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Objective stress exposure, the impartial level of threat, and perceptions of stress, the level of threat a person endorses feeling, are important constructs in biopsychosocial research on internalizing psychopathology risk. One stress responsive physiological system that is integral to physical health and implication in mental health outcomes is the autonomic nervous system (ANS). Perceived stress is likely to be one mechanism (i.e., mediator) via which objective stress exerts an effect on ANS reactivity, but also the relationship between perceived stress and ANS reactivity may intensify as objective stress increases (i.e., moderation)—that is, potential moderated mediation in a three-variable system. The present study investigated the role of stress exposure and perceived stress in the activation of multimodal ANS indicators in 128 healthy undergraduates. Participants were randomized to three distinct objective levels of negative evaluation in a laboratory-based stress induction, variations of the Trier Social Stress Test (TSST): a non-stressful control ($n$ = 44), an intermediate ambiguously negative evaluative condition ($n$ = 46), and an explicit negative evaluative condition ($n$ = 38). Indicators of autonomic functioning, salivary alpha amylase (sAA), systolic and diastolic blood pressure (SBP, DBP) and heart rate (HR) were measured repeatedly to gauge TSST reactivity. For each of the four ANS indicators (sAA, SBP, DBP, and HR), I hypothesized that I would observe significant moderated mediation in a three-variable system (a relatively novel statistical approach), in which increasing objective negative evaluation level (1) directly predicts increased reactivity in ANS indicators, (2) is mediated by perceived stress in the pathway to ANS reactivity, and (3) also moderates the relationship between perceived stress and ANS indicators,

such that the relationship between perceived stress and ANS indicators strengthens as negative evaluation level increases. Findings indicated there was no evidence of moderated mediation. Whereas stress condition (objective negative evaluation level) was strongly related to our measurement of perceived stress (e.g., $t = 2.539$, $p = .011$), in contrast it was only weakly related to stress reactivity across ANS indicators, with statistically significant direct effects for only two of the four outcomes (SBP, DBP). Findings help guide future research with investigating more sensitive mediators and outcomes when using multiple manipulations/stress level conditions of the TSST.

# OBJECTIVE NEGATIVE EVALUATION LEVEL, PERCEIVED STRESS, AND AUTONOMIC REACTIVITY IN A LAB-BASED STRESS INDUCTION: A TEST OF MODERATED MEDIATION IN A THREE -VARIABLE SYSTEM

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# CHAPTER I: INTRODUCTION

## Objective Negative Evaluation Level, Perceived Stress, and Autonomic Reactivity in a Lab-based Stress Induction: A Test of Moderated Mediation in a Three-Variable System

Internalizing psychopathology, that is, depressive and anxious conditions, is highly prevalent and costly to society. Depression and anxiety pose significant burdens on various facets of society, including individuals, families, workplaces and the wider economy (Baxter et al., 2014; Chisholm et al., 2016; Chow et al., 2019; Doran et al., 2019). One of the most well replicated findings in the psychiatric literature is that stressful experiences precipitate onsets of internalizing psychopathology (for a review see Vrshek-Schallhorn et al., 2019). Notably, major stressful life events and chronic stress significantly predict depression onset (Monroe et al., 2007). Moreover, experiencing a greater number of stressful events and reporting high levels of perceived stress over long periods of time are both associated with worse mental health outcomes (Crosswell & Lockwood, 2020). There is evidence that stress precipitates anxiety disorders as well, though this area is less well-documented (Kendler et al., 2003; Spinhoven et al., 2010). Although there are well-replicated findings that stressful experiences predict internalizing psychopathology, the mechanisms by which this occurs is less clear.

One potentially fruitful approach to understand individual differences in stress responding is to probe autonomic nervous system (one branch of the peripheral nervous system) responses to stress, given that it is integral to physical health (Cohen et al., 2007; Epel et al., 2018), implicated in mental health (Cohen et al., 2007; Hammen, 2005) and is stress-responsive. In particular, lab-based stress inductions offer an opportunity to deliver a controlled, objective "dose" of stress (i.e., an impartially determined level of threat) and to immediately monitor acute ANS responding across multiple indicators. Previously tested variations of one such lab-based

stressor, the Trier Social Stress Test (TSST), employ different degrees of stress severity including a non-stressful control, an intermediate level, and an explicit negative evaluation condition (Vrshek-Schallhorn, Ditcheva, & Sapuram, 2018). Perceived stress (the level of threat a person *feels*) during the TSST is likely to be one mechanism (i.e., mediator) via which the level of objective stress exerts an effect on ANS reactivity. Additionally, the relationship between perceived stress and ANS reactivity might intensify as objective lab-based stress increases (i.e., moderation).

# CHAPTER II: LITERATURE REVIEW

## Autonomic Nervous System (ANS) Indicators

The ANS is a division of the peripheral nervous system that operates primarily unconsciously, regulating body functions such as heart rate (HR) and blood pressure (BP) (Joyner et al., 2010). The ANS consists of two subdivisions: the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS) (Ernsberger & Rohrer, 2018). Within the ANS, heart rate is modulated by both the SNS and PNS (Zhong et al., 2007). When sympathetic activity dominates the ANS, HR and BP increase (Joyner et al., 2010). Conversely, when parasympathetic activity predominates in the ANS, this is associated with decreased HR and the tendency for BP to decline.

### HR and Systolic and Diastolic BP

BP is operationalized first as systolic BP (SBP), which measures the pressure in the arteries when the heart contracts, and second as diastolic BP (DBP), which measures the pressure in the arteries when the heart rests between contractions (e.g., Woody et al., 2018). Numerous studies of objective lab-based stress exposures like the TSST conclude that HR, SBP, and DBP increase during the lab-based stress exposure protocol and return to approximately baseline levels after the protocol has terminated (Crosswell & Lockwood, 2020; Nater et al., 2005; Hellhammer & Schubert, 2012; Vors et al, 2018; Birkett, 2011; Allen et al., 2017).

Almost no research, however, has evaluated whether perceived stress predicts HR, SBP and DBP in the TSST. One study examining perceived measurements of stress before, during, and after the TSST, found that stress perception significantly predicted increased heart rate *during* the TSST though not before or after the test (Hellhammer & Schubert, 2012), which highlights the importance of capturing reactivity in HR and other ANS indicators. Compared to

other physiological stress response biomarkers, to my knowledge, there are no studies that examine the correspondence between perceived stress and SBP/DBP in an induced lab-based acute stress environment.

### Salivary Alpha Amylase (sAA)

Salivary alpha-amylase (sAA) is an enzyme produced by acinar cells in the salivary glands and is widely regarded as a biomarker for the sympathetic nervous system (SNS) (Nater & Rohleder, 2009). Under stress, sAA correlates with plasma levels of norepinephrine, the main neurotransmitter for the SNS (Thoma et al., 2012). While the acinar cells are innervated by both the SNS and PNS, under perturbation such as psychosocial stress, sAA is modulated by the SNS (Hoyt & Zimmermann, 2020; Nater & Rohelder, 2009). In response to psychological stress, the activation of the SNS stimulates beta adrenergic receptors on salivary acinar cells in the oral cavity (for a review see: Hoyt & Zimmermann, 2020). In prior work, sAA activity has increased in response to physiological and acute psychosocial stressors such as exercise (Gilman et al., 1979; Nexø et al., 1988; Chatterton et al., 1996; Nater & Rohleder, 2009), written examinations (Chatterton et al., 1996; Bosch et al., 1996; Nater & Rohleder, 2009), and laboratory-induced stressors (Bosch et al., 2003; Nater & Rohleder, 2009). Past research has found exposure to the TSST (as an objective stress exposure) induces significant increases in sAA (Crosswell & Lockwood, 2020; Kudielka et al., 2007). Similarly, increased HR has been positively related to sAA increase in the TSST (Almela et al., 2011).

## Stress Conceptualization

### Objective Stress Exposure

Objective stress exposure refers to an unpleasant circumstance, and the unbiased level of threat or negative impact within that unpleasant circumstance, in an organism's environment.

This may include acute life events (e.g., death of a loved one), ongoing strain (e.g., chronic financial deprivation), more minor daily hassles (e.g., parking ticket), or even lab-based exposure to negative evaluation by others. Objective stress has been measured in a variety of ways including contextual threat interviews and lab-based stress inductions. In contextual threat interviews, raters blind to the emotional response to an event, rate its negative, long-term threat for the average person (Brown & Harris, 1978). Lab-based stress inductions involve a consistent "dose" that is applied to everyone in the same condition. By contrast, many stress assessment approaches focus on an individual's self-reported perceived stress (Föhr et al., 2015), and even common stressful life event checklists capture a mixture of perceived and objective stress because of individual differences in item interpretation. The present study utilized a lab-based stress induction to capture objective stress exposure.

**Perceived Stress**

Perceived stress refers to an individual's feelings or thoughts about how much stress they are under, drawing from aspects of cognitive and emotional responses such as attention, perception, and appraisal of the stress exposure. Measures of perceived stress can reflect not only the influence of objective stressors, but also the subjective appraisal of objective circumstances (Christensen et al., 2019). Perceived measures of stress are influenced by a wide range of factors related to the individual, such as psychological symptoms, concurrent mood states, personality dispositions, past personal experiences, coping strategies, one's interpretation of life events and environmental pressures (Christensen et al., 2019; Upchurch et al., 2015). Each person reacts differently to stressors resulting in different perceptions of stress. Characteristics of perceived stress measurements demonstrate important individual, social, and cultural differences, denoting a unique aspect to the experience perceived by each individual (García-Sesnich et al., 2017).

Furthermore, self-reports of perceived stress related to a specific stressor or to one's life circumstances are one of the simplest ways to measure stress response (Crosswell & Lockwood, 2020). Importantly, because objective stress and perceived stress constructs emerged from distinct—and sometimes even conflictual—research traditions, their interplay in influencing physiological responses to acute stress is insufficiently examined.

Although one study found that perceived stress levels predict sAA responding to lab-based stress (Wiegand et al., 2018) other studies have failed to find a relationship (Juster et al., 2012). A 2012 qualitative review found that of 358 TSST publications identified, only 49 studies had at least one subjective emotional measure before, during, or after the TSST that also reported physiological and emotional associations. Of these 49 studies, only one examined the relationship between a measure of perceived stress and sAA (using a sample of pregnant women), which was not supportive of an association (Campbell & Ehlert, 2012). These findings indicate that at least up until these authors' search window closure of August 2011, perceived stress and its relationship to ANS indicators in a lab-based stress setting, has not been a well-studied topic. While there is some evidence that perceived stress has been linked to increased cortisol secretion (Juster et al., 2012), perceived stress levels predicting sAA response using a lab-based stress induction including the TSST has not received as much attention.

Following Campbell & Ehlert's review, however, several studies have demonstrated the association between perceived stress and activation of the SNS under conditions of lab-based stress (Hellhammer & Schubert; 2012; Föhr et al., 2015) and in samples with coronary artery disease or heightened risk (Yang et al., 2015; Huang et al., 2011). Despite this relationship and the positive association between objective stress exposures and physiological arousal, few studies (including those using the TSST) examine the association between perceived stress and

heart rate in a population of healthy adults. To my knowledge, as noted earlier, only one study has found evidence that stress perception during the TSST significantly predicted increased HR (Hellhammer & Schubert, 2012). Similarly, while many TSST studies collect SBP and DBP data, there are no studies that examine their relationship with perceived stress. These gaps and discrepancies in the literature, combined with a compelling theoretical rationale, indicate a need to test the relationships between objective stress, perceived stress, and reactivity in ANS-related indicators further.

## The Trier Social Stress Test

The Trier Social Stress Test (TSST) is a well-validated, standardized, robust lab-based stress-induction paradigm in which participants give a speech and perform mental arithmetic in front of judges to induce an array of emotional and physiological stress responses (Kirschbaum et al., 1993). The TSST reliably elicits several measures of physiological stress responses for the majority of participants and triggers a significant increase in stress perception during and after the TSST as compared to baseline values of momentary stress perception (Crosswell & Lockwood, 2020; Hellhammer & Schuber, 2012). sAA, SBP, DBP, and HR all significantly increase following TSST exposure (Almela et al., 2011; Nater et al., 2005; Nater et al., 2006; Woody et al., 2018), with mean heart rate gaining approximately 15-25 beats per minute from baseline to peak, immediately following the TSST (Kudielka et al., 2007). A condition similar to our intermediate task produced an approximately 25-35 mmHg point increase in SBP and 10-15 mmHg point increase in DBP in comparison to a Trier more similar to our control which produced a 15-25 mmHg point increase in SBP and a 17-23 mmHg point increase in DBP (Woody et al., 2018).

Theory and emerging evidence suggest that a critical element of the TSST is the potential for negative evaluation by the judges observing the speech and mental arithmetic task performance. Social self-preservation theory posits that human beings strive to uphold their social status and social esteem (Dickerson & Kemeny, 2004). This predicts that threats to one's social self (perhaps uniquely so) will be accompanied by psychological and physiological responses (Dickerson, Gruenewald, & Kemeny, 2004). Notably, in a study by Woody et al., 2018, negative evaluation, not cognitive load, predicted cortisol and cardiovascular reactivity. This further aligns with emerging evidence regarding real life stressful life events: stressors that involve an element of interpersonal stress and social rejection are among the strongest proximal risk factors for future depression and elevations in inflammatory biomarkers (Epel et al., 2018; Slavich & Irwin 2014; Vrshek-Schallhorn, 2015). Negative evaluation in an experimental setting is meant to mimic what one might experience in a real-life negative evaluation scenario, but without any long-lasting consequences. In real life scenarios, negative evaluation can have significant implications because such scenarios typically involve social evaluations and/or rejection with continual social interactions (Dickerson & Kemeny, 2004). Thus, utilization of the TSST in a lab-based setting may be one of the closest ways to emulate realistic negative evaluation, and per the social self-preservation theory, has the potential to elicit a threat to the social self.

The original TSST induction uses cool, neutral observer judges, with the potential for participants to infer negative evaluation—that is, intentionally ambiguous negative evaluation. However, a relatively novel variant of the TSST utilizes explicit negative evaluation by judges (Way & Taylor, 2010). As argued in the literature, (Vrshek-Schallhorn, Ditcheva, & Sapuram, 2018; Vrshek-Schallhorn et al., 2019) the explicit negative evaluation may be theoretically

valuable as well, because internalizing disorders are characterized by elevated levels of social sensitivity (Slavich et al., 2010; Vrshek-Schallhorn et al., 2015). In Vrshek-Schallhorn et al.'s 2018 study with three levels of negative evaluation almost identical to the current proposal, cortisol reactivity significantly increased as stress condition increased, and moreover, the explicit negative evaluative condition provoked a significantly greater cortisol response than the intermediate condition of the TSST. One 2004 meta-analysis reporting on 208 laboratory studies demonstrated that the relationship between evaluation level and cortisol reactivity is greater in protocols with social evaluative aspects than in those without them (Dickerson & Kemeny, 2004). Given this evidence, inclusion of an explicitly negative evaluative manipulation in a lab-based stress induction may be an instrumental addition as it can further illuminate pathways to internalizing outcomes by eliciting greater physiological and psychological responses.

Further, while numerous studies use the TSST as an objective form of stress exposure, very few studies use three conditions/levels of difficulty, especially an explicit negative evaluative threat condition, and most only use one (the original TSST), using repeated measures to treat each participant as their own control. There may be advantages for power and interpretability to using more than two levels of negative evaluation, which could better approximate a dimensional variable. Thus, the present study employed three conditions with increasing levels of negative evaluation—a control without evaluation, an intermediate condition with ambiguous evaluation, and an explicit negative evaluation condition.

## Using Stress to Understand ANS Reactivity: Moderated Mediation in a Three-Variable System

Examining the interplay of both objective stress exposure and perceived self-report measures is likely to offer insights, as they measure distinct constructs, and objective stress

exposure has been shown to influence perceived stress (Christensen et al., 2019; Hellhammer & Schubert, 2012). Objective negative evaluation level may act on ANS reactivity through its action on perceived stress (i.e., mediation). Further, the relationship of perceived stress to ANS reactivity may also strengthen as objective negative evaluation level increases (i.e., moderation). The strengthening of this relationship may be due to the notion that under low stress conditions, ANS outcomes are likely driven by factors other than objective stress (i.e., cardiovascular health), which only becomes a more salient factor at higher levels. One recently emerging statistical tool for examining such hypotheses is moderated mediation in a three-variable system (Goldstein et al., 2021). In this model, a single variable (Predictor X) is both moderating and being mediated by a Third Variable M in a single analytic model on Outcome Y (Goldstein et al., 2021). This approach permits parsimoniously testing both the potential for (a) objective negative evaluation level (Predictor X) *acting through* perceived stress (mediator M) on ANS reactivity (Outcome Y), and (b) objective negative evaluation level *moderating* the effect of perceived stress on ANS reactivity (Figures 1 and 2).

## The Present Study

No prior studies have investigated whether objective stress exposure may exert its effects on ANS reactivity via perceived stress level, and whether the mediating pathway from perceived stress to ANS might also be strengthened (i.e., moderated) by objective stress level. The current study examined healthy undergraduates' ANS responses to three levels of negative evaluation in a lab-based stress induction: a non-evaluative, putatively non-stressful Control manipulation, an Intermediate manipulation with ambiguous negative evaluation, and an explicit negative evaluative Challenge condition. I applied an emerging statistical method that allows testing the negative evaluation level as both a moderator and mediated variable (via perceived stress)

simultaneously in a three-variable system predicting four ANS outcomes in separate models: sAA, SBP, DBP, and HR. I hypothesized that (1) perceived stress would mediate the relationship between negative evaluation level and ANS indicators, sAA, HR, SBP and DBP, in separate models, and that (2) as the level of negative evaluative threat of the TSST intensified, the association between perceived stress and ANS indicators (sAA for SNS, HR and SBP and DBP for ANS broadly) would increase.

# CHAPTER III: METHODS

## Participants

Data for the present study came from a larger investigation that examined predictors of biomarker responding to lab-based stress among healthy young adults aged 18 to 30 (analytic *N* = 128; negative evaluative TSST *n*= 38; Intermediate TSST; *n*= 46 Control *n*= 44). See Table 1 for sample demographics across condition. Participants were recruited from the University of North Carolina Greensboro's (UNCG) psychology research pool. Individuals who reported current use of nicotine, hormonal birth control, prescription psychotropic medications, or corticosteroid medications on a pre-screening measure were excluded from participation. Additionally, a diagnosis of a chronic health condition such as asthma or diabetes, blood pressure and heart rate readings with systolic ≥160 or diastolic ≥100 upon screening, uncorrected deficit in vision or hearing, a first language other than English (to ensure that the lab-based stress induction was not more stressful for some than for others), diagnosis with a learning disability, and history of head trauma were also exclusionary criteria. If it was determined by the Structured Clinical Interview for the DSM-IV, non-patient edition (SCID-I/NP; First et al., 2001) that a participant met criteria for a provisional or a preliminary diagnosis of a current major depressive episode, then that participant was assigned to the non-stressful control condition, as not to expose them to any additional stress. Additionally, research has provided evidence that depression appears to alter biomarker responses to stress inductions (Slavich & Irwin, 2014). These individuals were excluded from the present analyses due to non-randomization. Of the 142 participants consented, seven participants were excluded from analyses for current depression, six were excluded because they consented but withdrew from the study (e.g., during the TSST),

and one participant was excluded from analyses for evidence of extreme outlier biomarker values.

## Materials & Measurements

### Structured Clinical Interview for the DSM-IV (SCID-I/NP)

The SCID-I/NP is a semi-structured clinical interview designed to yield psychiatric diagnoses consistent with the DSM-IV/DSM-IV-TR (First et al., 2001). Only the major depression section was administered.

### Modified Primary Appraisal Secondary Appraisal Scale (PASA)

To measure perceived stress, the present study utilized the primary appraisal scale of the Primary Appraisal and Secondary Appraisal scale (PASA; Gaab et al., 2005). Following similar work examining cortisol in a lab-based stress induction (Juster et al., 2012), this measure was administered immediately following the initial TSST instructions and the 5-minute anticipatory speech preparation period, so that participants would be sufficiently familiar with their condition, including the confederate judge's demeanor and with instructions for the first task, but would not yet have completed either primary TSST activity, the speech or the math. The PASA measures two anticipatory cognitive appraisal constructs: Threat and Challenge (Primary Appraisal) and Self-concept of Own Abilities and Control Expectancy (Secondary Appraisal) (Gaab et al., 2005). Primary Appraisal captures the evaluation of the situation with respect to the amount of threat or challenge, while Secondary Appraisal captures perceived available coping strategies (Gaab et al., 2005). A modified version of the PASA consists of 14 items such as "I do not feel threatened by this situation," "I find this situation very unpleasant," and "This situation scares me," and is measured on a 6-point Likert scale from 1 (strongly disagree) to 6 (strongly agree). The modification from the original version involved items 7 and 11 in which the word

"interview" was changed to "situation" to better fit the TSST. Prior work with the PASA suggests that the Primary Appraisal scale measuring evaluation of threat is most relevant to physiological reactivity in the TSST (Juster et al., 2012) and this construct also has greater face validity as a measure of perceived stress; therefore, the present study used only the primary threat appraisal scale as an indicator of perceived stress. In a prior sample, internal consistency for the Primary Appraisal subscale administered early in the TSST was $\alpha$=0.80 (Juster et al., 2012).

**Socioeconomic Status**

The participants' parents' average of the level of education on an ordinal survey item was computed as an indicator of SES for covariation. One parent's education level was used when both were not available.

**Trier Social Stress Test (TSST)**

The TSST is a laboratory-based stress induction designed to reliably exposure participants to a controlled dose of stress to examine the physiological, endocrine, and affective consequences in human research participants (Kirschbaum et al., 1993). Three conditions representing variations on the original TSST were used in the current study: a non-stressful control condition, an intermediate ambiguously negative evaluative condition similar to the original TSST, and an explicit negative evaluative condition (Vrshek-Schallhorn, Ditcheva, & Sapuram, 2018). All conditions shared several procedures in common. Participants across all conditions were instructed to draw a speech topic number from a box, though topics were constant within condition. Each condition allowed five minutes of task preparation and anticipation, then five minutes of extemporaneous speaking on a pre-assigned topic, and then five minutes of mental arithmetic. The arithmetic portion of the task consisted of sequentially

subtracting 13 from 2,017 as quickly and accurately as possible. The experimenter informed participants that if they miscalculated, the experimenter would point out their mistake and they would have to start all over again. For both tasks across all three conditions, participants were told during the consent process that they would be videotaped for this portion of the study. However, in all three cases no such recording occurred.

The conditions also differed in significant ways including speech topic, whether or not the participants were told they were being evaluated, number of judges, and the judge's behavior. In the control condition the speech topic was neutral, describing advice people could follow for healthy living. In the intermediate condition the speech topic was moderately self-evaluative, discussing what plans they would enact if chosen for a student leadership position and why they think these actions are important. In the negative evaluative condition, the speech topic was the most self-evaluative, advocating for why they would they make a good candidate for a student government position.

Regarding judges, the control condition had none except for the experimenter who sat off to the side largely out of the participant's view. The experimenter did not engage with the participant or make eye contact during the either portion of the task and alleged to be preparing paperwork for the next session. Participants in the control condition were told they would not be evaluated on their performance and that the experimenter would only like them to complete the tasks. In the intermediate difficulty condition, the tasks were performed in front of one confederate judge who maintained a "neutral but interested" demeanor, without engaging in behaviors associated with either negative or positive evaluation. Participants in the intermediate condition were told they were not being evaluated on their performance. In the negative evaluative condition, there were two confederate judges, one male and one female, who provided

subtle negative non-verbal feedback in accordance with a behavioral script. The female-identifying confederate judge followed a behavioral script designed to appear bored (i.e., checking watch, sighing, and staring off into the distance) while the male-identifying confederate judge followed a behavioral script designed to appear dissatisfied (i.e., furrowing brow, scribbling notes, and making an X on a piece of paper). Participants in the negative evaluative condition were told that the judges would evaluate their performance.

**Post-Challenge Questionnaire**

After the TSST, the participants answered four questions for overall perceived evaluation, perceived negative evaluation and perceived positive evaluation. If respondents denied that perceived they had been evaluated at all, which was the first question, then they did not complete the positive or negative evaluation items.

**ANS and Cardiovascular Stress reactivity**

Salivary Alpha Amylase (sAA) was assayed from saliva collected via passive drool at four points in the protocol: at baseline immediately prior to the TSST (+0 minutes), immediately following the TSST (+30 minutes), following completion of two cognitive tasks not analyzed here (+65 minutes), and debriefing and additional recovery (+80 minutes). Preliminary analyses indicate that sAA recovered to baseline on average after the 3rd sample, consistent with prior work (Laurent et al., 2016; Nater, 2009); thus, only the first 3 sAA values will be used in the present analyses to measure sAA reactivity. Blood pressure and heart rate (SBP, DBP, and HR) were measured with the participant sitting down at all measurements, via a GE Dinamap V100 hospital grade digital blood pressure monitor. SBP, DBP and HR were measured twice in a row in conjunction with saliva sampling; the average of the two results at each timepoint were used in analyses to enhance accuracy. I inspected data to determine whether to use all four timepoints, or

fewer points, for the remaining ANS indicators as for sAA. Preliminary inspection of repeated measures indicated that, HR, SBP, and DBP reactivity completed (i.e., values returned approximately to baseline) across all conditions by the third measure; this, repeated measures 1, 2, and 3 but not 4 were used for all outcomes.

## Procedure

Upon arrival, participants were seated and their blood pressure and heart rate were measured via a blood pressure monitor to determine eligibility status prior to consent with a maximum of two attempts to meet criteria. Following informed consent, the depression section from the Structured Clinical Interview for the DSM-IV (SCID-I/NP, First et al., 2001) was administered to assess for current and past depression. The first saliva sample was collected. Next, participants completed one of three conditions of a modified TSST, described above: a non-stressful control condition, a moderate intermediate condition, or an explicit negative evaluative condition. Participants signed up to the study blind to the study conditions schedule for each session. Afterwards, participants were fully debriefed regarding the deception and provided signed permission to use their data.

Critically, the PASA was administered following the TSST instructions and the 5-minute speech preparation period. The TSST instructions were delivered by the experimenter (for Control participants) or confederate judge(s) (for Intermediate and Challenge participants). This retains the temporal precedence of the initial predictor, condition or negative evaluation level, before measurement of the mediator perceived stress, and mediator before the reactivity outcome measures.

## Analytic Plan

### Preliminary Analyses

#### *Group Equivalent Checks*

For group equivalent checks (to screen for major failures of randomization), I conducted a Chi Square test with Condition as a between-subjects factor to test for differences by group in gender identity, and minoritized race and ethnicity. I also tested for group differences based on SES and on the baseline dimensional physiological variables (HR, SBP, DBP, and sAA) using a one-way ANOVA.

#### *Manipulation Checks*

As a manipulation check, I conducted a one-way ANOVA using linear contrasts for negative evaluation level on the extent to which participants felt evaluated during the TSST including perceived overall evaluation, negative evaluation, and positive evaluation, measured with one question immediately following the TSST. I used a linear contrast for condition, so that the 0, 1, and 2 codes for control, intermediate, and negative evaluative conditions were treated as a single dimension rather than three separate groups.

### Primary Analyses

In my primary analyses, I coded condition as control = 0, intermediate = 1, and negative evaluative = 2; prior research work using almost identical manipulations supports this coding (Vrshek-Schallhorn, Ditcheva, & Sapuram, 2018). All biomarker variables were inspected for outliers within timepoint and condition (considering all conditions together for baseline before treatment of conditions diverged); modest outliers were winsorized to three standard deviations above the mean, and extreme outliers were evaluated on a case-by-case basis for retention. A total of n = 3 values were winsorized for sAA and total of n = 2 values were winsorized for DBP,

and none for HR or SBP. An additional three extreme outliers were evaluated for retention. Two of these outliers reflected a blood pressure machine malfunction where the research assistant noted that the participant moved excessively; these datapoints were excluded from analyses. This did not affect the overall n because two measurements were taken at each timepoint, and I was able to use the one measurement instead of averaging the two timepoints. One additional participant was removed from sAA analyses after natural log transformation (noted below) exacerbated a modest outlier (-3.16 standard deviations from mean), leaving n = 127 for sAA analyses only and 128 in all other models.

Next, to operationalize physiological reactivity, I used the Area Under the Curve with Respect to Increase (AUCi) metric, which is a commonly used indicator of reactivity calculated over repeated measures (Preussner et al., 2003), and which removes influence of the baseline level to the extent this is possible. AUCi variables were then z-scored for analyses to place them on a similar scale with other variables to facilitate convergence of analyses in Mplus. I used a natural log transformation with sAA only as the data showed notable positive skew. Following this transformation, all AUCi variables were sufficiently normal, and levels of skewness were in the acceptable range. Both negative evaluation level (condition) and perceived stress, as indicated with the PASA, were grand-mean centered before entry in the model. Given that I tested four models, I did not correct for multiple tests. I interpreted $p$-values $\leq .05$ as statistically significant. For HR, SBP and DBP, if one of the two timepoints was missing, I used the one instead of averaging the two timepoints prior to calculate AUCi variables, which require all datapoints for consistency across participants. For the PASA, there was no missing data, so no maximum likelihood estimation was needed (Muthén & Muthén, 2017).

For this study, I used MPlus Version 8 to test the moderated mediation hypothesis in a three-variable system with Negative Evaluation Level (X), Perceived Stress from the PASA (M) and ANS Reactivity Indicators (Y) in separate models for sAA, HR, SBP and DBP (Model 74; Stride et al., 2015). This approach provides regression path estimates for each path (see Figures 1 and 2) and calculates the conditional indirect effect using a model constraint where the relationship between the indirect effect and the moderator is linear (Hayes, 2015), providing bias correct bootstrapped confidence intervals for the indirect effect at specified levels of the X variable (with bootstrapping 10,000 times), from which I reported the 95% confidence intervals. Because model constraints in Mplus generate only unstandardized model results, I reported all unstandardized effects. I also adapted the code from Model 4a (Stride et al., 2015) to obtain an estimate of the overall indirect effect in addition to conditional indirect effects, since Model 74 does not provide this. Models provided estimates of the conditional indirect effect of negative evaluation on ANS outcomes, at the control, intermediate, and explicit negative evaluative levels of X. I did not report the individual condition levels in the model (low, medium, and high), if the interaction between X and M was not significant or approaching significance, treating these as post-hoc tests. In the event of significant moderation, I interpreted the *p*-values and the bootstrapped 95% confidence intervals of the unstandardized conditional indirect effects at control, intermediate, and negative evaluative conditions to indicate whether there was mediation at different levels of X.

**Covariates**

As a follow-up to primary models that produce significant findings, I covaried constructs thought to impact stress reactivity, including minoritized race/ethnicity (Upchurch et al., 2015), gender (Kirschbaum, Wust, & Hellhammer, 1992), and parental education to index

socioeconomic status (Hackma et al., 2012). Considering the current study's statistical approach, covariates were only reported when a significant prediction of the outcome was obtained. If mediation and moderation was not significant, those models did not add in covariates.

**Power Considerations**

Although the available sample is already established, I conducted a sensitivity analysis to probe power in a similar dataset. To estimate power for this model, I ran a Monte Carlo simulation (e.g., Thoemmes, MacKinnon, & Reiser, 2010) in Mplus version 8 (Muthen & Muthen, 2017) using parameters obtained from an older dataset with similar variables available. In this older study, participants ($N$ = 162) were randomized to two levels of the TSST (control and explicit negative evaluative condition) rather than three conditions. In the older dataset, perceived level of evaluation was measured with one item following the TSST (i.e., the manipulation check) in contrast to the present study in which perceived stress was measured at the beginning of the TSST after speech preparation. In the older dataset, sAA reactivity was operationalized as Area Under the Curve with Respect to Increase (AUCi) from samples taken at baseline (0 minutes), after the instructions (+5 minutes), after the TSST (+20 minutes), and after recovery (+45 minutes). The model tested the effect of condition acting through perceived evaluation to predict sAA reactivity and tested the condition as a moderator. When simulated 10,000 times with 128 participants (the expected number available in the present study), the indirect effect was expected to reach significance 100% of the time, and the Stress x Perceived Evaluation interaction on sAA reactivity was expected to reach significance 38.7% of the time. However, it is reasonable to expect that power would be better for this test in the present dataset because the objective stress variable will better approximate a dimension, which is likely to enhance power for the Objective Stress x Perceived Stress interaction, and because the timing of

perceived stress measurement (early in the TSST) is preferable for generating a larger effect size. As such, power for the present model was likely in the acceptable range.

# CHAPTER IV: RESULTS

## Descriptive Statistics

### Preliminary Analyses

There were no group differences based on gender ($F(2, 128) = .067$, $p = .967$), minority status ($F(2, 128) = .977$, $p = .614$), or SES ($F(2, 128) = 1.247$, $p = .291$). One-way ANOVA's revealed no significant differences in baseline sAA ($F(2, 126) = 2.887$, $p = .059$), HR ($F(2, 127) = 2.713$, $p = .070$), SBP ($F(2, 127) = .272$, $p = .762$), and DBP ($F(2, 127) = .026$, $p = .974$). See Table 2 for all sample characteristics across TSST condition.

### Validity of TSST Stress Paradigm

As expected, linear contrasts conducted via one-way ANOVAs revealed significant differences across condition of overall perceived evaluation ($F(2, 123) = 14.392$, $p < .001$), positive evaluation ($F(2 ,106) = 13.709$, $p < .001$), and negative evaluation ($F (2, 105) = 19.974$, $p < .001$), such that the negative evaluative condition reported greater overall perceived evaluation, less perceived positive evaluation, and greater perceived negative evaluation (Table 3).

### Zero-order Correlations

Zero-order bivariate correlations were examined among stress condition, perceived stress, covariates, and dependent variables and are presented in Table 4. Overall, the correlations among sAA, HR, SBP and DBP were not as strong as expected, except for SBP and DBP which were moderately correlated.

## Primary Model Results

Discussed below, the following four models were tested in MPlus to test the moderated mediation hypothesis in a three-variable system with TSST Level of Severity (X), Perceived Stress from the PASA (M) and ANS Reactivity Indicators (Y) in separate models for sAA, HR, SBP and DBP. To visualize the results, graphs in figures 5, 7, 9, and 11, show the pattern of change from each stress condition with each ANS outcome. A visualization of the relationship between PASA and stress condition is presented in figure 3. Following the criterion for reporting covariates only when obtaining a significant prediction of the outcome, only DBP has covariates added to the model.

### TSST Level of Severity x Perceived Stress Predicting sAA Reactivity

Whereas the objective stress exposure condition predicted perceived stress as expected ($t$ = 2.539, $p$ = .011), there was not a significant direct path from stress condition to the outcome, sAA AUCi ($t$ = 1.077, $p$ = .282), nor a significant path from perceived stress to sAA reactivity ($t$ = -.125, $p$ = .900). Further, results from the conditional indirect effect indicated no significant relationship between sAA reactivity and the interaction term XM, which denotes the interaction effect of the X variable (stress condition) with perceived stress as the mediator variable ($t$ = .167, $p$ = .868), and no overall mediation effect was observed ($t$ = -.117, $p$ =.907). Results for this model and a visualization of stress condition, perceived stress and sAA reactivity (AUCi), are presented in Figures 4 and 5.

### TSST Level of Severity x Perceived Stress Predicting HR Reactivity

Objective stress exposure condition predicted perceived stress as expected (t = 2.539, $p$ = .011); however, there was not a significant direct path from stress condition to the outcome, HR AUCi ($t$ = .734, $p$ = .463), nor a significant path from perceived stress to HR reactivity ($t$ = .727,

$p$ = .467). There was also no significant overall mediation effect ($t$ = .635, $p$ = .525) and no significant Stress Condition x Perceived Stress interaction effect, ($t$ = .157, $p$ = .875). Results for this model and a visualization of stress condition, perceived stress and HR reactivity (AUCi) are presented in Figures 6 and 7.

**TSST Level of Severity x Perceived Stress Predicting SBP Reactivity**

Results from this conditional indirect effect indicated that TSST objective stress exposure condition directly predicted SBP reactivity ($t$ = 2.394, $p$ = .017), and the objective stress exposure condition predicted perceived stress as expected ($t$ = 2.540, $p$ = .011). No other significant findings emerged, however. There was no significant relationship between Perceived Stress and SBP reactivity ($t$ = .458, $p$ = .647), nor was there a significant interaction between TSST level of severity and perceived stress predicting SBP reactivity ($t$ = 1.069, $p$ = .285), nor was there an overall mediation effect (t = .418, $p$ = .676). Results for this model and a visualization of stress condition, perceived stress, and SBP reactivity (AUCi) are presented in Figures 8 and 9.

**TSST Level of Severity x Perceived Stress Predicting DBP Reactivity**

Results from this conditional indirect effect indicated that TSST objective stress exposure condition directly predicted DBP ($t$ = 2.771, $p$ = .006) and the objective stress exposure condition predicted perceived stress as expected ($t$ = 2.539, $p$ = .0011). There was no significant path from perceived stress to DBP reactivity ($t$ = -1.518, $p$ = .129). Similarly, there was neither a significant interaction between TSST level of severity and perceived stress predicting DBP reactivity ($t$ = .873, $p$ = .382), nor an overall mediation effect ($t$ = -1.242, $p$ = .214). Results indicated a significant main effect between male sex predicting increased DBP (t = 2.397, $p$ =

.017). This model and a visualization of stress condition, perceived stress, and DBP reactivity (AUCi) are presented in Figures 9 and 11.

# CHAPTER V: DISCUSSION

The current study investigated the role of objective stress exposure level and perceived stress in the activation of the ANS in a healthy, non-depressed undergraduate sample. The study examined ANS responses to three levels of negative evaluation in a lab-based stress induction: a non-evaluative, non-stressful control condition, an intermediate condition with ambiguously negative evaluation, and a challenge condition with explicit negative evaluation. Using an emerging statistical method, where negative evaluation level (the condition) served as both a moderator and a mediated variable, I hypothesized that I would observe significant moderated mediation in a three-variable system, in which increasing objective negative evaluation level (1) directly predicts increased ANS reactivity indexed by sAA, HR, SBP and DBP, (2) is mediated by perceived stress in the pathway to ANS reactivity, and (3) simultaneously moderates the relationship between perceived stress and ANS indicators, such that the relationship between perceived stress and ANS indicators strengthens as negative evaluation level increases. Although there was no support for moderated mediation in a three-variable system, the current study demonstrated a significant relationship between stress condition (TSST level of severity) and an indicator of perceived stress (the PASA), as well as a significant relationship between stress condition and SBP and DBP reactivity, but not that of other sAA and HR indicators. Furthermore, perceived stress did not predict any ANS outcomes. Overall, there were few direct effects, no conditional indirect effects, and no moderated indirect effects.

## Consideration of the Moderated Mediation in a Three-Variable System

As researchers we often spend time distinguishing and clarifying the differences between moderators and mediators, and their respective roles in separate analytic models. Maintaining this level of diligence is good practice considering the essential roles moderators and mediators

play in informing clinical research. Nevertheless, historically, it is not uncommon for a single variable to act as a moderator in some studies while serving as a mediator in other studies of the very same constructs (Grant et al., 2006), which increases researcher "degrees of freedom" regarding how to test and report findings.

Rather than maintain the perspective that moderation and mediation are distinct concepts never to be tested together, Goldstein et al., (2021) suggests we treat them as components of a three-variable system that can be examined in the same analytic model within a single study. Still, a distinct variable that functions as both a moderator and mediator simultaneously in a single analytic model is a novel statistical approach that has rarely been reported. Despite its novelty, Goldstein et al., (2021) illustrates the value of this method and extrapolates on the reality of this approach through the lens of developmental psychopathology. For instance, there is evidence that neuroticism may mediate event exposure leading to depression (Kercher, Rapee, & Schniering, 2009; Wetter & Hankin, 2009) but may also moderate the impact of the event on depressive outcomes (Kendler, Kuhn & Prescott, 2004; Vinkers et al., 2014). Similarly, in Gene x Environment (G x E) studies there is evidence to suggest a genetic variable may interact with an environmental stressor to predict psychopathology, while simultaneously predicting the environmental stressor with which it interacts through stress generation processes (Goodyer et al., 2009; Hayden et al., 2010).

Considering the dynamic functions of moderators and mediators, and the evidence for their activity in a single analytic model, it is not conceptually unreasonable to consider moderated mediation in a three-variable system. Furthermore, if results reveal a co-occurrence of moderation and mediation in a three-variable system, the effects should not be separated since they are both contributing to the outcomes (Goldstein et al., 2021). The current study sought to

take Goldstein's approach, and permit testing both the potential for (1) objective negative evaluation level (Predictor X) *acting through* perceived stress (mediator M) on ANS reactivity (Outcome Y), and (2) objective negative evaluation level *moderating* the effect of perceived stress on ANS reactivity. No prior study has used this statistical approach investigating whether objective stress exposure may exert its effects on ANS reactivity via perceived stress level, and whether the mediating pathway from perceived stress to ANS might also be strengthened (i.e., moderated) by objective stress level.

### Stress Condition Weakly Related to ANS Outcomes

Contrary to many research findings, stress condition did not reliably predict most ANS outcomes. In fact, stress condition only had a significant main effect predicting SBP and DBP, and no other ANS indicators. This is noteworthy as both BP indicators were impacted by objective stress condition and were the only variables in the model to offer significance. Not only have few studies implemented multiple stress manipulations of the TSST, to my knowledge, the current study is the first to evaluate the effect of multiple stress condition levels on SBP and DBP. Despite these significant effects, there was considerable overlap between the bootstrapped 95% confidence intervals of the betas for direct effects to all the ANS outcomes (significant and non-significant), suggesting that the main effect of stress condition predicting SBP and DBP were unlikely to be "significantly different" from the other nonsignificant effects. These relatively weak (if significant) main effects of stress condition predicting SBP and DBP may also help explain why there is unlikely to be an interaction effect (XM) to detect in these models.

Considering the vast research on the TSST reliably eliciting significant increases in sAA, HR, SBP and DBP following TSST exposure (Almela et al., 2011; Nater et al., 2005; Nater et al., 2006; Woody et al., 2018), the results were somewhat surprising. I expected stress condition to

have at least a small and significant influence on most of the AUCi outcome variables, but this was not the case. Notably, the weak association between stress condition and sAA reactivity was particularly unexpected considering prior TSST research where sAA group differences (change over time within person in a single TSST condition) tend to be significant (Bosch et al., 2003; Crosswell & Lockwood, 2020; Kudielka et al., 2007; Nater & Rohleder, 2009). Although there has been significantly less research examining two or more group manipulations of the TSST and sAA responding. Inspection of the mean sAA AUCi values by condition suggested that, while there was overall growth in the expected direction as condition intensified, there was no increase between the intermediate and the more novel explicit negative evaluative condition. In a three-group stress manipulation, if the middle and highest groups behave similarly, then they will not fit a linear effect that anticipates continued growth from the middle to the highest condition. As a result, this trend diminishes the effects of the condition variable modeled linearly. Inspection of the mean HR AUCI values by condition suggested that although there was growth in the expected direction between the control and negative evaluative condition, the intermediate condition did not have the intended effect.

These findings suggest that sAA may not be particularly sensitive to increases in severity beyond the intermediate condition. However, given the HR AUCI results, it may be possible that these ANS indicators were not as sensitive to our study's TSST manipulations and/or the different stress manipulations were not a great fit for the dependent variables. Although these findings were rather unexpected, ultimately, there are no prior studies examining an explicit negative evaluative condition with autonomic outcomes.

### Perceived Stress Failed to Mediate Stress Condition on ANS Indicators

Across all four models, perceived stress was neither significant as an overall mediator nor did perceived stress significantly predict any of the ANS outcomes: sAA, HR, SBP and DBP. Even though prior work with the PASA suggests that Primary Appraisal—a component of the scale measuring evaluation of threat—is most relevant to physiological reactivity in the TSST (Juster et al., 2012), there are very few studies that evaluate whether perceived stress predicts these autonomic indicators in the TSST. Among the few studies that do report the relationship between sAA, HR, SBP and DBP with perceived stress, there are mixed findings. While some studies found stress perception to significantly predict increased HR during the TSST (Hellhammer & Schuber, 2012; Lackschewitz et al., 2008), other TSST studies found no significant correlation (Hofmann, 2006; Lerner et al., 2007). Further, the few studies that examine the correspondence between perceived stress and SBP or DBP in a lab-based stress induction report no significant relationship between the self-reported emotion measures and BP (Lerner et al., 2007; Cohen et al., 2000). Regarding sAA, the literature is scarce among healthy adult populations and contains mixed findings with one study reporting perceived stress levels predicting sAA responding to lab-based stress (Wiegand et al., 2018) while another study failing to find a relationship (Balodis et al., 2010). Given the lack of empirical evidence for perceived stress predicting ANS outcomes in an objective lab-based stress exposure, it may not be entirely surprising that no significant conditional indirect effects were observed. Further, the relative lack of significant effects of stress predicting the outcome variables makes it difficult to study mechanisms (mediation) because there is no variance to draw from.

### Stress Condition Predicted Perceived Stress

Consistent with previously published studies, stress condition significantly predicted perceived stress across all models in this sample. This finding was not surprising considering that numerous studies report the TSST reliably elicits a significant increase in stress perception. Furthermore, the PASA measure of perceived stress has good face validity, containing aspects of individuals' beliefs and an anticipated stressor such as the TSST (Juster et al., 2012). While many studies draw the connection between the TSST as an objective form of stress exposure predicting elevated stress perception, most studies only use the original TSST manipulation with one stress condition. Very few studies use three conditions/levels of difficulty, especially an explicit negative evaluative condition.

### Covariate and ANS Outcome

Although not the focus of hypotheses, male sex predicted higher levels of DBP. These findings were not surprising as there is ample evidence in the literature to support men having higher cardiovascular risk than women (Kelly & Jones, 2014; Palatini, 2001).

## Strengths & Limitations

The present study utilized a lab-based stress induction to capture objective stress exposure, using a virtually unique explicit negative evaluative condition and a relatively novel statistical approach, moderated mediation in a three-variable system. Further, this study addressed gaps and discrepancies in the literature such as bringing to light and expanding upon the often-conflated constructs of perceived and objective stress and their interplay in influencing physiological responses. The current study also examined perceived stress and its relationship to various ANS outcomes—another insufficiently explored topic in the stress literature.

While the study had several strengths, it had limitations as well. First, although the sample size (N = 128) is considered relatively large for studies employing the TSST, it may have been too small to obtain significant effects given smaller than anticipated effect sizes. Second, even though we are seeing significant effects of condition on SBP and DBP in the predicted direction, they are considered small effect sizes. Overall, there is not a strong relationship between stress condition and the ANS dependent variables. It may be the case that the ANS outcomes in the current study are not the strongest indicators of stress, especially considering other empirically supported ANS markers such as the pre-ejection period (PEP) which indexes sympathetic functioning (Krohova et al., 2017) and RSA which indexes parasympathetic functioning (Tonhajzerova et al., 2016). However, these both require more sophisticated psychophysiological measurement such as impedance cardiography which was not possible in the present study.

## Future Directions

Future stress research would benefit from continued probing of both objective stress exposure and perceived self-report measures as they are likely to offer insight into how different ANS biomarkers are related to and fit in with these constructs. Researchers in this line of work may consider more sensitive mediators than the PASA or more sensitive outcome variables such as PEP or RSA. Moreover, if investigating the effects of stress on the ANS, it may be useful to consider whether using multiple TSST stress manipulations is a good fit with the other variables in the model. In those studies, with the TSST measuring ANS outcomes, researchers may want to consider using blood pressure as a biomarker given both SBP and DBP were the only variables in our model that offered significance and had the most linear results. Furthermore, SBP and DBP are the least utilized biomarkers in this type of work.

Developmental psychopathology GxE studies and studies involving TSST or other lab-based inductions should consider the moderated mediation framework given the flexibility of single variables to act as both moderators and mediators in different studies. In GxE studies and others involving lab-based stressors, it is not uncommon for a single variable to take on multiple relationships. As a final point, future research may want to consider alternative statistical approaches to the AUCi, if anticipated main effects are sparse. Perhaps a growth curve analysis would be a more sensitive option for future work to obtain greater precision when testing the main effect of stress.

## Conclusion

Taken together, I report that while stress condition significantly predicted perceived stress as expected, in general, stress condition was weakly associated with ANS outcomes, only producing statistically significant direct effects for SBP and DBP. Despite obtaining significance, these effects were relatively weak, likely explaining why there was no evidence of moderated mediation. The current study is one of few studies that examines multiple stress manipulations of the TSST including a negative evaluative condition and is the first study to do so probing the relationship between stress condition and blood pressure. These findings help guide future lab-based stress research, providing significant evidence for underutilized biomarkers (SBP and DBP) and considerations for future studies to include more sensitive mediators and outcome variables when using multiple manipulations/stress level conditions of a lab-based stressor.

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APPENDIX A: TABLES

**Table A1. Sample Demographics Across Condition**

| | Control | Intermediate | Negative Evaluative |
|---|---|---|---|
| N (128) | 44 | 46 | 38 |
| Gender | | | |
| Male | 50% | 52% | 53% |
| Female | 50% | 48% | 47% |
| BIPoC | 64% | 65% | 55% |
| Race/Ethnicity | | | |
| Native American/Alaskan Native | 2% | 0% | 0% |
| Asian/Pacific Islander | 14% | 4% | 5% |
| Black/African American | 23% | 39% | 37% |
| Hispanic/Latinx | 5% | 2% | 3% |
| White (Non-Hispanic) | 36% | 35% | 45% |
| Biracial | 14% | 17% | 11% |
| Other | 7% | 4% | 0% |

**Table A2. Sample Characteristics Across TSST Condition**

| | Control<br>Mean (SD) | Intermediate<br>Mean (SD) | Negative Evaluative<br>Mean (SD) |
|---|---|---|---|
| Age | 9.42 (1.68) | 18.96 (1.19) | 19.47 (1.72) |
| SES Index | 5.09 (1.86) | 5.14 (1.52) | 5.63 (1.69) |
| Baseline sAA | 113.70 (75.31) | 152.03 (115.90) | 110.29 (66.61) |
| Baseline HR | 68.19 (9.43) | 73.23 (10.53) | 70.80 (10.39) |
| Baseline SBP | 112.30 (11.07) | 112.13 (13.11) | 110.42 (13.58) |
| Baseline DBP | 67.67 (10.09) | 68.01 (8.35) | 68.12 (8.91) |

**Table A3. Manipulation Checks**

| | Condition | N | Mean | Standard Deviation |
|---|---|---|---|---|
| Perceived Evaluation | -1 | 41 | 2.05 | 0.773 |
| | 0 | 46 | 2.87 | 1.002 |
| | 1 | 37 | 3.05 | 0.88 |
| | Total | 124 | 2.65 | 0.988 |
| Positive Evaluation | -1 | 33 | 2.52 | 0.755 |
| | 0 | 39 | 1.92 | 0.807 |
| | 1 | 35 | 1.54 | 0.741 |
| | Total | 107 | 1.98 | 0.858 |
| Negative Evaluation | -1 | 32 | 1.69 | 0.644 |
| | 0 | 39 | 2.21 | 0.864 |
| | 1 | 35 | 2.86 | 0.733 |
| | Total | 106 | 2.26 | 0.887 |

**Table A4. Zero-Order Correlations: Predictors, Covariates, and Outcomes**

| | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* | *9* |
|---|---|---|---|---|---|---|---|---|---|
| ***1. Condition*** | -- | | | | | | | | |
| ***2. PASA*** | *-.003* | -- | | | | | | | |
| ***3. sAA***[a] | *-.004* | *-.009* | -- | | | | | | |
| ***4. HR***[a] | *0.067* | *0.159* | *0.027* | -- | | | | | |
| ***5. SBP***[a] | *.211** | *-0.059* | *-.009* | *.001* | -- | | | | |
| ***6. DBP***[a] | *0.140* | *.60* | *0.017* | *0.178** | *0.558*** | -- | | | |
| ***7. Male Sex*** | *0.021* | *-.087* | *-.086* | *0.081* | *0.124* | *0.195** | -- | | |
| ***8. BIPoC*** | *-0.066* | *.112* | *0.113* | *-0.162* | *0.115* | *0.120* | *-0.088* | -- | |
| ***9. SES*** | *0.125* | *-0.40* | *-.037* | *-0.059* | *0.087* | *0.032* | *0.127* | *0.059* | -- |

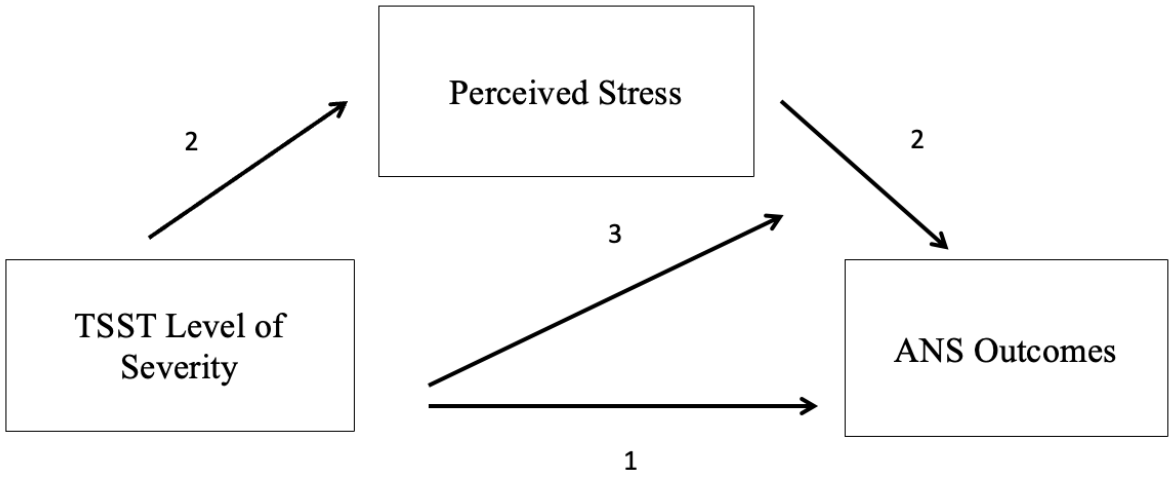
[a]Measured in $AUC_i$

*Correlation is significant at $p < .05$

**Correlation is significant at $p < .01$

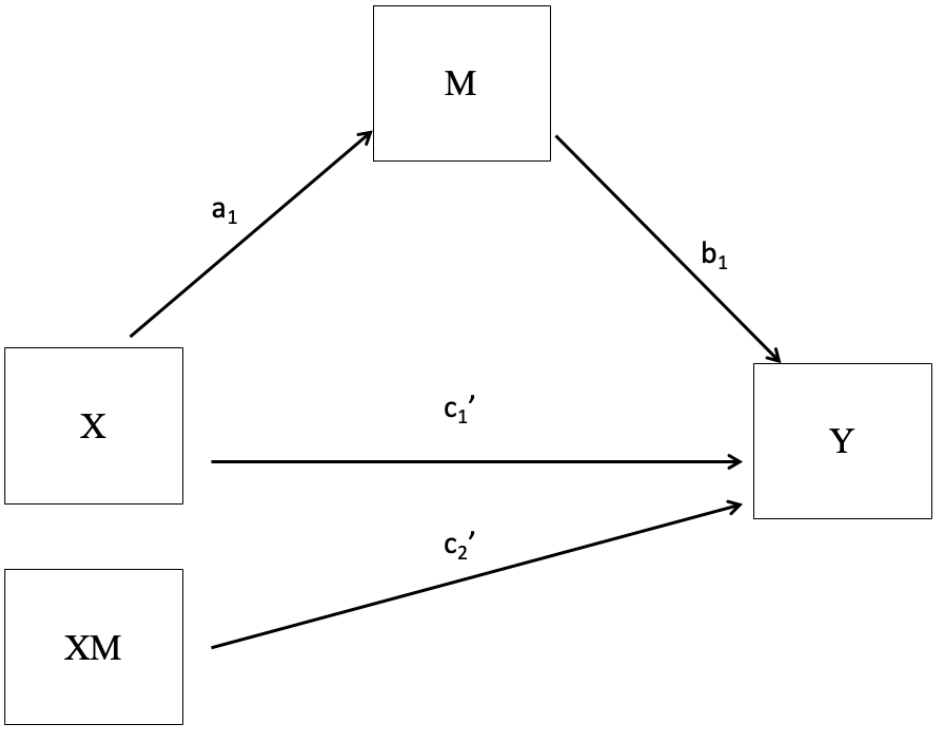
APPENDIX B: FIGURES

**Figure B1. Visualization of Three-Variable System**



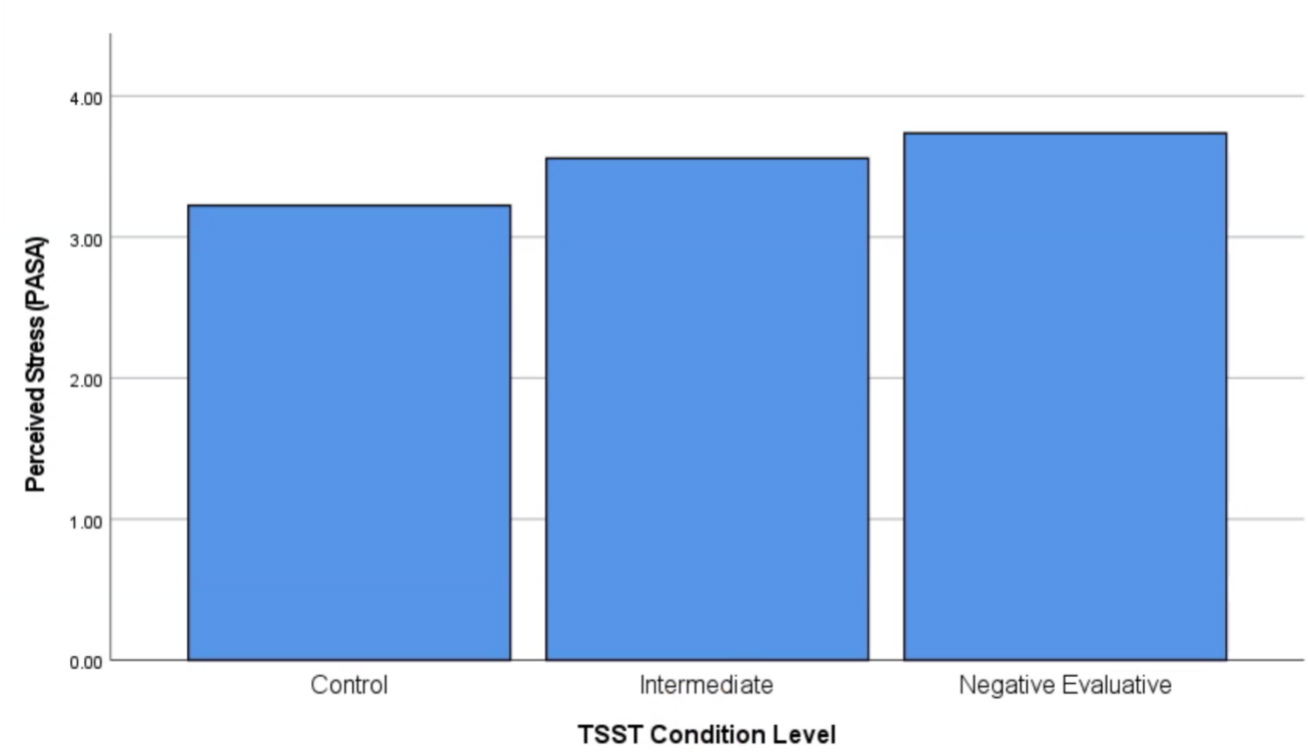

A moderated mediation hypothesis in a three-variable system with TSST Level of Severity as (X), Perceived Stress from the PASA as (M) and ANS Reactivity Indicators as (Y).

**Figure B2. Mathematical Model of Three-Variable System**



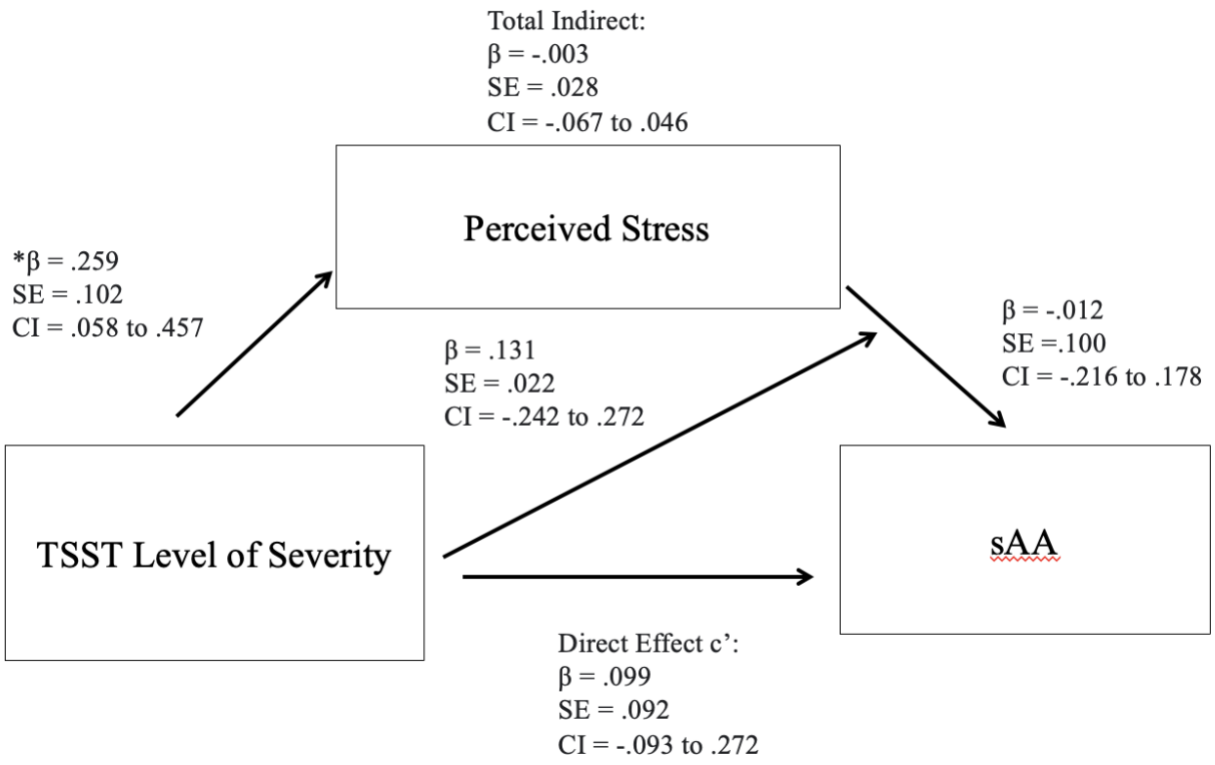

Stride et al., (2015) statistical model depicting a moderated mediation hypothesis in a three-variable system where $c_1$' is the direct effect adjusted for mediation.

**Figure B3. Perceived Stress and TSST Condition Level**



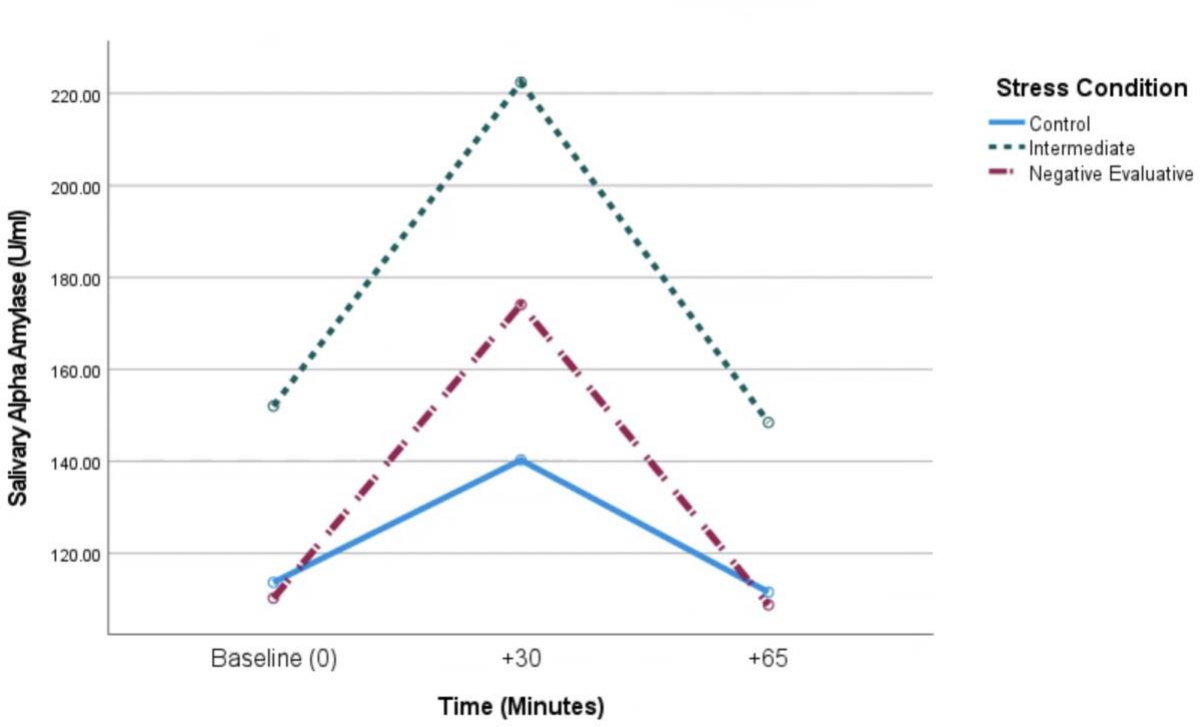

A visualization of the relationship between Perceived Stress (PASA) and TSST Condition Level. The negative evaluative condition reported greater overall perceived evaluation, less perceived positive evaluation, and greater perceived negative evaluation.

**Figure B4. A Visualization of Stress Condition, Perceived Stress and sAA Reactivity (AUCi)**



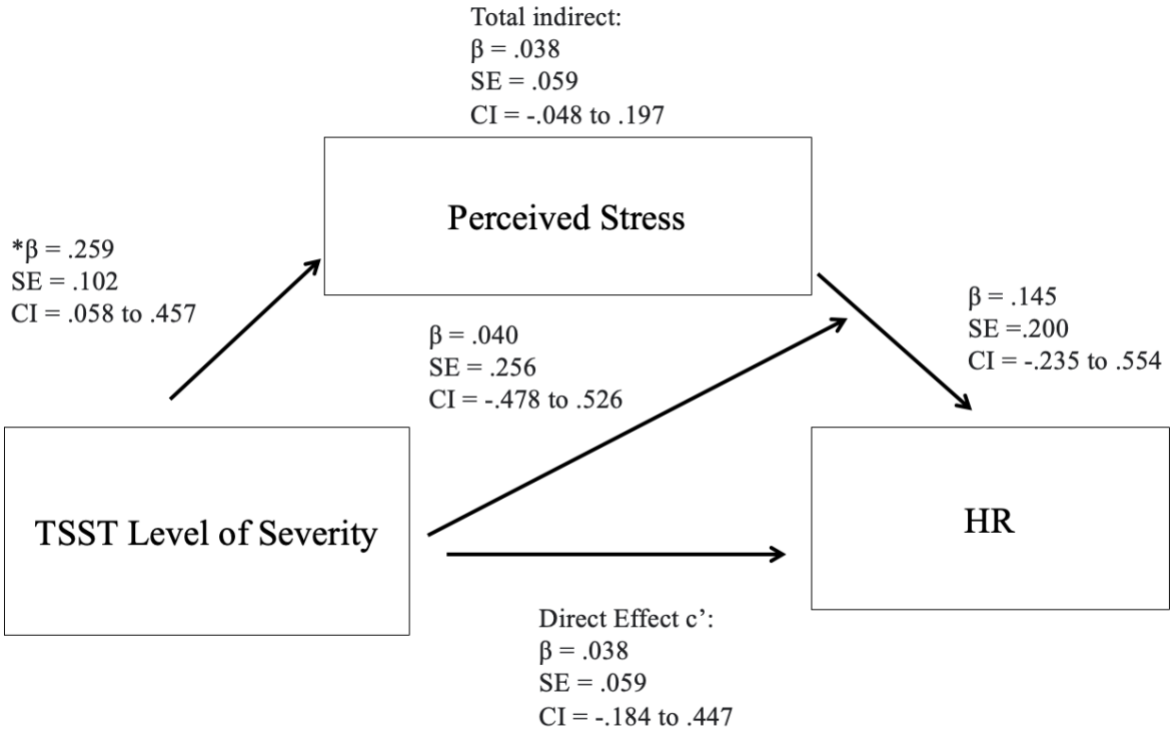

Results of Moderated Mediation Predicting sAA. CI = 95% bootstrapped confidence intervals (10,000 samplings).

**Figure B5. sAA Levels Across TSST Conditions**



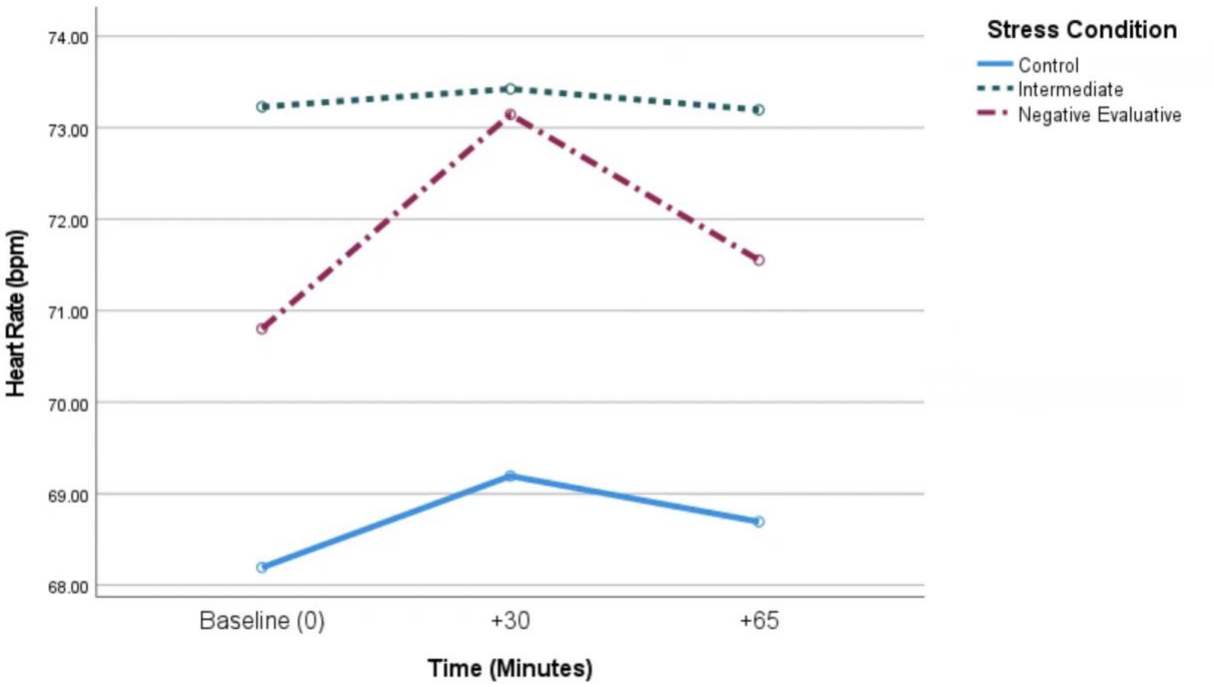

Samples included in the sAA calculation were collected at baseline immediately prior to the TSST (+0 minutes), immediately following the TSST (+30 minutes), and +65 minutes from baseline. sAA was measured in U/ml.

**Figure B6. A Visualization of Stress Condition, Perceived Stress and HR Reactivity (AUCi)**



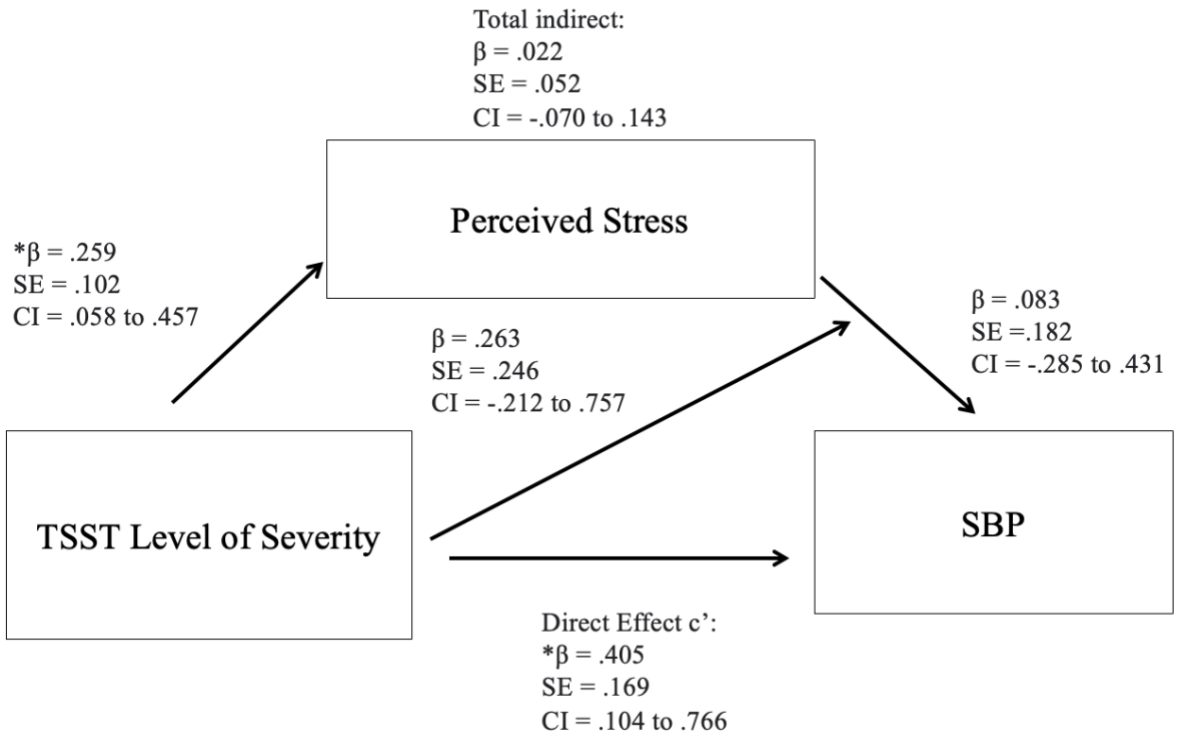

Results of Moderated Mediation Predicting HR. CI = 95% bootstrapped confidence intervals (10,000 samplings).

**Figure B7. HR Levels Across TSST Conditions**



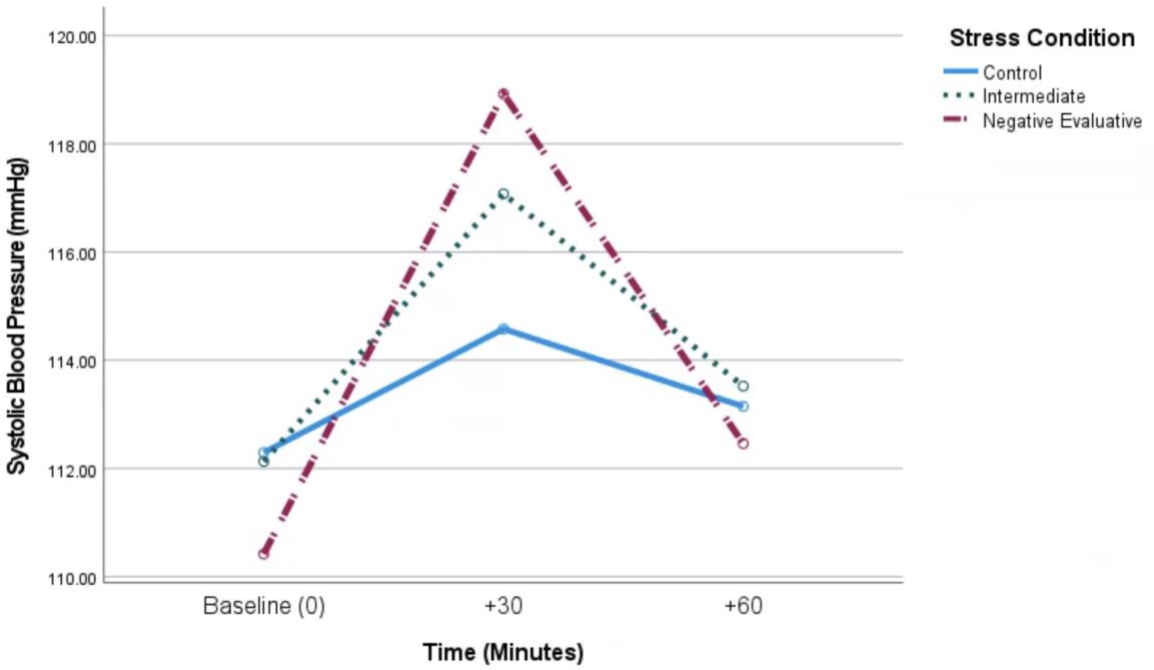

**S**amples included in the HR calculation were collected at baseline immediately prior to the TSST (+0 minutes), immediately following the TSST (+30 minutes), and +65 minutes from baseline. HR was measured in bpm.

**Figure B8. A Visualization of Stress Condition, Perceived Stress and SBP Reactivity (AUCi)**



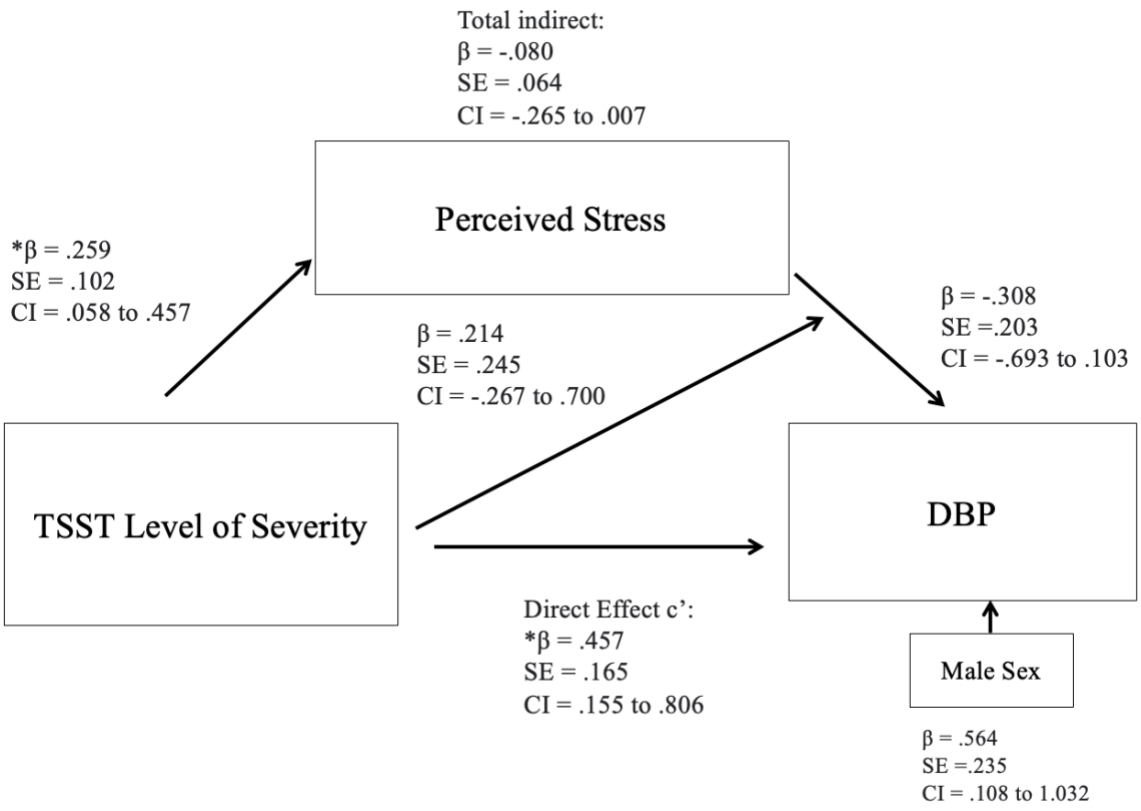

Results of Moderated Mediation Predicting SBP. CI = 95% bootstrapped confidence intervals (10,000 samplings).

**Figure B9. SBP Levels Across TSST Conditions**



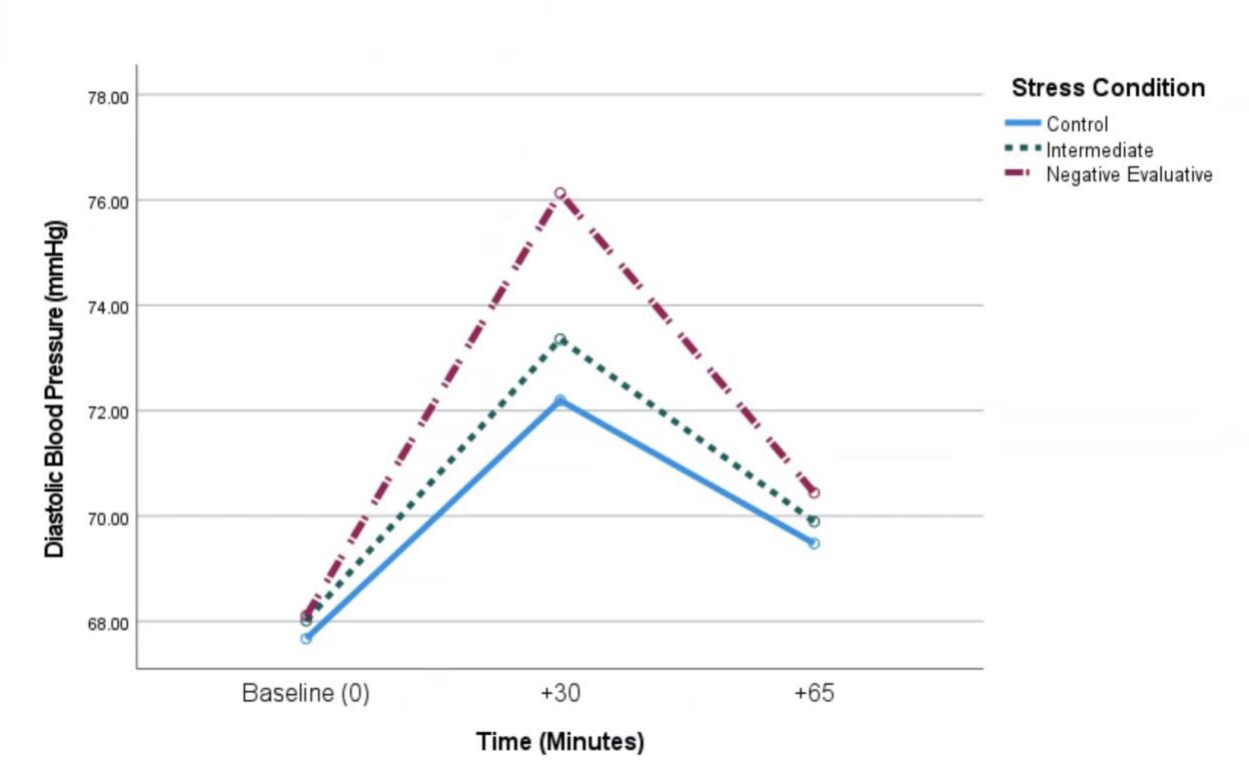

Samples included in the SBP calculation were collected at baseline immediately prior to the TSST (+0 minutes), immediately following the TSST (+30 minutes), and +65 minutes from baseline. SBP was measured in mmHg.

**Figure B10. A Visualization of Stress Condition, Perceived Stress and DBP Reactivity (AUCi)**




Results of Moderated Mediation Predicting DBP. CI = 95% bootstrapped confidence intervals (10,000 samplings).

**Figure B11. DBP Levels Across TSST Conditions**




Samples included in the DBP calculation were collected at baseline immediately prior to the TSST (+0 minutes), immediately following the TSST (+30 minutes), and +65 minutes from baseline. DBP was measured in mmHg.