Inverse association between stress induced cortisol elevations and negative emotional reactivity to stress in humans

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Abstract

Greater cortisol reactivity to stress is often assumed to lead to heightened negative affective reactivity to stress. Conversely, a growing body of evidence demonstrates mood-protective effects of cortisol elevations in the context of acute stress. We administered a laboratory-based stressor, the Trier Social Stress Test (TSST), and measured cortisol and emotional reactivity in 68 adults (48 women) between the ages of 25-65. In accordance with our pre-registered hypothesis (https://osf.io/t8r3w) and prior research (Het et al., 2012)[1], negative affective reactivity was inversely related to cortisol reactivity assessed immediately after the stressor. We found that greater cortisol response to acute stress is associated with smaller increases in negative affect, consistent with mood-protective effects of cortisol elevations in response to acute stress.

Key Words: cortisol, glucocorticoids, emotion, negative affect, stress, TSST

The relationship between the cortisol and the emotional response to stress has long been a topic of great interest.[1] A difficult topic to study naturalistically, "stressful" situations induce elevations in both glucocorticoids (i.e., cortisol in humans) and negative affect, such as anxiety or feeling threatened.[2] Because of the situational correspondence between cortisol elevation and negative affect, the magnitude of cortisol and negative affective stress responses have been assumed to be positively correlated.

Studies that have systematically examined the relationship between emotion and cortisol elevation during stress have produced mixed results.[1,3] In fact, multiple studies investigating acute stressors demonstrate mood-protective effects of cortisol. [1,4,5] For instance, pharmacological administration of cortisol prior to a stressor has anxiolytic or mood buffering effects in multiple placebo-controlled studies.[4–6] In addition, an investigation that pooled participants across multiple studies employing the Trier Social Stress Test (TSST)[7] demonstrated an inverse relationship between stress-induced cortisol and negative affect (Het et al., 2012).[1] Thus, evidence is accruing to support mood-protective effects of both exogenous and endogenous cortisol elevation in relation to acute stress.

Mood-protective effects of transient cortisol elevations contrast with pervasive notions of cortisol's detrimental effects. Modern conceptualizations are dominated by knowledge of the deleterious effects of chronic cortisol elevations.[8] The psychologically adaptive effects of transient cortisol elevations in response to acute stress are less well-known.[8]

We conducted a TSST as part of a larger NIH-funded project examining individual differences in emotional and stress responses. Our goal is the targeted analysis of cortisol and affective reactivity to the TSST. We hypothesized that we would replicate the inverse relationship between cortisol and negative affective responses to the TSST observed in prior research.[1]

Method

Participants

Participants were recruited through online postings, flyers, and emailed invitations to University of Wisconsin-Madison employees and Survey of the Health of Wisconsin participants.

Informed consent was obtained from 77 participants between the ages of 25-65 (mean age ± SD = 40.4 ± 12.9; 48 women, 28 men, 1 nonbinary; 3 American Indian, 6 Asian, 5 Black, 3 multiracial, and 60 white; 4 Hispanic, 73 non-Hispanic). Exclusionary criteria included medication changes in the previous 4 weeks, neurological disorders, pregnancy, chronic infectious disease or cancer, mania or substance use disorder, and use of antipsychotics, mood stabilizers, or systemic steroids. Study procedures were approved by the University of Wisconsin-Madison Health Sciences IRB. All participants provided written informed consent.

The pandemic halted in-person data collection in March 2020 for the larger NIH-funded project from which the current investigation is drawn. When in-person data collection resumed in February 2021, changes were made to mitigate the risk of infection, including changes to TSST procedures. Therefore, the larger project now includes a Cohort 1 (with 77 participants enrolled prior to March 2020) and a Cohort 2 (ongoing). The analyses presented herein include data from Cohort 1. Hypotheses and the data analysis plan were pre-registered on the Open Science Framework (https://osf.io/t8r3w) prior to analysis.

Data collection

Trier Social Stress Test

The Trier Social Stress Test (TSST), a social evaluative stressor,[7] was administered during a laboratory visit at approximately 2:00 pm and consisted of a five-minute each preparatory period, speech, and mental arithmetic test spoken into a microphone in front of a panel of judges and video camera. See Figure 1 for a schematic of the TSST and measures.

We measured state affect prior to the preparatory period and following mental arithmetic using the state version of the Positive and Negative Affect Schedule (PANAS-now).[9] Participants also rated self-reported stress on a 1-10 scale at baseline and immediately following the TSST. See Table 1 for self-report means.

Cortisol response was assessed using saliva from Salivette® cortisol tubes (Sarstedt, Nümbrecht, Germany). We were interested in initial cortisol reactivity to the stressor and therefore focused on samples acquired immediately post-TSST and 10 min post-TSST with respect to baseline

levels. Additional samples were acquired during the recovery period following the TSST and will be used in the future to examine cortisol stress recovery as an aspect of the larger project. See Figure 1 and Table 1 for mean cortisol levels for time points included in analyses reported here.

Participant characteristics

Prior studies have shown that the relationship between cortisol and psychological variables differs as a function of participant characteristics.[5,10] For the current project, we assessed depression symptoms using the Center for Epidemiologic Studies Depression Scale (CES-D)[11], anxiety symptoms using the trait anxiety scale of the State-Trait Anxiety Inventory (STAI)[12], and age on the day of the TSST. The investigation is not designed to test differences based on gender or race. However, we created binary variables of self-endorsed sex assigned at birth (female; not female) and race (self-endorsed as Black, Indigenous, and/or Asian; self-endorsed as White) to confirm that primary findings remained significant when including either of these in the model.

Data processing

Emotional Response to the TSST

Our pre-registration (https://osf.io/t8r3w) identified the PANAS-now negative affect (NA) scale[9] as our primary index of emotional response to the TSST. We used the difference of post-TSST NA minus pre-TSST NA to index change in NA. Our pre-registration identified two secondary psychological measures, PANAS-now positive affect (PA)[9] and self-reported stress. As with NA, we use the post-TSST minus pre-TSST differences as indices of change in PA and self-reported stress.

Salivary cortisol processing

Cortisol samples were stored at -80° C until analysis. Salivary cortisol was measured using an ElectroChemiLuminescence immunoassay (ECLIA; Roche, Basel, Switzerland). The inter-assay and intra-assay coefficients of variation were 13.2% and 10.3%, respectively. Five participants were unable or unwilling to provide saliva samples at baseline or after the stressor. An additional three participants had a saliva sample of insufficient volume for the assay. We

removed data from one outlier, who had a cortisol response at 10 min post-TSST greater than our pre-registered criteria for an outlier of three standard deviations from the mean, resulting in a total sample of 68 participants with sufficient cortisol data for analysis. The distributions of the cortisol data were approximately normal. Transformations were not applied because they over-corrected and created negative skew.

To index the cortisol response to the TSST, we computed area under the curve with respect to increase (AUCi).[13] Four participants were missing valid cortisol data from the immediate post-TSST (one participant) or the 10 min post-TSST sample (three participants). For these participants, we imputed values from the two neighboring sample timepoints using linear interpolation, which was preferrable to multiple imputation given the rapidly changing cortisol levels in the TSST.[14] Using this method, we retained 68 participants for analysis. We were primarily interested in cortisol increase from baseline to the sample when self-report was assessed concurrently, i.e., immediately post-TSST. This AUCi value is perfectly correlated with and therefore mathematically identical to the difference of post-TSST minus baseline cortisol levels.

Data Analysis

Analyses were conducted using SAS Enterprise Guide version 8.3 update 2. As described above, we restricted analysis to cortisol samples at baseline, immediately post-TSST, and 10 min post-TSST to address the relationship between cortisol and affective reactivity to acute stress. Using general linear modeling analysis techniques, we tested the relationship between emotional response and cortisol AUCi. We conducted post-hoc multiple regression with the three cortisol time points (baseline, post-TSST, 10 min post-TSST) as simultaneous predictors of emotional response to clarify the magnitude and direction of the relationship between cortisol level and emotional response at each time point, while accounting for baseline levels. For multiple regression analyses, we corrected for multiple comparisons (i.e., the inclusion of multiple unique cortisol time points) using an alpha level of 0.005 required to meet significance. We also explored whether results held after adjusting for variance related to age, depression, or anxiety

severity. In addition, we tested whether results held when sex assigned at birth or race were included in the model.

Results

Salivary cortisol, negative affect, and self-reported stress increased from baseline to post-TSST, *p*s < 0.001, but there was no change in positive affect (see Table 1). Increase in NA was inversely related to AUCi from baseline to post-TSST, $r(67) = -0.24$, $p = 0.049$ (see Figure 2). However, the increase in cortisol through 10 min post-TSST (i.e., AUCi including baseline, post-TSST, and 10 min post-TSST) was not significantly related to change in NA, $p = 0.12$. Post-hoc multiple regression analysis, including baseline cortisol in the model, also demonstrated that cortisol levels immediately post-TSST were significantly inversely related to increase in NA (see Table 2). However, cortisol levels at 10 min post-TSST were positively related to increase in NA but not significantly so after correction for multiple comparisons (see Table 2). Inclusion of age, depression severity, or anxiety severity in the model did not change the pattern of results (see Supplemental Material). When sex was included in the model, the significant inverse relationship between AUCi (from baseline to post-TSST) and increase in NA remained, $F(3,64) = 4.14$, $p = 0.046$. When race was included in the model, the inverse relationship between AUCi (from baseline to post-TSST) and increase in NA remained but was no longer significant, $F(3,64) = 3.91$, $p = 0.052$.

Change in PA was not related to TSST-evoked cortisol. The relationship between increase in cortisol measured immediately post-TSST and increase in self-reported stress level was in the same direction as findings for NA (i.e., an inverse relation), but was not significant, $r(65) = -0.22$, $p = 0.07$.

Discussion

We found that negative emotional reactivity was inversely related to endogenous cortisol reactivity measured immediately after the TSST. This finding supported our hypothesis that greater stress-induced acute cortisol increases are associated with smaller increases in negative affect. This is consistent with prior research investigating acute stress suggestive of mood-protective effects of cortisol elevations.[1,4,5]

In the current study, the inverse relationship between cortisol and NA was apparent for cortisol measured concurrently with NA, immediately after the TSST. The timing of these findings differs from Het *et al*.'s prior research demonstrating an inverse relation between negative affect and cortisol elevation at 10 minutes after a stressor.[1] Unfortunately, we did not assess NA at 10 minutes after the stressor and therefore additional research is needed to fully replicate Het *et al.*[1] Future research should address whether the relationship between affect and cortisol depends on timing of measurement with respect to a stressor. This is particularly important because prior research demonstrates that neural and molecular effects of acute stress depend on timing.[15] For instance, Joëls has demonstrated time-varying effects of stressors on neural function in humans and rodents, which are related to rapid non-genomic effects at corticosteroid receptors (largely mineralocorticoid receptor-dependent) and delayed genomic effects (largely glucocorticoid receptor-dependent).[15] In addition, research in humans demonstrates that timing and affective chronometry are key. For example, Hellhammer and Schubert showed that perceived stress measured during but not after stress was positively related to the magnitude of cortisol response.[3] Thus, while the current project extends prior research suggestive of an *inverse* association between heightened cortisol and negative affective response measured immediately after a stressor,[1] future research should address whether this association varies depending on the timing of responses with respect to the onset, duration, and cessation of an acute stressor.

The mounting evidence demonstrating mood-buffering effects of stress-evoked cortisol elevations[1,4,5] opposes popular conceptions of cortisol as associated with or even causing negative affective responses to stress.[8] Multiple studies now demonstrate that emotional and cortisol reactivity to acute stress are more dissociable than is often assumed.[1,4,5] This more nuanced picture of possible mood-buffering effects of cortisol responses to acute stress has important ramifications for "stress reduction" and emotion regulation practices, especially in the context of acute stressors. Cortisol reactivity to a stressor may promote adaptive emotion regulation, which is consistent with prior research demonstrating adaptive neural and cognitive effects of transient cortisol elevations.[8,16] These findings are in line with theoretical models positing that robust yet transient physiological reactivity is adaptive within the context of

emotionally provocative events.[17] The biological and psychological aspects of stress reduction are clearly more complex than a simple dampening of stress physiology.[1,8]

The relation between cortisol and affective function is relevant for mental health conditions.[16,18] While the relation between immediate responses to the TSST and longer-term changes in affect is unknown, psychological benefits of transient cortisol elevation have been demonstrated in a variety of clinical contexts and conditions.[16,19] Intervention focused on transiently enhancing the cortisol signal is an important area for future clinical investigation.[16] Emotional aspects of "stress reduction" may at times be supported with transient elevations in cortisol, consistent with mood-buffering effects of interventions involving "eustress" such as exercise.[20]

Limitations and Future Directions

The project from which the current analyses were drawn was hindered by the COVID-19 pandemic, which brought in-person data collection to a halt. The current report addresses findings for the participants brought into the laboratory prior to the pandemic-related national emergency, and analyses are restricted to TSST reactivity. We are retaining cortisol samples later in the TSST recovery period for future analyses because the time course of emotional recovery is a key aspect of the larger project. The goal of the current project is a limited analysis and a focused replication test of prior TSST cortisol and NA reactivity findings.[1] Though this analysis is limited, we believe it is important to publish pre-registered replications of prior research and to debunk misconceptions about the relationship between cortisol and emotional reactivity to acute stress.

The current analyses are limited in that we address only cortisol and not autonomic nervous system reactivity to stress, which has been shown to be buffered by exogenous cortisol in one study[5], but to correlate positively with NA after stress in another.[1] The complex inter-relations among cortisol, autonomic, and affective responses to stress is an exciting area for future research.

Prior research has demonstrated that relationships between cortisol elevations and psychological processes vary as a function of individual differences in personal characteristics

and psychopathology.[5,10,16] We demonstrated that results largely held when including several relevant variables in the model, including depression symptoms, anxiety symptoms, age, sex assigned at birth, and race. However, we lacked the power to adequately test whether these personal characteristics moderate relationships between cortisol and affective reactivity to stress. The current report should not be taken as evidence of a lack of such moderation, which should be addressed in future research.

Conclusion

As hypothesized in our pre-registration, we found that greater stress-induced cortisol increases are associated with smaller increases in negative affect in response to acute stress. The results partially replicate findings of Het et al.,[1] though the timing of results differs somewhat and should be addressed in future research. The findings add to a growing body of evidence suggestive of mood-protective effects of acute cortisol elevations, and demonstrate that emotional and cortisol reactivity to acute stress are more dissociable than is often assumed.[1,4,5] These findings may have important mental health implications and suggest that investigation of behavioral and/or pharmacological interventions aimed at transiently enhancing the cortisol signal is an exciting area for future research.

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Data Availability Statement

Data are shared and available through the National Institute of Mental Health Data Archive.

Author Contributions

SMS, MAR, RJD are key personnel and designed the R01-funded project from which this investigation is drawn, including methods and procedures related to the TSST. ALB, ECN, LG, & SMS collected and processed data. SMS, AJF, EH, MAR, DWG, & HCA planned analyses for TSST data. HCA analyzed the data, with assistance from DWG, SMS, & ALB. HCA wrote the first draft and all authors provided input on the manuscript.

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Table 1. Cortisol levels, affect, and self-reported stress with respect to the TSST.

Variable	Baseline	Post-TSST	10 min Post-TSST	Statistic	*p*-value
Salivary cortisol	8.34 (3.33)	13.14 (6.2)	14.0 (6.9)	39.24	.0001
Negative Affect	14.9 (4.9)	18.3 (7.2)	n/a	5.39	.0001
Positive Affect	30.2 (8.0)	29.6 (8.6)	n/a	0.89	*n.s.*
Self-reported Stress	2.5 (1.5)	5.4 (2.8)	n/a	7.63	.0001

Note: Values are presented as mean (standard deviation). Units for cortisol are nmol/L. Affect was assessed with the PANAS-now, which provides indices of positive and negative affect that range from 10 to 50.[9] Self-reported stress was measured with the question "How stressed are you right now?" rated on a 10-point scale. Self-report measures were not administered at 10 min post-TSST. For cortisol, the *F* statistic is presented for a one-way ANOVA including the three time points: baseline, immediately after the TSST (post-TSST), and ten minutes after the TSST (10 min post-TSST). For self-report measures, *t*-value statistics are presented for analysis of change from prior to the TSST to immediately post-TSST. As shown in the table, cortisol levels, negative affect, and self-reported stress increased with respect to the TSST, but there was no change in positive affect.

Table 2. Multiple regression predicting increase in negative affect (NA) in response to the TSST.

Variable	Parameter Estimate	Increment in R^2	t-value	p-value
Baseline cortisol	0.07	0.01	0.34	n.s.
Post-TSST cortisol	-0.70	0.11	-2.88	0.005
10 min Post-TSST cortisol	0.42	0.06	2.13	0.037

Note: This follow-up multiple regression analysis shows that salivary cortisol measured immediately after the TSST ("post-TSST cortisol") was inversely associated with and accounted for 11% of the variance in the increase in negative affect, even with baseline cortisol and 10 min post-TSST cortisol included in the model. The relation between salivary cortisol measured 10 minutes after the TSST (10 min post-TSST) and increase in negative affect is positive, but not significant after correction for multiple comparisons.

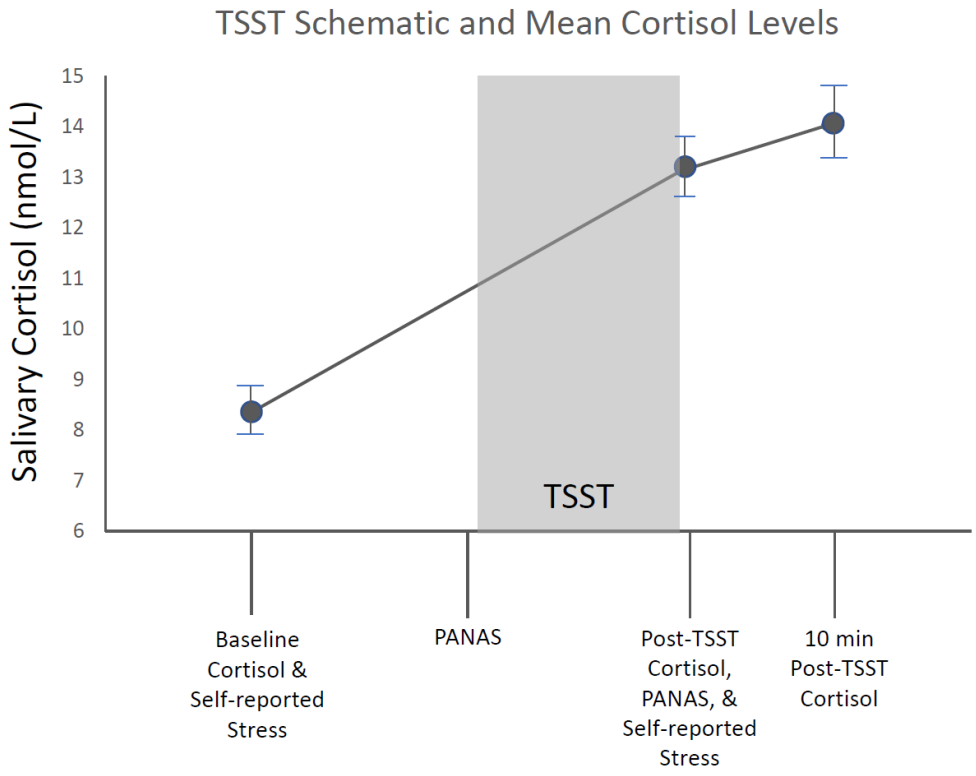

Figure 1.

Figure 1 Caption: Schematic of TSST and measures. The TSST[7] consisted of 5 min of anticipation, 5 min speech, and 5 min mental math periods. The current report addresses salivary cortisol taken at baseline, immediately post-TSST, and 10 min post-TSST. The PANAS-now[9] was administered immediately pre-TSST and immediately post-TSST. The question "How stressed are you now?" was assessed at baseline and immediately post-TSST. No self-report measures were administered at 10 min post-TSST. Mean cortisol levels at each time point are depicted.

Figure 1 Alt Text: Concise listing of experimental procedures with a line graph showing the increase in cortisol levels from baseline to after the stressor task.

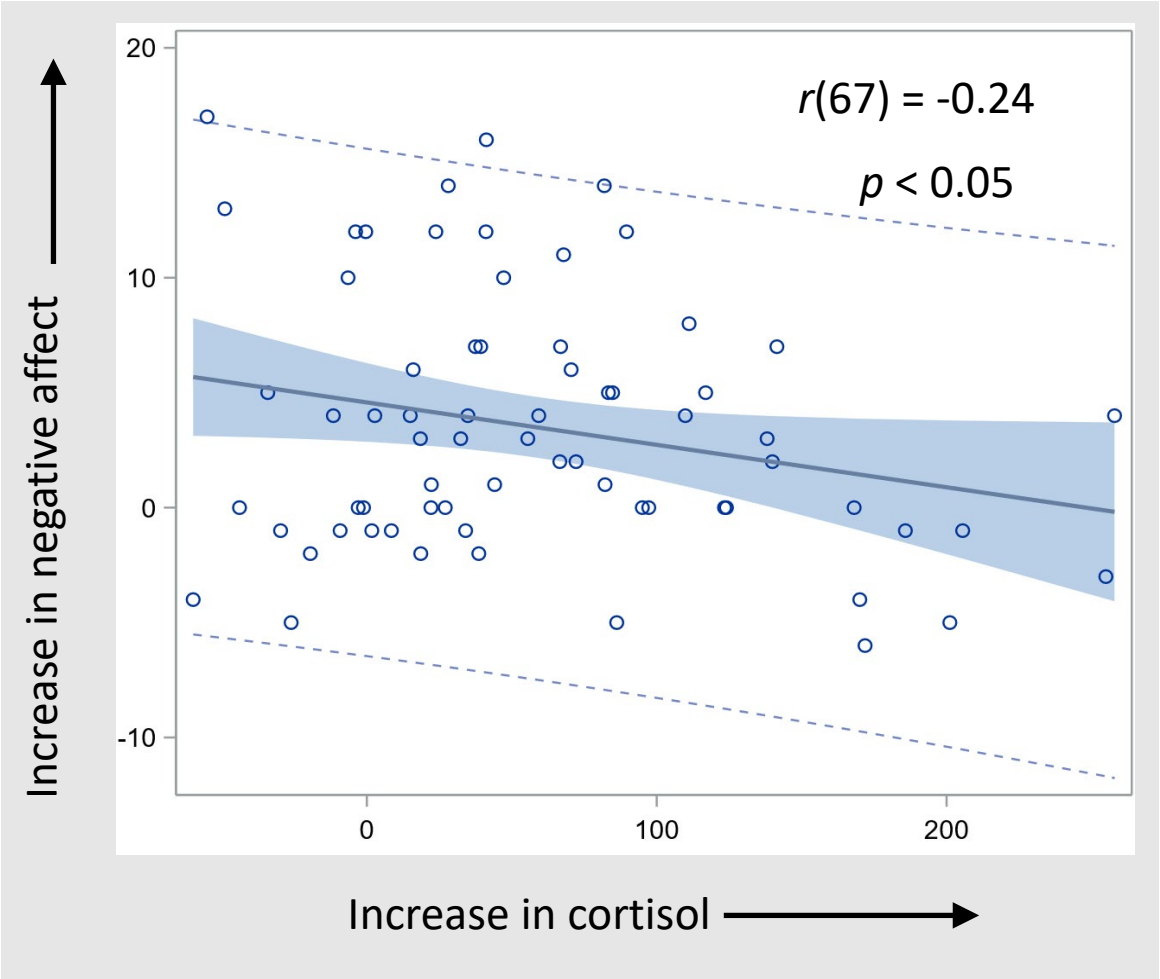

Figure 2.

Figure 2 Caption: Greater stress-induced cortisol increases are associated with smaller increases in negative affect during stress. Specifically, increase in cortisol (AUCi; original units in nmol/L) is inversely correlated with increase in negative affect assessed using the PANAS immediately after the TSST with respect to baseline, r(67) = -0.24, p < 0.049.

Figure 2 Alt Text: Scatter plot of the inverse relationship between the increase in cortisol and the increase in negative affect.

Figure Captions and Alt Text

Figure 1.

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Figure 2 Alt Text: Scatter plot of the inverse relationship between the increase in cortisol and the increase in negative affect.

Supplemental Material

Supplemental multiple regression analyses show that including age, depression severity (measured with the CES-D), or trait anxiety (measured with the STAI) does not change the pattern of results (see tables below). With each of these variables in the model, increase in NA in response to the TSST remains inversely related to post-TSST cortisol levels immediately after the TSST. This relation is not observed for 10 min post-TSST cortisol, which is positively related to increase in NA in response to the TSST but not significantly so after correction for multiple comparisons.

Supplemental Table 1. Multiple regression predicting increase in negative affect (NA) in response to the TSST with age in the model.

Variable	Parameter Estimate	Increment in R^2	*t*-value	*p*-value
Age	0.04	0.01	0.80	*n.s.*
Baseline cortisol	0.05	0.00	0.20	*n.s.*
Post-TSST cortisol	-0.66	0.10	-2.98	0.004
10 min Post-TSST cortisol	0.39	0.05	1.99	0.052

Supplemental Table 2. Multiple regression predicting increase in negative affect (NA) in response to the TSST with trait anxiety (measured with the STAI) in the model.

Variable	Parameter Estimate	Increment in R^2	*t*-value	*p*-value
Trait Anxiety	0.11	0.05	1.87	0.066
Baseline cortisol	0.10	0.00	0.48	*n.s.*
Post-TSST cortisol	-0.77	0.13	-3.67	0.0005
10 min Post-TSST cortisol	0.51	0.09	2.77	0.007

Supplemental Table 3. Multiple regression predicting increase in negative affect (NA) in response to the TSST with depression severity (measured with the CES-D) in the model.

Variable	Parameter Estimate	Increment in R^2	*t*-value	*p*-value
Depression Severity	0.22	0.12	2.95	0.005
Baseline cortisol	0.04	0.00	0.20	*n.s.*
Post-TSST cortisol	-0.65	0.08	-2.80	0.007
10 min Post-TSST cortisol	0.41	0.05	2.00	0.050