J Neurophysiol 120: 11–22, 2018.
First published March 14, 2018; doi:10.1152/jn.00064.2018.

RESEARCH ARTICLE | *50 Years of Microneurography: Insights into Neural Mechanisms in Humans*

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

Seth W. Holwerda,[1,6] Rachel E. Luehrs,[1] Allene L. Gremaud,[1] Nealy A. Wooldridge,[1] Amy K. Stroud,[2] Jess G. Fiedorowicz,[2,3,4,6] Francois M. Abboud,[4,5,6] and Gary L. Pierce[1,6,7]

[1]*Department of Health and Human Physiology, University of Iowa, Iowa City, Iowa;* [2]*Department of Psychiatry, University of Iowa, Iowa City, Iowa;* [3]*Department of Epidemiology, University of Iowa, Iowa City, Iowa;* [4]*Department of Internal Medicine, University of Iowa, Iowa City, Iowa;* [5]*Department of Molecular Physiology and Biophysics, University of Iowa, Iowa City, Iowa;* [6]*Abboud Cardiovascular Research Center, University of Iowa, Iowa City, Iowa; and* [7]*Fraternal Order of Eagles Diabetes Research Center, University of Iowa, Iowa City, Iowa*

Submitted 24 January 2018; accepted in final form 9 March 2018

Holwerda SW, Luehrs RE, Gremaud AL, Wooldridge NA, Stroud AK, Fiedorowicz JG, Abboud FM, Pierce GL. Relative burst amplitude of muscle sympathetic nerve activity is an indicator of altered sympathetic outflow in chronic anxiety. *J Neurophysiol* 120: 11–22, 2018. First published March 14, 2018; doi:10.1152/jn.00064.2018.—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 yr) and 18 healthy control subjects with low or no anxiety (CON; 8 men, 10 women, 39 ± 3 yr) were studied. Baseline MSNA and 24-h 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% arbitrary units (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 ($P = 0.01$) anxiety (State-Trait Anxiety Inventory), independent of age, sex, and body mass index. 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.

NEW & NOTEWORTHY Relative burst amplitude of muscle sympathetic nerve activity in response to acute mental and physiological stress is selectively augmented in individuals with chronic anxiety, which is a prevalent condition that is associated with the development of cardiovascular disease. Augmented sympathetic burst amplitude occurs with chronic anxiety in the absence of common comorbidities. These findings provide important insight into the relation between anxiety, acute stress and sympathetic activation.

anxiety; blood pressure; mental stress; MSNA

INTRODUCTION

Anxiety is the most common mental health problem in the United States, occurring in ~18% of adults per year (Kessler et al. 2005). Anxiety is also predictive of later incidence of hypertension (Jonas et al. 1997) and coronary heart disease (Kawachi et al. 1994; Roest et al. 2010). 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 (Esler et al. 2008; Rosengren et al. 2004). Alteration in the autonomic nervous system resulting in sympathetic overactivity is characteristic of hypertension- and stressor-induced cardiac events (Brotman et al. 2007; Meredith et al. 1991). 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 brain stem essential in regulating sympathetic outflow (Cassell and Gray 1989; Rauch et al. 2003; Wallace et al. 1992). Brain areas involved with stress responses such as the periaqueductal gray are also associated with changes in sympathetic nerve activity (Sverrisdóttir et al. 2014). Electrical stimulation of the dorsolateral periaqueductal gray in humans leads to increased muscle sympathetic nerve activity (MSNA) burst amplitude but not burst frequency (Sverrisdóttir et al. 2014). Burst amplitude and frequency of multiunit MSNA are important characteristics of sympathetic drive because they reflect the firing pattern of single-unit sympathetic neurons. Increased firing probability of single-unit sympathetic neurons increases multiunit burst frequency and amplitude. Latent single-unit neu-

Address for reprint requests and other correspondence: G. L. Pierce, Dept. of Health and Human Physiology, College of Liberal Arts and Sciences, Univ. of Iowa, 412 FH, Iowa City, IA 52242 (e-mail: gary-pierce@uiowa.edu).

rons can be recruited and fire (typically once per burst of MSNA) to increase multiunit burst frequency, but they may also fire in synchrony and 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 (Lambert et al. 2008; Macefield and Wallin 1999; Murai et al. 2006) (For supplementary description of sympathetic discharge patterns in humans, the reader is directed to recent expert reviews: Macefield and Wallin 2017; Shoemaker 2017). Interestingly, patients with intense forms of anxiety such as panic disorder exhibit augmented MSNA burst amplitude but not burst frequency during panic attacks (Wilkinson et al. 1998). 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 three or four times during a single burst of MSNA (Lambert et al. 2006, 2008). 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 (Sverrisdóttir et al. 1998, 2000), 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 (Fairfax et al. 2013b, 2013c). 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 (Lambert et al. 2010). 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 multiunit MSNA in individuals with moderate or high chronic anxiety and control subjects with low or no anxiety at rest and during sympathoexcitatory stress. Given that previous data suggest anxiety-induced augmentation of MSNA burst amplitude in patients with panic disorder (Wilkinson et al. 1998), we hypothesized that individuals with chronic anxiety would exhibit augmented MSNA burst amplitude responses to a sympathoexcitatory stimuli (cold stress) compared with control subjects with low or 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.

METHODS

All experimental procedures and protocols conformed to the Declaration of Helsinki and were approved by the University of Iowa Institutional Review Board (Project No. 201409782). Each subject received verbal and written explanation of the study objectives, measurement techniques, and risks and benefits associated with the investigation before providing written informed consent.

Subjects

A total of 36 participants were studied (age range: 25–63 yr). Eighteen healthy participants with moderate/high anxiety (ANX; 8 men, 10 women) based on anxiety assessments (see *Anxiety assessments*) and 18 control subjects 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 was not controlled for menstrual cycle phase because previous studies demonstrated that there is no effect of menstrual cycle phase on MSNA and blood pressure (BP) responses to acute mental stress (Carter and Lawrence 2007). A urine pregnancy test confirmed the exclusion criterion of pregnancy. All participants visited the laboratory for screening and written informed consent before 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 (Spitzer et al. 2006), was used in part for study entry criteria. A GAD-7 score $\geq$ 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. Fiedorowicz), who performed a structured diagnostic interview (M.I.N.I-International Neuropsychiatric Interview) before participation in the study. Anxiety was also assessed with the State-Trait Anxiety Inventory (STAI) surveys among all participants (Spielberger et al. 1970). 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 to 80. Anxiety and depressive symptoms were also assessed with the Beck Anxiety Inventory (BAI) and Beck Depressive Inventory (BDI-II) surveys (Beck et al. 1988, 1996).

Muscle sympathetic nerve activity. Multiunit postganglionic MSNA was recorded with the standard microneurographic technique, as previously described (Holwerda et al. 2016a, 2016b; Vallbo et al. 1979). 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; Univ. 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 were acquired at a frequency of 1,000 Hz with a PowerLab data acquisition system (ADInstruments) and analyzed with 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) with a 12-MHz linear-array Doppler probe in pulsed-wave mode with an insonation angle of 60°. Blood flow was calculated as the product of mean blood velocity (cm/s) and cross-sectional area (cm^2) and multiplied by 60 (ml/min). Mean blood flow was divided by beat-to-beat mean 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 (Carter et al. 2005a, 2005b).

Cardiorespiratory measures. Heart rate (HR) was determined from lead II of the three-lead ECG. Beat-to-beat BP was estimated with finger photoplethysmography (Nexfin), and arm cuff BP was estimated with electrosphygmomanometry over the brachial artery. Respiratory movements were monitored with 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 (AU) 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 ($\dot{V}E$). Estimated $\dot{V}E$ was not quantified during mental stress because participants were verbally communicating with the researcher administering the mental stress task.

Ambulatory 24-h BP. Noninvasive ambulatory 24-h BP was obtained with oscillometric SpaceLabs 90207 monitors (SpaceLabs) (O'Brien et al. 1991). SpaceLabs monitors were programmed to obtain BP readings at intervals of 30 min during the day from 0600 to 2300 h and at night every 60 min from 2200 to 0600 h. Participants were instructed to record their activities and sleep periods for the 24-h monitoring period. At least 10 daytime readings and 5 nighttime readings and at least 80% successful readings of planned measurements over the 24 h were required (Lane-Cordova et al. 2018). Average values for systolic, diastolic, and mean BP and BP variability (standard deviation) were determined from individual 24-h recordings. Daytime (awake) and nocturnal (sleeping) BP were adjusted to the nearest hour based on each participant's written record of his/her activities and sleep periods for the 24-h monitoring period. The percentage of nocturnal systolic BP dipping was calculated as [(nocturnal systolic BP – daytime systolic BP)/daytime systolic BP] $\times$ 100. Two ANX participants elected to not wear the 24-h 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 0700 and 0900 after an overnight fast. Participants were instructed to refrain from medication use the morning of the study (1 ANX participant was taking a selective serotonin reuptake inhibitor, and another ANX participant was taking a tricyclic antidepressant). Participants were also requested to abstain from caffeinated beverages the morning of the study and from 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 22–24°C. Upon arrival, a venous catheter was inserted into the antecubital vein 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 three ANX and four 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. 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-h 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 (Victor et al. 1987). 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 before 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"). The 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. After 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 three- or four-digit number (e.g., 1,547 minus 13), and a new number from which to begin subtracting was given every 20–30 s (Anderson et al. 1987, 1991). 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 did not speak to participants during the 2-min baseline period before 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/100 heartbeats), 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 the 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 (Macefield and Wallin 2017). 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 three largest bursts, and expressing all other burst amplitudes as a percentage of the maximum burst height as previously described by our laboratory and others (Fairfax et al. 2013b; Holwerda et al. 2016a, 2016b; Lambert et al. 2006; Vranish et al. 2018). 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 means ± SE. Statistical comparisons of baseline variables between ANX and CON were made by using *t*-tests and analysis of covariance (ANCOVA) to adjust for age, sex, and body mass index (BMI) where indicated. Statistical analyses of physiological responses to stress were made with two-way repeated-measures (RM) 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 with partial correlation. Data were analyzed with SigmaPlot 13 (Systat Software), 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 with 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 ($P = 0.03$).

Table 1. *Subject characteristics*

	ANX (n = 18)	CON (n = 18)	P Value
Sex (men/women)	8/10	8/10	
Age, yr	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-h 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-h 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-h diastolic BP, mmHg	74 ± 2	73 ± 1	0.79
24-h 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. ANX, subjects with chronic anxiety; CON, control subjects with low/no anxiety; BMI, body mass index; HDL, high-density lipoprotein; HTN, hypertension; BP, blood pressure; systolic BP dipping = [1 − (nighttime systolic BP/daytime systolic BP)] × 100; SD, standard deviation; STAI, State-Trait Anxiety Inventory.

No differences were observed between ANX and CON in resting HR (P = 0.23) and mean BP (P = 0.26). Twenty-four-hour ambulatory BP and BP variability were also similar between ANX and CON (both $P > 0.05$) (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/wk: ANX 265 ± 44 vs. CON 277 ± 40, P = 0.83) [12-mo avg. based on Modifiable Activity Questionnaire (Kriska et al. 1990, 1993; Vuillemin et al. 2000) and American College of Sports Medicine physical activity guidelines (Haskell et al. 2007)].

Resting Sympathetic Activity

Examples of individual resting MSNA recordings from five ANX participants and five CON participants of the same sex and comparable age are presented in Fig. 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/100 heartbeats, 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: 1,155 ± 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/100 heartbeats, 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: 1,142 ± 132 AU/min, P = 0.14). No relation between MSNA and depression was observed (e.g., MSNA burst incidence vs. BDI: R = 0.06, P = 0.74, adjusted for age, sex, and BMI). Plasma norepinephrine concentration tended to be higher in ANX compared with CON (ANX: 361 ± 39 vs. CON: 232 ± 24 pg/ml, P = 0.05). The correlations 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 with CON during the 2-min baseline before cold stress (P = 0.03) (Fig. 2*A*). 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) (Fig. 2*C*), suggesting an enhanced sympathetic anticipatory response. The overall increase in relative MSNA burst amplitude during cold stress and 2-min recovery was significantly greater among ANX compared with CON (2-way RM ANOVA; 2-min baseline, cold stress, 2-min recovery, P = 0.02) (Fig. 2*A*). After controlling for age, sex, and BMI, measures of anxiety were moderately correlated with relative MSNA burst amplitude during cold stress (see Fig. 5*A*). In contrast, MSNA burst frequency during the 2-min baseline immediately before cold stress was similar to the 10-min resting baseline and was not significantly different between ANX and CON (P = 0.17) (Fig. 3*A*). 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 baseline, cold stress, 2-min recovery, $P > 0.05$) (Fig. 3, *A*–*C*). Ratings of discomfort of the cold stress reported by participants (scale 0–10) were not significantly higher among ANX compared with 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 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 beats/min, P = 0.19). In response to cold stress, ANX and CON demonstrated similar peak increases in HR (ANX: Δ24 ± 3 vs. CON: Δ23 ± 4% beats/min, 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 (Fig. 4*A*). No sig-

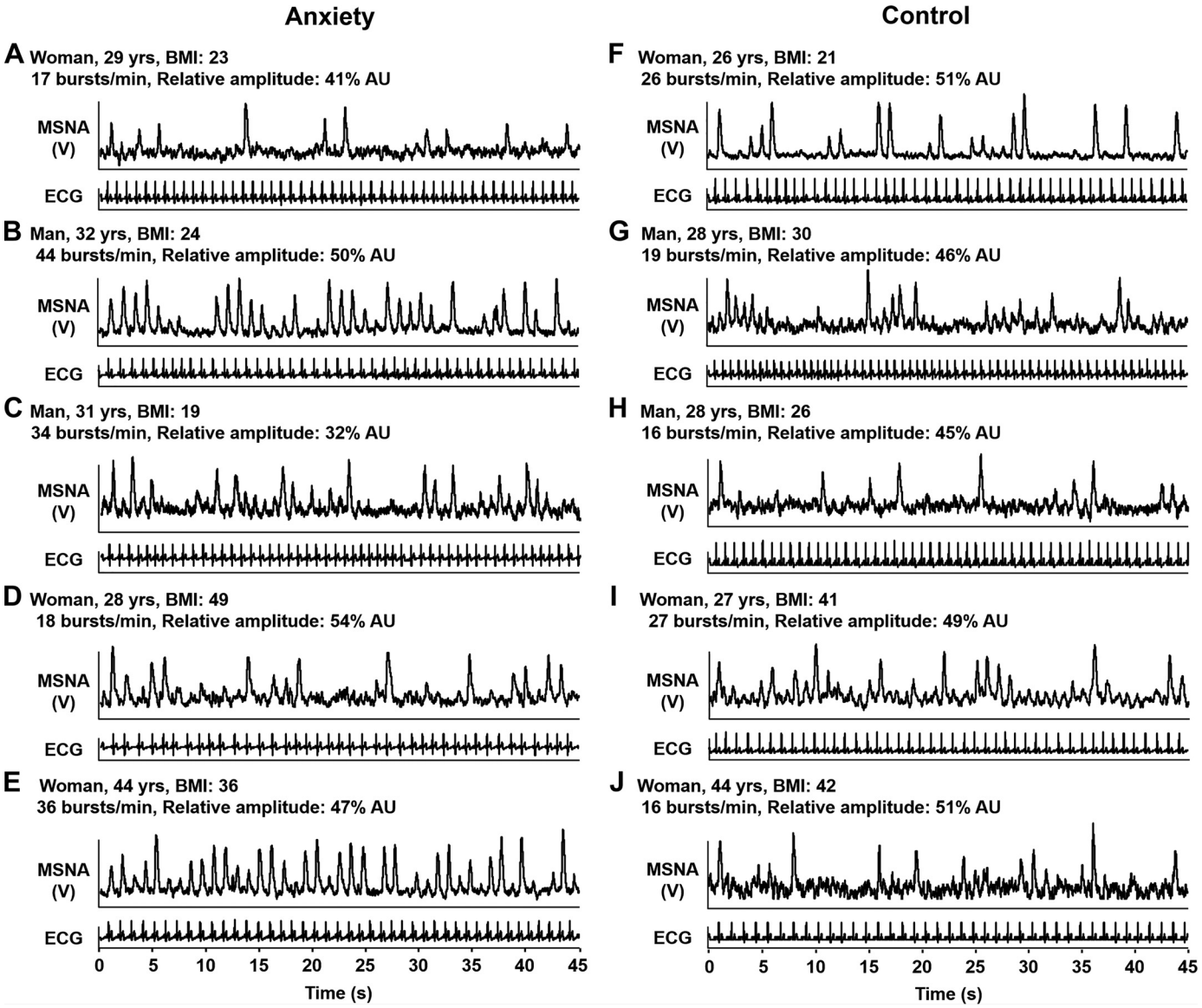

Fig. 1. Example baseline recordings (45 s) of muscle sympathetic nerve activity (MSNA) and electrocardiogram (ECG) in 5 individuals with moderate/high anxiety (*A–E*) and 5 control subjects with low/no anxiety (*F–J*). AU, arbitrary units; BMI, body mass index.

nificant difference between ANX and CON was observed in the increase in estimated $\dot{V}_E$ compared with the 10-min baseline (2-min baseline preceding cold stress: ANX $\Delta 7 \pm 6\%$ vs. CON $\Delta 13 \pm 7\%$; 2-min cold stress: ANX $\Delta 52 \pm 24\%$ vs. CON $\Delta 42 \pm 16\%$, $P = 0.89$), suggesting that 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 with CON ($P = 0.04$) (Fig. 2*B*). 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 (Fig. 2*C*), 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 baseline, mental stress, 2-min recovery, $P = 0.02$) (Fig. 2*B*). In contrast, MSNA burst frequency and incidence were lower among ANX compared with CON during mental stress (2-way RM ANOVA; 2-min baseline, cold stress, 2-min recovery) (Fig. 3, *D–F*). As a result, MSNA total activity tended to be lower among ANX compared with CON during mental stress (Fig. 3*F*). Relative MSNA burst amplitude responses to mental stress were strongly correlated with both "state" and "trait" anxiety and remained strongly correlated after adjustment for age, sex, and BMI (Fig. 5*A*, *I* and *II*), whereas measures of anxiety were not related to MSNA burst incidence (Fig. 5*B*, *I* and *II*). Similar correlations were seen for BAI ($R = 0.51$, $P = 0.01$). However, measures of depression (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).

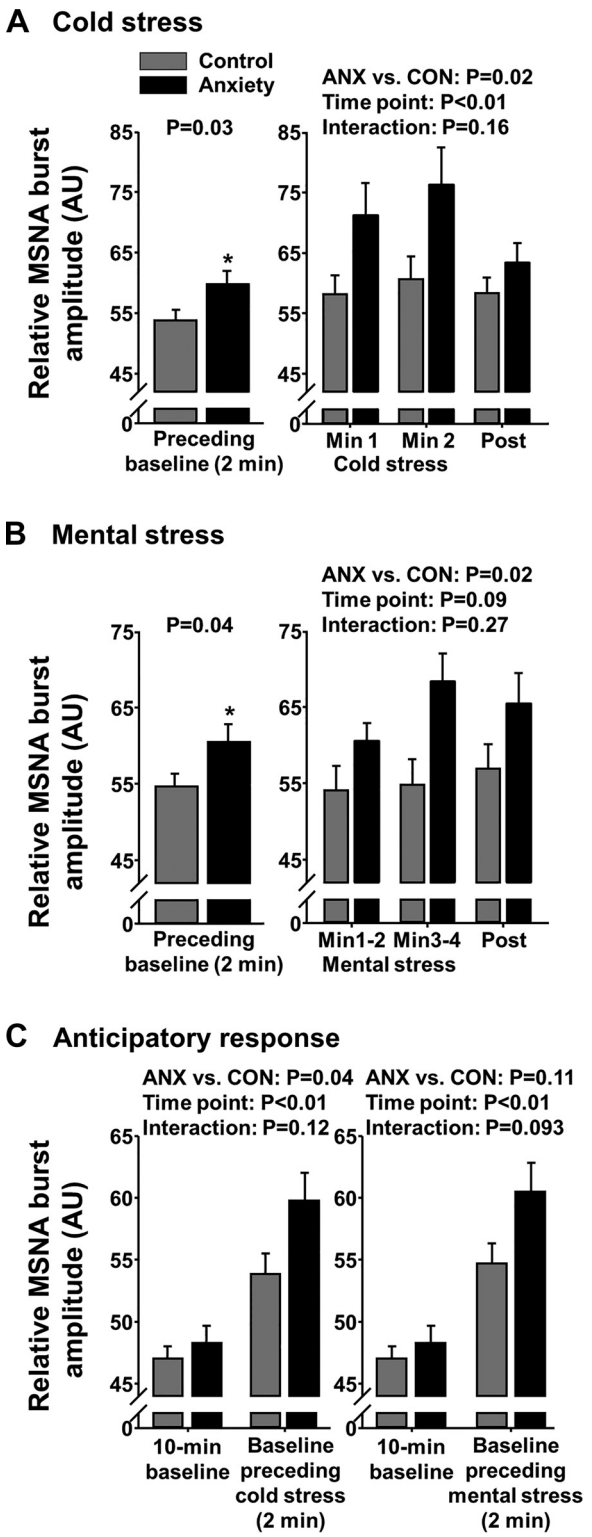

Fig. 2. Mean summary data of relative muscle sympathetic nerve activity (MSNA) burst amplitude in control subjects with low/no anxiety and individuals with moderate/high anxiety during a 2-min baseline and during and after 2 min of cold stress (*A*; control: $n = 18$, anxiety: $n = 18$) and 4 min of mental stress (*B*; control: $n = 15$, anxiety: $n = 13$). Also shown is the anticipatory response in relative MSNA burst amplitude during the 2-min baseline periods before cold and mental stress compared with the resting 10-min baseline period at the beginning of the study (*C*). Data are expressed as means ± SE. AU, arbitrary units.

Cardiovascular Responses to Mental Stress

A small but significant increase in HR was observed during the 2-min duration immediately preceding mental stress compared with the 10-min resting baseline (ANX: $\Delta 2 \pm 1$ vs. CON: $\Delta 3 \pm 1$ beats/min, $P < 0.001$), 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 in HR (ANX: $\Delta 20 \pm 3\%$ vs. CON: $\Delta 28 \pm 5\%$, $P = 0.19$), systolic BP (ANX: $\Delta 8 \pm 1\%$ vs. CON: $\Delta 9 \pm 2\%$, $P = 0.67$), and mean BP (ANX: $\Delta 10 \pm 1\%$ vs. CON: $\Delta 12 \pm 2\%$, $P = 0.38$).

DISCUSSION

This comprehensive study of sympathetic neural and cardiovascular responses to acute stress among individuals with chronic anxiety reveals three important findings. First, multiunit 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 control subjects with low or no anxiety, while increases in MSNA burst frequency and incidence were similar between groups. In contrast to our hypothesis, sympathetic vasoconstriction in response to elevated MSNA was not greater among individuals with chronic anxiety compared with control subjects, 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.

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 (Lambert et al. 2006, 2010; Villacres et al. 1987; Wilkinson et al. 1998), but no studies have demonstrated alteration in multiunit MSNA in individuals with chronic anxiety. A study of 13 individuals with panic disorder did not demonstrate alterations in multiunit MSNA responses to laboratory-based mental stress (Wilkinson et al. 1998). 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 (Carter et al. 2008; Carter and Goldstein 2015; Carter and Ray 2009; El Sayed et al. 2016; Fonkoue and Carter 2015) and independent of age (Ng et al. 1994) and sex (Jones et al. 1996). 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 (Wilkinson et al. 1998). 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

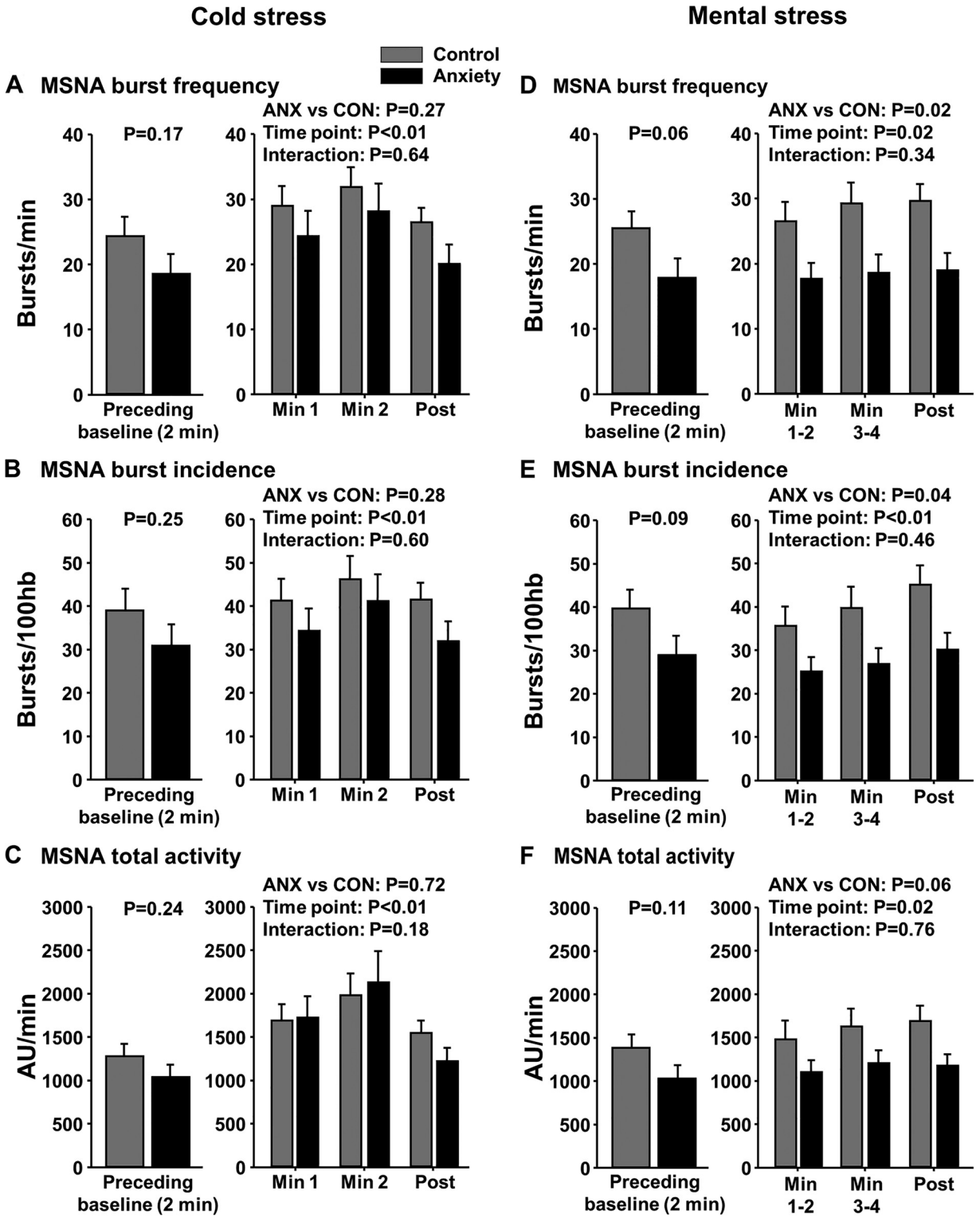

Fig. 3. Mean summary data of muscle sympathetic nerve activity (MSNA) burst frequency (*A* and *D*), MSNA burst incidence (*B* and *E*), and MSNA total activity (*C* and *F*) in control subjects with low/no anxiety and individuals with moderate/high anxiety during a 2-min baseline and during and after 2 min of cold stress (control: $n = 18$, anxiety: $n = 18$) and 4 min of mental stress (mental arithmetic) (control: $n = 15$, anxiety: $n = 13$). Data are expressed as means ± SE. AU, arbitrary units; hb, heartbeats.

present study extend results of previous investigations by demonstrating that alteration in multiunit 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.

MSNA burst frequency overall tended to be less among participants with chronic anxiety compared with control sub-

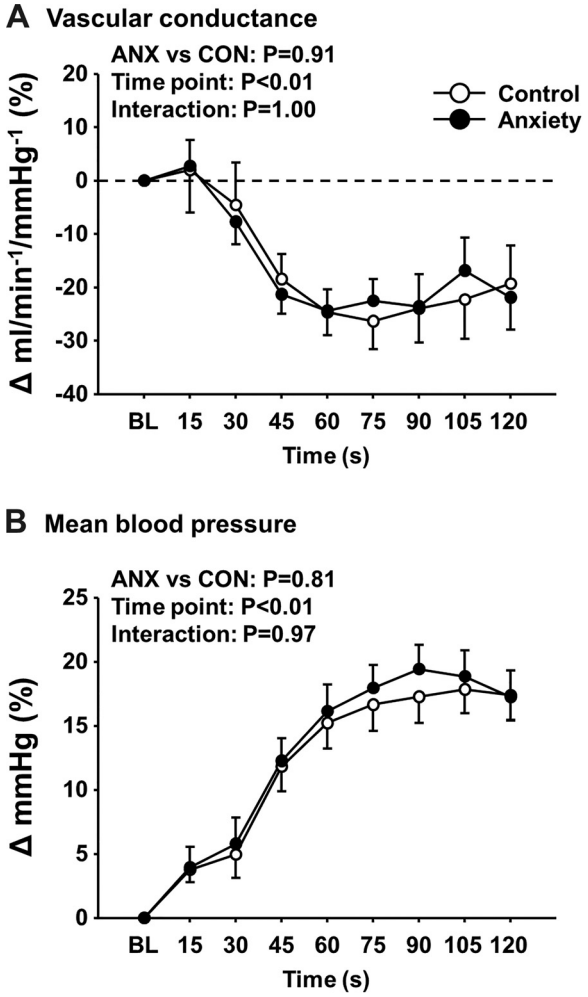

Fig. 4. Mean summary data of % change in brachial artery conductance (*A*; control: $n = 18$, anxiety: $n = 17$) and mean arterial blood pressure (*B*; control: $n = 18$, anxiety: $n = 18$) during 2 min of cold stress in control subjects with low/no anxiety and individuals with moderate/high chronic anxiety. Brachial artery conductance could not be collected in 1 participant with high anxiety. Data are expressed as means ± SE. BL, baseline.

jects. This was surprising given previous studies demonstrating a relation between anxiety symptoms and greater plasma norepinephrine concentration (Hughes et al. 2004). However, it should be noted that MSNA total activity, which reflects total sympathetic vasoconstrictor activity (burst frequency × mean burst amplitude), was similar between participants with anxiety and control subjects in response to cold stress, which was the more potent sympathoexcitatory 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 (Hjemdahl et al. 1989). 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 (Lambert et al. 2010). Brain regions such as the amygdala play an important role in anxiety and have descending neural pathways to areas of the brain stem that are involved in regulating sympathetic outflow (Cassell and Gray 1989; Rauch et al. 2003; Wallace et al. 1992). 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) (Kienbaum et al. 2001). 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 (Durocher et al. 2011; Saha 2005). 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 (Virtanen et al. 2003). However, no studies have directly examined baroreflex control of sympathetic nerve activity in this population.

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 before 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 before 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 (Seifert et al. 2013). 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 (Fairfax et al. 2013b, 2013c), 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 control subjects. 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 that in control subjects. Second, since MSNA was recorded from the leg (peroneal nerve) and not from the 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 (Fairfax et al. 2013b), whereas the

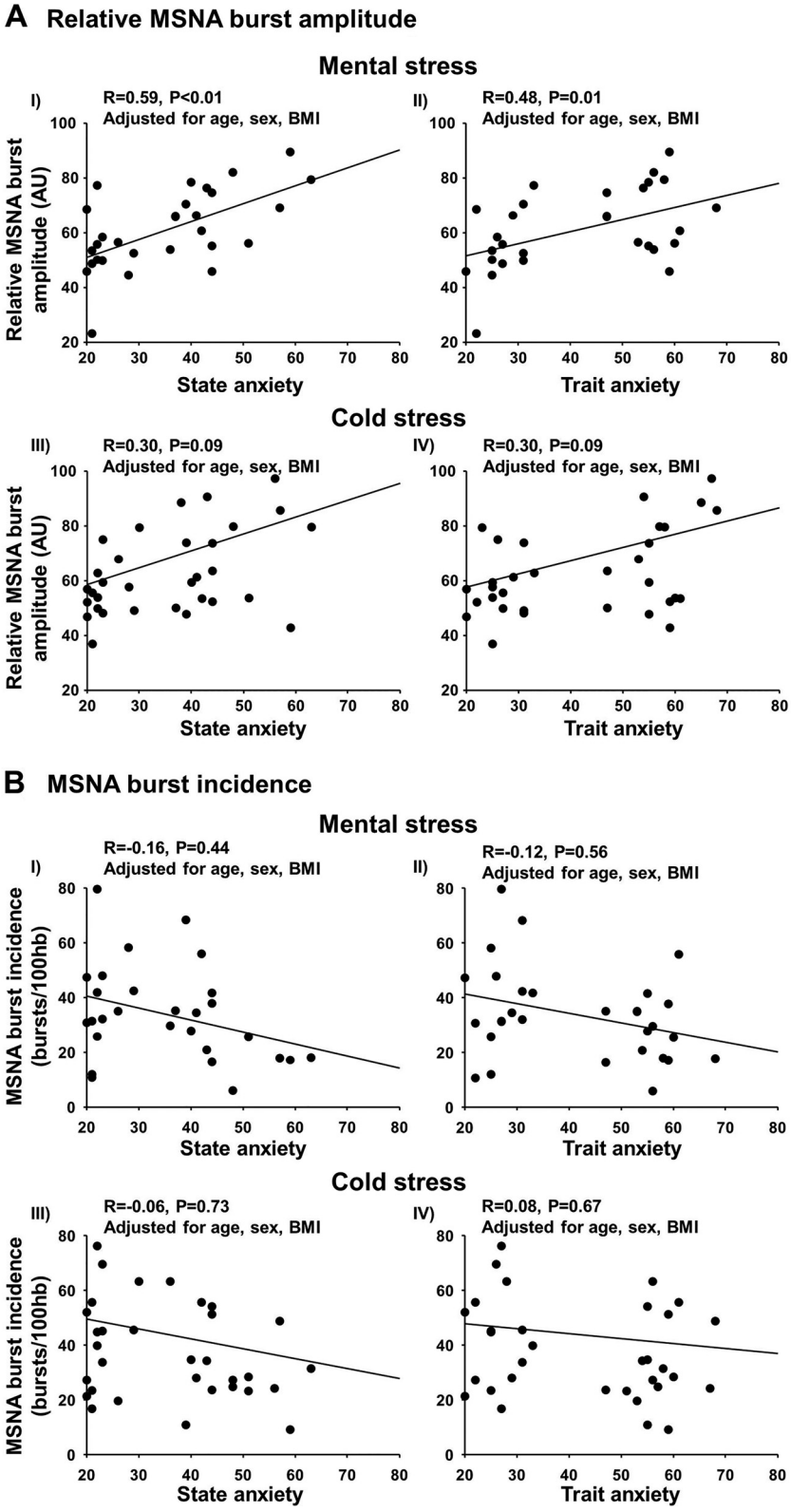

Fig. 5. Correlational analyses between measures of anxiety (State-Trait Anxiety Inventory) and relative muscle sympathetic nerve activity (MSNA) burst amplitude (*A*) and MSNA burst incidence (*B*) during mental stress (*I* and *II*; mental arithmetic; control: $n = 15$, anxiety: $n = 13$) and cold stress (*III* and *IV*; control: $n = 18$, 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. BMI, body mass index.

graded effects of MSNA burst amplitude on brachial artery conductance are moderate in comparison (Fairfax et al. 2013a). The difference in sensitivity to MSNA burst amplitude in the leg vs. the arm may be related to greater α-adrenergic receptor density or sensitivity in the leg compared with the arm (Pawelczyk and Levine 2002). 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 respon-

siveness. Seminal studies have shown parallel increases in MSNA and cardiac norepinephrine spillover during mental and physiological stress (Wallin et al. 1992), 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 HR 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.

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 (Grassi et al. 2009), left ventricular hypertrophy (Schlaich et al. 2003), and diastolic dysfunction (Grassi 2010). 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 (Macefield et al. 1994). This becomes important because such irregular single-unit sympathetic firing has previously been associated with a higher rate of norepinephrine spillover from the heart (Lambert et al. 2011), reflecting greater sympathetic influence and stress on the heart. Indeed, anxiety is particularly associated with fatal coronary heart disease (Kawachi et al. 1994; Roest et al. 2010). However, it remains unclear whether alteration 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 multiunit 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 control subjects with low or no anxiety, independent of age, sex, and BMI. However, local 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 multiunit MSNA in individuals with chronic anxiety.

ACKNOWLEDGMENTS

We acknowledge the University of Iowa Institute for Clinical and Translational Science Clinical Research Unit staff for assistance during studies.

GRANTS

This work was supported in part by a Iowa Cardiovascular Interdisciplinary Research Fellowship (T32HL007121) (S. W. Holwerda), American Heart Association Grants 17POST33440101 (S. W. Holwerda) and 13SDG143400012 (G. L. Pierce), and National Institutes of Health Grants P01 HL-014388-48 (F. M. Abboud, G. L. Pierce, J. G. Fiedorowicz) and U54 TR-001356 (University of Iowa).

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

S.W.H., J.G.F., F.M.A., and G.L.P. conceived and designed research; S.W.H., R.E.L., A.L.G., N.A.W., and A.K.S. performed experiments; S.W.H. analyzed data; S.W.H., J.G.F., F.M.A., and G.L.P. interpreted results of experiments; S.W.H. prepared figures; S.W.H. drafted manuscript; S.W.H., R.E.L., A.L.G., J.G.F., F.M.A., and G.L.P. edited and revised manuscript; S.W.H., R.E.L., A.L.G., N.A.W., A.K.S., J.G.F., F.M.A., and G.L.P. approved final version of manuscript.

REFERENCES

Anderson EA, Sinkey CA, Mark AL. Mental stress increases sympathetic nerve activity during sustained baroreceptor stimulation in humans. *Hypertension* 17: III43–III49, 1991. doi:10.1161/01.HYP.17.4_Suppl.III43.

Anderson EA, Wallin BG, Mark AL. Dissociation of sympathetic nerve activity in arm and leg muscle during mental stress. *Hypertension* 9: III114–III119, 1987. doi:10.1161/01.HYP.9.6_Pt_2.III114.

Beck AT, Steer RA, Brown GK. *Beck Depression Inventory Manual* (2nd ed.). San Antonio, TX: Psychological Corporation, 1996.

Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: psychometric properties. *J Consult Clin Psychol* 56: 893–897, 1988. doi:10.1037/0022-006X.56.6.893.

Brotman DJ, Golden SH, Wittstein IS. The cardiovascular toll of stress. *Lancet* 370: 1089–1100, 2007. doi:10.1016/S0140-6736(07)61305-1.

Carter JR, Cooke WH, Ray CA. Forearm neurovascular responses during mental stress and vestibular activation. *Am J Physiol Heart Circ Physiol* 288: H904–H907, 2005a. doi:10.1152/ajpheart.00569.2004.

Carter JR, Durocher JJ, Kern RP. Neural and cardiovascular responses to emotional stress in humans. *Am J Physiol Regul Integr Comp Physiol* 295: R1898–R1903, 2008. doi:10.1152/ajpregu.90646.2008.

Carter JR, Goldstein DS. Sympathoneural and adrenomedullary responses to mental stress. *Compr Physiol* 5: 119–146, 2015. doi:10.1002/cphy.c140030.

Carter JR, Kupiers NT, Ray CA. Neurovascular responses to mental stress. *J Physiol* 564: 321–327, 2005b. doi:10.1113/jphysiol.2004.079665.

Carter JR, Lawrence JE. Effects of the menstrual cycle on sympathetic neural responses to mental stress in humans. *J Physiol* 585: 635–641, 2007. doi:10.1113/jphysiol.2007.141051.

Carter JR, Ray CA. Sympathetic neural responses to mental stress: responders, nonresponders and sex differences. *Am J Physiol Heart Circ Physiol* 296: H847–H853, 2009. doi:10.1152/ajpheart.01234.2008.

Cassell MD, Gray TS. The amygdala directly innervates adrenergic (C1) neurons in the ventrolateral medulla in the rat. *Neurosci Lett* 97: 163–168, 1989. doi:10.1016/0304-3940(89)90157-2.

Durocher JJ, Klein JC, Carter JR. Attenuation of sympathetic baroreflex sensitivity during the onset of acute mental stress in humans. *Am J Physiol Heart Circ Physiol* 300: H1788–H1793, 2011. doi:10.1152/ajpheart.00942.2010.

El Sayed K, Macefield VG, Hissen SL, Joyner MJ, Taylor CE. Rate of rise in diastolic blood pressure influences vascular sympathetic response to mental stress. *J Physiol* 594: 7465–7482, 2016. doi:10.1113/JP272963.

Esler M, Eikelis N, Schlaich M, Lambert G, Alvarenga M, Dawood T, Kaye D, Barton D, Pier C, Guo L, Brenchley C, Jennings G, Lambert E. Chronic mental stress is a cause of essential hypertension: presence of biological markers of stress. *Clin Exp Pharmacol Physiol* 35: 498–502, 2008. doi:10.1111/j.1440-1681.2008.04904.x.

Fairfax ST, Holwerda SW, Credeur DP, Zuidema MY, Medley JH, Dyke PC 2nd, Wray DW, Davis MJ, Fadel PJ. The role of α-adrenergic receptors in mediating beat-by-beat sympathetic vascular transduction in the forearm of resting man. *J Physiol* 591: 3637–3649, 2013a. doi:10.1113/jphysiol.2013.250894.

Fairfax ST, Padilla J, Vianna LC, Davis MJ, Fadel PJ. Spontaneous bursts of muscle sympathetic nerve activity decrease leg vascular conductance in resting humans. *Am J Physiol Heart Circ Physiol* 304: H759–H766, 2013b. doi:10.1152/ajpheart.00842.2012.

Fairfax ST, Padilla J, Vianna LC, Holwerda SH, Davis MJ, Fadel PJ. Influence of spontaneously occurring bursts of muscle sympathetic nerve activity on conduit artery diameter. *Am J Physiol Heart Circ Physiol* 305: H867–H874, 2013c. doi:10.1152/ajpheart.00372.2013.

Fonkoue IT, Carter JR. Sympathetic neural reactivity to mental stress in humans: test-retest reproducibility. *Am J Physiol Regul Integr Comp Physiol* 309: R1380–R1386, 2015. doi:10.1152/ajpregu.00344.2015.

Grassi G. Sympathetic neural activity in hypertension and related diseases. *Am J Hypertens* 23: 1052–1060, 2010. doi:10.1038/ajh.2010.154.

Grassi G, Arenare F, Pieruzzi F, Brambilla G, Mancia G. Sympathetic activation in cardiovascular and renal disease. *J Nephrol* 22: 190–195, 2009.

Haskell WL, Lee IM, Pate RR, Powell KE, Blair SN, Franklin BA, Macera CA, Heath GW, Thompson PD, Bauman A. Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Med Sci Sports Exerc* 39: 1423–1434, 2007. doi:10.1249/mss.0b013e3180616b27.

Hjemdahl P, Fagius J, Freyschuss U, Wallin BG, Daleskog M, Bohlin G, Perski A. Muscle sympathetic activity and norepinephrine release during mental challenge in humans. *Am J Physiol Endocrinol Metab* 257: E654–E664, 1989. doi:10.1152/ajpendo.1989.257.5.E654.

Holwerda SW, Restaino RM, Manrique C, Lastra G, Fisher JP, Fadel PJ. Augmented pressor and sympathetic responses to skeletal muscle metaboreflex activation in type 2 diabetes patients. *Am J Physiol Heart Circ Physiol* 310: H300–H309, 2016a. doi:10.1152/ajpheart.00636.2015.

Holwerda SW, Vianna LC, Restaino RM, Chaudhary K, Young CN, Fadel PJ. Arterial baroreflex control of sympathetic nerve activity and heart rate in patients with type 2 diabetes. *Am J Physiol Heart Circ Physiol* 311: H1170–H1179, 2016b. doi:10.1152/ajpheart.00384.2016.

Hughes JW, Watkins L, Blumenthal JA, Kuhn C, Sherwood A. Depression and anxiety symptoms are related to increased 24-hour urinary norepinephrine excretion among healthy middle-aged women. *J Psychosom Res* 57: 353–358, 2004. doi:10.1016/S0022-3999(04)00064-9.

Jonas BS, Franks P, Ingram DD. Are symptoms of anxiety and depression risk factors for hypertension? Longitudinal evidence from the National Health and Nutrition Examination Survey I Epidemiologic Follow-up Study. *Arch Fam Med* 6: 43–49, 1997. doi:10.1001/archfami.6.1.43.

Jones PP, Spraul M, Matt KS, Seals DR, Skinner JS, Ravussin E. Gender does not influence sympathetic neural reactivity to stress in healthy humans. *Am J Physiol Heart Circ Physiol* 270: H350–H357, 1996. doi:10.1152/ajpheart.1996.270.1.H350.

Kawachi I, Sparrow D, Vokonas PS, Weiss ST. Symptoms of anxiety and risk of coronary heart disease. The Normative Aging Study. *Circulation* 90: 2225–2229, 1994. doi:10.1161/01.CIR.90.5.2225.

Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* 62: 593–602, 2005. [Erratum in *Arch Gen Psychiatry* 62: 768, 2005.] doi:10.1001/archpsyc.62.6.593.

Kienbaum P, Karlsson T, Sverrisdottir YB, Elam M, Wallin BG. Two sites for modulation of human sympathetic activity by arterial baroreceptors? *J Physiol* 531: 861–869, 2001. doi:10.1111/j.1469-7793.2001.0861h.x.

Kriska AM, Knowler WC, LaPorte RE, Drash AL, Wing RR, Blair SN, Bennett PH, Kuller LH. Development of questionnaire to examine relationship of physical activity and diabetes in Pima Indians. *Diabetes Care* 13: 401–411, 1990. doi:10.2337/diacare.13.4.401.

Kriska AM, LaPorte RE, Pettitt DJ, Charles MA, Nelson RG, Kuller LH, Bennett PH, Knowler WC. The association of physical activity with obesity, fat distribution and glucose intolerance in Pima Indians. *Diabetologia* 36: 863–869, 1993. doi:10.1007/BF00400363.

Lambert E, Dawood T, Schlaich M, Straznicky N, Esler M, Lambert G. Single-unit sympathetic discharge pattern in pathological conditions associated with elevated cardiovascular risk. *Clin Exp Pharmacol Physiol* 35: 503–507, 2008. doi:10.1111/j.1440-1681.2008.04905.x.

Lambert E, Dawood T, Straznicky N, Sari C, Schlaich M, Esler M, Lambert G. Association between the sympathetic firing pattern and anxiety level in patients with the metabolic syndrome and elevated blood pressure. *J Hypertens* 28: 543–550, 2010. doi:10.1097/HJH.0b013e3283350ea4.

Lambert E, Hotchkin E, Alvarenga M, Pier C, Richards J, Barton D, Dawood T, Esler M, Lambert G. Single-unit analysis of sympathetic nervous discharges in patients with panic disorder. *J Physiol* 570: 637–643, 2006. doi:10.1113/jphysiol.2005.100040.

Lambert EA, Schlaich MP, Dawood T, Sari C, Chopra R, Barton DA, Kaye DM, Elam M, Esler MD, Lambert GW. Single-unit muscle sympathetic nervous activity and its relation to cardiac noradrenaline spillover. *J Physiol* 589: 2597–2605, 2011. doi:10.1113/jphysiol.2011.205351.

Lane-Cordova AD, Kalil GZ, Wagner CJ, Sindler AL, Fiedorowicz JG, Ajibewa T, Haynes WG, Pierce GL. Hemoglobin A1c and C-reactive protein are independently associated with blunted nocturnal blood pressure dipping in obesity-related prediabetes. *Hypertens Res* 41: 33–38, 2018. doi:10.1038/hr.2017.82.

Macefield VG, Wallin BG. Firing properties of single vasoconstrictor neurones in human subjects with high levels of muscle sympathetic activity. *J Physiol* 516: 293–301, 1999. doi:10.1111/j.1469-7793.1999.293aa.x.

Macefield VG, Wallin BG. Physiological and pathophysiological firing properties of single postganglionic sympathetic neurons in humans. *J Neurophysiol* 119: 944–956, 2018. doi:10.1152/jn.00004.2017.

Macefield VG, Wallin BG, Vallbo AB. The discharge behaviour of single vasoconstrictor motoneurones in human muscle nerves. *J Physiol* 481: 799–809, 1994. doi:10.1113/jphysiol.1994.sp020482.

Meredith IT, Broughton A, Jennings GL, Esler MD. Evidence of a selective increase in cardiac sympathetic activity in patients with sustained ventricular arrhythmias. *N Engl J Med* 325: 618–624, 1991. doi:10.1056/NEJM199108293250905.

Murai H, Takata S, Maruyama M, Nakano M, Kobayashi D, Otowa K, Takamura M, Yuasa T, Sakagami S, Kaneko S. The activity of a single muscle sympathetic vasoconstrictor nerve unit is affected by physiological stress in humans. *Am J Physiol Heart Circ Physiol* 290: H853–H860, 2006. doi:10.1152/ajpheart.00184.2005.

Ng AV, Callister R, Johnson DG, Seals DR. Sympathetic neural reactivity to stress does not increase with age in healthy humans. *Am J Physiol Heart Circ Physiol* 267: H344–H353, 1994. doi:10.1152/ajpheart.1994.267.1.H344.

O'Brien E, Mee F, Atkins N, O'Malley K. Accuracy of the SpaceLabs 90207 determined by the British Hypertension Society protocol. *J Hypertens* 9: 573–574, 1991. doi:10.1097/00004872-199106000-00016.

Pawelczyk JA, Levine BD. Heterogeneous responses of human limbs to infused adrenergic agonists: a gravitational effect? *J Appl Physiol (1985)* 92: 2105–2113, 2002. doi:10.1152/japplphysiol.00979.2001.

Rauch SL, Shin LM, Wright CI. Neuroimaging studies of amygdala function in anxiety disorders. *Ann NY Acad Sci* 985: 389–410, 2003. doi:10.1111/j.1749-6632.2003.tb07096.x.

Roest AM, Martens EJ, de Jonge P, Denollet J. Anxiety and risk of incident coronary heart disease: a meta-analysis. *J Am Coll Cardiol* 56: 38–46, 2010. doi:10.1016/j.jacc.2010.03.034.

Rosengren A, Hawken S, Ounpuu S, Sliwa K, Zubaid M, Almahmeed WA, Blackett KN, Sitthi-amorn C, Sato H, Yusuf S; INTERHEART investigators. Association of psychosocial risk factors with risk of acute myocardial infarction in 11119 cases and 13648 controls from 52 countries (the INTERHEART study): case-control study. *Lancet* 364: 953–962, 2004. doi:10.1016/S0140-6736(04)17019-0.

Saha S. Role of the central nucleus of the amygdala in the control of blood pressure: descending pathways to medullary cardiovascular nuclei. *Clin Exp Pharmacol Physiol* 32: 450–456, 2005. doi:10.1111/j.1440-1681.2005.04210.x.

Schlaich MP, Kaye DM, Lambert E, Sommerville M, Socratous F, Esler MD. Relation between cardiac sympathetic activity and hypertensive left ventricular hypertrophy. *Circulation* 108: 560–565, 2003. doi:10.1161/01.CIR.0000081775.72651.B6.

Seifert F, Schuberth N, De Col R, Peltz E, Nickel FT, Maihöfner C. Brain activity during sympathetic response in anticipation and experience of pain. *Hum Brain Mapp* 34: 1768–1782, 2013. doi:10.1002/hbm.22035.

Shoemaker JK. Recruitment strategies in efferent sympathetic nerve activity. *Clin Auton Res* 27: 369–378, 2017. doi:10.1007/s10286-017-0459-x.

Spielberger CD, Gorsuch RL, Lushene RE. *Manual for the State-Trait Anxiety Inventory (Self-Evaluation Questionnaire)*. Palo Alto, CA: Consulting Psychologists, 1970.

Spitzer RL, Kroenke K, Williams JB, Löwe B. A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch Intern Med* 166: 1092–1097, 2006. doi:10.1001/archinte.166.10.1092.

Sverrisdóttir YB, Green AL, Aziz TZ, Bahuri NF, Hyam J, Basnayake SD, Paterson DJ. Differentiated baroreflex modulation of sympathetic nerve activity during deep brain stimulation in humans. *Hypertension* 63: 1000–1010, 2014. doi:10.1161/HYPERTENSIONAHA.113.02970.

Sverrisdóttir YB, Rundqvist B, Elam M. Relative burst amplitude in human muscle sympathetic nerve activity: a sensitive indicator of altered sympathetic traffic. *Clin Auton Res* 8: 95–100, 1998. doi:10.1007/BF02267819.

Sverrisdóttir YB, Rundqvist B, Johannsson G, Elam M. Sympathetic neural burst amplitude distribution: a more specific indicator of sympathoexcitation in human heart failure. *Circulation* 102: 2076–2081, 2000. doi:10.1161/01.CIR.102.17.2076.

Vallbo AB, Hagbarth KE, Torebjörk HE, Wallin BG. Somatosensory, proprioceptive, and sympathetic activity in human peripheral nerves. *Physiol Rev* 59: 919–957, 1979. doi:10.1152/physrev.1979.59.4.919.

Victor RG, Leimbach WN Jr, Seals DR, Wallin BG, Mark AL. Effects of the cold pressor test on muscle sympathetic nerve activity in humans. *Hypertension* 9: 429–436, 1987. doi:10.1161/01.HYP.9.5.429.

Villacres EC, Hollifield M, Katon WJ, Wilkinson CW, Veith RC. Sympathetic nervous system activity in panic disorder. *Psychiatry Res* 21: 313–321, 1987. doi:10.1016/0165-1781(87)90015-1.

Virtanen R, Jula A, Salminen JK, Voipio-Pulkki LM, Helenius H, Kuusela T, Airaksinen J. Anxiety and hostility are associated with reduced baroreflex sensitivity and increased beat-to-beat blood pressure variability. *Psychosom Med* 65: 751–756, 2003. doi:10.1097/01.PSY.0000088760.65046.CF.

Vranish JR, Holwerda SW, Young BE, Credeur DP, Patik JC, Barbosa TC, Keller DM, Fadel PJ. Exaggerated vasoconstriction to spontaneous bursts of muscle sympathetic nerve activity in healthy young black men. *Hypertension* 71: 192–198, 2018. doi:10.1161/HYPERTENSIONAHA.117.10229.

Vuillemin A, Oppert JM, Guillemin F, Essermeant L, Fontvieille AM, Galan P, Kriska AM, Hercberg S. Self-administered questionnaire compared with interview to assess past-year physical activity. *Med Sci Sports Exerc* 32: 1119–1124, 2000. doi:10.1097/00005768-200006000-00013.

Wallace DM, Magnuson DJ, Gray TS. Organization of amygdaloid projections to brainstem dopaminergic, noradrenergic, and adrenergic cell groups in the rat. *Brain Res Bull* 28: 447–454, 1992. doi:10.1016/0361-9230(92)90046-Z.

Wallin BG, Esler M, Dorward P, Eisenhofer G, Ferrier C, Westerman R, Jennings G. Simultaneous measurements of cardiac noradrenaline spillover and sympathetic outflow to skeletal muscle in humans. *J Physiol* 453: 45–58, 1992. doi:10.1113/jphysiol.1992.sp019217.

Wilkinson DJ, Thompson JM, Lambert GW, Jennings GL, Schwarz RG, Jefferys D, Turner AG, Esler MD. Sympathetic activity in patients with panic disorder at rest, under laboratory mental stress, and during panic attacks. *Arch Gen Psychiatry* 55: 511–520, 1998. doi:10.1001/archpsyc.55.6.511.