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Negative affect as a predisposing factor for cortisol release after an acute stress—the impact of unpleasant priming

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Abstract

Glucocorticoids have a key role in stress responses. There are, however, substantial differences in cortisol reactivity among individuals. We investigated if affective trait and mood induction influence the reactivity to psychological stress in a group of 63 young adults, male ($n = 27$) and female ($n = 36$), aged *ca.* 21 years. On the experimental day the participants viewed either a block of pleasant or unpleasant pictures for 5 min to induce positive or negative mood, respectively. Then, they had 5 min to prepare a speech to be delivered in front of a video-camera. Saliva samples were collected to measure cortisol, and questionnaire-based affective scales were used to estimate emotional states and traits. Compared to basal levels, a cortisol response to the acute speech stressor was only seen for those who had first viewed unpleasant pictures and scored above the average on the negative affect scale. There were no sex differences. In conclusion, high negative affect associated with exposure to an unpleasant context increased sensitivity to an acute stressor, and was critical to stimulation of cortisol release by the speech stressor.

Keywords: *Gender, HPA axis, International Affective Picture System, negative affect, acute stress, salivary cortisol*

Introduction

Personality and temperament traits have long been viewed as crucial variables through which stressors can impact on psychobiological systems, predisposing individuals to disease. Negative affect, a pervasive disposition that manifests itself as the tendency to experience negative emotions (Watson et al. 1988), is a personality-related construct with a putative biological basis (Whittle et al. 2006) and a strong association with abnormal reactivity to stress and increased vulnerability to illness (Kiecolt-Glaser et al. 2002).

Neuroendocrine systems are supposed to play an important role in the causal pathway linking negative affective styles, stress and compromised physical and mental health (McEwen 2002; van Eck et al. 1996). The hypothalamus–pituitary–adrenal (HPA) axis, in particular, is a key component of the neuroendocrine response to stress, its activation leading to the secretion of glucocorticoids and the promotion of wide-ranging changes in the organism. Psychological stressors are long recognized as consistently altering cortisol secretion. A recent meta-analysis of laboratory studies on acute stressors and cortisol responses by

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Dickerson and Kemeny (2004) revealed that among other stressors, performance tasks characterized by social evaluative threat and/or uncontrollability trigger significant elevations in cortisol levels. The authors acknowledged that although some studies showed the role of personality variables in modulating cortisol responses, others have failed. They argued that to identify reliable patterns of cortisol responses to specific situations it would be necessary to match the relevant susceptibility factors with the particular emotional context. The present work aims to address this proposal.

One of the instruments most widely employed to modulate emotional contexts in experimental settings is the International Affective Picture System (IAPS) (Lang et al. 1999). This standard catalog consists of thousands of pictures ranging from very unpleasant to very pleasant ones. Several studies have recorded autonomic and somatic reactions (Bradley et al. 2001; Fachinetti et al. 2006) as well as neuroendocrine responses (Codispoti et al. 2003) to emotional modulation by viewing these pictures. Blocked presentation of pictures with similar affective valence from the IAPS catalogue produces sustained emotional and behavioral reactions that persist during the inter-picture intervals and even after the exposure to the pictures has ceased (Azevedo et al. 2005; Smith et al. 2005; Pereira et al. 2006). Reactions to the pleasant and unpleasant pictures were hypothesized to reflect activation of the underlying brain circuits that mediate appetitive or defensive motivational behavior, respectively (Bradley et al. 2001). Indeed, neuroimaging studies contrasting the stimulation by affective vs. neutral pictures revealed the activation of various brain regions including several limbic structures (Mourão-Miranda et al. 2003; Sabatinelli et al. 2005; Urry et al. 2006).

Here, we investigated whether the pre-activation of the appetitive or defensive motivational systems by the presentation of affective pictures would influence the relationship between affective predispositions and stress reactivity. The chosen stressor was a speech stress task, which was designed to be of moderate intensity (without verbal interaction) in order to allow for both up and down regulation of a cortisol response. We hypothesized that: (1) a blocked presentation of unpleasant pictures prior to a speech stress task would potentiate the cortisol stress response, while the viewing of pleasant pictures would attenuate the expected cortisol release, and (2) that affective predispositions would modulate the magnitude of this response.

Methods

Participants were an initial 72 undergraduate students of the Federal University of Rio de Janeiro (Brazil). They were medication-free (except for the females using oral contraceptives), non-smokers and reported no psychological disorder. All subjects gave written, informed consent and were informed of their right to discontinue their participation at any time. The study protocol was approved by the ethics committee of the Federal University of Rio de Janeiro.

In preparation for the study, participants were asked to refrain from alcohol intake during the previous 24 h, from caffeine intake and excessive physical activity on the day of study, and from consuming food and beverages other than water during the 1 h prior to the experiment. All experimental sessions commenced between 1:00 and 3:00 PM to control for diurnal changes in cortisol secretion and lasted for approximately 2 h. Volunteers had a 30 min period of adaptation before they were taken into a different room where the experiment was conducted. During this same period, psychological measures were assessed using questionnaires. Participants rated their trait affect on the Positive Affect and Negative Affect Schedule (PANAS trait version, Watson et al. 1988), their anxiety trait on the State and Trait Anxiety Inventory (STAI-T, Spielberger et al. 1983) and their resilience trait on the Ego-Resilience Scale (ER89, Block and Kremen 1996).

For affective pre-activation, participants were allocated randomly to either of two groups. One group was assigned to exposure to pleasant pictures ("pleasant-primed"), and the other to unpleasant pictures ("unpleasant-primed"). Affective pictures were selected from the IAPS (Lang et al. 1999)*. The pleasant set comprised 40 pictures that primarily included photographs of families and babies, nature scenes, puppies, sports and romance. The 40 pictures in the unpleasant set consisted of mutilated bodies, human and animal attack, pollution and accidents. Each picture was presented for 5 s with an inter-picture interval of 2 s. The presentation of the sequence of pictures lasted 5 min and at the end participants rated the valence and arousal of the viewed block using the Self-Assessment Manikin Scale (Lang et al. 1999). Next, participants had 5 min to read a text about a neutral theme and prepare a free speech about it. Speech delivery had a mean duration of 5.8 (±1.44) min. The whole session was videotaped and participants were told that the experimenters were monitoring them remotely through the camera. Additionally, the speech task instructions

*IAPS numbers for unpleasant pictures in the sequence of presentation used here are: 3530, 6260, 6350, 3500, 6313, 6560, 6570, 6312, 1050, 1120, 1300, 1930, 1303, 1321, 1220, 1931, 3060, 3110, 3130, 3170, 3000, 3053, 3064, 3030, 9600, 9910, 9920, 9921, 9911, 9912, 9611, 9620, 9300, 9320, 9290, 9373, 9390, 9340, 9560, 9410; for pleasant pictures are: 5000, 5760, 5780, 5830, 5600, 5200, 5260, 5982, 2070, 2340, 2360, 2311, 2345, 2341, 2057, 2260, 1460, 1750, 440, 1710, 1920, 1721, 1463, 1722, 8190, 8200, 8210, 8400, 8180, 8370, 8490, 8185, 4660, 4533, 4532, 4599, 4641, 4640, 4250, 4608.

stated that the performance tape would be later evaluated by senior researchers.

Salivary cortisol is considered to be a reliable and valid measure of unbound ("free") cortisol concentration in the plasma. Three unstimulated saliva samples were collected during the experimental session by the use of a cotton roll under the tongue for 5 min. Samples were collected at −25 min (basal), +25 min (stress) and +40 min (recovery) relative to the start of preparing the speech. Saliva was extracted from the cotton roll by centrifugation at 5000 rpm for 10 min. Cortisol concentrations were determined by radioimmunoassay using a commercial kit (CORT-CT2, Cis-Bio). To reduce error due to intra-assay variation, all samples of one subject were analyzed in the same assay.

Participants rated their current mood (state affect) on the PANAS state version (Watson et al. 1988) four times during the experimental session: after adaptation, after pre-activation with pictures, after speech delivery and at the end of the session.

Participants in the "pleasant-primed" and the "unpleasant-primed" groups were independently assigned to "low" or "high" sub-groups based on a mean-split of each trait score (pleasant-primed, mean ± SD: 18.4 ± 5.64; unpleasant-primed: 17.6 ± 5.25). Gender differences in cortisol reactivity were analyzed with a mixed design ANOVA, with Greenhouse–Geisser correction, with TIME (basal, stress, recovery) as within-subject factor and PRIMING (pleasant, unpleasant) and GENDER (men, women) as between-subject factors. Influence of menstrual cycle phase on cortisol reactivity was analyzed with a mixed design ANOVA with Greenhouse–Geisser correction with TIME (basal, stress, recovery) as within-subject factor and PRIMING (pleasant, unpleasant) and CYCLE PHASE (luteal, follicular) as between-subject factors. Distribution of women using contraceptive pills in the two priming groups was analyzed by χ^2 test. Modulation of cortisol concentration was evaluated using a mixed design ANOVA with Greenhouse–Geisser correction with TIME (basal, stress, recovery) as within-subject factor and PRIMING (pleasant, unpleasant) and TRAIT (high, low) as between-subject factors. *Post hoc* tests were performed with Tukey's HSD. Spearman correlation analyses were used to evaluate the relationship between traits and cortisol response. Comparisons of the ratings for the block of pictures on the valence and the arousal dimensions were conducted separately using the Mann–Whitney *U*-test. Age and body mass index were compared between the two priming groups, for men and women separately, with Student's *t*-test for independent samples. Modulation of the positive and of the negative affect state through the session was analyzed for each group with one-way ANOVAs with Greenhouse–Geisser correction with TIME (adaptation, pre-activation, speech, end) as a single factor. Statistical tests were conducted at the 0.05 level of significance.

Results

Data analysis only included the participants ($N = 63$; Table I) who provided assayable volumes of saliva samples at the three critical time points for cortisol assays (basal, stress and recovery). The pleasant-primed and unpleasant-primed groups did not differ significantly with respect to age (men: $t = 0.04$, $p = 0.96$; women: $t = 1.06$, $p = 0.29$) and body mass index (men: $t = -0.96$, $p = 0.34$; women: $t = 0.04$, $p = 0.96$) (Table I). The investigation of differences in cortisol responses for male and female participants through the TIME × PRIMING × GENDER mixed design ANOVA revealed no main-effect for GENDER ($F(1,59) = 2.544$, $p = 0.11$) nor interaction of GENDER with the other factors (TIME and PRIMING). Further, in women volunteers, the mixed design ANOVA (TIME × PRIMING × CYCLE PHASE) revealed no main effect for CYCLE PHASE ($F(1,31) = 0.003$; $p = 0.95$) nor interaction with the other factors (TIME and PRIMING). Only one-third of the sample were using oral contraceptive pills, and they were evenly distributed between the priming groups (pleasant: 7 out of 21; unpleasant: 5 out of 14; $\chi^2 = 0.02$, df = 1, NS). Analyses with the group of women were conducted with 35 subjects since one participant failed to report both the last menstrual period and the use of contraceptives. Based on these results, all subsequent analyses were then conducted grouping men and women together.

As expected, valence ratings for the pleasant block of pictures were significantly different from those of the unpleasant one ($t = 11.42$, $p < 0.001$). On the arousal dimension, the comparison of the ratings of the two groups of participants did not reach statistical significance ($t = -1.92$, $p = 0.06$).

There was a significant main effect for the modulation of the negative affect state for the pleasant-primed ($F(3,99) = 8.324$, $p < 0.001$, $\varepsilon = 0.63$) and unpleasant-primed ($F(3,81) = 7.454$, $p < 0.001$, $\varepsilon = 0.76$) groups, as well as for the positive affect state in the former ($F(3,99) = 9.8$, $p < 0.001$, $\varepsilon = 0.56$) and in the latter ($F(3,81) = 19.707$,

Table I. Sample characteristics by gender.

	Pleasant primed		Unpleasant primed	
	Men	Women	Men	Women
Sample size	13	21	14	15
Age (years)	21.5 (2.91)	21.5 (3.28)	22.1 (3.82)	21.7 (3.49)
BMI (kg/m^2)	22.3 (2.14)	22.2 (2.13)	22.3 (2.11)	22.2 (2.06)

Data are mean ± SD. BMI, body mass index.

Table II. Positive and negative affect state (PANAS-S).

Time	Basal (adaptation)	Pre-activation (picture viewing)	Stress (speech)	End (recovery)
Pleasant primed				
PA	28.5 ± 6.97[a]	25.8 ± 9.08[b]	26.5 ± 8.40[c]	22.6 ± 8.71[abc]
NA	15.7 ± 6.74[a]	11.9 ± 3.95[ab]	15.5 ± 6.34[bc]	12.1 ± 2.60[c]
Unpleasant primed				
PA	27.4 ± 7.06[abc]	22.8 ± 6.95[a]	21.6 ± 7.23[b]	20.1 ± 8.03[c]
NA	14.0 ± 4.11[a]	14.7 ± 4.71[b]	15.0 ± 4.73[c]	11.9 ± 2.46[abc]

Data are mean ± SD. PA: positive affect. NA: negative affect. The presence of the same superscript letter denotes the existence of statistically significant difference ($p < 0.05$) between two time conditions. PA and NA are analyzed separately.

$p < 0.001$, $\varepsilon = 0.89$) groups. Negative affect decreased significantly after exposure to pleasant pictures ($p = 0.002$) and increased after the stress task ($p = 0.003$). On the other hand, subjects who viewed unpleasant pictures showed a significant reduction in positive affect after exposure to the pictures ($p = 0.001$). Both groups showed a significant decrease in the negative and in the positive affect at the end of the experiment. One participant from the unpleasant-primed group was excluded from the analysis of affect modulation because he failed to fill out one of the PANAS-state scales correctly. Table II summarizes the results.

The mixed design ANOVA (TIME × PRIMING × TRAIT) revealed a significant TIME effect for the cortisol concentrations ($F(2,118) = 16.24$, $p < 0.001$, $\varepsilon = 0.62$). More importantly, there was a significant interaction between TIME, TRAIT-negative affect and PRIMING ($F(2,118) = 4.32$, $p < 0.05$, $\varepsilon = 0.62$). Only those with high negative affect and unpleasant-primed significantly increased salivary cortisol concentrations after the acute stress ($p = 0.0005$). Participants with low negative affect and unpleasant-primed as well as those pleasant-primed presenting either high or low negative affect trait did not show a significant increase in cortisol concentration after the stress task. Figure 1 depicts the mean values of salivary cortisol at each time point for each group.

Importantly, for the participants primed with unpleasant pictures, the cortisol response (stress minus basal) correlated with the scores on the negative affect trait scale ($r = 0.61$; $p < 0.05$). That is, increased negative affect was associated with greater

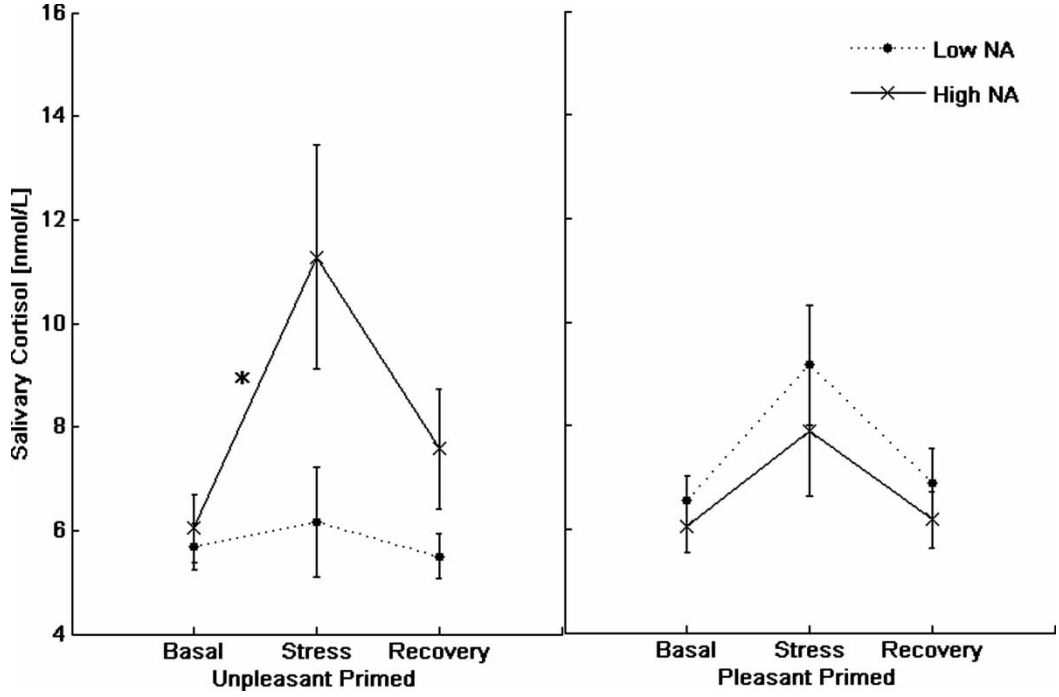

Figure 1. Priming vs. negative trait. Mean salivary free cortisol concentration (± SEM) is depicted at three time points relative to the start of the preparation of a speech: −25 min (basal), + 25 min (stress), and +40 min (recovery). Results for the unpleasant-primed group ($n = 29$; high NA: $n = 11$) are shown at the left side and those for pleasant-primed ($n = 34$; high NA: $n = 15$), at the right side. High NA participants are represented by solid lines and low NA participants, by dashed lines. Only the unpleasant-primed group with high NA significantly increased salivary cortisol ($p < 0.05$). NA: negative affect.

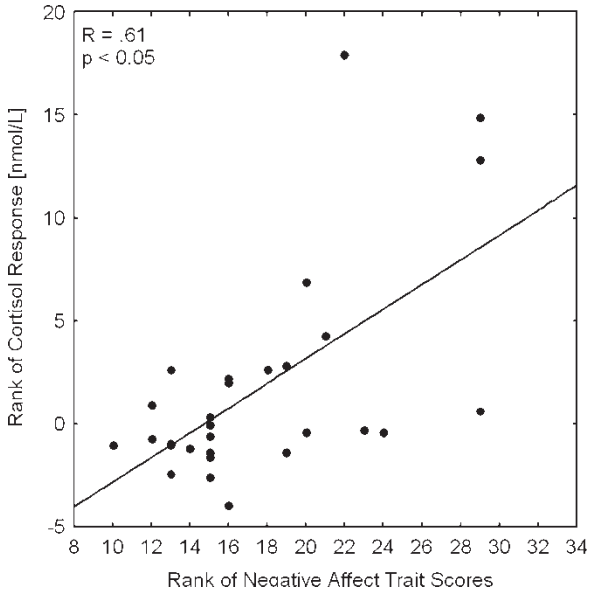

Figure 2. Dispositional negative affect vs. cortisol response for unpleasant-primed participants ($n = 29$). The scatter plot depicts the relation between the magnitude of cortisol response (stress minus basal) and the rank ordering of negative affect trait as measured by the Positive Affect and Negative Affect Schedule—Trait (PANAS-T).

cortisol reactivity to the stress task (Figure 2). In contrast, for the pleasant-primed group no such association was found ($r = -0.29$; $p > 0.05$).

Discussion

To the best of our knowledge, this is the first study to evaluate whether the pre-activation of the appetitive or defensive systems would affect the relationship between personality traits and the HPA axis response to an acute stress. We showed that, depending on the degree of individual negative affectivity, previous exposure to stimuli with opposite hedonic valence differentially impacts on the amplitude of the cortisol response to a subsequent stress task. We found that prior exposure to unpleasant pictures led to a significant increase in the salivary cortisol response to a stress task specifically in individuals with high negative affect trait. This effect was not found for those primed with pleasant pictures, irrespective of their affective predisposition. These findings strongly suggest that affective contextual settings may operate in tandem with individual predispositions in modulating the neuroendocrine response to acute stress. As seen in Figure 1, our data suggest that the combination of high negative affect and unpleasant priming accounts for most of our findings.

Several stress-related neural circuits are known to influence the HPA axis (Urry et al. 2006). The amygdala is one of the key structures involved in the affective modulation by unpleasant pictures (Davidson 2003; Mourao-Miranda et al. 2003; Sabatinelli et al. 2005), and animal studies showed that amygdala activation enhances the secretion of glucocorticoids (Herman et al. 2005). Some studies had further demonstrated that individual variability could modulate the activation of the amygdala by unpleasant pictures (Phan et al. 2005; Sabatinelli et al. 2005). In particular, a recent study found that increased signal intensity in the amygdala in response to unpleasant vs. neutral pictures (selected from the IAPS) correlated robustly with higher levels of dispositional negative affect as measured by PANAS (see Figure 6 in Davidson 2003). This observation provides an explanatory framework for our findings. In the present study, we found a significant correlation between the cortisol response and PANAS negative affect exclusively in participants primed with unpleasant pictures from IAPS. Our correlation dovetails with the neuroimaging results described above. It could be hypothesized that among the unpleasant-primed participants, those with higher dispositional negative affect would have the amygdalae sensitized by the pictures facilitating the release of cortisol in response to the subsequent speech stressor.

The possibility cannot be excluded that the exposure of some of our volunteers to pleasant pictures may have acted "protectively", attenuating the endocrine response to stress. It has been suggested that viewing of pleasant pictures pre-activates the appetitive system (Bradley et al. 2001). Indeed, a recent study showed that pleasant stimuli (social support) are a consistent way of blunting the cortisol response to a speech stress task (Heinrichs et al. 2003). Future studies including a control group submitted to priming with neutral pictures could clarify if pleasant priming indeed exerts an attenuating effect.

Despite the many existing lacunae in the knowledge about the factors that influence physiological reactivity to psychological stress, it is likely that our findings will have clinically relevant implications. A substantial body of literature supports the existence of a strong association between the physiological changes of chronic stress (including overactivation of the sympathetic nervous system and alterations of the HPA axis) with adverse physical and mental health outcomes (McEwen 2002; Rohleder et al. 2003; Herman and Seroogy 2006). Identifying the temperamental traits and the underlying mechanisms that predispose individuals to the negative consequences of stress may well be one of the critical steps required in order to develop successful preventive strategies.

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