

Stress-Induced Cortisol Level Elevations Are Associated With Reduced Negative Affect After Stress: Indications for a Mood-Buffering Cortisol Effect

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Objective: Stress is associated with increased negative affect and activation of the sympathetic nervous system and of the hypothalamic-pituitary-adrenal axis. However, the relationship between these stress systems and negative affect is incompletely understood. We therefore investigated positive and negative affects in relationship with salivary cortisol and salivary α -amylase (sAA) levels in a large sample of participants exposed to a psychosocial stressor or a control condition. **Methods:** Cortisol and sAA levels from five studies with a total sample size of 232 participants were reanalyzed using hierarchical linear modeling. In these studies, we measured affective responses to the Trier Social Stress Test (TSST) and its control condition (placebo TSST) with the Positive and Negative Affect Schedule. **Results:** An inverse relationship between cortisol and negative affect was observed across all participants ($\beta_{06} = -0.13, p = .002$). Higher level of negative affect was associated with lower mean cortisol levels 10 minutes after the TSST or the control condition. When the two conditions were tested separately, the effect was significant in the stress condition ($\beta_{06} = -0.05, p = .02$) but not in the control condition ($\beta_{06} = -0.0008, p > .05$). In contrast to the results for cortisol, a positive relationship was found between sAA and negative affect within the stress condition ($\beta_{06} = 0.10, p = .005$). **Conclusions:** The present findings suggest that cortisol is associated with an attenuated negative emotional arousal in response to acute stress, whereas sAA levels seem to reflect the degree of negative emotional arousal. Together with previous pharmacological studies, these data seem to support the hypothesis of mood-buffering effects of cortisol. **Key words:** affect, cortisol, emotion, stress, sympathetic nervous system, hypothalamic-pituitary-adrenal axis.

GC = glucocorticoid; HLM = hierarchical linear modeling; HPA = hypothalamic-pituitary-adrenal axis; KS = Kolmogorov-Smirnov; PANAS = Positive and Negative Affect Schedule; P-TSST = placebo TSST; sAA = salivary α -amylase; SNS = sympathetic nervous system; TSST = Trier Social Stress Test.

INTRODUCTION

Modern conceptualizations view stress from a systemic point of view, namely, as a condition in which expectations do not match the current or anticipated perceptions of the internal or external environment. This discrepancy between what is observed or sensed and what is expected or programmed elicits patterned compensatory responses, which are collectively interpreted as the stress response (e.g., references (1–3)). The two prominent physiological systems that mediate the stress response are the fast-reacting sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal axis (HPAA), which reacts more slowly. The former system allows short-term adaptation to challenging conditions within a few seconds through the release of epinephrine and norepinephrine and through direct innervation of target tissues (4). The latter system further facilitates adaptation through the release of glucocorticoids (GCs). Among other effects, the main human GC cortisol is able to permeate the blood-brain barrier, where it influences the activity of the central nervous system by activating central corticosteroid receptors (4,5). Because stress is typically associated with changes in affect (6–8), a considerable

number of studies have investigated the effects of cortisol on emotional processes. For instance, van Eck et al. (9) found that negative affective states were positively associated with elevated cortisol levels at baseline immediately before and after a laboratory speech task. These studies seem to suggest that cortisol is associated with increased negative affect. However, other studies have found a significant effect of stress on negative affect while reporting no significant correlation between cortisol levels and negative affective state. Buchanan et al. (10), for example, investigated the relationship between cortisol and negative and positive affects while manipulating the participants' affect with different methods (speech stressor versus humorous video). Both negative affect and cortisol level increased on the "stress day" and decreased on the "video day." However, correlations between cortisol and negative affect were not significant.

Pharmacological studies have revealed that, on the one hand, cortisol administration has hardly any effects on mood when the experiment is conducted in resting conditions (e.g., references (11–13)). On the other hand, however, there are reports on the influence of cortisol on mood when it is administered in arousing conditions. For instance, patients with phobic disorders reported less anxiety after treatment with cortisol 1 hour before confrontation with a phobic stimulus in contrast to placebo-treated patients (14). Putman et al. (15) observed that cortisol increased risky decisions in a motivated decision task, which was interpreted by the authors as suggestive of anxiolytic properties. We recently found that oral application of cortisol before psychosocial stress leads to a reduced negative affective state in contrast to placebo treatment (16). Therefore, we concluded that elevated cortisol concentrations before, during, or after stressful situations might help to cope with the emotional load of the situation by preventing an emotional overshoot. This conclusion is in accordance with the assumption of compensatory cortisol effects (17) and with McEwen's (18) allostatic load model. This model suggests that an inadequate stress response facilitates emotional disturbances (19–21).

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In addition, our previous findings (16) support the idea that cortisol might act differentially on mood, depending on the level of stress. In resting situations, it may have an activating effect with no other changes in mood, but during arousing situations, it might function to prevent extreme decreases in mood, which might be interpreted as a protective effect (see also Reuter (12)).

Taken together, the current literature suggests that associations between acute cortisol elevations and negative affective state exist, but findings are still quite heterogeneous. This might reflect a nonlinear relationship and the fact that most previous studies have used rather small samples with limited power. In addition, there are few reports on the association between cortisol and positive affect (22,23). The hypothesis of mood-protective cortisol effects has so far been predominantly supported by pharmacological studies. Thus, we set out to explore the relationship between cortisol and affect in a psychosocial laboratory stress situation. Based on our previous study (16), we expected an inverse relationship between posttreatment affect scores and cortisol levels (i.e., a reduced cortisol response is associated with stronger increase in negative affect); especially in the stress condition, this relationship should be stronger because of higher cortisol levels. We had no expectations concerning the relationship between positive affect and cortisol response because there are too few studies on this topic. With regard to SNS activity, studies show that there is a positive covariance between SNS activity and affective state, that is, the stronger the affect state, the stronger the SNS activity (e.g., references (24–27)). Thus, we expected a positive association between markers of SNS activity and affective state after stress.

METHODS

Participants

The present reanalysis is based on data from five studies conducted in the psychological laboratories of the University of Bielefeld and the Ruhr University Bochum, Germany. All of the included studies investigated the effects of acute stress on memory and were conducted from April 2006 to June 2009. Results regarding the effects of the stressor on memory have been published for two of those five studies (8,28). The age range of the included participants was 18 to 40 years. All participants underwent a brief medical and psychological examination before testing to check for the following exclusion criteria: a) a body mass index (BMI) outside the reference range between 18 and 26 kg/m²; b) menstrual period during the time of testing; c) psychiatric, endocrine, or cardiovascular diseases or other specific chronic diseases; d) an intake of psychoactive drugs, β -blockers, gonadal steroids (hormonal contraceptives), or GCs; e) previous experience with the stress protocol (see the next section); and f) physical exercise, smoking (>6 cigarettes per day), and consumption of anything but water for 1 hour before testing. The total sample consisted of 232 adults (mean [standard deviation] age = 24.14 [0.3] years; 84 women and 148 men). The female sample consisted of women during the luteal or follicular phase of their menstrual cycle. All studies were conducted in the morning (9 AM to 12 noon). Study protocols were approved by the ethics committee of the German Psychological Association (Deutsche Gesellschaft für Psychologie), and all participants provided written informed consent.

Study Protocol

All studies used a randomized controlled design and took place on 1 day only, except for Study 5 (see the last part of this section). After arrival at the laboratory, the participants in Studies 1 to 4 were asked about their current well-being, signed a written informed consent, and were seated in a quiet room. Twenty-five minutes later, the first saliva sample (baseline) was obtained, and

participants were asked to answer a mood questionnaire (see “Affect Measurement” section). Participants were randomly assigned and exposed to either the Trier Social Stress Test (TSST (29)) or to its control version (placebo TSST [P-TSST] (30)). Immediately after treatment, posttreatment samples (+1 minute) were collected. At the same time, participants answered the mood questionnaire again. The third and fourth saliva samples were obtained 10 and 25 minutes, respectively, after TSST or P-TSST. Cognitive testing was conducted between Saliva Samples 3 and 4. Participants in Study 5 had a similar protocol but had visited the laboratory 1 day before testing to learn words that they had to recall during the cognitive testing session on the main study day (28).

Stress and Control Procedure

The TSST protocol takes 15 minutes and consists of a 5-minute preparation period, a 5-minute free speech, and a 5-minute mental arithmetic task. The TSST was performed similarly to the description provided by Kirschbaum et al. (29) and has been shown to be highly effective in eliciting an HPA response (1). In addition, the TSST has been found to be effective in inducing negative affect (6,8,31). As a control condition, the P-TSST was performed as described by Het et al. (30). It is similar to the TSST in physical and mental demands (speech and math task alone in a room) but lacks the stress-inducing components of the TSST (social evaluative threat and uncontrollability; see Dickerson and Kemeny (1) and Het et al. (30) for details).

Saliva Sampling and Biochemical Analyses

Saliva was collected to obtain free cortisol levels and salivary α -amylase (sAA) activity as markers of HPA and SNS activity, respectively (32,33). The samples were obtained using Salivette sampling devices (Sarstedt, Nümbrecht, Germany). Cortisol levels were measured using a commercially available immunoassay with chemiluminescence detection (IBL, Hamburg, Germany). sAA activity was measured by using a quantitative enzyme kinetic method, as described elsewhere (32). Interassay and intra-assay coefficients of variation were lower than 10% for both assays.

Affect Measurement

Participants filled out the Positive and Negative Affect Schedule (PANAS (34)) at baseline and immediately after cessation of the stressor. The PANAS is a standardized, reliable, and valid measure for the current affective state (35) and consists of 10 items for positive affect (e.g., interested, enthusiastic) and 10 items for negative affect (e.g., upset, ashamed). Participants were asked to rate the items on a 5-point scale ranging from 1 = “very slightly or not at all” to 5 = “extremely.” The ratings were averaged to obtain separate scores for positive and negative affects.

Statistical Analyses

Group differences in descriptive variables (sex, age, and BMI) and post-treatment and pretreatment subjective affect ratings were analyzed using Pearson χ^2 test, Student's *t* test, and Mann-Whitney *U* test, respectively. Data on endocrine responses were analyzed for outliers using *z* scores and were checked for goodness of fit to a normal distribution by using the Kolmogorov-Smirnov (KS) test. In the case of a significant KS test, data were log transformed, and the following analyses were conducted with the log-transformed data. These statistical analyses were performed by IBM SPSS 18 (Chicago, IL) for MAC OS X (Apple Inc., Cupertino, CA).

Time-dependent between-group differences in the neuroendocrine reaction and the relationship among cortisol levels, sAA levels, and current affective state were analyzed using two-level hierarchical linear modeling (HLM-2) and three-level HLM (HLM-3). In the within-person (i.e., Level 1) models, log-transformed cortisol and sAA levels were estimated as a function of time since study entry (coded in real time in minutes; to test for linear effects) and time since study entry squared (coded in real time in minutes squared; to test for curvilinear effects). Because cortisol concentrations peak 20 to 30 minutes after the onset of a stressor, we anticipated the cortisol peak at 10 minutes after the end of the TSST (see Dickerson and Kemeny (1) and Kirschbaum et al. (29,33) for further information). We thus centered the time variable at that point. For sAA, main changes were expected 1 minute after the treatment (time variable was centered at that point). To detect between-group differences in neuroen-

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ocrine responses, we used HLM-2 and estimated the neuroendocrine responses as a function of experimental condition (i.e., Level 2 predictor). The relevant coefficients reported (β_{ij}) refer to changes at important (centered) points in time (i.e., +10 minutes and +1 minute) and indicate mean level differences between the groups (β_{0j}), group differences in the instantaneous rate of change (slope, β_{1j}), and group differences in the curvature (acceleration, β_{2j}) at these points in time. The resulting Level 1 model for cortisol and sAA as outcome variables (j) was

$$\text{hormone levels}_{ij} = \pi_{0j} + \pi_{1j}(\text{time centered})_{ij} + \pi_{2j}(\text{time centered}^2)_{ij} + e_{ij}.$$

At Level 2, the influence of the condition on both outcome variables (j) was modeled as follows:

$$\begin{aligned} \pi_{0j} &= \beta_{00j} + \beta_{01j}(\text{condition})_j + r_{0j}, \\ \pi_{1j} &= \beta_{10j} + \beta_{11j}(\text{condition})_j + r_{1j}, \text{ and} \\ \pi_{2j} &= \beta_{20j} + \beta_{21j}(\text{condition})_j + r_{2j}. \end{aligned}$$

The influence of person-specific variables including the posttreatment affective reaction to the outcome variables (cortisol and sAA) were analyzed using HLM-3. According to our hypotheses, the poststress affect scores should be predicted by the poststress cortisol and sAA levels; that is, the poststress affect scores should be the outcome variables. However, HLM needs at least three points of time measurement (36), and only one prestress affect score and one poststress affect score were available. Thus, we predicted the cortisol levels and the sAA levels using the poststress affect scores (inverse prediction) and assumed, according to our hypotheses, that high poststress affect scores predict low cortisol levels. For each participant, the outcome values of cortisol and sAA were predicted as a function of time (i.e., Level 1) and the following covariates (i.e., Level 2): age, BMI, smoking (yes versus no), sex (male versus female), and posttreatment PANAS score for positive and negative affects. The experimental condition (TSST versus P-TSST) was considered on Level 3. The relevant coefficients reported (γ_{ijk}) by HLM-3 refer to changes at +10 minutes (cortisol) or at +1 minute (sAA) after the TSST and to differences between the groups. As in HLM-2, they indicated mean level differences at relevant time points (γ_{0jk}), differences in the instantaneous rate of change (slope, γ_{1jk}), and differences in the curvature (acceleration, γ_{2jk}) at these points in time. The resulting Level 1 model for both outcome variables (k) was

$$\text{hormone levels}_{ijk} = \pi_{0jk} + \pi_{1jk}(\text{time centered})_{ijk} + \pi_{2jk}(\text{time centered}^2)_{ijk} + e_{ijk}.$$

At Level 2, the influence of the covariates on cortisol and sAA was modeled as follows:

$$\begin{aligned} \pi_{0jk} &= \beta_{00k} + \beta_{01k}(\text{age})_{jk} + \beta_{02k}(\text{BMI})_{jk} + \beta_{03k}(\text{smoking})_{jk} \\ &+ \beta_{04k}(\text{sex})_{jk} + \beta_{05k}(\text{posttreatment positive affect score})_{jk} \\ &+ \beta_{06k}(\text{posttreatment negative affect score})_{jk}, \\ \pi_{1jk} &= \beta_{10k} + \beta_{11k}(\text{age})_{jk} + \beta_{12k}(\text{BMI})_{jk} + \beta_{13k}(\text{smoking})_{jk} \\ &+ \beta_{14k}(\text{sex})_{jk} + \beta_{15k}(\text{posttreatment positive affect score})_{jk} \\ &+ \beta_{16k}(\text{posttreatment negative affect score})_{jk} + r_{1jk}, \text{ and} \\ \pi_{2jk} &= \beta_{20k} + \beta_{21k}(\text{age})_{jk} + \beta_{22k}(\text{BMI})_{jk} + \beta_{23k}(\text{smoking})_{jk} \\ &+ \beta_{24k}(\text{sex})_{jk} + \beta_{25k}(\text{posttreatment positive affect score})_{jk} \\ &+ \beta_{26k}(\text{posttreatment negative affect score})_{jk} + r_{2jk}. \end{aligned}$$

At Level 3, the influence of the condition on cortisol and sAA was modeled as follows:

$$\begin{aligned} \beta_{00k} &= \gamma_{000}; \beta_{01k} = \gamma_{010}; \beta_{02k} = \gamma_{020} + v_{02k}; \beta_{03k} = \gamma_{030}; \beta_{04k} = \gamma_{040}; \\ \beta_{05k} &= \gamma_{050} + \gamma_{051}(\text{condition})_k; \beta_{06k} = \gamma_{060} + \gamma_{061}(\text{condition})_k; \beta_{10k} = \gamma_{100}; \\ \beta_{11k} &= \gamma_{110}; \beta_{12k} = \gamma_{120} + v_{12k}; \beta_{13k} = \gamma_{130}; \beta_{14k} = \gamma_{140}; \beta_{15k} = \gamma_{150} + \end{aligned}$$

$$\begin{aligned} \gamma_{151}(\text{condition})_k; \beta_{16k} &= \gamma_{160} + \gamma_{161}(\text{condition})_k; \beta_{20k} = \gamma_{200}; \beta_{21k} = \gamma_{210}; \\ \beta_{22k} &= \gamma_{220}; \beta_{23k} = \gamma_{230}; \beta_{24k} = \gamma_{240}; \beta_{25k} = \gamma_{250} + \gamma_{251}(\text{condition})_k; \\ \beta_{26k} &= \gamma_{260} + \gamma_{261}(\text{condition})_k. \end{aligned}$$

All growth curve models were estimated using HLM 6.08 (Scientific Software International, Inc, Lincolnwood, IL). Estimation of variance components is produced by HLM 6.08 and reported by using intraclass correlation coefficient (ICC (37,38)) and R^2 (coefficient of determination (36)). For comparison with HLM results, Spearman correlation coefficients were calculated among the cortisol output 10 minutes after the TSST, the sAA output 1 minute after the TSST, and the posttreatment affect scores within the TSST group. Overall level of significance was defined as $p < .05$.

RESULTS

Description of the Sample

The stress group (TSST exposure) consisted of 118 participants. There were 75 male participants within this group. The control group (P-TSST exposure) consisted of 114 participants with 73 male participants. The groups differed neither in their gender distribution ($\chi^2 = 0.94, df = 1, p > .05$) nor in the distribution of menstrual cycle phases ($\chi^2 = 0.07, df = 2, p > .05$). Furthermore, there were no between-group differences with regard to age ($t = 0.33, df = 230, p > .05$), BMI ($t = 0.27, df = 228, p > .05$), and frequency of smoking ($\chi^2 = 2.21, df = 1, p > .05$). After outlier analysis, one female participant and one male participant of the control group were excluded because of extremely high values ($z \geq 3.29$) in the endocrine parameters.

Endocrine Stress Responses

The KS test revealed that cortisol, as well as sAA, data deviate from the normal distribution significantly (all p values $< .05$). Thus, data on endocrine responses were log transformed. Figure 1 shows that both groups did not differ in their baseline salivary cortisol levels. After the treatment, the stress group showed higher cortisol levels than the control group. As expected, there was a peak in salivary cortisol concentrations 10 minutes after treatment with the TSST. HLM-2 revealed across all participants and all sampling times a mean log-transformed cortisol level of $\beta_{00} = 0.098 (\pm 0.02$ standard error of the mean [SEM]), which is significantly different from zero ($t = 46.48, df = 229, p < .001$). Ten minutes after the stress or the control condition, there was a significant difference between

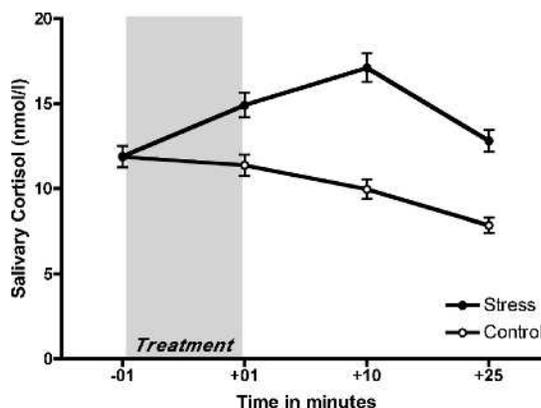


Figure 1. Mean (\pm standard error of the mean) free salivary cortisol responses (untransformed values). Time refers to the time of treatment. The Trier Social Stress Test group showed a maximum of free salivary cortisol concentrations at 10 minutes after the cessation of the stressor.

the two experimental conditions for the mean cortisol levels ($\beta_{01} = 0.20$ [± 0.03 SEM], $t = 6.74$, $df = 229$, $p < .001$). The coefficient β_{01} indicates that, when the predictor “condition” increases by 1 unit (i.e., going from control [= 0] to stress condition [= 1]), the mean log-transformed cortisol level (intercept) increases by 0.20 units. That is, the stressed participants had higher mean cortisol levels 10 minutes after the treatment than the participants of the control group. The mean slope of time estimates the relation between time and cortisol levels and was $\beta_{10} = -0.005$ (± 0.0004 SEM, $t = -11.46$, $df = 229$, $p < .001$). β_{10} indicates the mean slope of time across all participants 10 minutes after the treatment. When the predictor “time” increases 1 unit, the mean cortisol level decreases by 0.005 units. Again, there was a significant group difference ($\beta_{11} = 0.003$ [± 0.0005 SEM], $t = 5.50$, $df = 229$, $p < .001$). That is, as group increases 1 unit (going from control [= 0] to stress condition [= 1]), the slope increases by 0.003 units. This indicates that the slope was steeper in the TSST group. Similarly, the mean curvature was $\beta_{20} = -0.00008$ (± 0.00002 SEM, $t = -3.21$, $df = 229$, $p = .002$), and there was a group difference as well ($\beta_{21} = -0.0002$ [± 0.00003 SEM], $t = -6.07$, $df = 229$, $p < .001$).

Figure 2 shows the results for sAA. Both groups showed similar baseline values and differed in their concentrations of sAA 1 minute after the treatment. Whereas the control group showed only a small increase in sAA concentrations, the stress group displayed an increase of nearly 100%. HLM-2 revealed a mean sAA level of $\beta_{00} = 1.61$ (± 0.04 SEM, $t = 42.01$, $df = 226$, $p < .001$) and a significant mean level group difference ($\beta_{01} = 0.14$ [± 0.05 SEM], $t = 2.67$, $df = 226$, $p = .008$) 1 minute after the stress or the control condition. When the predictor condition increases by 1 unit, the mean sAA level (intercept) increases by 0.14 units, indicating higher mean sAA levels in the stress group than in the control condition 1 minute after the treatment. The mean slope across all participants did not differ from zero and was $\beta_{10} = 0.0008$ (± 0.0009 SEM, $t = 0.91$,

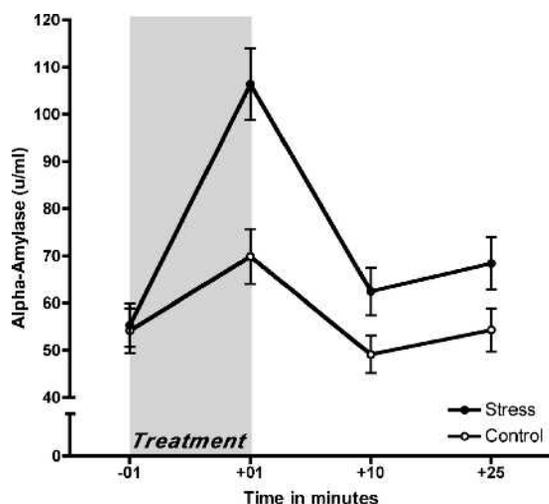


Figure 2. Mean (\pm standard error of the mean) salivary α -amylase levels (untransformed values). Time refers to the time of treatment. The Trier Social Stress Test group showed a maximum at +1 minute, whereas the control group displayed only a small increase.

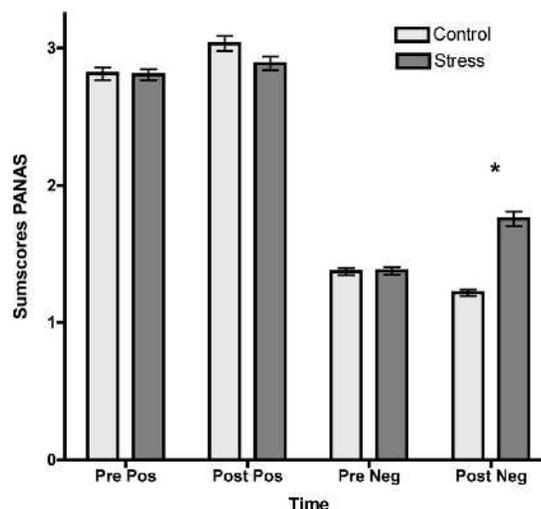


Figure 3. Mean (\pm standard error of the mean) pretest and posttest scores of the positive (Pos) and the negative (Neg) sum scores of the Positive and Negative Affect Schedule (PANAS) for both groups. A significant ($p < .05$) difference was found between the groups on the negative affect dimension only in posttest scores (indicated by the asterisk).

$df = 226$, $p = .37$). However, there was a significant group difference with $\beta_{11} = 0.004$ (± 0.001 SEM, $t = 3.68$, $df = 226$, $p = .001$), indicating a stronger slope in the stress condition. The mean curvature across all participants was $\beta_{20} = -0.0001$ (± 0.00005 SEM, $t = -2.11$, $df = 226$, $p = .035$), and there was a group difference as well ($\beta_{21} = -0.0003$ [± 0.00006 SEM], $t = -4.41$, $df = 226$, $p < .001$).

Affective Responses

The affect scores in each group as measured by the PANAS are shown in Figure 3. The affect scores were summarized in the two dimensions of the PANAS (positive affect and negative affect). On both dimensions, the groups did not differ in pretreatment scores (p values $> .10$). On the negative affect dimension, we found a substantial increase in the posttreatment scores of the stress group and a decrease in the control group. The Mann-Whitney U test on this dimension revealed a significant group difference assessed after the treatment ($z = -7.7$, $p < .001$).

Relationship Between Affect and Cortisol

As shown in Figure 4, HLM revealed in both groups associations of low posttreatment negative affect scores and high cortisol levels.

Analysis with HLM-3 revealed a significant influence of the Level 2 predictor “posttreatment negative affect” on the mean cortisol levels 10 minutes after the stress or the control condition with $\beta_{06} = -0.13$ (± 0.04 SEM, $t = -3.11$, $df = 863$, $p = .002$). The coefficient β_{06} refers to all participants and indicates a decrease in mean cortisol levels by 0.13 units when the predictor posttreatment negative affect increases by 1 unit. This indicates an association of high negative affect scores and low cortisol levels across the entire sample 10 minutes after the treatment. When the Level 3 predictor condition is considered additionally, there was again a significant influence on the mean

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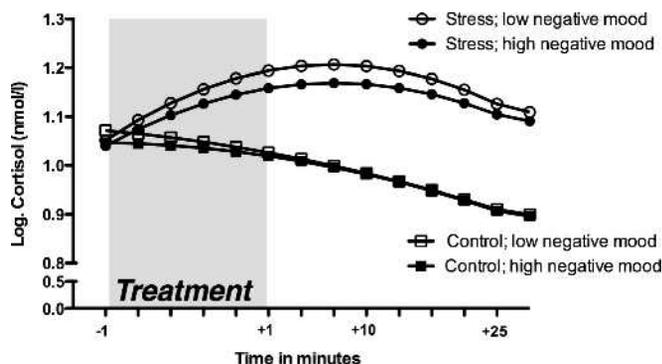


Figure 4. Log-transformed salivary cortisol levels of both groups during the course of the experiments related to negative affective state after the treatment as modeled using hierarchical linear modeling (see “Results” section). High cortisol levels are associated with low negative affect scores (below the 25th percentile) after stress exposure, whereas high negative affect scores (above the 75th percentile) predict lower stress-induced cortisol levels. No relationship between cortisol and negative affect is detectable in the control group. Note that the percentiles were chosen for graphical purposes only. They were not included in the hierarchical linear modeling analysis.

cortisol levels ($\gamma_{061} = 0.10 [\pm 0.04 \text{ SEM}]$, $t = 2.57$, $df = 863$, $p = .01$). This effect indicates that the association between posttreatment negative affect and cortisol differs between the two groups. The positive sign of γ_{061} indicates that the association is more pronounced in the stress group. There was no significant effect neither on the slope nor on the acceleration considering the predictors posttreatment negative affect ($\beta_{16} = 0.0003 [\pm 0.002 \text{ SEM}]$, $t = 0.14$, $df = 217$, $p = .89$; $\beta_{26} = 0.00002 [\pm 0.0002 \text{ SEM}]$, $t = 0.08$, $df = 217$, $p = .94$) and condition ($\gamma_{161} = 0.0003 [\pm 0.002 \text{ SEM}]$, $t = 0.15$, $df = 217$, $p > .88$; $\gamma_{261} = 0.00004 [\pm 0.0002 \text{ SEM}]$, $t = 0.22$, $df = 217$, $p = .83$).

To further investigate the HLM-3 findings, the association of high negative affect scores and low cortisol levels across the entire sample was analyzed further using HLM-2 separately for both conditions. For this purpose, the HLM-3 model, shown in the “Statistical Analyses” section, was reduced by elimination of Level 3. The resulting model was conducted separately with the data of stress, and control, group. For the stress condition, a decrease in mean cortisol levels by 0.05 units was observed when the predictor posttreatment negative affect increases by 1 unit ($\beta_{06} = -0.05 [\pm 0.02 \text{ SEM}]$, $t = -2.4$, $df = 442$, $p = .02$). For the control condition, no significant association between posttreatment negative affect scores and mean cortisol levels was found ($\beta_{06} = -0.008 [\pm 0.05 \text{ SEM}]$, $t = -0.15$, $df = 426$, $p = .90$). When examining these data using the Spearman correlation coefficient, an inverse relationship was found within the stress condition between cortisol levels at 10 minutes after the TSST and posttreatment negative affect ($r = -0.061$, $p = .52$), which, however, was not significant.

Concerning “posttreatment positive affect” as a predictor, we descriptively observed the same results as for posttreatment negative affect, but HLM-3 detected no significant effects across the entire sample 10 minutes after the treatment ($\beta_{05} = -0.02 [\pm 0.02 \text{ SEM}]$, $t = -1.06$, $df = 863$, $p = .28$). Both predictors (posttreatment negative affect and condition) had no effects neither on the slope ($\beta_{15} = -0.0006 [\pm 0.001 \text{ SEM}]$,

$t = -0.57$, $df = 217$, $p = .57$; $\gamma_{151} = 0.0008 [\pm 0.001 \text{ SEM}]$, $t = 0.77$, $df = 217$, $p = .44$) nor on the acceleration ($\beta_{25} = -0.00002 [\pm 0.0002 \text{ SEM}]$, $t = 0.08$, $df = 217$, $p = .94$; $\gamma_{251} = 0.00004 [\pm 0.0002 \text{ SEM}]$, $t = 0.22$, $df = 217$, $p = .83$). However, HLM-2 analysis for the stress condition revealed a decrease in mean cortisol levels by 0.04 units when the predictor posttreatment positive affect increases by 1 unit ($\beta_{05} = -0.04 [\pm 0.02 \text{ SEM}]$, $t = -2.0$, $df = 442$, $p = .05$). For the control condition, posttreatment positive affect scores were not associated with mean cortisol levels ($\beta_{05} = 0.01 [\pm 0.02 \text{ SEM}]$, $t = 0.59$, $df = 426$, $p = .56$).

Relationship Between Affect and sAA

HLM revealed a positive association between sAA levels and affect scores in both conditions. As shown in Figure 5, stressed participants who had high posttreatment negative affect scores displayed higher sAA levels. Accordingly, participants with a low posttreatment score in negative affect showed low sAA levels.

However, analysis with HLM-3 revealed no significant effect for the predictor posttreatment negative affect on the mean sAA levels of the entire sample ($\beta_{06} = 0.10 [\pm 0.07 \text{ SEM}]$, $t = 1.38$, $df = 863$, $p = .17$). Adding the Level 3 predictor condition to the Level 2 predictor posttreatment negative affect had again no significant influence on the mean sAA levels ($\gamma_{061} = -0.04 [\pm 0.07 \text{ SEM}]$, $t = -0.53$, $df = 863$, $p = .60$). There was no significant effect neither on the slope nor on the acceleration considering the predictors posttreatment negative affect ($\beta_{16} = 0.001 [\pm 0.004 \text{ SEM}]$, $t = 0.28$, $df = 219$, $p = .78$; $\beta_{26} = -0.00003 [\pm 0.0003 \text{ SEM}]$, $t = -0.10$, $df = 219$, $p = .92$) and condition ($\gamma_{161} = 0.0002 [\pm 0.004 \text{ SEM}]$, $t = 0.06$, $df = 219$, $p = .96$; $\gamma_{261} = 0.000005 [\pm 0.0003 \text{ SEM}]$, $t = 0.02$, $df = 219$, $p = .99$).

HLM-2 analysis for the stress condition only revealed an increase in mean sAA levels by 0.10 units when posttreatment negative affect increases ($\beta_{06} = 0.10 [\pm 0.04 \text{ SEM}]$, $t = 2.9$, $df =$

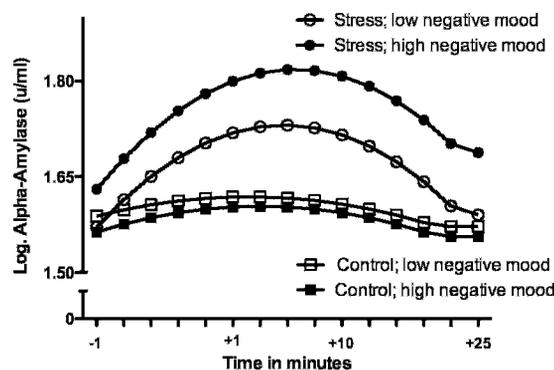


Figure 5. Log-transformed salivary α -amylase (sAA) levels of both groups during the course of the experiments related to negative affective state after the treatment as modeled using hierarchical linear modeling (see “Results” section). High sAA levels are associated with high negative affect scores (above the 75th percentile) in the stress group, whereas low negative affect scores (below the 25th percentile) predict low sAA levels. No relationship between sAA levels and negative affect is detectable in the control group. Note that the percentiles were chosen for graphical purposes only. They were not included in the hierarchical linear modeling analysis.

434, $p = .005$). For the control condition, HLM-2 analysis detected no significant association between mean sAA levels and posttreatment negative affect ($\beta_{06} = -0.05 [\pm 0.09 \text{ SEM}]$, $t = -0.60$, $df = 414$, $p = .55$). Spearman correlation coefficients revealed a similar pattern, indicating a positive, although not significant, relationship between sAA levels immediately after the TSST and posttreatment negative affect within the stress group ($r = 0.14$, $p = .13$).

The predictor posttreatment did not influence the mean sAA levels of all participants ($\beta_{05} = -0.02 [\pm 0.03 \text{ SEM}]$, $t = -0.87$, $df = 863$, $p = .37$). After adding the predictor condition, however, there was an effect on the mean sAA levels 1 minute after the treatment of $\gamma_{051} = 0.07 (\pm 0.03 \text{ SEM})$, $t = 2.18$, $df = 863$, $p = .03$. γ_{051} indicates a significant difference in the relationship between posttreatment positive affect and condition between the two conditions. There were no significant effects for the slope ($\beta_{15} = -0.0001 [\pm 0.002 \text{ SEM}]$, $t = -0.08$, $df = 219$, $p = .94$; $\gamma_{151} = 0.0009 [\pm 0.002 \text{ SEM}]$, $t = 0.47$, $df = 219$, $p = .64$) and the acceleration ($\beta_{25} = 0.00001 [\pm 0.0001 \text{ SEM}]$, $t = 0.09$, $df = 219$, $p = .93$; $\gamma_{251} = -0.00009 [\pm 0.0001 \text{ SEM}]$, $t = -0.63$, $df = 219$, $p = .53$). For the stress condition, HLM-2 analysis revealed an increase of mean sAA levels by 0.09 units when posttreatment positive affect increases ($\beta_{05} = 0.09 [\pm 0.03 \text{ SEM}]$, $t = 2.6$, $df = 434$, $p = .009$). The opposite relationship was observed in the control condition ($\beta_{05} = -0.08 [\pm 0.03 \text{ SEM}]$, $t = -2.28$, $df = 414$, $p = .02$). The latter finding indicates a significant decrease of mean sAA levels by 0.08 units when posttreatment positive affect increases within the control condition.

Other Covariates and Estimation of Variance Components

Other covariates implemented in the models also had effects on cortisol and sAA mean levels. HLM-3 revealed a trend effect of the predictor “smoking” on mean cortisol levels at 10 minutes after the stress or the control condition across all participants ($\beta_{03} = -0.05 [\pm 0.03 \text{ SEM}]$, $t = -1.89$, $df = 863$, $p = .06$). β_{03} indicates that smokers displayed lower cortisol levels by 0.05 units. For “age,” also, a trend was found across all participants with $\beta_{01} = -0.005 (\pm 0.003 \text{ SEM})$, $t = -1.72$, $df = 863$, $p = .09$ indicating that increasing age is associated with decreasing mean log-transformed cortisol levels by 0.005 units 10 minutes after the treatment. “Sex” had a significant influence on mean cortisol levels 10 minutes after the treatment across the entire sample as well ($\beta_{04} = 0.04 [\pm 0.02 \text{ SEM}]$, $t = 2.06$, $df = 863$, $p = .04$). Men displayed higher mean cortisol level than women. For “BMI,” no effect was found ($\beta_{02} = -0.008 [\pm 0.004 \text{ SEM}]$, $t = -2.021$, $df = 863$, $p = .98$). Effects of sex ($\beta_{04} = 0.20 [\pm 0.04 \text{ SEM}]$, $t = 5.22$, $df = 863$, $p < .001$) and age ($\beta_{01} = 0.01 [\pm 0.005 \text{ SEM}]$, $t = 2.85$, $df = 863$, $p = .005$) on mean sAA levels 1 minute after the treatment were detected. Men and older participants displayed overall higher mean sAA levels 1 minute after the stress or the control condition. Neither BMI ($\beta_{02} = -0.010 [\pm 0.007 \text{ SEM}]$, $t = -1.35$, $df = 863$, $p = .91$) nor smoking ($\beta_{03} = -0.06 [\pm 0.05 \text{ SEM}]$, $t = -1.35$, $df = 863$, $p = .17$) was a significant predictor of sAA response 1 minute after the treatment. Furthermore, neither the slopes

nor the accelerations of the curves were influenced by the predictors age, BMI, smoking, or sex (all p values $> .05$) in this rather homogenous group.

An estimated 27.3% (ICC) of the total variation in cortisol levels is attributable to differences between the participants. Conversely, approximately 72.7% of variation is attributable to within-subject variation. Approximately 64% (R^2) of within-subject variation in cortisol levels is attributable to the total effects of time; the rest remains unexplained. There is an increase of explained between-subject variation of approximately 8% to 35.4% (R^2) when significant Level 2 predictors and condition as Level 3 predictor are considered in contrast to the HLM model without any predictors. Regarding sAA, 22.2% (ICC) of the total variation is attributable to between-subject variation; that is, 77.8% is attributable to within-subject variation. However, only 16.2% (R^2) of within-subject variation in sAA levels is attributable to the total effects of time. The explained between-subject variation increased to 40.6% (R^2) when significant Level 2 predictors and condition as Level 3 predictor are considered in contrast to the null predictor model.

DISCUSSION

In the present study, we investigated the relationship among cortisol, sAA, and current affective state in a healthy sample exposed to a stress (TSST) or a control (P-TSST) condition. In line with previous TSST studies, an activation of both main stress systems was observed, with participants in the TSST group displaying higher cortisol and sAA levels (29,39–41). Moreover, the P-TSST induced no HPAA activation and only a mild sAA response, an effect similar to previous reports using this type of control condition (e.g., Schoofs et al. (8), Het et al. (30)).

Although only at a trend level, we observed an attenuating effect of smoking on the HPAA, which is also in line with previous reports (e.g., Kudielka et al. (40), Rohleder and Kirschbaum (42)). The effect of a more pronounced salivary cortisol response to the TSST in men in contrast to women has also been shown previously (40). Furthermore, we observed that male participants displayed overall higher levels of sAA. This finding is in line with some (30,43) but not all previous studies (44,45). The effect of age on acute sAA responses (older participants displayed higher sAA levels) is in need to be replicated. Actually, one recent study found an attenuated sAA response in older adults in comparison to children and young adults (46).

With regard to current affective state, the participants in the stress group reported more negative affect after the experimental manipulations, which is again in line with other studies (6,8,16,31). Using HLM, we detected relationships among cortisol, sAA, time, posttreatment affect, and experimental condition. As expected, we found a positive relationship between sAA levels and high posttreatment affect scores within the stress condition. There are many studies showing that emotional arousal is associated with heightened SNS activity (e.g., references (24–27,47–50)). sAA seems to be associated with enhanced emotional arousal after stress, which could point to an association between increased amygdala activity and sAA (50–52).

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The positive association between sAA levels and high post-treatment affect scores seems to be mediated by stress exposure because we could not observe such a positive relationship within the control condition. In contrast, within the control condition, an increase in positive affect was associated with a decrease in sAA levels, whereas there was no association between negative affect and sAA levels. This might indicate that positive and negative affects are linked differently to sAA levels when there is no stress-related arousal.

Cortisol was found to have an inverse relationship to post-treatment negative affect scores for the entire sample (see $\beta_{06} = -0.13$ in the "Relationship Between Affect and Cortisol" section). That is, high negative affect scores after both treatments (TSST or P-TSST) were related to low cortisol levels 10 minutes after the treatment. In other words, low poststress negative affect ratings occurred in parallel with high poststress cortisol levels. A similar relationship between posttreatment cortisol levels and posttreatment positive affect scores was observed for the entire sample at a descriptive level without reaching any statistical significance (see $\beta_{05} = -0.02$ in the "Relationship Between Affect and Cortisol" section). These relationships were stronger, and using HLM-2, both reached statistical significance within the stress condition but not in the control condition. Thus, we found a significant inverse relationship between cortisol and posttreatment affect scores within the stress condition ($\beta_{06} = -0.05$ for negative affect and $\beta_{05} = -0.04$ for positive affect), whereas there was no significant association between these variables within the control condition ($\beta_{06} = -0.008$ for negative affect and $\beta_{05} = 0.01$ for positive affect). These analyses were focused on the point of maximum cortisol concentrations (usually 10 minutes after stress exposure as described by Dickerson and Kemeny (1), Kirschbaum et al. (29), and Kirschbaum and Hellhammer (33)). The inverse relationships between cortisol and posttreatment affect scores, especially within the stress condition, support our hypothesis of a mood-protective effect of cortisol. High stress-induced cortisol levels might help to cope with the emotional load of a given situation, as has already been described for other systems, for example, the immune system (17). Cortisol seems to downregulate the activity in emotional circuits, thereby helping to reinstate homeostasis. Conversely, participants with high affect scores showed lower cortisol output. Downregulation of emotional arousal might be attenuated in these participants. A blunted cortisol response to an emotional stressor might be associated with a vulnerability to stress-associated affective disturbances (18). This could indicate that stress-induced cortisol elevations are associated with reduced emotional arousal independent of valence. Thus, whereas heightened sAA levels were associated with higher emotional arousal after stress, the opposite pattern became apparent for cortisol.

These observational findings are in line with our hypothesis, which was formerly based on pharmacological cortisol studies only (e.g., references (12,14–16)). One reason why previous observational studies (10,14,16) could not detect a correlational relationship may lie in the lack of power due to relatively small sample sizes or in the use of statistical methods that were un-

suitable for detecting relationships within time series. The latter case may be the reason why we could not detect significant relationships between cortisol and posttreatment affect scores when using the Spearman correlation coefficient. The standard correlation coefficients are suited to detect simple linear relationships by considering one point in time only. However, they fail to find significant associations in case of a nonlinear relationship because the influence of time and other covariates is not considered. Growth-curve modeling, such as HLM, is an appropriate statistical technique for nested repeated-measures data because these procedures allow simultaneous modeling of the effects of time, time-varying, and non-time-varying covariates on the outcome measures (36,53,54). Furthermore, HLM, in contrast to other procedures (e.g., analysis of variance with repeated measures, autoregressive integrated moving average models, or analyses of standard correlation coefficients), makes no assumptions about sphericity or the nature of the correlation between the residuals. Another advantage of HLM is that participants who provided missing cortisol data points can be included, as long as the participants provide enough data to model the shape of cortisol across the investigated time span. In contrast, standard correlation coefficients decrease in statistical power because of a reduction in sample size.

In the context of our current evidence for a mood-buffering cortisol effect, the work of Schlotz et al. (55) is of interest. They recently used time-lagged cross-correlations between cortisol and subjective psychological responses to explain why standard correlations between the HPA and psychological ratings are often so low. Using the TSST, they found a positive time-lagged cross-correlation ($0.48 \leq r \leq 0.54$) as a main effect, indicating that subjective psychological responses to the TSST usually precede HPA responses to this treatment. In addition, of interest for the present work are additional findings from this study (55) indicating that cortisol levels were negatively correlated with subsequent reports of anxiety and activation ($-0.42 \leq r \leq -0.36$). The latter finding indicates that high cortisol levels preceding affective changes were associated with lower and later levels of anxiety and activation (55). This finding is in line with a protective effect cortisol on mood after stress.

In another study (56), the relationship between trait suppression and reappraisal as natural emotion regulation techniques, on the one hand, and cortisol reactivity to an acute social stress task, on the other hand, was analyzed. Both trait suppression and reappraisal predicted more pronounced cortisol responses to the stress task, with participants scoring higher on suppression or reappraisal exhibiting larger cortisol reactivity. Similarly, rumination, which can be considered to be an alternative emotion regulation method in the sense of suppression, although unsuccessful in most cases, seems to be associated with elevated cortisol levels in response to an acute stress task (57). These studies suggest that certain emotion regulation strategies are associated with heightened cortisol reactivity to an acute stressor. The reduction of emotional arousal in challenging situations such as the TSST is important to manage the given situation appropriately because high level of emotional arousal seems to disturb cognitive performances (e.g., Dolcos and McCarthy (58)).

It is important to emphasize that we do not postulate that cortisol prevents negative affect per se. Rather, we hypothesize that stress is always associated with an increase in negative affect initially leading to a positive correlation between affect and cortisol. However, the amount of cortisol released seems to reduce or buffer the magnitude and/or the duration of negative affect, as apparent in a negative correlation at later post-stress points in time (see $\beta_{06} = -0.13$ [HLM-3 model] and $\beta_{06} = -0.05$ [HLM-2 model for the stress group only] in the “Relationship Between Affect and Cortisol” section). Our study does not address the underlying mechanisms of the observed associations. Based on the literature, we assumed that cortisol might alter cognitive processing of emotional information through changing selective attention (59), increasing avoidance behavior (60,61), reducing the activity of the hippocampus on emotional memory retrieval, or affecting the ventral prefrontal cortex and the orbitofrontal cortex, which are important regions in affect regulation (62–65). Interestingly, these brain structures are sensitive to GCs because there is a high density of corticosteroid receptors within these structures (66–69). However, the influence of other neurotransmitters such as dopamine or central corticotropin-releasing hormone might be important as well (70–73). Using functional magnetic resonance imaging, it has been shown that the stress-associated sensitization of the amygdala is counteracted by hydrocortisone, rapidly leading to reduced amygdala responsivity to emotional faces (74). It is suggested that this normalization in amygdala activity is related to an amygdala–prefrontal cortex connection (75). The normalization could be critical for avoiding an overshoot in amygdala activity during or after stress and could enable adequate recovery (74) and cognitive performance (58).

Our results need to be discussed within the coping framework as well. The model by Lazarus and Folkman (76) seems to make opposite predictions to those suggested by our results. It assumes that stressors associated with strong emotional responses lead to perceptions of threat (primary appraisal) and uncontrollability (secondary appraisal). This leads to a stronger negative affective response, which is followed by a more pronounced SNS and HPAA response. Thus, the biologic stress response is theorized to be driven by strong negative affect or distress. This model does not contradict our results. Our hypothesis of mood-buffering cortisol effects starts later in the course of time, namely, at the point at which cortisol levels are already high. Cortisol feedback to the central nervous system not only seems to reduce HPAA activity but also, in a similar fashion, might help to reestablish emotional well-being. We propose that an intense acute stressor causes a strong SNS and HPAA activation. As shown in one study, the HPAA activity seems to be driven by the anticipation of uncontrollability (77). In the short run, high levels of SNS and HPAA activities are related to high emotional arousal and typically to negative emotions. This explains why several researchers have found positive correlations between cortisol and negative affect (e.g., Schoofs et al. (8), van Eck et al. (9)). However, later on, an inverse relationship between cortisol and negative affect seems to occur. In this sense, high stress-associated cortisol levels could

be helpful by facilitating positive reappraisal processes or other effective strategies of emotional regulation (emotion-based coping process (76)).

Finally, the fact that this inverse relationship was found for both affective qualities within the stress condition should be discussed. Previous studies observed that pretreatment application of cortisol on healthy participants (12,16) or on patients with phobia (14) reduced the negative impact of stress on self-reported mood or anxiety, but an effect on positive affective states has not yet been reported (e.g., Reuter (12), Soravia et al. (14), Het and Wolf (16)). It thus remains to be established whether cortisol specifically buffers negative emotional arousal or has broader unspecific effects leading to reduced emotional arousal in general.

The results of this study need to be placed in a somewhat heterogeneous empirical picture. There are studies reporting positive or null correlations between cortisol and negative affect (e.g., Kudielka et al. (6), van Eck et al. (9)). Having said this, some of these studies used an observational open-field design (e.g., Smyth et al. (78)), or they found positive relationships between cortisol and specific negative emotions (e.g., loss and shame (79)) in participants who had been exposed to chronic stress (e.g., war, abuse, assault, or caregiving). It is conceivable that associations observed under chronic conditions differ from those found in a healthy sample exposed to acute stress. We suggest that HPAA activity and its effects on body and mind are different under conditions of acute and chronic stress due to long-term effects of “wear and tear” (e.g., changes in receptor sensitivity (18,80)). It is possible that the cortisol output during acute stress supports emotional coping, a feature that might get lost during chronic stress and chronic HPAA hyperactivity (18,21) as for example in major depression (72).

A nonsignificant relationship between cortisol and negative affect was found (1) in a large and important meta-analysis. One explanation for the discrepancy between our result and the results of this study could lie in differences in timing with regard to the stressors and the affect measurement. The meta-analysis (1) integrated different stressors with different durations and different tasks. In our studies, we always investigated the same stressor. Besides this, the meta-analysis included studies that obtained data on negative affect and distress with different questionnaires. In contrast to this, we used the same questionnaire (PANAS) in all included studies. These different approaches might lead to differences in variance and might explain why we found an effect in contrast to Dickerson and Kemeny (1).

A limitation of our study is the affect measurement used in the present study. The PANAS (34) does not break down the affective ratings into specific emotional states. It would be most interesting to learn whether similar associations would be observed if specific emotional qualities were focused on, for example, fear (14,81) or shame (82). Another limitation of our study is the lack of trait questionnaires. For example, neither chronic stress nor depression was assessed. Given that depression has been associated with high level of negative affect and blunted cortisol reactivity (83–86), variability in trait depression

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could have influenced our results. A broader assessment of potential moderators could have helped to explain the substantial individual variance in the obtained measures of affect. An additional limitation of this study is the number of affect measurements. We only had two points of time of affect measurement, but at least three sampling times are needed for modeling curves with HLM (36). Thus, we could not predict the course of mood over time using the levels of cortisol, which would have been more according to our theoretical framework. We actually used the inversed prediction and reasoned that, if cortisol and affect are related in an inverse relationship, high affect scores after stress exposure must be associated with low poststress cortisol levels. Therefore, all analyses were centered to points in time with high hormone levels. Future studies should investigate the associations between affect and cortisol by measuring affective responses at more than two points of time ideally always in parallel to obtained saliva samples as described in Schlotz et al. (55). Finally, it should be mentioned that poststress negative affect scores were rather low in general (<2 on a 5-point Likert scale; Fig. 3), which is in line with previous studies by our group using the PANAS in combination with the TSST (8,30,87,88). Thus, the associations reported in this article occur in the context of experimentally induced mild to moderate increases in negative affect in young healthy participants. It remains to be established whether similar associations can be detected in the context of more severe changes in negative affect occurring in real-life situations (e.g., major life events) and/or in pathological conditions.

Taken together, we provide further evidence for an inverse relationship between cortisol levels and affective state with this reanalysis. Our correlative findings support findings from pharmacological studies on cortisol's influence on affective states after stress exposure (e.g., Soravia et al. (14), Het and Wolf (16)). In contrast to participants with low cortisol levels, participants with high cortisol levels reported lower level of negative affect, especially after the exposure to stress. In general, it seems that the cortisol output in the context of stress supports the normalization not only of peripheral systems but also of central emotional circuits. Cortisol seems not to prevent affective changes per se but seems to reduce emotional arousal and to reinstate "emotional homeostasis" after stress.

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