

Article

Trier Social Stress Test Elevates Blood Pressure, Heart Rate, and Anxiety, But a Singing Test or Unsolvable Anagrams Only Elevates Heart Rate, among Healthy Young Adults

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Abstract: The Trier Social Stress Test (TSST) is a psychosocial stressor that effectively stimulates the stress response but is labor and time intensive. Although other psychological stressors are often used experimentally, none are known to comparably elevate stress. Two stressors that may potentially elevate stress are a singing task (ST) and unsolvable anagrams, but there are not enough data to support their effectiveness. In the current experiment, 53 undergraduate males and females (mean age = 21.9 years) were brought into the laboratory, and baseline blood pressure, heart rate, self-rated anxiety, and salivary cortisol were recorded. Then, participants were randomly assigned to one of three stress conditions: TSST ($n = 24$), ST ($n = 14$), or an unsolvable anagram task ($n = 15$). Stress measures were taken again after the stressor and during recovery. The TSST significantly elevated systolic blood pressure, diastolic blood pressure, heart rate, and self-rated anxiety from pre-stress levels, replicating its stress-inducing properties. However, the ST and unsolvable anagrams only elevated heart rate, indicating that these methods are not as able to stimulate physiological or psychological stress. Overall, results indicate that out of these three laboratory stressors, the TSST clearly engages the stress response over the ST or unsolvable anagrams.

Keywords: stress; laboratory stressors; TSST; singing task; unsolvable anagrams



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1. Introduction

Human research on stress often employs acute laboratory stressors to induce various elements of the stress response in participants. One major difficulty in this area of research is that many different techniques are used to induce stress, with little evidence of how psychological or physiological stress markers are comparable between them. In addition, the most reliable and replicated laboratory stressors are labor and time intensive, making them unfeasible for many studies. The purpose of the current research was two-fold: first, to compare markers of stress between three psychological stressors to identify if there is a comparable stimulation of stress, and second, to use these data to establish reliability for a relatively easy way to stimulate acute stress in a laboratory setting.

Acute laboratory stressors are often subdivided as either psychological or physical stressors. Psychological stressors are those that induce stress mentally, without a physically taxing component (i.e., math, Stroop test, or unsolvable anagrams), whereas physical stressors elevate stress through either physical exertion (e.g., exercising on a stationary bicycle) or a physically challenging task (e.g., the cold pressure test). Psychological and physical stressors can have distinct outcomes on behavior. For example, psychological stressors elevate energy intake [1,2]; however, the use of a physical stressor, such as the cold pressure test, does not reliably elevate energy intake [3]. Furthermore, it is well established that individuals with high depressive symptomology have a blunted cardiovascular and hormonal response to a psychological stressor; however, this blunted response is no longer observed when a physical stressor is used [4]. As further evidence that psychological

and physical stressors have separable responses, individuals who have an elevated stress response to a psychological stressor will not necessarily have a stress reaction to a physical stressor [5]. Therefore, it is often the case that a study will require stress stimulation through a psychological, and not a physical, manipulation.

One of the most commonly used psychological stressors is the Trier Social Stress Test (TSST), which was established by Kirschbaum, Pirke, and Hellhammer [6]. This requires participants to give a speech in front of a panel of judges and then complete a serial subtraction test. It combines both uncontrollability with social-evaluative threat. The TSST reliably elevates blood pressure and heart rate almost immediately, stimulates cortisol release, which peaks in saliva approximately 30 min after the stressor initiation, and increases self-reported state anxiety (for review see [7]). Although the TSST is reliable in elevating physiological and psychological measures of stress [6,8], it is labor and time intensive. It requires the involvement of three confederates acting as judges, in addition to the participant and research assistant. Furthermore, the procedure requires substantial administrative work in terms of training assistants and scheduling. Therefore, many studies that aim to elevate stress through a psychological stressor use an alternative method that is less labor and/or time intensive.

Several modified versions of the TSST have been studied as potentially less labor-intensive options (for review see [7]). The use of either an imagined or virtual committee is a salient option that would eliminate the need for an in-person panel of judges. Indeed, a virtual panel has been shown to elevate cortisol reactivity; however, this was more effective in highly immersive paradigms and even then, it did not elevate stress to the degree of the traditional TSST [9]. In addition, performing a speech to an imagined committee behind a two-way mirror did not stimulate salivary cortisol to the level of an in-person audience [10]. A group assessment, where multiple participants perform speeches in front of the committee, has also been assessed as a time-saving option. In this paradigm, participants who engaged in the TSST as a group did have a substantial elevation in salivary cortisol, although this was not compared to the traditional TSST format [11]. A group assessment may certainly be an option for certain experimental designs, but many studies require evaluating each participant individually and therefore would not be able to use a group TSST option. Clearly, there is still a need for an acute psychological laboratory stressor that presents fewer drawbacks than the TSST but is still able to stimulate a reliable stress response.

There are several previous studies that have compared between various types of laboratory stressors in healthy adults. Many of these compare psychological to physical stressors [12–15], and therefore, they do not address the issue of needing specifically a psychological stressor that is effective in inducing the stress response. Others determine the effectiveness of the stressor by comparison to participants' pre-task baseline but not to a known effective stressor, such as the TSST [12,14,16–18]. In addition, several psychological stressors have already been shown to be less effective than the TSST. Both mental arithmetic and the Stroop test have been shown to induce only a weak stress response. Mental arithmetic has been shown to elevate negative mood but not salivary cortisol or heart rate [13]. In contrast, the Stroop test elevated self-reported stress and heart rate but did not stimulate an elevation in salivary cortisol, unlike the TSST during the same study [15]. Therefore, it still needs to be established if there is a psychological stressor that is comparable to a reliable laboratory stressor.

The use of unsolvable anagrams is a commonly used stressor that has not had its effects well researched. Although this is a widely used task [19–21], there is only limited evidence of its success in stimulating the stress response. The use of unsolvable anagrams to induce stress is a likely candidate, because there is some research that indicates attempting unsolvable anagrams elevates physiological measures of stress, including blood pressure, heart rate [22,23], and galvanic skin response [24]. In addition, the unsolvable anagram task has the element of uncontrollability in that the task cannot be completed, which prevents the participant from obtaining a salient goal [20], which are critical components

to previously known stressors (for review see [25]). To the best of our knowledge, there is not any study that has determined if attempting unsolvable anagrams stimulates an elevation in cortisol. Therefore, the current study has explored whether an unsolvable anagram task elevates cortisol or if the elevation of stress markers is comparable to that induced by the TSST.

Laboratory studies have also found promising results in using singing as an acute stressor. Anticipation of singing “The Star-Spangled Banner” was shown to elevate heart rate and skin conductance [26]. In this study, participants were in a room with two confederates, and it is not known if the presence of the confederates added significantly to the elevation in stress markers. In addition, only limited measures of stress were taken. Reschke-Hernandez, Okerstrom, Edwards, and Tranel [27] had participants engage in either the traditional TSST or a modified version where participants sang instead of giving a speech. They found comparable elevations of self-rated stress and salivary cortisol. These studies indicate that singing may be a successful way to elevate stress markers. However, it has yet to be determined if a singing task (ST) would be effective in the absence of a committee. Certainly, having an evaluative component is necessary for the effectiveness of the TSST [28], but it is possible that the use of a video camera would be similarly stress-inducing. Indeed, giving a speech in front of a video camera elevates heart rate, but this has yet to be compared to the TSST with a full committee [16]. Therefore, the current study aimed to determine if a ST completed in front of a video camera would stimulate a stress response comparable to that of the TSST.

Although many previous studies have employed acute stressors, several key factors are missing from this literature. First, it is unknown if the commonly used stressor of unsolvable anagrams or the relatively new ST stimulate cortisol. Second, these potentially very useful laboratory stressors have yet to be compared to the TSST. Considering the importance of establishing a purely psychological stressor that is less cumbersome to employ than the TSST, the purpose of the current study was twofold: to assess the stress response in reaction to several promising laboratory stressors that previously have been understudied in this regard, and to compare their stress-inducing properties to that of the TSST. To this aim, three methods of inducing acute stress were compared: an ST, unsolvable anagrams, and the TSST. Measures of stress included blood pressure, heart rate, self-rated anxiety, and salivary cortisol. It was hypothesized that all stress procedures would elevate psychological and physiological measures of stress comparable to the TSST.

2. Materials and Methods

2.1. Participants

Participants were 53 male and female undergraduate students enrolled in psychology classes (see Figure 1 for the reporting checklist). Potential participants were recruited through class announcements asking students to participate in a study that was “examining bodily changes during difficult tasks”. Participants qualified for inclusion if they were between the ages of 18 and 50, were not a regular nicotine user (defined as smoking less than 10 or more cigarettes or e-cigarettes in a single day in the past six months), did not take any daily medications (not including birth control), were not pregnant, and did not have a diagnosis of major depression, anxiety, an eating disorder, an endocrine disorder, or a metabolic disorder (see Table 1 for participant demographics). Participants were compensated through extra credit and were entered into a raffle for a \$50 gift card. All procedures were approved by the Institutional Review Board.

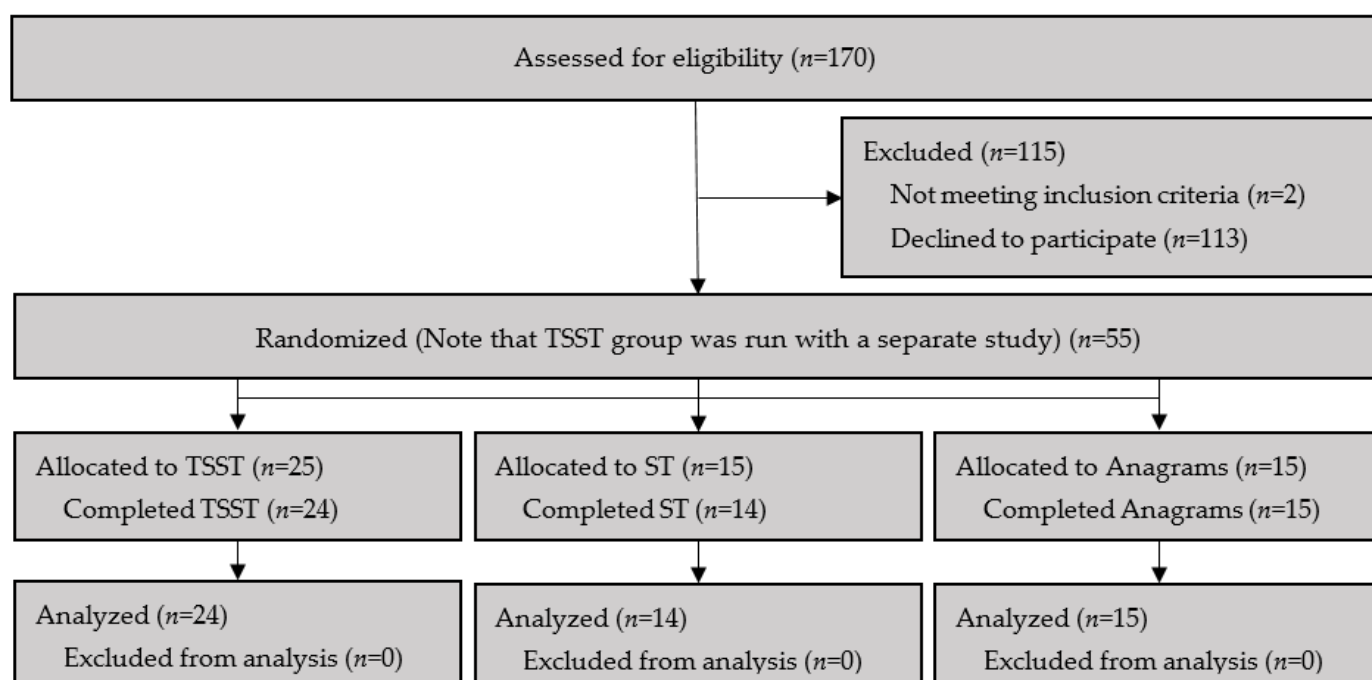


Figure 1. Reporting checklist for participants.

Table 1. Participant descriptive characteristics.

	Descriptive Characteristics			
	Overall	TSST	ST	Anagrams
Age in Years [Mean (SD)]	21.9 (3.6)	21.5 (3.9)	22 (2.1)	23.4 (4.2)
BMI (kg/m ²) [Mean (SD)]	27.4 (6.3)	26.5 (7.0)	26.4 (4.4)	29.6 (6.4)
Females [N (%)]	45 (84.9)	21 (87.5)	12 (85.7)	12 (80)
Males [N (%)]	8 (15.1)	3 (12.5)	2 (14.3)	3 (20)
Ethnicity [N (% of condition)]				
Latinx	8 (15)	4 (16.7)	3 (21.4)	1 (6.7)
Race [N (% of condition)]				
Black/African American	29 (55)	13 (54.2)	8 (57.1)	8 (15.3)
White/Caucasian	14 (26)	4 (16.7)	5 (35.7)	5 (33.3)
Asian	3 (6)	2 (8.3)	0 (0)	1 (6.7)
Other/Unspecified Race	7 (13)	5 (20.8)	1 (7.1)	1 (6.7)

2.2. Experimental Design

2.2.1. Baseline Procedures

The experiment was run as a between-subjects design. Participants were told that the study aim was to determine psychological and physiological responses to a variety of tests. Potential participants were screened via phone or text message and then scheduled for a 1 h laboratory visit. Start times were between 12:00 and 4:30 pm, to avoid the morning elevation of cortisol. Participants were told not to eat or drink anything except water for 2 h before their visit start time and not to consume any alcohol for 12 h before their visit start time. Upon arrival, compliance with intake instructions was verified verbally, and written consent was obtained. Then, participants were instructed to rinse their mouth out with water to assist with later saliva samples. After, participants sat quietly for 15 min. During this time, instrumental jazz was played softly, and popular magazines were provided.

After the rest period, baseline measures were taken, including salivary cortisol, blood pressure, heart rate, and self-rated anxiety (0 min). Then, participants were assigned to one of two experimental stress conditions, ST ($n = 14$) or unsolvable anagrams ($n = 15$). Participants in the third condition, the TSST ($n = 24$), were part of a larger study that was conducted during the same time frame. Participant inclusion/exclusion requirements, baseline measurements, and experimental design were identical between both experiments. Only the participants that underwent the control condition in the TSST experiment were used for the current analyses. Therefore, all procedures were identical for participants included in the three experimental condition except for the stress manipulation itself. After task instructions, blood pressure and heart rate were taken a second time (5 min). Then, participants underwent their designated stress task.

2.2.2. TSST Procedure

The TSST condition was modified from Kirschbaum et al. [6] and Klatzkin et al. [29]. Participants were told that they were going to undergo a mock job interview, but unlike an ordinary interview, they were going to give a self-promoting speech to a panel of committee members. Participants were told that their goal was to convince the committee that they were the best candidate for their ideal job. After the instructions, they were given a five-minute anticipatory period, during which they were provided with a blank piece of paper and pen and told to prepare their speech. After 5 min, participants had their notes removed and were brought into a separate room where there was a panel of three committee members (research assistants wearing white laboratory coats) seated at a table with a video and voice recorder. Participants were instructed to stand in front of the table and give their oral presentation for five minutes. If participants stopped talking at any time during the presentation period, the judges reminded them that they still had time left and asked them follow-up questions (e.g., "Where do you see yourself in 10 years?"). After the self-promoting speech, participants completed a serial subtraction task for five minutes, during which they were asked to count backwards from 2000 by 7. When an incorrect answer was given, participants were told, "Stop, mistake, start over at 2000, please."

2.2.3. ST Procedure

The procedure for the ST was modified from Brouwer and Hogervost [26], which found an elevation in both skin conductance and heart rate while incorporating key aspects of the TSST. Those in the ST condition were informed that they would sing karaoke in front of a video camera (in order to increase the perception of social-evaluative threat). Then, participants were given lyrics to five commonly known nursery rhymes and told that they had 5 min to decide which two songs they would like to sing (so as to replicate the anticipatory stress from the TSST). They were informed that they would not be able to refer to the lyrics during the singing portion. After the 5 min anticipatory period, participants were instructed to stand on an indicated line on the floor directly in front of the computer screen. A video camera and voice recorder were turned on next to the computer in an identical fashion to the TSST procedure. They were instructed to begin singing when prompted by the computer and sang consecutively for approximately 5 min.

2.2.4. Unsolvable Anagram Procedure

The unsolvable anagram condition was based on Zellner et al. [30] because this procedure was shown to elevate psychological stress and stimulate a change in behavior. Those in the anagram condition were told that they had 10 min to complete eight anagrams and that they would receive a \$50 gift card if they were able to solve all the anagrams correctly. However, four of the anagrams were unsolvable.

2.2.5. Post-Stressor Measurements

At the completion of the stress task, blood pressure, heart rate, and self-rated anxiety were measured again (15 min from baseline for the ST and Unsolvable Anagrams, 20 min

for the TSST). Then, participants were given several popular magazines and told to sit quietly. Blood pressure, heart rate, and self-rated anxiety were measured one final time (25 min). Salivary cortisol elevates approximately 20–30 min after the start of a stressor [6]; therefore, a salivary sample was collected again 20 min and 30 min after the start of the stress task. After the post-stress measures, participants filled out a demographic questionnaire, which asked for details on race, ethnicity, gender, and age, had their height and weight measured, and were debriefed on the experiment (see Figure 2 for timeline).

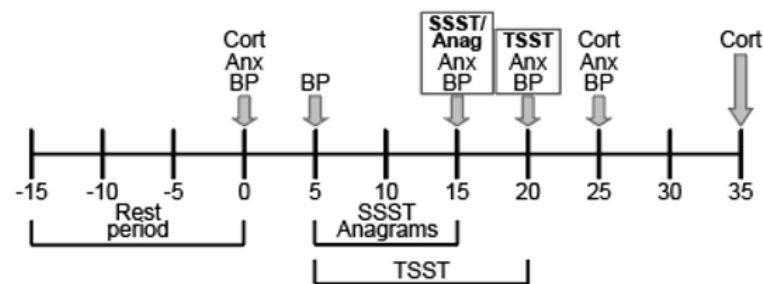


Figure 2. Experimental timeline.

2.3. Measures

2.3.1. Cardiovascular Measures

Blood pressure and heart rate were measured with an automatic upper arm blood pressure monitor (Omron Series 10; Omron Healthcare Inc. (Mississauga, ON, CA, USA)). During blood pressure/heart rate measures, participants were told to sit comfortably with their feet flat on the ground and legs uncrossed. They were instructed to either roll up their sleeve or take their arm out of their sleeve. If a tight lightweight shirt was worn, then the blood pressure cuff was placed over their shirt.

2.3.2. Self-Rated Anxiety Measure

The Profile of Mood States (POMS; McNair, 1981) was used to measure self-rated state anxiety. The POMS was designed to measure transient mood states and has been previously used to measure anxiety elevation in response to a laboratory stressor, and it has been shown to correlate with stress-induced cortisol release [8]. Although the POMS includes measures for several mood states, for the purposes of this study, it was only used to assess anxiety. Therefore, participants answered how much they identified with the following adjectives at that moment: tense, shaky, on edge, panicky, relaxed, uneasy, restless, nervous, and anxious, on a scale from 0 to 4. Scores from all nine adjectives were added to get a participant's final anxiety score. It took participants approximately one minute to complete this questionnaire.

2.3.3. Salivary Cortisol Analysis

Cortisol can be successfully measured in saliva [6]; therefore, salivary cortisol was used as a primary outcome of stress responsivity. During saliva collection, participants were provided with a 1.5 mL microcentrifuge tube affixed with a salivary collection aid mouthpiece (Salimetrics, Carlsbad, CA, USA). They were instructed to tip their head forward and fill the collection tube up to the indicated line (500 μ L) without forcing the production of saliva. Salivary samples were immediately frozen at -20°C until they were analyzed for cortisol. Cortisol was measured with a salivary cortisol enzyme immunoassay kit per the assay protocol (Salimetrics, Carlsbad, CA, USA). In brief, samples were thawed, vortexed, and centrifuged at 1500 g for 15 min (Eppendorf model 5414D centrifuge). Standards, controls, and saliva samples were pipetted into the desired wells (25 μ L per well). A solution of Assay Diluent and Enzyme Conjugate was pipetted into every well (200 μ L per well). The plate was mixed on a plate rotator for 5 min at 500 rpm and then incubated at room temperature for 1 h. Then, plates were washed 4 times with wash buffer.

TMB Substrate Solution was added (200 μ L per well), and the plate was mixed for 5 min on a plate rotator and then incubated in the dark at room temperature for 25 min. Immediately after incubation was complete, Stop Solution was added to each well (50 μ L). Plates were mixed for 3 min and read at 450 nm with a Thermo Electron Multiskan EX (Thermo Fisher Scientific, Waltham, MA, USA) plate reader. Sample optical density was converted to μ g/dl with Multiskan software (Thermo Fisher Scientific, Waltham, MA, USA).

2.4. Statistics

Demographic variables were examined as potential covariates. Demographics included were age, gender, ethnicity, race, and body mass index (BMI). Demographic variables that were associated with any of the dependent variables at $p < 0.10$ were used as covariates in subsequent analyses of that dependent variable. Chi-squared analyses were run to determine group differences for categorical variables, and one-way ANOVAs were run to determine group differences for continuous variables.

All outcome measures of stress, including systolic blood pressure, diastolic blood pressure, heart rate, self-rated anxiety, and salivary cortisol, were each analyzed separately with a mixed-designs repeated-measures two-way ANOVA or ANCOVA with condition as the between-subjects variable and time as the within-subjects variable. For all F statistics, sphericity was determined with Mauchly's test of sphericity. If Mauchly's was significant, analyses were corrected with either Greenhouse–Geisser (if $\epsilon < 0.75$) or Huynh–Feldt (if $\epsilon > 0.75$). Any significant F values were further probed with Bonferroni post-hoc analysis. Since many people do not have a cortisol response to the TSST [8], we attempted to determine if there were a different number of individuals who had a cortisol response to the different stress manipulations. To this end, a secondary analysis of salivary cortisol was conducted. For this, participants were categorized as either stress responders (based on an elevation of cortisol of at least 0.1 μ g/dl after the stressor) or non-responders. For this analysis, a Chi-square was conducted with the number of responders in each stress condition used as the variable. IBM SPSS Statistics 24 (<https://www.ibm.com/analytics/spss-statistics-software>) (accessed on 19 March 2021) was used for all analyses with alpha set at 0.05.

3. Results

3.1. Participant Characteristics

The average age of participants was 21.9 years (SD = 3.6, range = 19–34). Gender distribution was 85% female and 15% male. Participant ethnic composition was 15% Latinx and race was 6% Asian, 55% Black/African American, 26% White/Caucasian, and 13% other or not specified. Participants had an average BMI of 27.4 (SD = 6.3, range = 18–45). Distribution across condition was analyzed to determine if participant characteristics were similar between the experimental groups. Age, gender, ethnicity, race, and BMI were not significantly different across experimental conditions (participant demographics are detailed in Table 1). Age, gender, ethnicity, race, and BMI were explored as potential covariates. There was no relationship between ethnicity, race, or BMI with any of the dependent variables. There was a correlation between age and systolic blood pressure ($r = 0.28$, $n = 45$, $p = 0.07$) and between gender and systolic blood pressure ($r = -0.35$, $n = 53$, $p = 0.007$), but there was no significant relationship between gender or age and any other dependent variable. Therefore, gender and age were used as covariates during the analysis of systolic blood pressure.

3.2. Cardiovascular Response to Stressors

It was determined if there was an interaction between condition and time on systolic blood pressure. The data violated the assumptions of sphericity ($\chi^2(5) = 15.06$, $p = 0.01$; $\epsilon = 0.92$) and the corrected output determined that there was a significant condition X time interaction on systolic blood pressure (Figure 3A; $F(5.5, 110) = 4.5$, $p = 0.001$; $\eta^2 = 22.4\%$). Post hoc analysis determined that after participants underwent the TSST, systolic blood

pressure increased during the second reading compared to baseline (for all means and standard deviations, see Table 2; $p < 0.001$), which occurred immediately before participants began their stress condition. Systolic blood pressure reduced after the stressor, during the third and fourth readings ($p = 0.003$, $p < 0.001$ compared to the second reading); however, it stayed elevated compared to baseline during the third reading ($p < 0.001$). There was no elevation of systolic blood pressure in participants that engaged in the ST or the unsolvable anagrams ($p > 0.05$ for all time points). Furthermore, there were no differences in systolic blood pressure between conditions at any time point ($p > 0.05$).

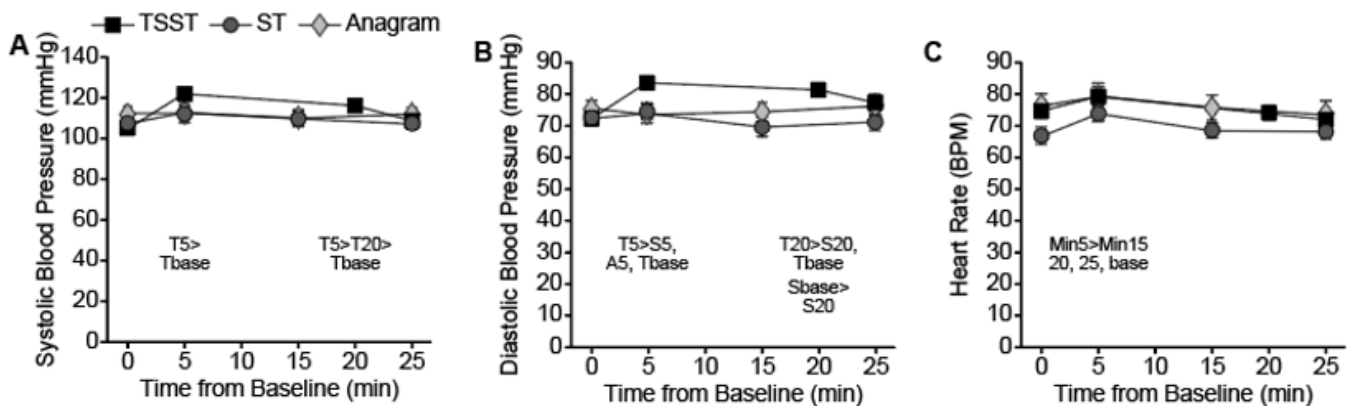


Figure 3. Cardiovascular response to laboratory stressors. (A) Systolic blood pressure was elevated during the TSST at 5 min and 20 min. (B) Diastolic blood pressure was also elevated during the TSST at 5 min and 20 min and suppressed during the ST at 20 min. (C) Heart rate in all conditions was elevated at 5 min. T = TSST, S = ST, A = anagram, base = baseline. All significant effects are $p < 0.05$.

Table 2. Means and standard deviations of outcome measures.

Measure	Cond	Baseline	Pre-Stress	Post-Stress 1	Post-Stress 2	Statistical Results
SBP M(SD)	TSST	105 (12.2)	121.8 (9.5) *	116 (10.3) *	108.8 (10.8)	CxT F(5.5, 110) = 4.5, $p = 0.001$; $\eta^2 = 22.4\%$
	ST	107.5 (10.9)	112 (16.8)	109.6 (14.5)	107.3 (10.6)	
	Anag	111.8 (14.8)	112.6 (14.6)	110.3 (14.3)	111.9 (14.5)	
DBP M(SD)	TSST	72.3 (8.7)	83.5 (9.3) *	81.3 (10.5) *	77.2 (13.3)	CxT F(4.9, 122.5) = 5.1, $p < 0.001$; $\eta^2 = 20.5\%$
	ST	72.6 (10.2)	74.5 (9.3) #	69.5 (11.4)	71.1 (10)	
	Anag	75.4 (9.7)	73.9 (12.1) #	74.3 (11.3)	76.3 (13.1)	
HR M(SD)	TSST	74.6 (11.3)	79.3 (15.6) *	74.1 (11.6)	71.9 (12.4)	Main of T F(1.9, 96.4) = 11.2, $p < 0.001$; $\eta^2 = 22.3\%$
	ST	66.9 (10.3)	73.9 (9.7) *	68.6 (9.4)	68.1 (8.7)	
	Anag	76.5 (13.5)	79.2 (16.6) *	75.7 (15)	73.8 (15.5)	
Anx M(SD)	TSST	6.4 (4.2)		10.3 (7.6) *	5.5 (4.2)	CxT F(3.5, 88.1) = 2.8, $p = 0.04$; $\eta^2 = 11.3\%$
	ST	7.8 (4.5)		8.4 (5.9)	5.3 (4.2) *	
	Anag	5.9 (4.1)		6.1 (5.6)	5.3 (4)	
Cort M(SD)	TSST	0.81 (0.4)		0.9 (0.4)	0.84 (0.6)	CxT ns F(3.2, 77.8) = 0.92, $p = 0.44$; $\eta^2 = 3.8\%$
	ST	0.66 (0.6)		0.65 (0.7)	0.72 (0.6)	
	Anag	0.77 (0.5)		0.72 (0.4)	0.63 (0.4)	

Cond = condition; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; Anx = self-rated anxiety; Cort = salivary cortisol; M = arithmetic mean; SD = standard deviation; TSST = Trier Social Stress Test; ST = singing test; Anag = unsolvable anagrams; CxT = condition by time interaction; T = time; ns = not significant. * indicates significant difference from baseline of the same condition. # indicates significance difference from TSST in the same time point.

Measures of diastolic blood pressure also violated the assumptions of sphericity ($\chi^2(5) = 24.19, p < 0.001; \epsilon = 0.82$). The corrected analysis found that similar to systolic blood pressure, there was a significant condition \times time interaction on diastolic blood pressure (Figure 3B; $F(4.9, 122.5) = 5.1, p < 0.001; \eta^2 = 20.5\%$). Diastolic blood pressure was elevated from baseline in participants that underwent the TSST during the second and third readings ($p < 0.001$ for both comparisons). There was no elevation of diastolic blood pressure in participants that underwent either the ST or the unsolvable anagrams; however, there was a decrease in diastolic blood pressure just after the ST was completed ($p = 0.04$ reading 3 compared to reading 2). When examining between condition effects, it was found that diastolic blood pressure was higher in the TSST condition compared to either the ST or the unsolvable anagrams during the second reading ($p = 0.03$ and $p = 0.02$ respectively). Diastolic blood pressure continued to be elevated in those who underwent the TSST compared to the ST during the third reading ($p = 0.01$).

Heart rate was also corrected for a violation of the assumption of sphericity ($\chi^2(5) = 40.75, p < 0.001; \epsilon = 0.64$). When heart rate was analyzed, a main effect of time was found (Figure 3C; $F(1.9, 96.4) = 11.2, p < 0.001; \eta^2 = 22.3\%$). Post hoc analysis revealed that heart rate was higher during the second reading than at any other time point ($p = 0.01$ compared to baseline and third reading, $p < 0.001$ compared to fourth reading). The interaction between condition and time on heart rate was not significant ($F(3.9, 96.4) = 0.6, p = 0.68; \text{Eta}^2 = 2.3\%$).

3.3. Self-Rated Anxiety in Response to Stressors

It was also determined if self-reported anxiety was affected by stressor condition. The self-reported anxiety analysis corrected for violation of sphericity ($\chi^2(2) = 11.88, p = 0.003; \epsilon = 0.88$) found a condition \times time interaction (Figure 4A; $F(3.5, 88.1) = 2.8, p = 0.04; \eta^2 = 11.3\%$). Further analysis showed that participants had an elevation in self-rated anxiety in response to the TSST, with anxiety higher immediately after TSST compared with baseline or compared to recovery ($p = 0.003$ and $p < 0.001$ respectively). However, there was not any elevation in self-rated anxiety after the ST or unsolvable anagrams ($p > 0.05$). Participants who engaged in the ST did have a suppression of anxiety during the recovery period compared to baseline and immediately after the task ($p = 0.04$ and $p = 0.03$ respectively). There were no differences between conditions at any time points ($p > 0.05$).

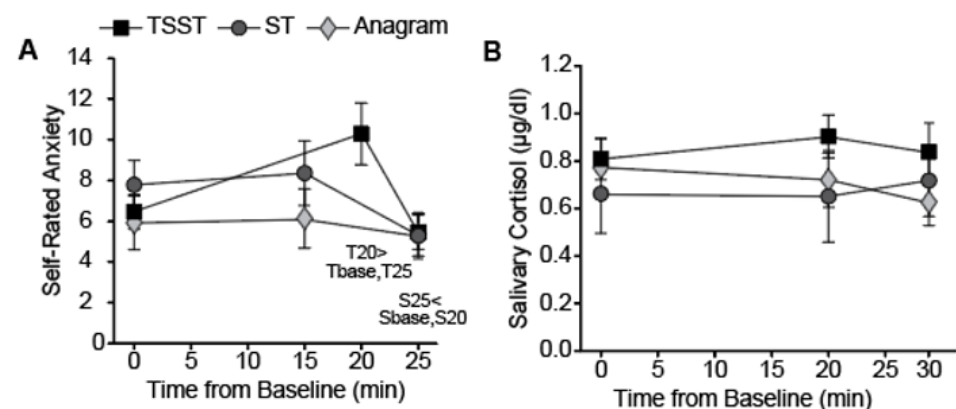


Figure 4. Self-rated anxiety and salivary cortisol in response to laboratory stressors. (A) Self-rated anxiety was elevated after the completion of the TSST and suppressed after the ST. (B) There was no change in salivary cortisol in response to the conditions. All significant effects are $p < 0.05$.

3.4. Salivary Cortisol in Response to Stressors

Salivary cortisol was measured to determine if there was an elevation stimulated by any of the laboratory stressors. Measures of salivary cortisol violated sphericity assumptions and were corrected with Huynh–Feldt ($\chi^2(2) = 18.1, p < 0.001; \epsilon = 0.81$). Analysis revealed that there was no significant effect of stressor on salivary cortisol (Figure 4B;

$F(3.2, 77.8) = 0.92, p = 0.44; \eta^2 = 3.8\%$). It is often found that only a portion of participants respond to a laboratory stressor [8]. Therefore, participants who had an elevation in salivary cortisol of $0.1 \mu\text{g}/\text{dl}$ after the stressor (at either sample 2 or 3) were categorized as responders, while those who did not have an elevation in salivary cortisol were categorized as non-responders. There was no difference in the number of responders between condition (data not shown; $\chi^2(2, N = 51) = 0.36, p = 0.84$).

4. Discussion

This study compared stress reactivity in response to three laboratory stressors—the TSST, an ST, and unsolvable anagrams—and measured cardiovascular, hormonal, and self-report measures of stress. Overall, engaging in the TSST elevated systolic blood pressure, diastolic blood pressure, heart rate, and self-rated anxiety. However, participants in the ST or unsolvable anagrams condition only exhibited an elevation in heart rate. Clearly, these results indicate that the TSST outperforms these other two laboratory stressors on several measures of stress reactivity. Taken together with the other work that has been done in this area, it suggests that the TSST is currently the most reliable psychological stressor to use in laboratory-based stress research.

It is surprising that the ST and unsolvable anagrams did not elevate measures of stress, as was predicted. Previous research studies on similar STs have found elevated stress, but these studies either recorded limited stress measures [26] or were confounded by the presence of an evaluative committee [27]. This indicates the necessity of an in-person social evaluative component to a laboratory stressor. Furthermore, unsolvable anagrams have previously been shown to elevate blood pressure and galvanic skin conductance [22–24]. Outcome differences may be explained by participant characteristics. For example, Weidner et al. [23] found that stress reactivity was specific to individuals with high hostility. It may also be due to differences in the experimental procedure. For example, Messay and Marsland [22] found an elevation in blood pressure five minutes into the anagram task, whereas in the current study, blood pressure was measured at the completion of the task. Regardless of these subtle differences, the results clearly show that the TSST outperformed these other tasks in terms of stress induction.

Surprisingly, there was no significant elevation of cortisol in participants that were subjected to the TSST or either of the other stressors. Previously, the TSST has been shown to reliably elevate salivary cortisol [6]. It is likely that either participant characteristics or the experimental protocol was such that cortisol reactivity was blunted. The majority of participants in this study identified as a minority, and previous findings show that minorities have blunted cortisol stress reactivity [31]. Furthermore, the average household salary for families of students at this institute is approximately \$55,000 (nearly \$10,000 a year less than the neighboring state university), indicating that socioeconomic status may have been lower than in other undergraduate populations. Considering that socioeconomic status has been shown to predict blunted cortisol [32], this certainly could contribute to the null cortisol results. Furthermore, female menstrual cycle and contraceptive use were not controlled for in the current study. Females who use oral contraceptives or are in the follicular stage of their menstrual cycle have a lower cortisol response to the TSST than males or females in the luteal phase [33]. Considering that the majority of participants were women, this is a possible contributing factor to the blunted cortisol. Future studies will have to address the importance of participant characteristics in our population and hypothalamic–pituitary–adrenal axis responding to acute laboratory stressors. Participants were all undergraduate students; however, it is unlikely that their academic experiences would have contributed to the lack of cortisol responding, because previous studies with undergraduate students have found significant elevations of cortisol after the TSST [34].

It is also possible that some aspect of the TSST protocol prevented cortisol reactivity. However, this is less likely, because the protocol that was followed has been shown to elevate salivary cortisol at other institutions [29], and in the current study, there was an elevation of all cardiovascular measures in response to the TSST. It is also unlikely that the

timing of salivary samples missed a peak in cortisol, because previous studies have found that salivary cortisol peaks 30 min after the initiation of the stressor and then begins to drop back down to baseline [6,35].

One limitation of this study was that there was a larger sample size in the TSST ($n = 24$) condition than in the ST ($n = 14$) or unsolvable anagram ($n = 15$) conditions, allowing for the possibility that a negative test of significance may have been due to low power in those two conditions. To determine this possibility, a power analysis was conducted with G*Power Version 3.1.9.4 to estimate the sample size necessary to detect group differences based on the effect sizes found from the TSST condition. It was determined that with an effect size of 0.22, α set at 0.05, power set at 0.8, with three between-subjects groups and four within-subjects measures, a minimum of 13 participants would be necessary per group. Therefore, the current negative results are unlikely due to sample size and are more likely to signify that the two other conditions (ST and unsolvable anagrams) were simply not as stress inducing.

There are several other limitations to consider. First, the length of the stressor paradigm was longer for the TSST (approximately 15 min) than the ST or unsolvable anagrams (approximately 10 min). This allows for the possibility that if the ST or unsolvable anagrams had continued for an additional 5 min, there might have been a similar rise in stress reactivity. However, the timing of the rise in blood pressure argues against this possibility. The peak in blood pressure occurred directly after instructions were given for the TSST, but there is no similar rise after the instructions for the ST or the unsolvable anagrams. Furthermore, there are other stressors, including the cold pressure test, that reliably elevate hormonal and cardiovascular measures of stress after only a 2–5 min stressor [3,36].

Although the results from this study cannot be used to assume the ability of other laboratory stressors to stimulate the stress response, it is likely that the inclusion of a live committee is critical for the effects seen in response to the TSST. Indeed, modified versions of the TSST without the committee are less stressful. Gruenewald, Kemeny, Aziz, and Fahey [37] found that performing in front of an audience led to elevated heart rate and cortisol over performing alone. In addition, performing in front of an imagined audience sitting behind a one-way mirror does not stimulate cortisol to the extent of performing in front of an in-person committee [10]. In the current study, the social evaluative component was attempted by recording participants in the ST or unsolvable anagram conditions with a video camera and telling them that the recordings would later be evaluated. This method has been used previously to add a social evaluative component to the cold pressure test and does elevate salivary cortisol over the classical cold pressure test [38]. However, it does not seem that this was able to stimulate an acute stress response when compared to the TSST.

The TSST clearly elevated stress markers over the use of unsolvable anagrams or the ST. It is likely that the social evaluative threat introduced by the committee is critical for a robust elevation in the stress response. This work contributes to the field in several notable ways. First, there are clear advantages to using the TSST as an acute laboratory stressor because of its robust stimulation of the stress response. However, it needs to be carefully established which population and demographic measures may contribute to a blunted cortisol response. The current study will also allow stress researchers to make a more informed decision about which laboratory stressor to use depending on the specifications of their study. In addition, this work warns that a single measure of the stress response is not sufficient to establish whether a laboratory stressor is effective.

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