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An Online Trier Social Stress Paradigm to Evoke Affective and Cardiovascular Responses

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Impact statement: We demonstrate that the Trier Social Stress Test can evoke both affective and cardiovascular (heart rate and blood pressure) responses when delivered online and remotely. This protocol can be safely administered during the COVID-19 pandemic and could be used to include populations that may be unable to participate in laboratory and in-person protocols (e.g. rural populations, and those with limited transportation options).

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ABSTRACT

In response to the COVID-19 pandemic, prior studies have modified the Trier Social Stress Test to be conducted remotely. The current report aimed to extend these studies to test whether a remote Trier Social Stress Test (rTSST) can elicit (a) affective, (b) blood pressure, and (c) heart rate responses relative to a control condition and whether these responses were reliable when assessed one week later.

Participants ($N = 99$, 19.7 ± 3.5 years, 55% female) were randomized to a control or stress condition. Controls completed easier versions of the tasks with a single, friendly researcher. Stress participants performed more difficult versions of the task in front of two judges who participants believed were rating their performance. Blood pressure and heart rate were measured every two minutes throughout, while affect was assessed at baseline, after the final task, and following recovery. The rTSST was feasible to administer with minimal technical issues reported. Results suggest that lower positive affect and higher negative affect were reported during the tasks in the stress condition relative to controls. Similarly, stress participants had higher cardiovascular responses during the tasks relative to controls, except that blood pressure was not elevated during mental arithmetic in stress participants relative to controls.

Cardiovascular responses demonstrated good test-retest reliability when assessed one week later, especially when computed using area under the curve methods. Overall, a rTSST can be used to elicit affective and cardiovascular reactivity and provides an opportunity to include participants previously unreachable for in-person laboratory procedures.

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1. Introduction

Due to the coronavirus pandemic, research requiring in-person and psychophysiological assessments confer a heightened level of infectious and disease risk to both participants and experimenters. Psychophysiological research examining physiological responses to acute stressors are especially risky because of the necessity for close and prolonged indoor physical contact between experimenters and participants. However, acute stress protocols may be adapted for remote virtual data collection. One such protocol amenable to online adaptation is the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993; Kudielka, Hellhammer, & Kirschbaum, 2007). During the TSST, participants complete speech and mental arithmetic tasks in front of a small evaluative audience. Although the laboratory version of the TSST evokes robust affective and physiological reactions (Kudielka, Hellhammer, & Kirschbaum, 2007), this version requires a minimum of three staff members (two evaluative interviewers as an audience, and one experimenter to administer task instructions, etc.) in addition to the research subject in a single room. As a result, this resource-intensive laboratory version of the TSST carries notable potential coronavirus infection risks to both participants and researchers. In addition to being safer, a fully remote administration of a virtual TSST may allow for populations previously unable to attend laboratory sessions (*e.g.*, those with limited transportation or in remote locations) to engage in stress research, even after the era of COVID-19. This would increase the ability to collect data from participants who live further from academic institutions, which could increase the generalizability and inclusivity of research findings on acute stress (Kirschbaum, 2021). For these reasons, the current study aims to validate a completely remote version of the Trier Social Stress Test (rTSST).

The current study was conducted to test the validity and reliability of a rTSST for eliciting blood pressure, heart rate, and affective responses. Eligible participants were randomized into either a stress or control condition. All participants received a blood pressure monitor before their scheduled remote visit. During the remote visit, participants completed a series of tasks (delivering a speech and mental arithmetic) while taking their blood pressure and heart rate. Participants in the stress condition were

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invited to complete a second, slightly modified, remote stress task one week later to explore whether the stability of cardiovascular responses are comparable to those reported for in-person laboratory stress assessments (e.g., Kamarck et al., 1992; Cohen et al., 2000). After their final task, all participants were debriefed. The study aims, hypothesis, statistical analyses, and procedures were pre-registered prior to data collection. Pre-registration documents, complete study protocols, and supplementary information can be found on Open Science Framework (<https://osf.io/eg5dr/>). Pilot research that informed the design of the present study can also be found online on the study's Open Science Framework page under 'Study 1'.

2. Method

2.1 Participants

Participants ($N = 99$) ages 18 – 47 were recruited from the University of Pittsburgh undergraduate subject pool. Participants were deemed ineligible if they were currently pregnant, taking medications for cardiac arrhythmias, or reported a history of heart surgery, myocardial infarction, or stroke. Participants that self-reported symptoms consistent with COVID-19 were rescheduled until symptoms subsided. All participants reported access to a stable internet connection on either a desktop computer or laptop with a webcam. Participants received course credit for their participation.

2.2 General Procedures

2.2.1 Eligibility Visit

Participants attended a meeting on the videoconferencing platform, Zoom (Zoom Video Communications Inc., 2021), to (1) assess eligibility, (2) habituate participants to interacting with researchers over Zoom and (3) troubleshoot any technical issues. Participants signed up for the study online and received a confirmation email with the Zoom link and password. At this initial visit, eligible participants completed informed consent, were randomized to a condition, were scheduled for their study visit(s), and completed demographic questionnaires. Participants randomized to the stress condition were

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also instructed on how to setup their screen for their study visit (*e.g.*, viewing a stopwatch, Zoom in full screen, etc.) and any technical issues were identified and resolved. Stress participants were informed ahead of time that they would complete their task in a breakout room with two other researchers. All participants were compensated by course credit for completing a single virtual visit. Stress participants who completed a second study visit also received a \$20 gift card.

2.2.2 Remote Trier Social Stress Test

General Procedures. Prior to the rTSST study visit, the experimenter met participants outside, measured their arm circumference and gave them a bag that contained an Omron blood pressure monitor with a printer (Model: 7051T; Omron Healthcare, Inc; Kyoto, Japan), an appropriately sized blood pressure cuff, extra batteries, a sheet of paper to record cardiovascular measures, and alcohol swabs to clean study equipment. Participants also received an email containing Zoom login information for their scheduled rTSST visit.

Upon entry into the Zoom room, participants were assessed for adherence to the following pre-visit restrictions: No food in past 3 hours; no beverages (except water) in past 30 minutes; no caffeine in past 3 hours; no moderate to vigorous exercise in past 2 hours; no alcohol in past 12 hours; and no symptoms of viral illness or cold. Participants who did not meet the pre-visit restrictions were rescheduled. Participants were then taught to put on the blood pressure cuff and take cardiovascular readings. For each reading, the researcher present would ask the participant to take their blood pressure by hitting the ‘Start’ button on the monitor. Participants told the researcher their blood pressure for the practice reading and if values appeared outside of the normal range (SBP < 80 or > 130 mmHg; DBP < 50 or > 110 mmHg), the participant was asked to refit the cuff until values appeared stable and/or within normal limits. After the practice readings, the participants were informed that they did not need to recite or pay attention to their readings for the rest of the visit because the data would be collected from the monitor’s memory at the end of the visit. To take the readings, researcher told the participant when to hit ‘Start’ throughout the entire procedure. The participants then watched an educational video for 10 minutes while taking cardiovascular readings every two minutes. After baseline measures, participants

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prepared (5 min) and delivered a speech or speaking task (5 min), performed a mental arithmetic task (5 min), and watched a calming video during recovery (16 min). Following the recovery period, participants were directed to retrieve digitized values from the monitor's memory and record heart rate, systolic, and diastolic blood pressure, and the time each measurement was taken on a piece of paper. The piece of paper with the written cardiovascular measures was placed in the study bag so that it could be returned to the laboratory after the final study visit. A subset of the stress participants ($N = 25$) completed a second virtual session, approximately one week apart ($M = 6.6$ days, range = 2 to 7 days) with modified task instructions. Participants were debriefed on the true purpose of the study once they completed their final rTSST task. Following their final visit, participants returned to the laboratory where the experimenter met them outside to receive the study bag, with both parties masked.

Eligibility visit questionnaires. Demographic data were collected during the eligibility visit, including race/ethnicity, year in college, program(s) of study, parental education, perceived social standing for each parent and self, and English fluency (Is English your native language?). Trait positive and negative affect were also collected during the eligibility visit using the positive and negative emotional style questionnaire, asking participants how they felt 'in general' (Cohen et al., 2003). Finally, all participants completed the Adult Reading Questionnaire, which assesses perceived reading and language ability, and reading concerns that are consistent with dyslexia (Snowling et al., 2012). The Adult Reading Questionnaire was included because control participants were asked to read from a script during the task, which could be perceived as a stressor, particularly in individuals who perceive themselves as having a low reading ability.

Control condition. In the control condition, participants were on Zoom with a single researcher and the visit was not recorded. Participants were provided with a neutral script that was written at a third-grade reading level and asked to maximize the script so that it took up their entire computer screen, so that they did not see the experimenter. They were asked to read the script silently to themselves for 5 minutes (speech preparation) and then to read it out loud for 5 minutes (speech delivery). The researcher reassured participants that they were not being evaluated and that the purpose of the task was to

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understand the relationship between blood pressure and speaking. Participants then performed a mental arithmetic task that consisted of counting by 2s at a leisurely pace for 5 minutes. Participants were informed that they could start over from two if the numbers got too high, or if they lost track of which number they were on. The experimenter was friendly and cooperative during the visit.

Stress condition. After the baseline blood pressure readings, participants in the stress condition were informed that they would be asked to deliver a speech in front of two judges. They were then sent to a breakout room where two research assistants were waiting for them, named ‘Judge 1’ and ‘Judge 2’ on Zoom. Participants were then provided with instructions for the speech task – either a job interview (visit 1), or introducing themselves to a new class of twenty students (visit 2) and told that their speech would be recorded for evaluation of their performance and nonverbal behavior. They were given 5 minutes to prepare and 5 minutes to deliver their speech. If they finished their speech before the time limit, participants were told to continue with their speech and/or were asked follow-up questions by the judges. Following the speech task, participants performed serial subtraction by 13s, starting from 1022 (or 17s starting from 2023 for visit 2). They were told to work as fast as possible, while still answering accurately. If the participant made any mistakes, they were asked to start the task over. To make the task more difficult, participants who had five consecutively correct responses during serial subtraction were given a 10 second time limit. If they continued to answer five consecutive responses correctly, a 5 second time limit was then enforced. Following the serial subtraction task, participants were instructed to return to the main Zoom room for the recovery period. The research staff were evaluative during the visit and displayed neutral or negative facial expressions throughout the rTSST.

Task questionnaires. Measures of positive and negative affect were collected at the end of baseline, after mental arithmetic, and following recovery to assess affective reactivity to the rTSST. Positive and negative affect were assessed using adjectives from the positive and negative emotional style questionnaire to include affective states that differ in level of arousal and valence (Cohen et al., 2003). All responses were presented on a visual analogue scale (0-100). Task engagement was also assessed by five items that assessed task difficulty, motivation to perform well, effort exerted during the task, fatigue

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following the task, and how important it was to perform well on the task (results reported in Supplementary Materials).

2.4 Cardiovascular measures

Blood pressure and heart rate were collected once every two minutes throughout the Zoom protocol, as detailed above (see ‘General Procedures’). Once the monitor was returned, the cardiovascular measures and timing of each measure that was written by the participant was double checked against the monitor’s memory for accuracy. The monitor memory was then cleared, and the blood pressure monitor and cuff were thoroughly cleaned with disinfectant. Cardiovascular values were manually transcribed to the online database from the paper copies. To ensure data accuracy, a research assistant double checked that the database values matched the written copies. Cardiovascular data were reduced by averaging across the following periods: baseline, speech preparation, speech delivery, mental arithmetic task, and recovery. The eight recovery values were reduced to four measures by averaging every two measurements.

2.5 Post-task Questionnaire

All study participants completed a post-task questionnaire that assessed (1) if they believed the interviewers were evaluating them during the task; (2) if the task was more difficult or stressful to perform the tasks on Zoom (compared to in person); (3) how they felt about having the visit recorded; and (4) how difficult it was to take their own blood pressure. Participants also provided open-ended feedback regarding the study and how they thought performing the tasks online might compare to in person administration. The complete list of feedback questions and multiple-choice responses are presented in Table S1.

2.6 Statistical Analyses

Covariates. Any group differences at baseline were used as a covariate for all subsequent analyses to control for any inherent group differences that may affect the interpretation of the results. Group differences in demographic variables were tested using either a chi-square or t-test.

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Feasibility. To assess how feasible the protocol was to administer, participants provided feedback about the remote Trier Social Stress Test and researchers documented any issues that occurred during administration (*e.g.*, internet connection was unstable, participant was distracted by a family member part-way through the task, etc). Participant feedback and the frequency of various issues during testing are reported.

Validity. A semi-parametric two-way repeated measures MANOVA was used to assess differences in (1) affective and (2) cardiovascular responses to the rTSST between the stress and control conditions. Repeated measures MANOVAs simultaneously include multiple, highly correlated outcome variables in the model to reduce the number of statistical tests performed. All cardiovascular variables were correlated at $r \geq 0.50$ were included as dependent variables within the same model. Similarly, all affective variables that were correlated at $|r| \geq 0.50$ were included as dependent variables within the same model. The test statistic was calculated using a parametric bootstrap modified ANOVA-type statistic (Q_N) within the *r* package MANOVA.RM (Friedrich, Konietzschke, & Pauly, 2018). The benefit of this semi-parametric approach is that it does not assume multivariate normality or homogeneity of variance-covariance matrices. The modified ANOVA-type statistic (Q_N) and p -values are estimated by parametric bootstrapping procedures, such that no degrees of freedom are reported for results calculated using a modified repeated measures MANOVA. Any statistically significant findings at the conventional threshold of $p < 0.05$ were further examined by bivariate post-hoc comparisons with p -values adjusted for Bonferroni correction.

Test-retest Reliability of Reactivity. A subset of participants in the stress condition completed a second rTSST approximately one week after their first visit. Reactivity values were calculated separately for speech preparation, speech delivery, and mental arithmetic. Reactivity was calculated by the following methods: (a) delta change score from baseline, (b) percent change from baseline, (c) area under the curve with respect to ground, (d) area under the curve with respect to increase. During a pilot study (<https://osf.io/eg5dr/>; Study 1), the test-retest reliability of cardiovascular reactivity to the rTSST was low when reactivity was calculated using change scores and percent change methods ($ICC = .003 - .57$).

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Exploratory analyses suggested that cardiovascular reactivity calculated by area under the curve metrics were more reliable ($ICC = .34 - .84$). As such, various methods were used to calculate reactivity to determine which method(s) are most reliable. The test-retest reliability of reactivity was calculated by intraclass correlations (2-way mixed effects model with absolute agreement; Koo & Li, 2016).

Test-retest Reliability of Recovery. Past research has quantitatively demonstrated that measures of reactivity are fairly stable within an individual (*e.g.*, Manuck, 1994); however, this has not yet been done for recovery. Previous studies have *qualitatively* described conceptual differences between the various ways of calculating recovery (Linden et al., 1997), but no studies to the authors' knowledge have *quantitatively* assessed which measures of recovery are most stable. As such, recovery was calculated using the following methods: (a) delta change score from baseline, (b) delta change score from task; (c) percent change from baseline, (d) percent change from task, (e) area under the curve with respect to ground, and (f) area under the curve with respect to increase. The stability of recovery is assessed by calculating (1) the correlations between the methods for calculating recovery and (2) the intraclass correlation (2-way mixed effects model with absolute agreement) for all recovery methods in the subset of participants that performed a second rTSST. The equations for calculating for each measure can be found in Supplementary Materials.

Exploratory Analyses. The following exploratory analyses were pre-registered, and their rationales, analyses, and results are included in the Supplementary Materials for completeness: (1) Group differences in motivated engagement during the rTSST; (2) The relationship between trait positive affect and cardiovascular recovery; (3) The relationship between affective and cardiovascular reactivity.

3. Results

3.1 Participant Characteristics

The final sample consisted of 99 participants (19.7 ± 3.5 years; 54.5% female; 67.7% Caucasian and 65.7% first years in college); Control $n = 50$; Stress $n = 49$; See Table 1). Of the 49 participants allocated to the stress condition, half completed a second study visit ($n = 25$; see Figure 1 for study flow

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chart). There were no significant group differences in demographic characteristics, resting cardiovascular variables, or self-reported affect at baseline (Table 1). For this reason, no covariates were included in any subsequent statistical models.

3.2 Aim 1: Feasibility

Most study participants were able to setup their environment to be consistent with other participants (Table 2). A few study participants had another person in the room with them while completing the study (Control $n = 6$; Stress $n = 2$); had a window nearby that did not have a curtain to close to prevent them from looking outside during the task (Control $n = 1$; Stress $n = 1$); used an iPad during the visit instead of a laptop or desktop computer (Control $n = 2$); appeared to not pay attention to baseline videos (Control $n = 1$); had a pet in the room with them during the task (Stress $n = 1$); were unable to sit with their feet flat on the floor due to a recent surgery (Control $n = 1$); or experienced distractions during the task, such as background noise (Control $n = 4$; Stress $n = 4$). The feedback survey was administered to all participants and those completing the study twice completed a feedback survey at the end of each study visit. Interestingly, both stress and control participants reported that they believed the experimenters were evaluating them during the task (Control $n = 29$; Stress $n = 38$) even though the control participants were explicitly told that they were not being evaluated. Most participants, regardless of condition, reported that it was either ‘very easy’ (Control $n = 35$; Stress $n = 27$) or ‘easy’ (Control $n = 10$; Stress $n = 17$) to take their own blood pressure during the tasks. While most stress participants did not mind being recorded ($n = 21$), they did also indicate that recording the study visit made them more nervous ($n = 20$; full results reported in Table 2).

3.3 Aim 2: Validity

Positive and negative affect were weakly correlated (range $r = |.02 - .41|$; Table S2), so two separate ANOVAs were conducted: (1) vigor, wellbeing, and calm were entered together as dependent variables in one model and (2) anxiety, depression, and hostility were entered together into a second model. There was a main effect of group on positive affect ($Q_N = 26.6, p < .001$). Post-hoc analyses demonstrate that control participants reported being calmer and having more vigor relative to stress

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participants. There was also a main effect of time ($Q_N = 415.2, p < .001$), with less calm, and well-being during the task than baseline and recovery time points. There were no differences in vigor across time points. Finally, there was a group x time interaction ($Q_N = 81.2, p < .001$). Post-hoc analyses suggest that stress participants reported less positive affect, specifically being less calm and having lower wellbeing, during the task compared to control participants (see Table S3 for *post-hoc* comparisons and Figure 2).

Analysis of negative emotions revealed a main effect of group ($Q_N = 76.0, p < .001$), with control participants reporting less anxiety, depression, and hostility than stress participants. There was also a main effect of time ($Q_N = 299.2, p < .001$), showing that anxiety, depression, and hostility were higher during the task than baseline or recovery periods. Finally, there was a group x time interaction ($Q_N = 121.6, p < .001$), with post-hoc analyses showing more anxiety, depression, and hostility during the task among the stress than the control group. Participants in the stress condition also continued to report more hostility during the recovery period relative to control participants. See Figure 2 for graphs depicting changes in self-reported affect across time by condition and Table S4 for *post-hoc* analyses.

Of the participants who completed a remote visit, 87 participants had complete cardiovascular data (control $n = 47$; stress $n = 40$). Of note, there was a statistical trend towards stress participants having more dropped readings during the rTSST relative to control participants ($\chi^2(2, N = 99) = 3.55, p = .059$; dropped readings: control $n = 2$; stress $n = 9$). One control participant was identified as an outlier ($> 3SD$ above the mean) for blood pressure data and was not included in the blood pressure analyses; however, results were unchanged when the outlier was included in the analyses (see Supplementary Materials). Heart rate was only moderately correlated with both systolic ($r = .11$) and diastolic blood pressure ($r = .38$) at baseline (Table S2). As a result, two separate models were used to evaluate blood pressure and heart rate reactivity.

The first model included systolic and diastolic blood pressure as simultaneously dependent variables. There was a main effect of time ($Q_N = 245.3, p < .001$), with higher systolic and diastolic blood pressure during the speech and mental arithmetic task (but not during speech preparation) relative to baseline and recovery periods. There was also an effect of group ($Q_N = 33.8, p < .001$), indicating that

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stress participants had higher blood pressure relative to the control condition. Finally, there was also a significant group x time interaction ($Q_N = 31.2, p = .01$; Figure 3). Post-hoc analyses indicated that participants in the stress condition had higher blood pressure during speech preparation and speech delivery (but not during mental arithmetic) relative to control participants (See Table S5 for all *post-hoc* comparisons).

Analysis of heart rate revealed a main effect of time ($F(7, 602) = 73.8, p < .001$), with higher heart rate during the tasks relative to baseline and recovery periods. There was no main effect of group ($F(1, 86) = 3.3, p = .07$); however, there was a significant group x time interaction ($F(7, 602) = 17.9, p < .001$). Post-hoc analyses showed higher heart rate during speech preparation, speech delivery, and mental arithmetic among the stress group than the control group (Table S5 and Figure 3).

3.4 Ancillary Aim 1: Test-Retest Reliability of Cardiovascular Reactivity

Half of the stress participants ($n = 25$) completed a second study visit. One participant was dropped due to incomplete cardiovascular data. Four methods were used to calculate the test-retest reliability in cardiovascular reactivity across two study visits: change score, percent change from baseline, area under the curve with respect to ground, and area under the curve with respect to increase. In comparison to the other methods, area under the curve approaches demonstrated the highest reliability across all cardiovascular measures (all $ICCs > 0.60$, all $ps < .001$; Table S6). The one exception was that the test-retest reliability of systolic blood pressure reactivity was slightly lower than anticipated during the mental arithmetic task ($ICC_{\text{observed}} = .56-.58$; $ICC_{\text{expected}} = .60$). Averaging reactivity across the three task periods did not increase the reliability for any measure of cardiovascular reactivity (systolic blood pressure, diastolic blood pressure, or heart rate).

3.4 Ancillary Aim 2: Test-Retest Reliability of Cardiovascular Recovery

Cardiovascular recovery was calculated using six different methods to assess stability across study visits. Of the methods tested, only area under the curve measures demonstrated high test-retest reliability between the study visits for heart rate and blood pressure recovery: area under

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the curve with respect to ground (AUC_g; SBP: $ICC = .86$; DBP: $ICC = .89$; HR: $ICC = .91$), and area under the curve with respect to increase (AUC_i; SBP: $ICC = .83$; DBP: $ICC = .88$; HR: $ICC = .92$; Table S6). Unsurprisingly, area under the curve measurements were also highly correlated with one another ($r = .82-.99$). See Tables S7-S8 for correlations between all measures of recovery for blood pressure and heart rate, respectively.

4. Discussion

The rTSST resulted in significant affective and cardiovascular reactivity when compared with the control condition. Furthermore, we replicated our earlier findings (<https://osf.io/eg5dr/>; Study 1) that cardiovascular reactivity and recovery demonstrated high test-retest reliability when calculated by area under the curve measures. The rTSST protocol was feasible and few implementation problems were noted by research staff or participants.

The current findings are consistent with recently published work showing that rTSST protocols can elicit changes in self-reported negative affect (Gunnar et al., 2011; Eagle et al., 2021; Harvie et al., 2021), salivary cortisol and alpha amylase (Gunnar et al., 2011), heart rate (Eagle et al., 2021; Harvie et al., 2021), and heart rate variability (Eagle et al., 2021). We extended this work by demonstrating that a rTSST can also elicit blood pressure reactivity in response to the speech task, but not mental arithmetic. This is consistent with results of in-person TSST protocols, with higher heart rate and blood pressure responses to the speech task than the mental arithmetic task (*e.g.*, Al'absi et al., 1997). It is possible that social evaluation contributes to the larger cardiovascular reactivity to the speech task, which may elicit greater feelings of evaluation and social judgement on a topic that is personally salient. In support of this possibility, previous studies demonstrate that interpersonal tasks evoke stronger cardiovascular responses than tasks without an interpersonal component (Christensen & Smith, 1993; Girdler et al., 1990). Taken together, our results suggest that the speech task is a more provocative stimulus of cardiovascular reactivity than mental arithmetic.

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Results of test-retest reliability support the temporal stability of cardiovascular responses (reactivity and recovery) to the rTSST when assessed on two occasions separated by one week, particularly when assessed using area under the curve methods. The area under the curve method permits the inclusion of more raw data than other methods of assessing stability, such as change scores or percent change. In contrast, other methods of calculating reactivity and recovery do not yield information about the pattern of change across time, potentially missing some valuable aspects of individual differences in physiological stress responses (*e.g.*, habituation during the tasks). Importantly, these findings have not yet been validated in other types of stress tasks (cognitive or affective stressors) or across different settings (laboratory-assessed or MRI-based stress tasks). As such, future work is necessary to understand whether area under the curve measures of reactivity and recovery are consistently reliable across independent samples.

The current study extends previous research to demonstrate that rTSST protocols can be used to stimulate reliable affective and cardiovascular (blood pressure and heart rate) responses to challenge. To date, there are only three such studies that have created and implemented a rTSST (Harvie et al., 2021; Gunnar et al., 2021; Eagle et al., 2021). Gunnar and colleagues (2021) published the first study, showing reliable increases in cortisol and alpha-amylase, a marker of sympathetic activation, in response to rTSST in an adolescent sample. The second published study extended these findings to show reliable increases in heart rate and decreases in heart rate variability during the rTSST that did not habituate over a 12-week period (Eagle et al., 2021). Critically, neither of these studies included a control condition, making it difficult to determine whether reactivity related to aspects of the stress task (*e.g.*, social evaluation, time pressure, novelty) or other factors, such as rate and rhythm of speech, interacting with unfamiliar researchers on Zoom, or cognitive effort exerted during the task. To address this issue, Harvie and colleagues (2021) used a control condition to establish that their rTSST led to greater heart rate reactivity relative to a control task. Interestingly, this study used a smart phone application to measure heart rate by photoplethysmography, demonstrating that the rTSST can be administered cheaply and to a wider geographical audience, without the laboratory having to mail study equipment to participants (Harvie et

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al., 2021). In summary, although the literature on rTSSTs remains nascent, accumulating evidence suggests that laboratory stressors can be effectively delivered online without sacrificing the ability to evaluate their physiological sequelae.

The current study extends the findings from the rTSST literature in three important ways: by (1) including blood pressure responses to the rTSST, (2) replicating findings that the rTSST has good test-retest reliability, and (3) exploring the test-retest reliability of various measures of recovery. Although it is more common to collect cortisol and heart rate measures during the TSST, extending this work to include blood pressure may be of particular interest to researchers studying the relationship between stress and cardiovascular disease risk. In fact, blood pressure responses to acute laboratory stressors are prospectively associated with negative cardiovascular outcomes (Chida & Steptoe, 2010; Turner et al., 2020), particularly when paired with low heart rate reactivity (Brindle et al., 2016). Thus, validating a rTSST protocol for blood pressure responses to stress may allow researchers interested in understanding the relationship between psychological stress and cardiovascular disease risk to extend their work to populations they might not otherwise reach (*e.g.*, international, remotely rural, transportation issues, etc; Kirschbaum 2021). The second benefit of the current study is that we replicated prior work demonstrating that the rTSST paradigm elicits a cardiovascular response with high test-retest reliability (Eagle et al., 2020). Our findings are in line with prior work indicating that cardiovascular responses to psychological stress show a lesser degree of habituation relative to cortisol reactivity (Hughes, Lü, & Howard, 2018), suggesting that researchers can use rTSST paradigms for repeated measures of acute stress. Finally, a longstanding issue in the field of stress research is a lack of consensus on how to calculate physiological recovery following an acute stressor. In fact, many studies report collecting recovery without reporting on such findings, likely due, at least in part, to the lack of consensus on how to best measure recovery (Linden et al., 1997). Although there has been a qualitative review on the various modes of calculating recovery (Linden et al., 1997), no studies to date have quantitatively compared these methods. As such, the current study extends prior research by showing that area under the curve metrics are the most reliable measures of calculating recovery to a stressor. The aim of reporting these results is to generate further

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discussion and consensus in the field, and to inform common methods of calculating and reporting recovery that can be used to assess responses to a range of tasks.

While the current study was unable to compare responses to the rTSST with an in-person protocol, the magnitude of the responses elicited during the tasks are comparable to in-person reports. During in-person TSSTs, heart rate typically increases by 15-25 bpm during the task (Kudiekl, Hellhammer, & Kirschbaum, 2007). Previous rTSST paradigms elicit a heart rate response between 2-7 bpm, which is considerably lower than anticipated (Eagle et al., 2021; Harvie et al., 2021). By contrast the current rTSST protocol led to a larger increase in heart rate that more closely aligns with in-person reports (11-16 bpm). The current rTSST protocol deviated from the two previously published reports in a few ways that could have contributed to larger increases in heart rate. In the current protocol, participants were placed into a second virtual (breakout) room where they interacted with two judges while the main researcher stayed in the main, or original, virtual room. By contrast, both prior studies left the participant alone during the preparation period and kept the participant in the main virtual room during the entire procedure (Eagle et al., 2021; Harvie et al., 2021). Sending participants into a second virtual room with the judges could lead to a sense of novelty. In fact, previous work demonstrates that providing participants more time to habituate to the laboratory environment decrease cortisol responses to acute stress (Goodman, Janson, & Wolf, 2017). Thus, it is possible that remaining in the same virtual room throughout the protocol decreased the magnitude of HR responses in prior studies. Another difference between the current study and previously published rTSSTs is whether participants were able to view themselves during the study tasks. The current study asked stress participants to hide their own image so they could fully attend to the judges and were not distracted by their self-image or using it to help cope with the task. The two other rTSST paradigms that measured heart rate did not provide instructions regarding the participants' own video, potentially providing the participants with a means of diverting their attention from the judges.

In person, the TSST typically increases systolic blood pressure by 15-30 mmHg (al'Absi et al., 1997; al'Absi et al., 2012; Girdler et al., 1990) and diastolic blood pressure by 10-20 mmHg (al'Absi et al.,

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1997; al'Absi et al., 2012; Girdler et al., 1990). By comparison, virtual reality versions of the TSST show smaller blood pressure responses to the task than in-person reports (Δ SBP $\sim$ 10 mmHg; Hawn et al., 2015). Blood pressure reactivity in our version of the rTSST exhibited changes in systolic (11-20 mmHg) and diastolic (8-17mmHg) blood pressure that are between those reported for in-person and virtual reality TSST protocols. One potential explanation for smaller BP responses to the rTSST than the in-person version could be the comfort that comes from participating at home. In fact, 57% of the stress participants noted that they imagined the stress tasks would be more difficult and/or stressful in-person relative to an online task. As mentioned above, novelty is an important aspect of acute psychological stress paradigms that could have reasonably influenced the blood pressure responses during the rTSST.

4.1 Strengths and Limitations

The current study has several strengths, including the pre-registration of all data collection procedures and statistical analyses, assessing heart rate *and* blood pressure during the rTSST, evaluating test-retest reliability of the task, and measuring positive and negative affect along the arousal continuum. There were also limitations. First, the study used an undergraduate sample, making it unclear how the results might generalize to older samples. Second, all data were collected during the COVID-19 pandemic. As research demonstrates that chronic stress can influence acute stress reactivity (Chida & Hamer, 2008), it is plausible that lasting high levels of perceived stress, as a result of the pandemic, could have influenced the data. Third, due to the ongoing pandemic, we were unable to bring participants into the laboratory to compare reactivity to the remote protocol with that observe in response to the traditional version of the TSST. Fourth, although all stress participants were invited to complete a second study visit, half of the participants declined. Because participants self-selected to complete a second visit, it is possible that those who perceived the task as less stressful were more likely to participate in a second study visit, which could have influenced the estimated test-retest reliability.

4.2 Future Directions

In light of these limitations, future studies are needed to expand upon the current findings. The study findings suggesting that test-retest reliability of cardiovascular reactivity and recovery are highest

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when measured using area under the curve metrics should be tested in other samples and expanded to include additional stress paradigms (*e.g.*, in-person laboratory stressors, cognitive tasks, etc). In addition, once it is safe to perform the TSST again in person, it would be beneficial to determine whether cardiovascular response to the remote stressor are replicated in person. In continuing to develop and refine a rTSST paradigm that reliably evokes a cardiovascular response, stress researchers will be able to include populations that were previously unable to participate, making findings in the field more generalizable and inclusive.

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6. Author Notes

Conflicts of Interest: none

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7. Tables

Table 1.

Participant demographic information and baseline characteristics for the remote TSST (Study 2).

	Controls (<i>N</i> = 50) <i>M</i> (<i>SD</i>) or <i>N</i> (%)	Stress (<i>N</i> = 49) <i>M</i> (<i>SD</i>) or <i>N</i> (%)	Difference <i>t</i> or <i>X</i> ² <i>p</i>	
Demographic Information				
Age (years)	19.2 (1.19)	20.3 (4.82)	-1.56	.12
Sex				
Female	27 (54.0%)	27 (55.1%)	<0.01	1.0
Male	23 (46.0%)	22 (44.9%)		
Race				
Caucasian	34 (68.0%)	33 (67.3%)		
Asian	11 (22.0%)	10 (20.4%)	3.05	.55
African American	4 (8.0%)	2 (4.1%)		
Biracial	1 (3.1%)	2 (4.1%)		
Other	1 (3.1%)	2 (4.1%)		
Year in College				
Freshman	34 (68.0%)	31 (63.3%)		
Sophomore	10 (20.0%)	8 (16.3%)	4.05	.40
Junior	3 (6.0%)	5 (10.2%)		
Senior / Senior +	3 (6.0%)	5 (10.2%)		
Psychotropic Use				
Yes	6 (12.0%)	5 (10.2%)	< 0.01	1.0
No	44 (88.0%)	44 (88.9%)		
Trait Affect				
Positive Affect	28.8 (4.9)	29.7 (4.8)	-0.98	0.33
Negative Affect	19.2 (4.8)	18.4 (4.8)	0.87	0.39
Reading Scores				
Reading Ability	3.95 (2.1)	3.5 (2.0)	1.12	.27
Word Finding	3.82 (1.8)	3.37 (1.9)	1.22	.22
Dyslexia	0.36 (0.63)	0.47 (.66)	4.20	.38
Native Language - English				
Yes	42 (84.0%)	45 (91.8%)	0.79	.38
No	8 (16.0%)	4 (8.2%)		
Baseline Characteristics				
SBP (mmHg)	113 (12.0)	113 (11.2)	-0.01	.99
DBP (mmHg)	67.9 (9.37)	67.6 (9.4)	0.17	.11
HR (bpm)	71.5 (10.5)	72.7 (12.9)	-0.48	.63
State PA (0 – 100 scale)	53.2 (12.6)	56.1 (12.2)	-1.19	.24
State NA (0 – 100 scale)	18.6 (11.7)	15.4 (13.5)	1.26	.21

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Table 2.

Researcher documented issues that occurred during the remote TSST and participant responses to the feedback survey.

Issues Documented	N of Reports		
	Control	Stress V1	Stress V2
Technical Issues			
Zoom session was unable to be recorded	0	1	0
Poor internet connection	2	0	0
Older version of Zoom	0	1	0
Cuff was placed on dominant arm	0	0	1
Could not print blood pressure readings	1	1	0
Non-compliance			
Roommate was present during task	6	2	1
Pet was in the room	0	1	1
Used an iPad instead of laptop or desktop computer	2	0	0
Not paying attention during videos (baseline or recovery)	1	0	0
Not sitting with feet flat on the floor	1	0	0
Unable to cover nearby windows	1	1	0
Unknown distraction (<i>e.g.</i> , suspected phone use, background	4	4	1
<hr/>			
Participant Survey Responses	N (%) of Reports		
	Control	Stress	
<hr/>			
Did you believe that your performance was being evaluated?			
Yes	29 (58.0%)	38 (77.6%)	
No	13 (26.0%)	5 (10.2%)	
Unsure	8 (16.0 %)	6 (12.2%)	
How might these tasks compare to performing them in person?			
A lot worse in person	3 (6.0%)	5 (10.2%)	
A little bit worse in person	14 (28.0%)	23 (46.9%)	
No difference	25 (50.0%)	7 (14.3%)	
A little bit worse on Zoom	8 (16.0%)	11 (22.4%)	
A lot worse on Zoom	-	3 (6.1%)	
How easy or difficult was it for you to take your own blood pressure?			
Very easy	35 (70.0%)	27 (55.1%)	
Easy	10 (20.0%)	17 (34.7%)	
Neutral	5 (10.0%)	3 (6.1%)	
Difficult	-	2 (2.0%)	

8. Figure Captions

Figure 1. CONSORT flow chart describing the enrollment, recruitment, and retention of study participants for the remote TSST.

Figure 2. Changes in average aggregate (A) positive and (B) negative emotions, (C) vigor, (D) anxiety, (E) wellbeing, (F) depression, (G) calm, and (H) hostility during the remote rTSST. All affective variables were collected on a scale from 0 (low) to 100 (high). Error bars indicate 95% confidence intervals.

Figure 3. Physiological reactivity and recovery by condition. Error bars indicate 95% confidence intervals.

9. Figures

Figure 1.

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Figure 2.

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Figure 3.