANX: 48 ± 1% vs. CON: 47 ± 1% AU, P=0.50), and total activity (ANX: 850 ± 132 vs. CON: 1142 ± 132 AU/min, P=0.14). No relation between MSNA and depression was observed (e.g., MSNA burst incidence vs. Beck Depression Inventory: R=0.06, P=0.74, adjusted for age, sex, and BMI). Plasma norepinephrine concentration tended to be higher in ANX compared to CON (ANX: 361 ± 39 vs. CON: 232 ± 24 pg/mL, P=0.05). The correlation between plasma norepinephrine (log transformed) and resting MSNA burst frequency (R=0.19, P=0.33) and relative MSNA burst amplitude (R=0.13, P=0.49) were not statistically significant.

MSNA responses to cold stress: Relative MSNA burst amplitude was significantly greater among ANX compared to CON during the 2-min baseline prior to cold stress (P=0.03) (Figure 2A). The rise in relative MSNA burst amplitude during the 2-min baseline preceding cold stress occurred in ANX and CON; however, this rise was significantly greater among ANX compared with CON (P=0.04) (Figure 2C), suggesting an enhance sympathetic anticipatory response. The overall increase in relative MSNA burst amplitude during cold stress and 2-min recovery was

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significantly greater among ANX compared with CON (2-way RM ANOVA; 2-min BL, cold stress, 2-min recovery, P=0.02) (Figure 2A). After controlling for age, sex, and BMI, measures of anxiety were moderately correlated with relative MSNA burst amplitude during cold stress (Figure 5A). In contrast, MSNA burst frequency during the 2-min baseline immediately prior to cold stress was similar to the 10-min resting baseline and was not significantly different between ANX and CON (P=0.17) (Figure 3A). No significant differences between ANX and CON were observed in the increase in MSNA burst frequency, burst incidence and total activity during cold stress (2-way RM ANOVA; 2-min BL, cold stress, 2-min recovery, all P>0.05) (Figure 3A-C). Ratings of discomfort of the cold stress reported by participants (scale 0-10) were not significantly higher among ANX compared to CON (ANX: 5.3 ± 0.6 vs. CON: 6.6 ± 0.4, P=0.10).

Cardiovascular responses to cold stress: A small but significant increase in HR was observed during the 2-min duration immediately preceding cold stress when compared with the 10-min resting baseline (P=0.003), although the increase in HR was not significantly different between ANX and CON (ANX: Δ1 ± 1 vs. CON: Δ2 ± 1 bpm, P=0.19). In response to cold stress, ANX and CON demonstrated similar peak increases in heart rate (ANX: Δ24 ± 3% vs. CON: Δ23 ± 4% bpm, P=0.82), systolic BP (ANX: Δ16 ± 2% vs. CON: Δ17 ± 2% mmHg, P=0.77), and mean BP (ANX: Δ20 ± 2% vs. CON: Δ20 ± 2% mmHg, P=0.90). In contrast to our hypothesis, the decrease in brachial artery conductance in response to elevations in MSNA during cold stress was not greater among ANX compared with CON (Figure 4A). No significant difference between ANX and CON were observed in the increase in estimated minute ventilation (V_E) when compared to the 10-min baseline (2-min baseline preceding cold stress: ANX Δ7 ± 6% vs.

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CON Δ13 ± 7%; 2-min cold stress: ANX Δ52 ± 24% vs. CON Δ42 ± 16%, P=0.89), suggesting the pattern of augmented relative MSNA burst amplitude during cold stress among ANX was likely not a result of differences in respiration.

MSNA responses to mental stress: Similar to cold stress, relative MSNA amplitude during the 2-min baseline immediately preceding mental stress was greater among ANX compared to CON (P=0.04) (Figure 2B). The rise in relative MSNA burst amplitude during the 2-min baseline preceding mental stress (anticipation) compared with the 10-min baseline tended to be greater among ANX compared with CON (Figure 2C), although not statistically significant (P=0.11). Relative MSNA burst amplitude during mental stress was increased and significantly augmented among ANX compared with CON (2-way RM ANOVA; 2-min BL, mental stress, 2-min recovery, P=0.02) (Figure 2B). In contrast, MSNA burst frequency and incidence was lower among ANX compared with CON during mental stress (2-way RM ANOVA; 2-min BL, cold stress, 2-min recovery) (Figure 3D-F). As a result, MSNA total activity tended to be lower among ANX compared with CON during mental stress (Figure 3F). Relative MSNA burst amplitude responses to mental stress were strongly correlated with both "state" and "trait" anxiety, and remained strongly correlated after adjusting for age, sex, and BMI (Figure 5AI-II), whereas measures of anxiety were not related to MSNA burst incidence (Figure 5BII-II). Similar correlations were seen for Beck Anxiety Inventory (R=0.51, P=0.01). However, measures of depression (Beck Depression Inventory, BDI) were not significantly correlated with relative MSNA burst amplitude responses to mental stress (R=0.37, P=0.06, adjusted for age, sex and BMI).

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Cardiovascular responses to mental stress: A small but significant increase in HR was observed during the 2-min duration immediately preceding mental stress when compared with the 10-min resting baseline (ANX: Δ2 ± 1 vs. CON: Δ3 ± 1 bpm, P<0.001), although the increase in HR was not significantly different between ANX and CON (P=0.40). In response to mental stress, no significant differences between ANX and CON were observed for peak increases heart rate (ANX: Δ20 ± 3% vs. CON: Δ28 ± 5%, P=0.19), systolic BP (ANX: Δ8 ± 1% vs. CON: Δ9 ± 2%, P=0.67), and mean BP (ANX: Δ10 ± 1% vs. CON: Δ12 ± 2%, P=0.38).

Discussion

This comprehensive study of sympathetic neural and cardiovascular responses to acute stress among individuals with chronic anxiety reveals three important findings. First, multi-unit MSNA at rest was *not* elevated in healthy adults with chronic anxiety. Second, chronic anxiety was associated with augmented relative MSNA burst amplitude during anticipation of mental and physiological stress. Third, relative MSNA burst amplitude was further exaggerated during acute mental and physiological stress in individuals with chronic anxiety compared with controls with low/no anxiety while increases in MSNA burst frequency and incidence were not augmented with chronic anxiety. In contrast to our hypothesis, sympathetic vasoconstriction in response to elevated MSNA was not greater among individuals with chronic anxiety compared to controls as indicated by similar reductions in brachial artery conductance. These data demonstrate that relative MSNA burst amplitude, but not burst frequency or incidence, is selectively exaggerated in response to acute stress in individuals with chronic anxiety, while local vasoconstriction in the upper limb is not augmented.

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Studies of anxiety in individuals with hypertension and metabolic syndrome, and other anxiety disorders, such as panic disorder, demonstrate alteration in firing properties of individual sympathetic fibers (35, 36, 61, 67), but no studies have demonstrated alteration in multi-unit MSNA in individuals with chronic anxiety. A study of 13 individuals with panic disorder did not demonstrate alterations in multi-unit MSNA responses to laboratory-based mental stress (67). This is not surprising given the vast amount of variability in MSNA responsiveness to laboratory-based mental stress among individuals, which can make it difficult to detect group differences. Individuals may exhibit a rise or fall in MSNA burst frequency during mental stress independent of the perceived difficulty of the task (7, 8, 11, 14, 19), and independent of age (44) and sex (28). In panic disorder, relative MSNA burst amplitude has not been assessed during mental arithmetic; however, augmented MSNA burst amplitude has been observed during spontaneous panic attacks (67). In the present study, the increase in MSNA burst amplitude in response to mental stress was strongly correlated with anxiety scores independent of age, sex and BMI. Although anxiety and depression are closely linked, results demonstrated a weaker correlation between relative MSNA burst amplitude responses and quantitative measures of depression. The findings of the present study extend results of previous investigations by demonstrating that alteration in multi-unit MSNA in individuals with chronic anxiety manifests in response to mental and physiological stress, and that the alteration is a selective augmentation in MSNA burst amplitude rather than burst frequency or incidence.

MSNA burst frequency overall tended to be less among participants with chronic anxiety compared with controls. This was surprising given previous studies demonstrating a relation between anxiety symptoms and greater plasma norepinephrine concentration (26). However, it should be noted that MSNA total activity, which reflects total sympathetic vasoconstrictor

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activity (burst frequency × mean burst amplitude), was similar between participants with anxiety and controls in response to cold stress, which was the more potent sympatho-excitatory stimulus compared with mental stress. Thus, based on the calculation of MSNA total activity, participants with chronic anxiety increased MSNA total activity by relying extensively on burst amplitude. These findings are consistent with previous reports of a primary contribution of burst amplitude to the overall increase in MSNA during stress in healthy individuals (23). However, the contribution of MSNA burst amplitude to total sympathetic outflow during stress appears exaggerated in chronic anxiety. The mechanisms responsible for greater relative MSNA burst amplitude in chronic anxiety are not entirely clear. Active sympathetic fibers can increase firing rate within a burst of MSNA to increase burst amplitude. In this regard, greater incidence of multiple single-unit firing during a burst of MSNA has previously been correlated with higher trait and state anxiety (35). Brain regions such as the amygdala play an important role in anxiety, and have descending neural pathways to areas of the brainstem that are involved in regulating sympathetic outflow (12, 47, 65). Moreover, the arterial baroreflex is an important regulator of the occurrence of a sympathetic burst and the strength of a sympathetic burst (i.e., burst amplitude) (31). Evidence suggests that projections from the central nucleus of the amygdala can inhibit the arterial baroreflex and lead to increases in sympathetic activity during stress (13, 50). Although speculative, augmented relative MSNA burst amplitude during stress in chronic anxiety may potentially be attributed to exacerbated inhibition of sympathetic baroreflex control. Previous studies indicate alteration in cardiovagal baroreflex sensitivity in individuals with high anxiety (62); however, no studies have directly examined baroreflex control of sympathetic nerve activity in this population.

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Interestingly, relative MSNA burst amplitude was elevated during the 2-min duration of rest immediately preceding either mental or physiological stress, suggesting an anticipatory response to the stimuli. Indeed, concurrent increases in HR, albeit small, were observed preceding both mental and physiological stress. Importantly, relative MSNA burst amplitude immediately prior to mental and physiological stress was greater among individuals with anxiety. Since the timing of the stimulus was announced to each participant at the beginning of the 2-min duration prior to the stimulus, these data suggest that elevation in relative MSNA burst amplitude may indicate anticipation or apprehension. Previous studies have demonstrated an increase in measures of sympathetic activity in association with brain activity involved with anticipation of pain (52). However, no previous studies have reported anticipatory MSNA responses to cold stress or mental stress in humans, therefore additional investigations are needed to confirm whether chronic anxiety influences anticipatory sympathetic responses.

Sympathetic vasoconstriction and a subsequent decrease in vascular conductance is a target end organ response to acute increases in MSNA. Given previous evidence demonstrating an association between larger MSNA burst amplitude and greater vasoconstrictor responses (17, 18), we hypothesized that augmented relative MSNA burst amplitude among individuals with chronic anxiety would translate to greater decreases in brachial artery conductance. Contrary to our hypothesis, the reductions in brachial artery conductance were similar between participants with chronic anxiety and controls. There are several possible explanations for this observation. First, although the increase in relative MSNA burst amplitude in response to cold stress was significantly augmented among participants with chronic anxiety, the total rise in sympathetic vasoconstrictor activity (i.e., MSNA total activity) in response to cold stress was similar to controls. Secondly, since MSNA was recorded from the leg (peroneal nerve) and not from the

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arm, regional differences in sympathetic outflow (arm vs leg) during cold stress that may be a result of chronic anxiety cannot be completely ruled out. Finally, studies examining the influence of MSNA burst amplitude have demonstrated robust and dynamic effects on femoral artery conductance (17), whereas the graded effects of MSNA burst amplitude on brachial artery conductance are moderate in comparison (16). The difference in sensitivity to MSNA burst amplitude in the leg vs. arm may be related to greater α-adrenergic receptor density or sensitivity in the leg compared with the arm (46). Thus, it is plausible that the influence of greater MSNA burst amplitude on vascular conductance in individuals with chronic anxiety may be less when examining brachial artery conductance given the lower α-adrenergic receptor density or sensitivity in this region.

Additional target organ responses to elevations in sympathetic activity were also considered such as cardiac responsiveness. Seminal studies have shown parallel increases in MSNA and cardiac norepinephrine spillover during mental and physiological stress (66), suggesting that alterations observed in MSNA may also be reflected at the level of the heart. However, although not a direct measure of sympathetic outflow to the heart, peak changes in heart rate in response to acute stress were comparable between anxiety and control groups. Given the similarity in target organ responses, and that individuals in the present study with chronic anxiety also had low cardiovascular disease risk factor burden, it is tempting to speculate that augmented increases in MSNA burst amplitude may be a signature of anxiety that consequently becomes deleterious when comorbidities common to anxiety develop (e.g., hypertension, obesity, etc.). Future studies are warranted to determine whether augmented MSNA burst amplitude is associated with deleterious end-organ consequences in persons with anxiety and cardiovascular disease or cardiovascular disease risk factors.

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Perspectives

Sympathetic nerve firing is an important determinant of norepinephrine release from the nerve terminal and the end-organ response. Elevated MSNA is associated with target organ damage such as vascular remodeling (21), left ventricular hypertrophy (51), and diastolic dysfunction (20). Although resting MSNA appears normal, we demonstrate for the first time that the increase in relative MSNA burst amplitude is augmented during acute mental and physiological stress in chronic anxiety. Interestingly, studies have indicated that anxiety disorders may increase the firing pattern of active single-unit sympathetic fibers. Greater MSNA burst amplitude observed in chronic anxiety may reflect multiple firing of active single-unit neurons during a burst of MSNA. In healthy individuals, sympathetic neurons usually fire as a single spike once during a burst of MSNA independent of burst rate (41). This becomes important because multiple firing of active single-unit neurons has previously been associated with a higher rate of norepinephrine spillover from the heart (37), reflecting greater sympathetic influence and stress on the heart. Indeed, anxiety is particularly associated with fatal coronary heart disease (29, 48). However, it remains unclear whether alterations in MSNA with chronic anxiety sufficiently augments end-organ responses in the periphery (e.g., vasoconstriction, vascular remodeling), or is a marker of a preferential increase in sympathetic outflow to the heart. Further studies are warranted to examine the link between alterations in sympathetic firing and the marked increase in cardiac risk that is prevalent with anxiety.

In summary, the results from the present study demonstrate that multi-unit MSNA at rest is not elevated by chronic anxiety; however, relative MSNA burst amplitude is augmented in response to acute mental and physiological stress in individuals with chronic anxiety compared with controls with low/no anxiety, independent of age, sex and BMI. However, local

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vasoconstriction in the arm is not enhanced in parallel with greater relative MSNA burst amplitude responses. These data are the first to indicate an augmentation in multi-unit MSNA in individuals with chronic anxiety.

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

Author Contributions

Author contributions: S.W.H., J.G.F., G.L.P., F.M.A. conception and design of research; S.W.H., R.E.L., A.L.G., N.A.W., A.K.S. performed experiments; J.G.F. phenotyped anxiety and provided medical oversight during studies; S.W.H. analyzed data and prepared figures; S.W.H., J.G.F., G.L.P., F.M.A., J.G.F. interpreted results of experiments; S.W.H drafted manuscript; S.W.H., R.E.L., G.L.P., F.M.A., J.G.F. edited and revised the manuscript; S.W.H., R.E.L., A.L.G., N.A.W., A.K.S. G.L.P., F.M.A., J.G.F., approved the final version of the manuscript.

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Figure 1. Example baseline recordings (45 s) of muscle sympathetic nerve activity (MSNA) and electrocardiogram (ECG) in 5 controls with low/no anxiety and 5 individuals with moderate/high anxiety.

Figure 2. Mean summary data of relative muscle sympathetic nerve activity (MSNA) burst amplitude in controls with low/no anxiety and individuals with moderate/high anxiety during a 2-min baseline, and during and after 2 min of cold stress (Panel A, controls: n=18, anxiety: n=18) and 4 min of mental stress (Panel B, controls: n=15, anxiety: n=13). Also shown is the anticipatory response in relative MSNA burst amplitude during the 2-min baseline periods prior to cold and mental stress compared with the resting 10-min baseline period at the beginning of the study (Panel C). Data expressed as mean ± SEM.

Figure 3. Mean summary data of muscle sympathetic nerve activity (MSNA) burst frequency (Panel A), MSNA burst incidence (Panel B), and MSNA total activity (Panel C) in controls with low/no anxiety and individuals with moderate/high anxiety during a 2-min baseline, and during and after 2 min of cold stress (controls: n=18, anxiety: n=18) and 4 min of mental stress (mental arithmetic) (controls: n=15, anxiety: n=13). Data expressed as mean ± SEM.

Figure 4. Mean summary data of percent change in brachial artery conductance (Panel A, controls: n=18, anxiety: n=17) and mean arterial blood pressure (Panel B, controls: n=18, anxiety: n=18) during 2 min of cold stress in controls with low/no anxiety and individuals with moderate/high chronic anxiety. Brachial artery conductance was not able to be collected in 1 participant with high anxiety. Data expressed as mean ± SEM.

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Figure 5. Correlational analyses between measures of anxiety (State-Trait Anxiety Inventory) and relative muscle sympathetic nerve activity (MSNA) burst amplitude (Panels AI-AIV) and MSNA burst incidence (Panels BI-BIV) during mental stress (mental arithmetic, controls: n=15, anxiety: n=13) and cold stress (controls: n=18, anxiety: n=18). Data shown are MSNA responses during the latter half of mental stress (2 min avg.) and cold stress (1 min avg.) when peak responses tended to occur.

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Figure 1

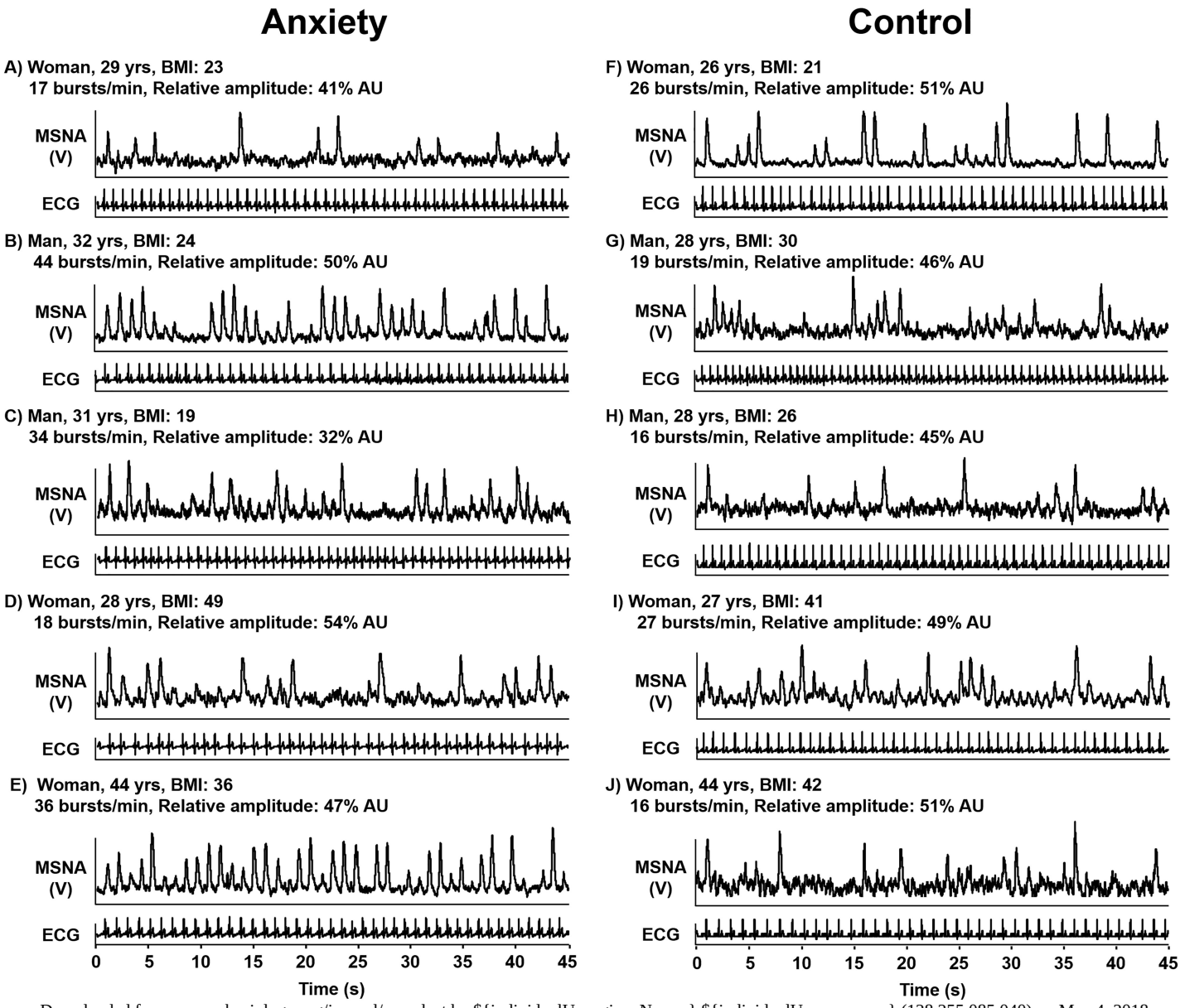

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Figure 2

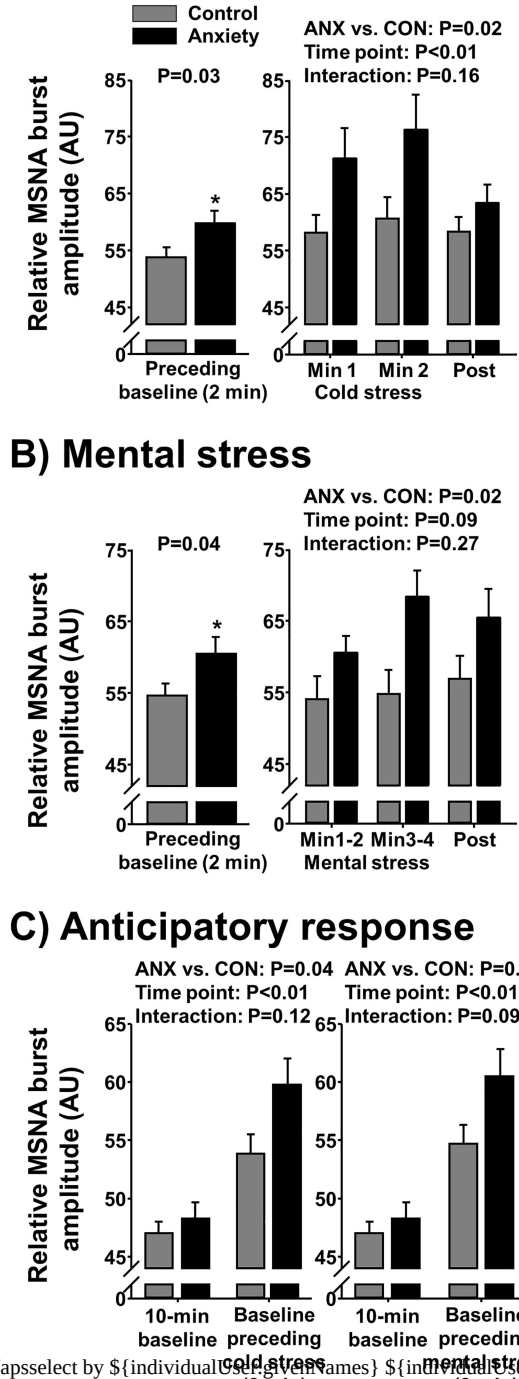

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Figure 3

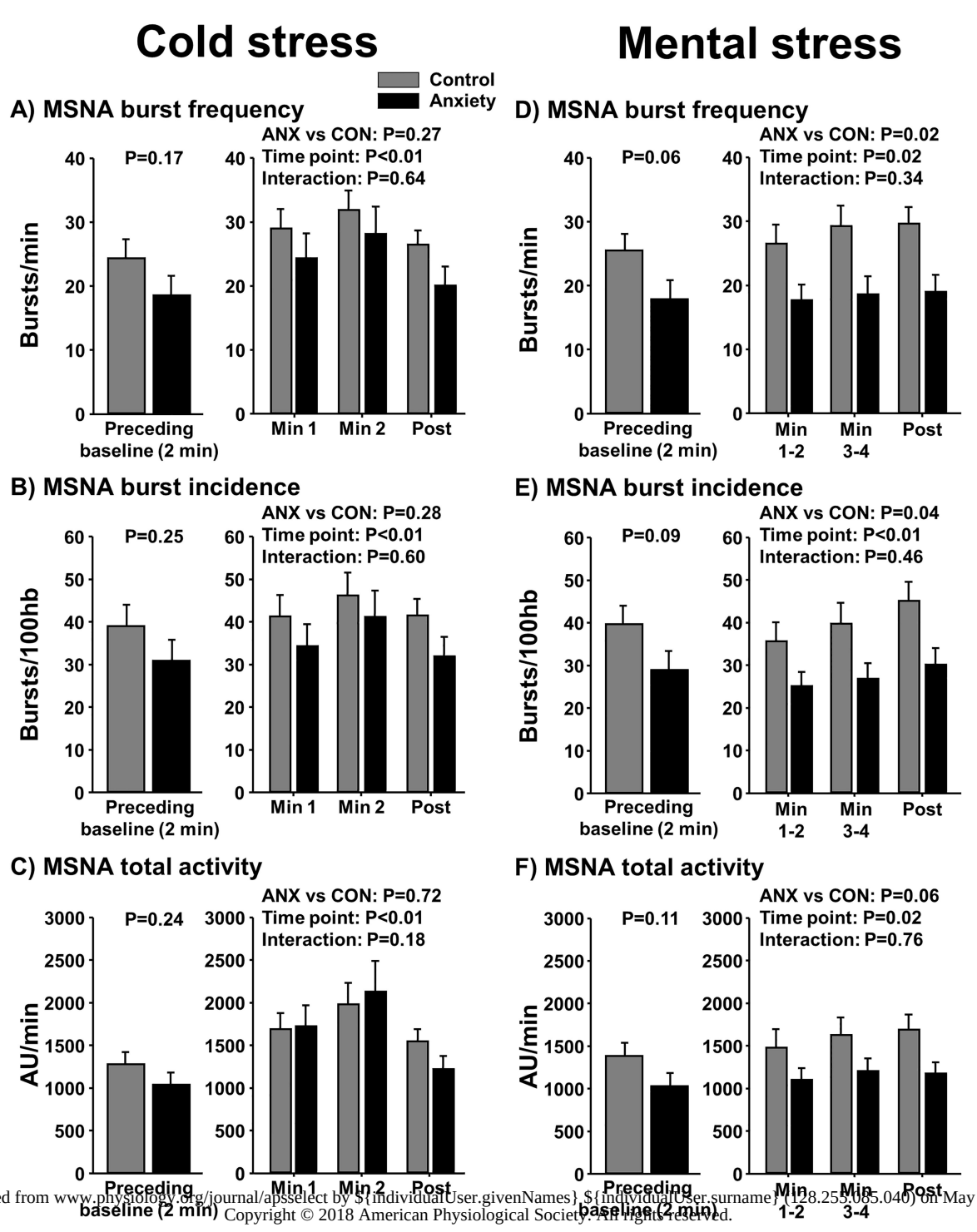

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Figure 4

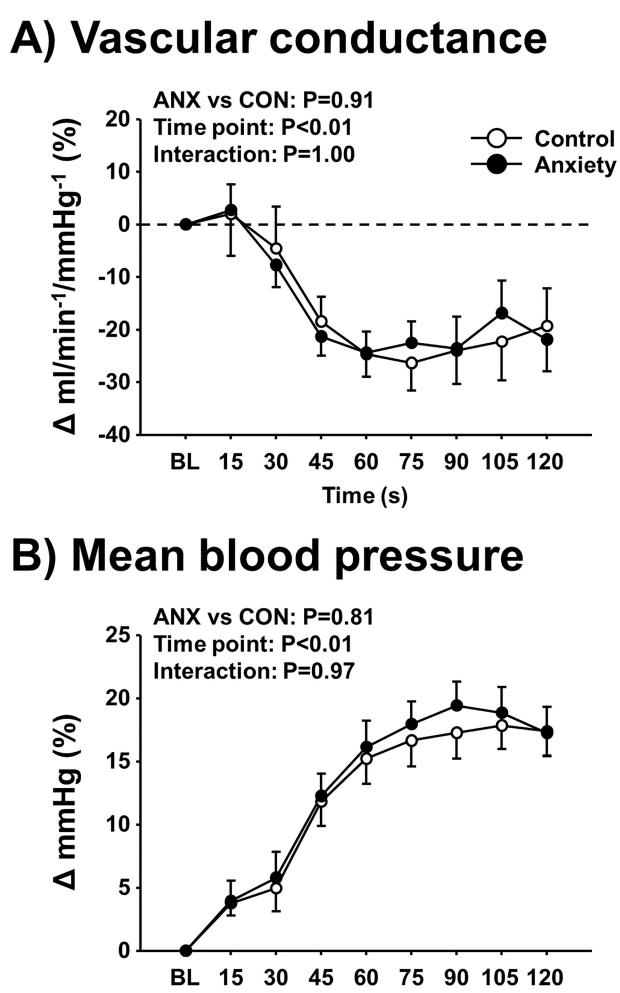

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Table 1. Subject characteristics

	ANX (n=18)	CON (n=18)	P value
Sex (men/women)	8/10	8/10	-
Age, years	32 ± 2	39 ± 3	0.08
Weight, kg	80 ± 5	88 ± 5	0.24
BMI, kg/m^2	27 ± 2	30 ± 1	0.18
Glucose, mg/dL	88 ± 2	97 ± 5	0.07
Insulin, μIU/mL	6.5 ± 0.9	9.8 ± 1.1	0.03
Triglycerides, mg/dL	89 ± 16	86 ± 9	0.86
HDL, mg/dL	57 ± 4	52 ± 4	0.36
Family history HTN	9/18	10/18	0.74
Cardiovascular variables			
Heart rate (beats/min)	59 ± 2	62 ± 2	0.23
Systolic BP (mmHg)	119 ± 3	120 ± 3	0.84
Diastolic BP (mmHg)	67 ± 2	72 ± 2	0.10
Mean BP (mmHg)	85 ± 2	88 ± 2	0.26
24-hr Ambulatory BP			
Daytime systolic BP (mmHg)	127 ± 3	126 ± 3	0.90
Nocturnal systolic BP (mmHg)	115 ± 3	109 ± 2	0.09
Systolic BP dipping (%)	9 ± 2	13 ± 1	0.02
24-hour systolic BP (mmHg)	124 ± 2	122 ± 2	0.65
Daytime diastolic BP (mmHg)	77 ± 2	77 ± 2	0.86
Nocturnal diastolic BP (mmHg)	63 ± 3	61 ± 1	0.46
24-hour diastolic BP (mmHg)	74 ± 2	73 ± 1	0.79
24-hour mean BP (mmHg)	91 ± 2	90 ± 2	0.42
Systolic BP variability (SD)	11.5 ± 0.7	11.8 ± 0.6	0.72
Mean BP variability (SD)	11.2 ± 0.5	10.7 ± 0.6	0.51
Anxiety assessments			
State Anxiety (STAI)	47 ± 2	25 ± 2	<0.01
Trait Anxiety (STAI)	57 ± 1	28 ± 2	<0.01
Beck Anxiety Inventory	18 ± 2	4 ± 1	<0.01
Beck Depression Inventory	21 ± 2	3 ± 1	<0.01

Values are means ± SE. BMI, body mass index; HDL, high density lipoprotein; HTN, hypertension; BP, blood pressure; Systolic BP dipping = [1-(nighttime SBP/daytime SBP)]×100; SD, standard deviation; STAI, State-Trait Anxiety Inventory.

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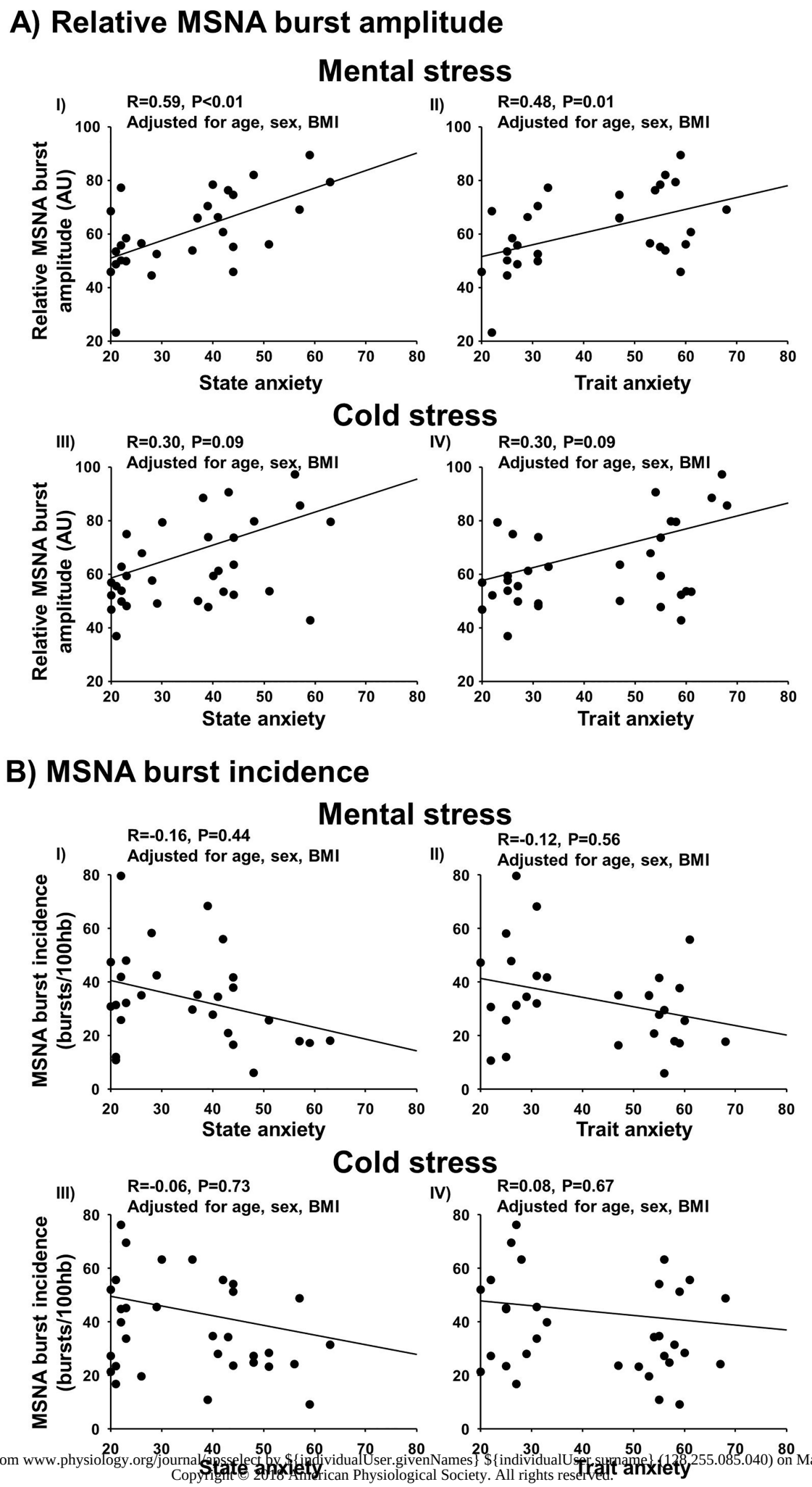
Figure 5



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