Neural and cardiovascular responses to emotional stress in humans

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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. First published September 10, 2008; doi:10.1152/ajpregu.90646.2008.—Sympathetic neural responses to mental stress are well documented but controversial, whereas sympathetic neural responses to emotional stress are unknown. The purpose of this study was to investigate neural and cardiovascular responses to emotional stress evoked by negative pictures and reexamine the relationship between muscle sympathetic nerve activity (MSNA) and perceived stress. Mean arterial pressure (MAP), heart rate (HR), MSNA, and perceived stress levels were recorded in 18 men during three randomized trials: 1) neutral pictures, 2) negative pictures, and 3) mental stress. MAP and HR increased during mental stress (Δ14 ± 2 mmHg and Δ15 ± 2 beats/min, P < 0.001) but did not change during viewing of negative or neutral pictures. MSNA did not change during viewing of neutral (Δ1 ± 1 burst/min, n = 16) or negative (Δ0 ± 1 burst/min, n = 16) pictures or during mental stress (Δ1 ± 2 burst/min, n = 13). Perceived stress levels were higher during mental stress (3 ± 0 arbitrary units) than during viewing negative pictures (2 ± 0 arbitrary units, P < 0.001). Perceived stress levels were not correlated to changes in MSNA during negative pictures (r = 0.10, P = 0.84) or mental stress (r = 0.36, P = 0.23). In conclusion, our results demonstrate robust increases in MAP and HR during mental stress, but not during emotional stress evoked by negative pictures. Although the influence of mental stress on MSNA remains unresolved, our findings challenge the concept that perceived stress levels modulate MSNA during mental stress.

Mental stress elicits a complex physiological response in humans. The two most common techniques used to evoke mental stress, mental arithmetic and the Stroop color word test, consistently increase heart rate (HR) and arterial blood pressure, but sympathetic neural responses are controversial. Most studies report an increase in leg muscle sympathetic nerve activity (MSNA) during mental stress (1–3, 5, 7, 19, 20), although some report no change (4, 24) or a decrease (10, 18). Others report that mental stress can result in an increase or no change in MSNA, depending on whether it is reported as burst frequency or amplitude (12, 13, 23).

It is unclear why some individuals have a neural response to mental stress while others do not. Callister et al. (3) performed a comprehensive study that examined the influence of task type, task difficulty, and the perception of stress on MSNA during mental arithmetic and the Stroop color word test. It was determined that MSNA was primarily influenced by the perception of stress, which was partly dependent on the absolute level of difficulty. The type of cognitive task (mental arithmetic vs. the Stroop color word test) and performance levels did not appear to influence MSNA responses (3). The findings by Callister et al. (3) are relevant, yet the concept that MSNA responses to mental stress are related to levels of perceived stress has not been confirmed.

Mental arithmetic is considered to be primarily a cognitive stress, requiring attentional and working memory resources. Whereas the neural and cardiovascular responses to mental stress are well documented (1–5, 7, 10, 18–20, 24), studies examining neural and cardiovascular responses to primarily an emotional stress have not been performed. One method for evoking emotional stress, without a confounding cognitive stress, is through the viewing of negative pictures (i.e., wounded child, murder victims, etc.). Kern et al. (15) reported that viewing negative pictures has a significantly reduced cognitive demand compared to mental digit tracking, a task that is significantly easier to perform than mental arithmetic. Although the use of negative pictures has been used throughout the psychological literature (8, 15–17), the impact of negative pictures on physiological responses remains unclear. Using functional magnetic resonance imaging techniques to examine the amygdala, Kensinger and Schacter (14) recently reported that viewing negative pictures produced similar activity as fear situations. Given that fear is a strong stressor, we hypothesized that viewing negative pictures would elicit physiological responses similar to responses produced by mental arithmetic.

Therefore, the primary purpose of the present study was to determine neural and cardiovascular responses to emotional stress evoked by negative pictures. We hypothesize that viewing negative pictures will increase arterial blood pressure, HR, and MSNA. A secondary purpose of the present study was to reexamine the relationship between MSNA and perceived stress levels. On the basis of the findings of Callister et al. (3), we hypothesize that increased levels of perceived stress will be associated with greater changes in MSNA.

METHODS

Subjects. Eighteen male subjects (mean ± SE: 22 ± 2 yr of age, 87 ± 3 kg body wt, 178 ± 1 cm height) participated in the study. All subjects were nonsmokers, were not taking any medication, and abstained from exercise, alcohol, and caffeine for ≥12 h before laboratory testing. All subjects attended an orientation session at which the study design and procedures were outlined. Approval for testing was granted through the Human Subjects Research Committee at Michigan Technological University, and all subjects signed a written informed consent before participating in the study.

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Experimental design. Each subject performed the following experimental trials in randomized order: 1) viewing of neutral pictures, 2) viewing of negative pictures, and 3) performing mental stress tests (via mental arithmetic). We measured MSNA, mean arterial blood pressure (MAP) and HR responses during all three trials. Each subject was lying prone throughout the entire experiment with his/her head resting comfortably in a massage table head piece. The laptop computer used to display the negative and neutral slides was placed ~80 cm below the subjects' eyes. Lighting was adjusted in the room to allow for clear viewing, and all subjects reported excellent visibility of the slides. Each trial was 9 min in length and included a 3-min baseline, 3-min intervention (i.e., neutral pictures, negative pictures, or mental stress), and 3-min recovery. Up to 5 min of nonrecorded recovery separated the trials to ensure variables were at baseline levels prior to beginning a new trial.

Picture slide shows were shown on a laptop computer using pictures from the International Affective Picture System (16). The neutral and negative picture slide shows each contained one blank slide and 59 pictures, and subjects viewed each picture for 3 s (180 s total viewing time). Pictures were chosen based on normative ratings of valence (i.e., emotional continuum of negative to positive) and arousal. One set was negative and highly arousing (e.g., a wounded child) and the other set was neutral in both valence and arousal levels (e.g., a table lamp). Using the International Affective Picture System (16), we found that independent samples t-tests confirmed the negative pictures were significantly more negative [t(116) = −3.107, P < 0.001] and more arousing [t(116) = 34.57, P < 0.001] than the neutral pictures.

Mental arithmetic was used to elicit mental stress. Mental arithmetic was selected instead of the Stroop color word test for two reasons: 1) mental arithmetic and the Stroop color word test produce similar neural and cardiovascular responses in humans (3), and 2) our laboratory has had more success stimulating cognitive stress using the mental arithmetic protocol (5, 7). Subjects were given a two- or three-digit number and were asked to perform serial subtraction using either number 6 or 7. Subjects answered verbally and were encouraged by an investigator to subtract very quickly. A new number to subtract from was provided every 5–10 s to make the task stressful. After a transition from baseline to mental stress in one of our subjects, this slight shift did not affect our burst frequency data, but did make it inappropriate to examine total MSNA activity in that subject. Therefore, our total MSNA data represent 12 subjects for the mental stress trial.

Statistical analysis. All data were statistically analyzed with commercial software (SPSS 15.0, SPSS, Chicago, IL). A repeated-measures ANOVA was used to examine MSNA, MAP, and HR during each of the three trials. Post hoc analysis was performed using least-significant-difference pairwise comparisons. Paired t-tests were used to compare perceived stress levels (negative vs. mental stress and neutral vs. negative). Pearson’s correlations were utilized to probe for relationships between perceived stress and MSNA. Means were considered significantly different when P < 0.05. Results are expressed as means ± SE.

RESULTS

Mean values for MSNA, MAP, and HR during the three experimental trials are presented in Table 1. Baseline values were not different across the three trials. MSNA did not change during neutral pictures (Δ1 ± 1 burst/min and Δ−22 ± 96 arbitrary units [AU]), negative pictures (Δ0 ± 1 burst/min and Δ−84 ± 76 AU), or mental stress (Δ1 ± 1 burst/min and Δ839 ± 644 AU). Figure 1 depicts representative neurograms from one subject during the negative picture and mental stress trials. Figure 2 demonstrates that MAP and HR significantly increased during mental stress (Δ14 ± 2 mmHg and Δ15 ± 2 beats/min) but did not change during negative picture (Δ0 ± 1 mmHg and Δ−2 ± 1 beats/min). Neutral pictures did not change MAP (Δ−1 ± 1) or HR (Δ0 ± 1 beats/min). Neutral

Table 1. Neural and cardiovascular variables recorded during the three trials

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Intervention</th>
<th>Recovery</th>
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<tbody>
<tr>
<td>MSNA, bursts/min</td>
<td></td>
<td></td>
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<tr>
<td>Neutral</td>
<td>13 ± 1</td>
<td>13 ± 2</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>Negative</td>
<td>12 ± 1</td>
<td>12 ± 1</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>MS</td>
<td>13 ± 2</td>
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<td>16 ± 3</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutral</td>
<td>77 ± 2</td>
<td>77 ± 2</td>
<td>77 ± 2</td>
</tr>
<tr>
<td>Negative</td>
<td>77 ± 2</td>
<td>77 ± 2</td>
<td>78 ± 2</td>
</tr>
<tr>
<td>MS</td>
<td>79 ± 2</td>
<td>93 ± 4*</td>
<td>83 ± 3</td>
</tr>
<tr>
<td>HR, beats/min</td>
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<tr>
<td>Neutral</td>
<td>68 ± 3</td>
<td>68 ± 3</td>
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</tr>
<tr>
<td>Negative</td>
<td>69 ± 3</td>
<td>67 ± 3</td>
<td>68 ± 3</td>
</tr>
<tr>
<td>MS</td>
<td>70 ± 3</td>
<td>86 ± 4*</td>
<td>69 ± 3</td>
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</table>

Values are means ± SE. MSNA, muscle sympathetic nerve activity [n = 16 for neutral and negative pictures, n = 13 for mental stress (MS)]; MAP, mean arterial pressure (n = 18); HR, heart rate (n = 18). *P < 0.001 vs. corresponding baseline value.
pictures were perceived as not stressful (score of 0) by all subjects. Figure 3 shows that although the negative pictures were perceived as stressful, mental stress was perceived as more stressful than the negative pictures (1.7 ± 0.2 vs. 3.0 ± 0.1 AU, *P < 0.001"). Figure 4 demonstrates that changes in MSNA were not correlated to perceived stress levels during either negative pictures (r = 0.10, *P = 0.84) or mental stress (r = 0.36, *P = 0.23).

**DISCUSSION**

Contrary to expectations, cognitive stress (i.e., mental arithmetic) and emotional stress (i.e., negative pictures) did not produce similar cardiovascular responses, nor were they reported as similarly stressful. One possible explanation is that the mental arithmetic task involved a higher cognitive load (mental calculations) and multiple sources of stress (task performance, time demand, and performance evaluation), whereas viewing negative pictures was a passive task (low cognitive

Fig. 1. Representative neurograms from 1 subject during negative pictures and mental stress. Muscle sympathetic nerve activity (MSNA) was similar during baseline and intervention for both trials.

Fig. 2. Heart rate (HR) and mean arterial pressure (MAP) during negative pictures and mental stress. HR and MAP were elevated during mental stress. *P < 0.001 vs. baseline.

Fig. 3. Perceived stress during negative pictures and mental stress. Subjects reported an increase in perceived stress during both negative pictures and mental stress but rated mental stress as significantly more stressful compared to negative pictures. *P < 0.001 vs. neutral pictures; †P < 0.001 vs. negative pictures. a.u., Arbitrary units.

Fig. 4. Perceived stress during negative pictures and mental stress. Subjects reported an increase in perceived stress during both negative pictures and mental stress but rated mental stress as significantly more stressful compared to negative pictures. *P < 0.001 vs. neutral pictures; †P < 0.001 vs. negative pictures. a.u., Arbitrary units.
was not the source, but the circumstance under which the arithmetic itself negative, would cause stress). To one condition of high emotional load (which, when high enough, would cause arousal and negative, we suspected that we had one condition of high cognitive load (which, when high enough, would cause stress). Our results suggest that cardiovascular responses depend on the source of stress.

Another possible explanation lies in the nature of the mental arithmetic task. Initially, our interpretation of the experience of mental arithmetic was that of a primarily cognitive task. Subjects were asked to actively listen to and calculate numbers. Compared with passively viewing pictures, this appeared to be a high-cognitive-load task. In contrast, our interpretation of viewing negative pictures was that of a primarily emotional task. Subjects were not asked to perform any mental task, and they were not told to memorize, evaluate, or interpret the pictures. They were asked only to view the pictures. Because the negative pictures had been normatively rated as highly arousing and negative, we suspected that we had one condition of high cognitive load (which, when high enough, would cause stress) and one condition of high emotional load (which, when negative, would cause stress).

In retrospect, it appears the mental arithmetic condition likely induced strong negative emotions. The arithmetic itself was not the source, but the circumstance under which the subjects had to perform the math was highly aversive. Although the math was described as “simple arithmetic,” the investigator providing the math problems was demanding and vocally loud, and the time pressure was substantial. Subjects tended to falter in completing the calculations. They were slow to respond or were cut off by the experimenter before they could state the solution. In other words, the circumstances were such that they “choked” under the pressure. It is our opinion that the subjects were probably feeling embarrassed by their inability to quickly perform “simple” math; thus, the situation became personally consequential. An embarrassing performance in front of an investigator and laboratory technicians should be cause for strong negative emotions.

Because our two stressors (mental arithmetic vs. negative pictures) were not of comparable intensity, we cannot make meaningful comparisons between the two trials. However, important physiological interpretations are available within each trial. Most notably, our subjects rated the negative picture trial as “stressful,” indicating that low levels of perceived emotional stress related to negative pictures do not alter cardiovascular control. This is quite different than the cardiovascular responses observed during mental arithmetic, which result in prompt cardiovascular excitation even at low levels of perceived stress (3). These findings suggest that HR and MAP are governed differently by various types of stress. More specifically, the source of the stress may play an important role.

The present study reports no change in MSNA during either emotional or mental stress. Although this study is the first to report MSNA responses to a task that is primarily an emotional stress, MSNA responses to mental stress are well documented. Anderson et al. (2) was the first to demonstrate that mental stress increases MSNA. Several investigators have confirmed (1, 3, 5, 7, 19, 20) and refuted (4, 10, 18, 24) this original data (2). To date, only one study has attempted to explain the discrepant MSNA responses observed during mental stress (3). Callister et al. (3) examined MSNA in 12 subjects during mental stress performed at six levels of increasing difficulty. Both the Stroop color word test and mental arithmetic were utilized to stimulate mental stress. It was determined that MSNA decreased during low levels of difficulty and stress but increased linearly during higher levels of difficulty and perceived stress. The type of task did not appear to influence these findings, as responses during the Stroop color word test and mental arithmetic were similar. The authors concluded that during mental stress, the stimulation of MSNA is governed primarily by the perception of stress (3).

Our results are inconsistent with the conclusions of Callister et al. (3). Despite perceiving the negative pictures as “stressful” and the mental arithmetic as “very stressful”, we did not observe any changes in MSNA. The perceived stress ratings during the mental stress trial in the present study were comparable to levels reported to increase MSNA in the Callister et al. (3) study. Additionally, we found no correlation between changes in MSNA and perceived stress levels during either mental stress or negative pictures. Our data challenge the concept that perceived stress levels modulate MSNA during mental stress. The relationship between perceived stress and MSNA deserves more attention.

There are some key differences between Callister et al. (3) and the present study that may help explain the contrasting conclusions. First, Callister et al. (3) examined MSNA using a
graded-stress protocol, whereas our subjects participated in three distinct trials designed to elicit varying stress responses when compared to one another. The design by Callister et al. (3) allowed the authors to monitor MSNA during increasing levels of difficulty and perceived stress but also resulted in a prolonged experimental protocol. Each trial included a 5-min baseline, six stress levels (5 min each), and a 10-min recovery; thus the entire protocol took 45 min per trial. Since subjects randomly performed both the Stroop color word test and mental arithmetic trials (separated by 5–15 min of additional rest to ensure a return to baseline levels), the entire experiment took ~2 h after location of the peroneal nerve (3). This prolonged experimental time frame necessitated the inclusion of time controls. However, only two time control subjects were included to ensure that changes over levels of task were not simply variations over time (3). It is possible that the changes in MSNA observed during increasing levels of task difficulty could have been influenced by time (i.e., 30 min of mental stress).

Another difference between Callister et al. (3) and the current study is the MSNA responsiveness of the subjects. Callister et al. (3) reported that 9 out of 12 subjects demonstrated increases in MSNA during mental arithmetic, whereas in the present study, only 6 out of 13 subjects demonstrated increases in MSNA during mental arithmetic. It is possible that the relationship between MSNA and perceived stress may be different in responders and nonresponders. We recently proposed that it may be appropriate to classify individuals performing mental stress tests into responders and nonresponders (4) and the present study highlights this need. To date, no studies have attempted this and the present study does not have enough subjects for such an analysis. If future studies are to attempt to classify individuals performing mental stress tests into responders and nonresponders, what would be the appropriate level to label a subject as a responder? On the basis of studies from our laboratory (5, 7) and others (1–3, 12, 13, 19, 20, 23), who have reported significant increases of MSNA during mental stress, we propose a responder be classified as an individual with an average MSNA increase of 3–4 bursts/min or greater. Probing for differences between responders and nonresponders may lend valuable insight into understanding the inconsistent MSNA responses to mental stress.

In conclusion, our results demonstrate robust increases in MAP and HR during mental stress, but not during emotional stress evoked by negative pictures. This finding suggests that cardiovascular excitation during mental stress is modulated primarily by cognitive stress, at least at low levels of stress. Although the influence of mental stress on MSNA remains unresolved, our findings challenge the concept that perceived stress levels modulate MSNA during mental stress.

**Perspectives and Significance**

Mental stress has been linked to several cardiovascular diseases, including myocardial ischemia (9, 21), hypertension (11), and atherosclerosis (22). It is unclear whether the cardiovascular complications associated with mental stress are due to emotional stress, cognitive stress, or both. Because mental arithmetic and the Stroop color word test elicit consistent and robust increases in HR and blood pressure, they will continue to serve as an important technique for inducing mental stress in humans. However, the current study highlights the need for more techniques and experimental procedures that allow researchers to distinguish between different types of stress (i.e., cognitive vs. emotional). Such techniques are available and are routinely used by psychologists but have not received as much attention from physiologists examining cardiovascular control. Distinguishing between emotional and cognitive stress responses may lead to a better understanding of the associations between mental stress and cardiovascular disease in humans.

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**GRANTS**

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