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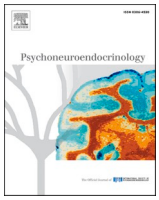

Does high perceived stress over the past month alter cortisol reactivity to the Trier Social Stress Test?

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ABSTRACT

Background: Theories highlight the important role of chronic stress in remodeling HPA-axis responsivity under stress. The Perceived Stress Scale (PSS) is one of the most widely used measures of enduring stress perceptions, and no previous studies have evaluated whether greater perceptions of stress on the PSS are associated with cortisol hypo- or hyperactivity responses to the Trier Social Stress Test (TSST).
Objective: To examine if high perceived stress over the past month, as measured by the PSS, alters cortisol and subjective acute stress reactivity to the TSST in healthy young adults.
Methods: Five studies across three laboratories involving healthy young adults (N = 585) were conducted. Participants were exposed to the TSST, and cortisol levels and subjective stress responses were measured. Studies 1–2 served as exploratory, and Studies 3–5 as explanatory, with pre-registered hypotheses.
Results: Higher PSS scores were consistently associated with greater acute subjective stress perceptions during the TSST across four out of five studies. Meta-analytic results revealed that higher perceived stress on the PSS was associated with blunted cortisol reactivity to the TSST. This cortisol hyporeactivity effect was more pronounced in studies using a combined speech and arithmetic TSST protocol compared to a speech-only protocol. Depressive symptoms did not significantly alter cortisol reactivity effects in these studies.
Conclusion: Persistent high perceived stress over the past month may be associated with greater acute stress perceptions and blunted cortisol reactivity to the TSST. These findings highlight the potential importance of persistent perceived stress in HPA-axis responses to acute stress in healthy young adults, with potential implications for understanding stress-related health risks. Further research is needed to explore the underlying mechanisms and extend findings to diverse populations.

1. Introduction

Cortisol reactivity to acute stress challenge is thought to be an important marker for health risks. For example, previous studies have shown that cortisol reactivity to acute challenge prospectively predicts incidence of upper respiratory tract infections during subsequent high stress periods (Cohen et al., 2002), is associated with dysregulated eating and obesity risk (Epel et al., 2000; Herhaus et al., 2020; Incollingo Rodriguez et al., 2015; van Strien et al., 2013), and may distinguish individuals who have a major depression or anxiety disorder diagnosis (Zorn et al., 2017). However, the direction of cortisol reactivity effects is highly variable, with studies showing that both hyper- and hypo-reactivity of cortisol to acute stress challenge on the Trier Social Stress Test (TSST) can be important for understanding health risks (Allen et al., 2014; Kudielka et al., 2009; Zorn et al., 2017). While previous studies have explored the role of cortisol reactivity moderators like sex and age (Allen et al., 2014; Hellhammer et al., 2009; Kudielka et al., 2009), very little published research has examined how persistent perceived stress alters cortisol reactivity responses to acute stress challenge, which is surprising given that leading theories highlight how enduring or chronic stress alters the responsivity of the HPA-axis. Here we report five studies which examine whether perceptions of stress over

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the past month, as measured by the Perceived Stress Scale (PSS) (Cohen et al., 1983), are associated with alterations in cortisol and subjective stress reactivity to the TSST in healthy young adult samples across three laboratories.

There has been extensive theoretical and empirical scholarship examining how chronic stress impacts the HPA-axis (Cohen et al., 1995, 2007; Melamed et al., 2006; Miller et al., 2007). In a now classic meta-analysis and review, Miller et al. (2007) showed that initial stressor exposures increase adrenocorticotropic hormone (ACTH) and cortisol levels, but as these stressors persist there is a rebound below baseline on daily cortisol output measures. Specifically, the number of months since a stressor first emerged was inversely associated with cortisol production (and cortisol production in response to a dexamethasone challenge), suggesting that longer chronic stress exposure duration is associated with hypocortisolism (Miller et al., 2007). While much of the work focusing on chronic stress and HPA-axis responses has focused on daily measures of cortisol (such as total daily cortisol output), studies which have looked at markers of chronic stress (e.g., early life stress, burnout, PTSD) and cortisol responses to acute stress challenge have concluded that there may be a cortisol hyporeactivity effect among chronically stressed participants (Kudielka et al., 2009; Metz et al., 2020; Young et al., 2021).

The Perceived Stress Scale (PSS) is one of the most widely used stress instruments, measuring self-reported perceptions that one's life feels unpredictable and uncontrollable over the past month (Cohen et al., 1983). Previous studies show that higher scores on the PSS predict a broad range of stress-related health risks, including a greater risk for upper respiratory tract infections (Cohen et al., 1999), greater mortality risk after an acute myocardial infarction (Arnold et al., 2012), and greater likelihood of utilizing mental health services (Prior et al., 2018). Despite the widespread use of the PSS and the TSST in scientific studies, to our surprise there has been no published research examining whether higher perceived stress over the past month moderates acute cortisol reactivity responses to the TSST. In contrast to studies looking at other measures of enduring stress (e.g., early life stress, burnout, PTSD) which show a cortisol hyporeactivity response, this effect is mixed when looking at more general measures of life stress. In a meta-analytic review of wide-ranging laboratory stress reactivity studies, Chida and Hamer (2008) showed that measures of general life stress had no effect on measures of HPA-axis reactivity, and that this effect might skew toward hyperreactivity responses. One strength of using the PSS relative to previous studies looking at a broad range of background stressors is that it more carefully measures background perceptions of stress over the past month as a common metric of background stress.

Here we report five studies (conducted in three independent laboratories) which examine the role of PSS in moderating cortisol and subjective stress reactivity to the TSST in healthy young adults. The TSST is a widely used laboratory stress challenge task, which reliably increases salivary measures of cortisol, increases blood pressure and heart rate, and increases acute subjective stress perceptions (Allen et al., 2014, 2017; Kirschbaum et al., 1993). The TSST provides an optimal way to explore how PSS moderates both subjective stress perceptions and cortisol reactivity responses, and the possibility that individuals who have higher perceived stress over the past month have *higher* acute stress perceptions (Epel et al., 2018) but blunted cortisol reactivity to the TSST. There are now many adaptations to the original TSST paradigm (Kirschbaum et al., 1993) for different experimental contexts, some of which are represented in the five studies reported here. In two initial discovery studies (Studies 1–2) we first explored whether there was a relationship between PSS and stress reactivity to the TSST. In these studies, we found that PSS was associated with greater subjective stress perceptions and blunted cortisol reactivity to the TSST. We then pre-registered our protocols and hypotheses that greater perceived stress on the PSS would be associated higher stress perceptions, but blunted cortisol reactivity response to the TSST, and then tested these predictions in three independent datasets (Studies 3–5) (https://osf.io/b2m4q). Depressive symptomatology (such as that measured with the Center for Epidemiological Studies Depression Scale, CES-D) (Lewinsohn et al., 1997) is positively associated with the PSS and may be associated with blunted cortisol reactivity (Burke et al., 2005; Cohen et al., 1983), so we also report supporting analyses exploring whether depressive symptomatology moderates acute cortisol reactivity to the TSST.

2. Methods

2.1. Participants and study design

2.1.1. Overview

All participants provided written informed consent. Each study was approved by the host institution's Institutional Review Board. Table 1 displays the demographic characteristics of each sample. Descriptions of how salivary cortisol was assayed in each study are available in the online supporting materials.

2.1.2. Discovery Study 1 participants

The Study 1 sample was conducted at a private STEM-focused university in the mid-Atlantic region in 2016–2017. We recruited 163 healthy male and female young adult participants (56.4 % female) from nearby universities and the surrounding community using flyers, newspaper advertisements, and participant registries. Inclusion criteria for the participants were being between the ages of 18 and 30 years, fluent in English, mentally and physically healthy (i.e., no medical diagnosis of a cardiovascular, respiratory, chronic, or mental disease or disorder), not currently taking medications for a mental health problem, no previous participation in studies that used similar study protocols (e.g., TSST), not pregnant or nursing, and not currently taking hormonal birth control (i.e., oral contraceptives, hormonal intrauterine devices). We excluded one participant who elected to discontinue their participation in the study upon hearing the instructions for the TSST, and one participant for erratic behavior and responses during the study session that prevented us from administering the protocol in the standard manner.

2.1.3. Discovery Study 1 procedure

Interested participants completed a telephone screening questionnaire to confirm their eligibility in the study. Recruitment and screening materials explained that the study examined how individuals perform tasks and did not mention stress. Participants were asked to refrain from drinking alcohol or taking any illicit drugs for at least 24 hours before their study session. Participants arrived for study sessions one at a time and completed the sessions between 1400 h and 2000 h to control for diurnal variation in cortisol. Upon arrival, participants received more details about the study procedures and provided written informed consent. Participants were notified they would be video- and audio-recorded during the performance tasks. After completing an initial set of questionnaires, participants provided baseline cortisol saliva samples 40 minutes after arrival. They then completed a second set of questionnaires, including the 10-item PSS.

2.1.4. Discovery Study 1 TSST

Participants heard pre-recorded instructions for the upcoming speech performance task and were told they would be given instructions for the arithmetic task upon completing the speech. The instructions stated that the participants would give a five-minute speech as an applicant for the role of an administrative assistant, and that the speech would be given without the use of any notes or written materials in front of a panel of evaluators, who would evaluate the participants' speeches. Participants were given three minutes to mentally prepare their speech. Two evaluators dressed in white lab coats were trained to act in a neutral and non-accepting manner. Each evaluator interrupted the participants' speeches to provide 3–5 critical comments, while also writing notes and

Table 1
Demographic characteristics of the five samples.

Variable	Study 1		Study 2		Study 3		Study 4		Study 5	
	N	%	N	%	N	%	N	%	N	%
Age (M yrs ± SD)	22.17 (2.99)		19.40 (1.41)		29.52 (11.96)		19.81 (1.30)		19.88 (1.62)	
Gender (female)	92	56.4	53	43.4	92	67.2	66	48.9	56	72.7
Race										
White	89	54.9	17	13.9	70	51.9	17	12.6	21	26.9
Black	19	11.7	03	02.5	36	26.6	02	01.5	–	–
Pacific Isl	01	0.6	01	0.80	04	3.0	19	14.1	–	–
Asian	49	30.2	52	42.6	22	16.3	58	42.0	36	46.2
Mid Eastern	–	–	12	09.8	–	–	06	04.4	–	–
Filipino	–	–	10	08.2	–	–	10	07.4	–	–
Vietnamese	–	–	10	08.2	–	–	18	13.3	–	–
Multi/Other	04	2.5	17	13.9	03	2.2	–	–	21	26.9
Ethnicity										
Hispanic	05	3.1	12	09.8	03	02.0	05	03.7	14	17.9
PSS (M ± SD)	15.85 (6.31)		25.90 (6.18)		14.82 (7.37)		17.60 (5.77)		17.99 (5.92)	
TSST stress (M ± SD)	51.00 (22.90)		04.45 (1.77)		–	–	03.87 (1.78)		04.35 (1.84)	

checking boxes on a note sheet attached to the evaluators' clipboards. After participants completed the speech task, the evaluators exited the room as the experimenter provided instructions over an intercom for the participants to rate how stressed or anxious they felt on a 100-point visual analog self-report scale. Participants also squeezed a hand-held dynamometer (Creswell et al., 2019). The evaluators then returned to the room and provided instructions for the five-minute arithmetic task, which consisted of solving a mental calculation task by counting aloud backwards from 2083 in increments of 17. Participants were asked to begin again at 2083 after each mistake, and both evaluators provided 2–4 critical comments while once again making notes and checking boxes on their clipboards. After the participant completed the task, the evaluators exited the room and the experimenter once again instructed the participants over an intercom to express how stressed or anxious they felt by completing the visual analog self-report scale.

Following the task, participants were left alone in the experiment room for 5 minutes. After this recovery period, the participants completed a variety of questionnaires, most for other study purposes but including a health behaviors scale assessing the cups of caffeinated beverages the participants drink typically and on the day of the study session, smoking behaviors, typical alcohol consumption, and illicit or prescribed drugs taken on the day of the session. While the participants completed the questionnaires, the experimenter collected the second and third saliva samples 25 and 35 minutes after the start of the performance task. The final saliva sample was collected 60 minutes after the start of the TSST. Participants were then debriefed and informed of the study's aims, including the purpose of the TSST to reduce any distress experienced.

2.1.5. Discovery Study 2 participants and procedure

The second study was conducted at a public western U.S. university in 2006–2007. Participants (N = 114; 43.4 % female) were recruited through the university subject pool or fliers posted on campus. People who smoked, used oral contraception, were pregnant, did not generally wake up by 10:00 AM on weekdays, or had health conditions or took medications known to influence HPA axis functioning were excluded. To limit diurnal variations in cortisol, all study sessions took place in the afternoon or evening (between 12PM and 5:30PM) and lasted approximately two hours. The study procedure deviated from the standard TSST in that there was no arithmetic part to the performance task (speech only). Saliva was collected at 5 time points: baseline, then + 15, + 25, + 40, + 55 min after onset of the speech task.

2.1.6. Explanatory Study 3 participants and procedure

Study 3 was conducted at a public mid-Atlantic U.S. university from 2009 to 2011. Participants were 149 university employees (n = 81) and students (n = 68) recruited through poster and e-mail advertisements (67.2 % female). Study 3 procedures were the same as in Study 1. Saliva was collected at 5 time points: baseline, then + 10, + 20, + 30, + 45 min after TSST onset of the speech task.

2.1.7. Explanatory Study 4 participants and procedure

Study 4 was conducted at the same public western U.S. university as Study 2, during 2008–2009. Participants (N = 81; 48.9 % female) were recruited through the university subject pool or fliers posted on campus. Sample inclusion/exclusion criteria and study procedures were the same as Study 2, using a modified TSST with a speech task only. Saliva was collected at five time points: baseline, then + 10, + 20, + 35, + 55 min after onset of the speech task.

2.1.8. Explanatory Study 5 participants and procedure

Study 5 was conducted at the same public western U.S. university as Studies 2 and 4, with data collection occurring in 2006–2007. Participants (N = 44; 63.6 % female) were recruited through the university subject pool or fliers posted on campus. Participant and procedures were the same as Studies 2 and 4 (using a modified TSST with a speech task only), but the study procedure manipulated (by random assignment) the affective valence of the two TSST evaluators. Specifically, in the negative condition, both evaluators maintained stoic demeanors, as in the standard TSST; in the positive condition, both evaluators maintained positive demeanors, conveying interest and smiling in affirmation; and in the third condition, one evaluator was negative (stoic), the other positive. This paper focuses on the negative evaluation condition but analyses using all conditions are presented in the Supplementary Materials. The second difference from the standard TSST was that only the 5-min speech task was performed (and not the arithmetic task). Saliva was collected at 5 time points: baseline, then + 15, + 25, + 40, + 55 min after onset of the speech task.

2.1.9. Explanatory Study 5

This study was also conducted at a public western U.S. university in 2006. Participants (N = 34; 84.8 % female; n = 1 undeclared) were students recruited and screened as in the Study 4 sample, above. All study sessions took place in the afternoon or evening and lasted approximately two hours. This study differed from the standard TSST by experimentally manipulating the number of evaluators present in the 5-min speech task. In one condition there were 2 evaluators; in the second condition there was one evaluator. This paper focuses on the standard TSST two evaluator condition but analyses using both conditions are presented in the Supplementary Materials. An additional deviation from the standard TSST was that there was no arithmetic task, as in Study 2 and Study 4. Saliva collection was done at 5 time points: baseline, then + 15, + 25, + 40, + 55 min after onset of the speech task. PSS and perceived stressfulness of the speech task was measured as in the Study 4

sample. For purposes of analysis, Study 5 samples 5a and 5b were combined to increase statistical power.

2.2. Measures

2.2.1. Perceived stress

Chronic Background Perceived Stress was measured with the *Perceived Stress Scale* (PSS), a 10-item self-report measure of past-month personal stress perceptions on a 5-point Likert scale ranging from 0 (*not at all*) to 4 (*very often*) (e.g., "In the last month, how often have you felt that you were unable to control the important things in your life?") (Cohen et al., 1983). Relevant items were reverse-coded, and all items were summed to create a total perceived stress score (sample Cronbach's α =.85 in all studies), with higher scores reflecting higher stress.

Acute perceptions of stress in response to laboratory stressors were measured in Study 1 by two questions, one immediately after the math task and the other immediately after the speech task. The wording of the questions was identical: "How stressed do you feel RIGHT NOW on the scale with 0 being no stress at all and 100 being the most intense stress you've ever felt." Cronbach's α was.80 so the two questions were averaged for analysis. Acute stress perceptions were measured in Studies 2, 4, and 5 by one question after the TSST: "How stressful was the task?" and was rated on a 1 (*not at all*) to 7 (*very much*) scale. Acute stress perceptions were not measured in Study 3.

2.2.2. Depressive symptoms

We assessed depressive symptoms in Studies 2, 4, and 5 to determine whether PSS results are independent of potential effects of depressive symptoms. Depressive symptoms were assessed at baseline using the Center for the Epidemiological Scale-Depression (CES-D) (Radloff, 1977). This 20-item scale (sample Cronbach's α range =.81 to.83) asked participants to report on their depressive symptoms over the last week (e.g., "I felt that everything I did was an effort."). Items were answered on a 0 ("Rarely") to 3 ("Most or all of the time") scale and summed to create a total CES-D score, where higher scores indicated greater depressive symptoms (score range = 0 – 60). In Study 3, depressive symptoms were assessed using the Beck Depression Inventory (BDI) (Beck et al., 1988). Each item is rated on a 0–3 scale based on self-reported severity in the past two weeks (e.g., "I am so sad and unhappy that I can't stand it"). Items are summed to create a depressive symptoms score with higher scores reflecting more severe symptoms (score range = 0 – 63; sample Cronbach's α=.85).

2.2.3. Salivary cortisol

Study 1. Salivary cortisol was collected using a Salivette (Rommelsdorf, Germany). All Salivettes were frozen at −20°C in a locked and secure laboratory freezer. Participants kept the Salivette under their tongue for 2 min during each collection period and did not touch the sample with their hands. At the conclusion of the experiment, the samples were shipped on dry ice to a professional laboratory in Dresden, Germany specializing in cortisol measurement. At this laboratory, cortisol was measured using a chemoluminescence-immuno-assay with high sensitivity (Immuno-Biological Laboratories; IBL; Hamburg, Germany). Intra- and inter-assay coefficients of variation were below 10 %. Raw cortisol concentration values are reported in nmol/L.

Studies 2, 4, 5. Saliva samples were collected with Salivette swab collection devices in all three studies (Salimetrics, State College, PA). After collection, saliva samples were stored at −20°C until the end of each study enrollment period and then assayed in duplicate (results averaged) using commercially available enzyme-linked immunosorbent assays (Diagnostic Systems Laboratories [DSL; Study 2] and IBL (Study 4, Study 5). The DSL assays had a sensitivity of less than 0.012 μg/dl and inter-assay and intra-assay coefficients of variation (CV) of less than 8 %. The IBL assays had a sensitivity of 0.005 μg/dl, with average inter- and intra-assay coefficients of covariance of less than 10 %. Raw cortisol concentration values were converted to standard units (nmol/L).

Study 3. Saliva samples were collected with Salivette swab collection devices. After the session, samples were stored at −20 °C until batch processed. Samples were then thawed and centrifuged for 15 min at 1500 g at 10 °C. Cortisol was assayed using the Salimetrics competitive immunoassay method. Inter-assay CVwas 6.69—6.88 %, intra-assay CV was 3.88—7.12 %, and the sensitivity was < 0.007 μg/dl. The raw (untransformed) salivary cortisol values are presented in Supplementary Table 1 for each study (1 – 5) and time point of data collection in the TSST.

2.3. Statistical analysis

Multilevel modeling (mixed modeling) was used to test all questions pertaining to cortisol response. Preliminary analyses first tested for the presence of change in cortisol response over time, whether linear (time) or curvilinear (time × time, or time^2). Next, to test the hypothesis concerning PSS prediction of cortisol, primary interest was in the PSS × time^2 interaction effects on cortisol response. These analyses tested whether the curvilinear slope of cortisol over time was moderated by PSS score. Sensitivity analyses were performed to test whether the hypothesized PSS results remained significant after controlling for depressive symptoms at baseline.

Time points of saliva collection were coded 0–4 (0–3 in Study 1). Demographic covariates, namely sex, age, and race were included in the preliminary analyses. Race was dummy coded (0, 1) as White versus others, Asian versus others, etc. Results pertaining to the demographic variables are presented in the Supplementary Materials. All continuous variables were standardized before analysis to facilitate comparison of results across studies. Distributional assumptions concerning univariate normality, assessed via inspection of skewness and kurtosis were checked for all continuous variables. No transformations were required to achieve normality of distributions except for raw cortisol values, which were natural log transformed. No missing cortisol values were imputed. One outlying log cortisol value in Study 5 was removed. Ordinary least squares (OLS) regression models were used to test the hypothesis concerning baseline PSS score prediction of perceived stressfulness of the TSST. The same demographic variables as noted above were included in preliminary analyses and are presented in the Supplementary Materials. In all multilevel and OLS regression analyses, statistical significance was set at $p < .05$. Confidence intervals were set at 95 % probability.

3. Results

3.1. Preliminary analyses of cortisol and TSST stress perception outcomes

3.1.1. Perceived stress

A one-way ANOVA model tested whether PSS scores differed by study. This model revealed significant differences between studies [$F(4623) = 6.36, p < .0001$]. Tukey post-hoc tests showed that the Study 2, 4, and 5 PSS scores ($M = 18.07$, $SD = 6.45$; $M = 17.59$, $SD = 5.77$; and $M = 17.99$, $SD = 5.92$, respectively) were significantly higher than those of the Study 1 ($M = 15.85$, $SD = 6.31$) and Study 3 ($M = 14.82$, $SD = 7.38$), $ps < .05$.

3.1.2. Cortisol response

Initial analyses evaluated whether each study's TSST elicited robust salivary cortisol reactivity. Linear mixed models tested for a significant time × time (time^2), or curvilinear change (rise and fall) in cortisol across time points. As shown in Table 2, both the discovery studies (Studies 1 and 2) and the explanatory studies (Studies 3–5) elicited a significant time × time effect on salivary cortisol, such that there was a significant TSST-provoked rise and fall of cortisol over time. The studies varied considerably in magnitude of peak reactivity, which was defined as the difference in mean cortisol level between the baseline and the 20 or 25 min saliva collection points. In all five studies, the highest levels of

Table 2
Multilevel modeling of TSST cortisol response regressed on perceived stress and time (Studies 1–5).

Estimates	*Time*	*Time*Time*	*PSS*	*Time*PSS*	*Time*Time*PSS*
Discovery Study 1					
Estimate	1.03	−0.35	0.04	−0.26	0.06
Std Error	0.07	0.02	0.07	0.07	0.01
95 % CI lower	0.91	−0.39	−0.10	−0.38	0.02
95 % CI upper	1.17	−0.31	0.18	−0.13	0.10
P	**< .001**	**< .001**	0.60	**< 0.001**	**0.002**
Discovery Study 2					
Estimate	0.18	−0.04	0.13	−0.24	0.05
Std Error	0.07	0.02	0.09	0.07	0.02
95 % CI lower	0.05	−0.08	−0.06	−0.38	0.02
95 % CI upper	0.32	−0.01	0.30	−0.11	0.09
P	**0.01**	**0.01**	0.19	**< 0.001**	**0.001**
Explanatory Study 3					
Estimate	0.50	−0.11	−0.01	−0.01	0.03
Std Error	0.04	0.01	0.01	0.01	0.001
95 % CI lower	0.42	−0.13	−0.03	−0.03	0.0001
95 % CI upper	0.57	−0.09	0.01	−0.001	0.06
P	**< .001**	**< .001**	0.30	**0.04**	**0.046**
Explanatory Study 4					
Estimate	0.31	−0.09	−0.08	−0.003	0.01
Std Error	0.07	0.02	0.12	0.07	0.002
95 % CI lower	0.18	−0.12	−0.31	−0.18	−0.02
95 % CI upper	0.45	−0.06	0.16	0.01	0.05
P	**< .001**	**< .001**	0.52	0.65	0.50
Explanatory Study 5					
Estimate	0.006	−0.0002	0.001	−0.0004	−9.98E−6
Std Error	0.004	0.0001	0.004	0.003	0.0001
95 % CI lower	−0.002	−0.0003	−0.009	−0.01	−0.0001
95 % CI upper	0.01	−0.0001	0.007	0.004	0.0001
P	0.13	**0.004**	0.79	0.87	0.88

Notes. TSST = Trier Social Stress Test; PSS = Perceived Stress Scale. Estimates, standard errors and confidence intervals are based on standardized data. Bold values are significant at $P < .05$.

cortisol were found at this 20–25 min time point. Following Cumming (2011), the effect size of peak reactivity, *d*, was defined as the mean difference in cortisol level between the two time points divided by the average of their standard deviations. Discovery Study 1 and explanatory Study 3 showed large effect sizes (d =.85 and d =.84, respectively). The effect sizes for discovery Study 2 and explanatory Studies 4 and 5 were in the small-to-medium range: $d = .26$, $d = .44$, and $d = .14$, respectively (d_{mean} =.28).

3.1.3. TSST stress perceptions

We hypothesized in the preregistration that participants scoring higher in chronic background perceived stress on the PSS would report greater acute perceptions of stress experienced during the TSST. All studies (except Study 3) included an acute perceived stress perceptions measure immediately post-TSST (see Measures). Our hypothesis was supported in three of the four samples (and was marginally significant in the fourth sample), as follows: In Discovery Study 1, higher PSS score was related to greater acute stress perceptions to the TSST (b =.23, SE =.08, 95 % CI [.08,.39], p = .003). In Study 2 as well, higher PSS was associated with higher stress perception (b =.32, SE =.09, 95 % CI [.14,.49], p < .001). In Study 4, the effect was marginally significant (b =.23, SE =.12, 95 % CI [-.01,.47], p = .06). In Study 5, higher PSS was again related to higher acute stress perceptions (b =.33, SE =.12, 95 % CI [.09,.56], p = .01). In none of the studies was gender identification a moderator of the association between PSS score and stress perceptions (ps > .49).

3.2. Five study mini meta-analysis of PSS moderation of salivary cortisol reactivity

Similarities in procedures across the studies, including the cortisol outcome and use of the PSS, allowed for meta-analysis of effect sizes pertaining to the observed relations of PSS to log cortisol reactivity in the TSST. The meta-analytically derived summary mean effect across studies has greater precision than do single study results; thus, we first asked whether the effect size for the relations of interest were stable (and secondarily, statistically significant) across studies. Details on the model choice and specific hypotheses are given in the Supplementary Materials.

It was predicted that PSS would moderate cortisol reactivity over time, such that participants higher on PSS would show blunted salivary cortisol reactivity to the TSST. Consistent with this prediction, the overall PSS × time^2 interaction effect size was significantly greater than zero (Z = 2.06, p = 0.04), and overall small in magnitude d = .09 (ES 95 % CIs = [0.004, 0.17]. The heterogeneity among the included studies was evaluated using the Q-test. The results indicated no significant heterogeneity (Q(4) = 1.78, p = 0.775), suggesting that the effect sizes were not highly inconsistent across studies. Consequently, the pooled effect size is considered a reliable estimate of the overall effect. Specifically, as shown in Fig. 1, across five studies participants scoring higher on the PSS showed blunted cortisol reactivity over time, relative to participants with lower PSS scores. It is important to note, however, that there were differences in study design and individual study results are described below.

3.3. Discovery Studies 1 and 2

Prior to preregistering predictions (https://osf.io/b2m4q), we first conducted discovery research that explored whether the PSS moderated cortisol reactivity in two independent datasets using the standard TSST paradigm which included a speech and math task (and two performance evaluators). These two studies showed that PSS significantly moderated the time × time pattern of cortisol reactivity ($b = 0.06$, SE =.02, 95 % CI [0.02,.10], $p = .002$; and $b = 0.05$, SE =.02, 95 % CI [0.02,.09], $p = .001$, respectively). Higher PSS scores were associated with lower (blunted) cortisol reactivity to the TSST, relative to participants lower in PSS (see Table 2 and Fig. 2, panels A and B).

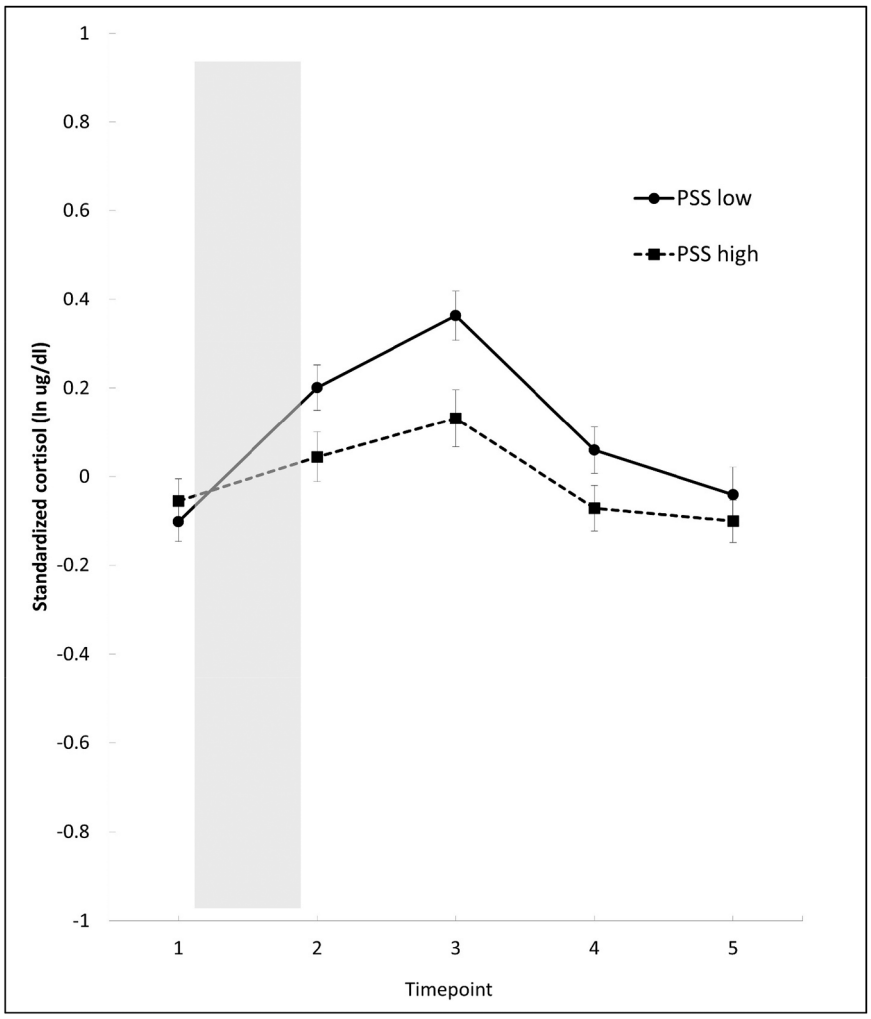

Fig. 1. Mean salivary cortisol responses to the Trier Social Stress Test across all five studies according to high and low perceived stress (± 1 *SE*).*Notes.* PSS = Perceived Stress Scale; dashed line = high PSS; solid line = low PSS). Across studies the task was performed in the time period between the first and second saliva collections. Cortisol values have been standardized.

3.4. Explanatory Studies 3-5

Analyses then tested the preregistered prediction that PSS would moderate salivary cortisol reactivity to the TSST in three independent datasets, Studies 3–5. Specifically, we tested the prediction that past-month perceived stress would predict blunted cortisol reactivity in the TSST, as was shown in the discovery datasets. In Study 3, this prediction was confirmed (b = 0.003, SE =.001, 95 % CI [0.0001,.01], p = .046; see Table 2 and Fig. 2, panel C). In Studies 4 and 5, which used modified versions of the TSST with a speech task only, the PSS effect was not statistically significant (b = 0.01, SE =.02, 95 % CI [-0.02,.01], p = .50; and b = −9.98E-6, SE =.0001, 95 % CI [-0.0001,.0001], p = .88, respectively; see Table 2 and Fig. 2, panels D and E).

In only Study 3 was gender identification a moderator of the interaction between PSS score and cortisol reactivity (p = .02; in the other studies, ps > .13). In Study 3, males with higher PSS scores showed higher cortisol reactivity than males with lower PSS scores. Females in Study 3 showed the higher stress – blunted cortisol reactivity pattern reported above.

3.5. Supporting analyses: Depressive symptomatology

It is possible that these associations of the PSS with cortisol reactivity might actually be attributable to depressive symptoms that often positively correlate with chronic perceived stress. To examine this hypothesis, we tested whether there was a depressive symptoms × time^2 interaction on cortisol reactivity, including the main effects of depression symptoms and of time, and the symptoms × time^2 interaction in the equation. There was no significant depressive symptomatology (as measured by CES-D in Studies 2,4,5, and BDI in Study 3) × time^2 interactions in the four studies where depressive symptomatology was assessed: Study 2 b = .0002, SE = .002, 95 % CI [-.004,.005], p = .94; Study 3 b = .01, SE = .01, 95 % CI [-.01,.04], p = .29; Study 4 b = .02, SE = .02, 95 % CI [-.02,.06], p = .28; and Study 5 b = -.0001, SE = .004, 95 % CI [-.003,.01], p = .19. Since baseline depressive symptomatology was not associated with cortisol response, it could not provide an alternative explanation for the association between PSS and cortisol responses in these TSST studies with young adults.

4. Discussion

In five studies across three different laboratories, the present work is the first to show that higher stress perceptions over the past month (measured by the PSS) were associated with greater acute perceptions of stress and blunted cortisol responses to the TSST. This is the first study to show that chronic feelings that one's life is unpredictable and uncontrollable over the past month are associated with alterations in HPA-axis responses to the TSST. Our mini meta-analysis of these five studies in

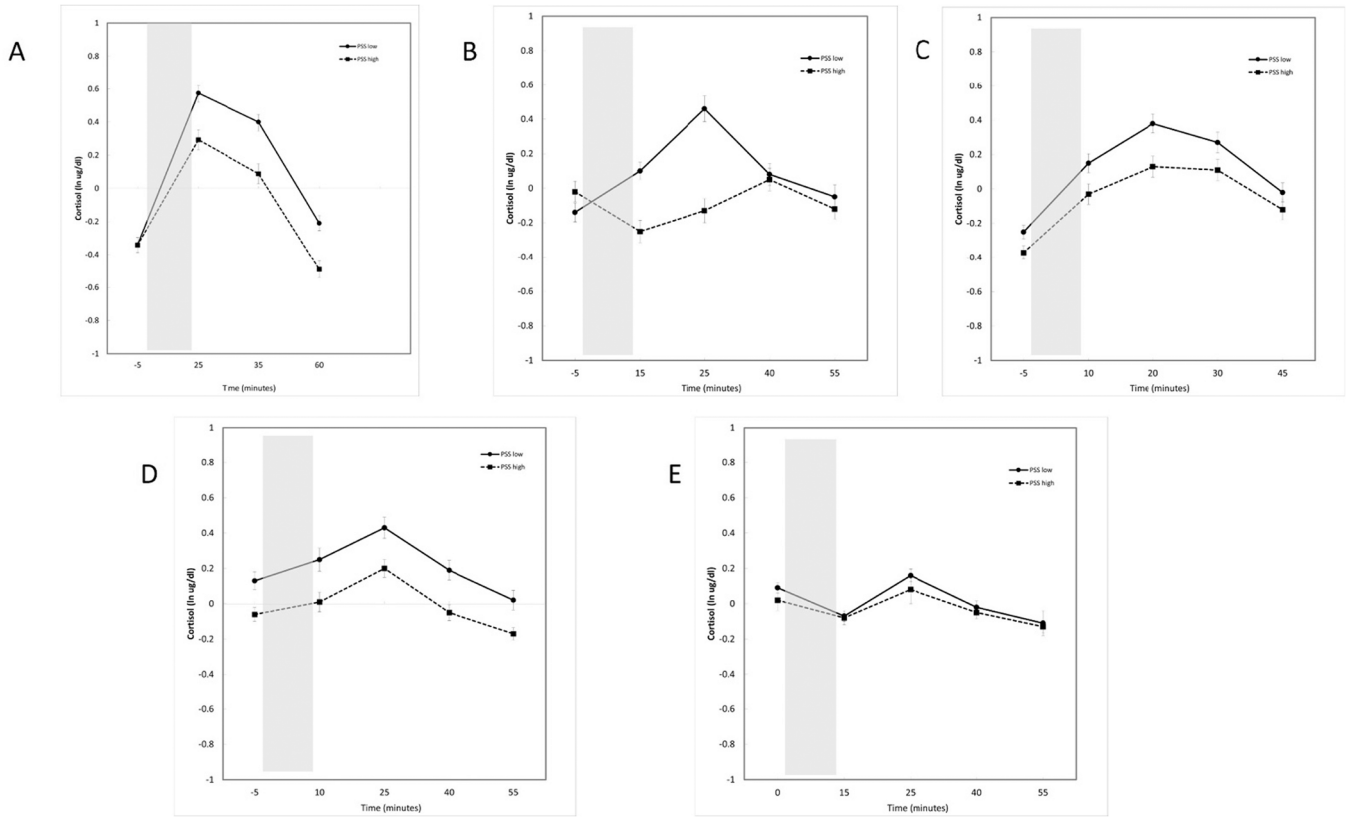

Fig. 2. Mean salivary cortisol responses to the Trier Social Stress Test according to high and low perceived stress (± 1 *SE*) in each of the five studies.*Notes*. PSS = Perceived Stress Scale; dashed line = high PSS; solid line = low PSS). Task was performed in the time period between the first and second saliva collections. Panel A = Discovery Study 1; panel B = Discovery Study 2; panel C = Explanatory Study 3; panel D = Explanatory Study 4; panel E = Explanatory Study 5. Cortisol values have been standardized.

young adult samples shows that the overall PSS cortisol TSST hyporeactivity effect is small, with some studies showing a more robust PSS effect (Studies 1, 2, 3) and other studies not showing a statistically significant effect (Studies 4,5). Broadly the findings here contribute to a somewhat mixed literature on the effects of chronic stress on TSST cortisol reactivity (cf. Gump and Matthews, 1999). While some meta-analytic reviews have suggested that measures of chronic stress blunt cortisol reactivity (Kudielka et al., 2009; Miller et al., 2007), others have suggested no effect (Chida and Hamer, 2008). The findings here support both accounts. Specifically, there was reliable peak cortisol responses and PSS cortisol hyporeactivity effects among studies that used a more standard speech and math task TSST protocol (Studies 1, 3), whereas there were smaller peak cortisol responses and mixed evidence for PSS cortisol hyporeactivity effects among studies that used a speech task only in the TSST (i.e., Study 2 showed a PSS hyporeactivity effect, but Studies 4 and 5 did not). The less robust cortisol reactivity effects of public speaking tasks (relative to the combination of public speaking plus cognitive tasks) has been known for some time (Dickerson and Kemeny, 2004), and the present work shows that this may help to explain the conditions under which measures of chronic stress are associated with a cortisol hyporeactivity response. The present work also suggests the value in conducting an updated meta-analysis of the literature looking at background stress effects on acute stress reactivity responses with these potential moderators in mind, especially since it has been some time since integrative reviews have been conducted on this topic (Gump and Matthews, 1999, Miller et al., 2007, Kudielka et al., 2009).

It is interesting to consider potential mechanisms linking PSS with cortisol hyporeactivity to the TSST. One possibility is that higher background perceptions of stress on the PSS reduce acute stress appraisals during the TSST performance tasks and in turn this drives reduced cortisol reactivity, but this was not supported by the data. Specifically, we observed that higher PSS was associated with *higher* acute stress perceptions during the TSST in four out of the five studies (the effect was marginally significant in the fifth study). The dissociation between PSS effects on (higher) acute stress perceptions and (lower) cortisol responses is consistent with a broader literature showing that acute stress perceptions and cortisol do not correlate strongly (Campbell and Ehlert, 2012), and in this case indicate that lower stress appraisals are not a good candidate mechanism for cortisol hyporeactivity effects. A second possibility is that the PSS is tapping into other stable individual difference factors that may better explain cortisol hyporeactivity effects to the TSST. Evaluating the many person-level measures associated with the PSS will be important in future studies, but our initial evaluation of depressive symptomatology (which is often highly positively correlated with PSS (Lee, 2012)) indicated that it was not a good candidate mechanism. It was perceived stress and not depressive symptomatology that was associated with cortisol hyporeactivity to the TSST. It would be interesting to explore whether other factors that covary with the PSS might serve as alternative explanations or mechanisms (e.g., locus of control, positive affect, neuroticism) in future research. Third, it is possible that chronic background perceptions of stress on the PSS over the past month reflect a time period long enough for the body to reset a new HPA-axis response to stress. Specifically, it is well known that the HPA-axis is regulated by negative feedback, such that cortisol suppresses CRH and ACTH by acting on central glucocorticoid receptors. As such, the PSS may be presenting a case of repeated cortisol counterregulation of the HPA-axis, which is consistent with some theories on the importance of longer term stressors on blunting HPA-axis activation over time (Miller et al., 2007).

Most of the research on the PSS has focused on how perceptions of stress over the past month associate with stress-related health risks, such

as a greater risk for upper respiratory tract infections (Cohen et al., 2002) or greater mortality risk after acute myocardial infarction (Arnold et al., 2012). The studies reported here show that the PSS is also associated with alterations in HPA-axis stress responsivity. It is unclear if cortisol hyporeactivity may be a mechanism for these stress related health and disease outcomes that co-vary with the PSS (cf. Cohen et al., 2012), but the present work suggests that cortisol reactivity could be an important mechanistic target to assess in future studies examining how chronic stress increases stress-related health risks.

This research has limitations and suggests some important new additional research directions. First, the present work is correlational, and does not tell us whether high ongoing background stressors play a causal role in reshaping HPA-axis responses to acute stress. It is unclear the direction of the effect and there would be high value in conducting new longitudinal studies which could help establish temporal precedence. For example, studies that compare participants undergoing long term high stress (relative to low stress periods) could test for alterations in HPA-axis reactivity after periods of high ongoing stress (e.g., Liston et al., 2009). Also, future experimental studies could compare a stress reduction intervention to a control intervention among high stress individuals with established cortisol hyporeactivity to acute stress challenge, and test whether a stress reduction intervention causally increases cortisol reactivity to the TSST in these participants. A second consideration of the present work is that these five studies examined perceived stress and stress responsivity in healthy young adults attending college, and it is possible that cortisol reactivity may be different in patient, older adult populations, or among those who are not pursuing college degrees (Kudielka et al., 2009). Thus, it is unclear how or whether these findings would extend to other groups. One strength of our methodological approach is that we adopted a discovery-explanatory research process, such that we conducted two discovery studies, then preregistered predictions, and then conducted analyses on three explanatory studies. This approach yielded support for the prediction that higher PSS is associated with greater acute subjective stress perceptions to the TSST, but mixed evidence for cortisol hyporeactivity effects. In the case of cortisol, while there was evidence supporting our cortisol hyporeactivity prediction in one explanatory study (Study 3), there was not supportive evidence for this prediction in the remaining two explanatory studies (Studies 4–5). The mini meta-analysis of all five studies suggests the presence of a cortisol hyporeactivity effect, although the overall magnitude of this effect is small (d=.08), and it will helpful if other groups (who have collected the PSS as part of their TSST studies) can further test the PSS cortisol hyporeactivity hypothesis.

5. Conclusions

The PSS and the TSST are two of the most widely used measures of stress and stress responsivity. Extending existing theories linking chronic stress with HPA-axis activation (Miller et al., 2007), the present work suggests that higher perceptions of stress over the past month are associated with lower cortisol reactivity to the TSST, especially when both the speech and math tasks are used. This research reveals a potentially important role for chronic stress perceptions in reshaping HPA-axis stress responsivity.

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CRediT authorship contribution statement

Sarah Wu: Writing – review & editing, Validation, Project administration, Investigation, Data curation. **Brian Chin:** Writing – review & editing, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Sally Dickerson:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Janine Dutcher:** Writing – review & editing, Validation, Supervision, Project administration, Formal analysis, Data curation, Conceptualization. **Peggy Zoccola:** Writing – review & editing, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sheldon Cohen:** Writing – review & editing, Supervision, Conceptualization. **Kasey Creswell:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **David Creswell:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Kirk Warren Brown:** Formal analysis.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used chatGPT in order to refine the writing of the abstract. After using this tool/service, the author(s) reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of Competing Interest

JDC is the Chief of Science at Equa Health, Inc, but otherwise the authors have no competing or conflicts of interests to declare.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2024.107256.

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