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Stress Response to the Functional Magnetic Resonance Imaging Environment in Healthy Adults Relates to the Degree of Limbic Reactivity during Emotion Processing

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Key Words
Emotion · Functional magnetic resonance imaging · Amygdala · Stress · Cortisol

Abstract
Background: Imaging techniques are increasingly being used to examine the neural correlates of stress and emotion processing; however, relations between the primary stress hormone cortisol, the functional magnetic resonance imaging (fMRI) environment, and individual differences in response to emotional challenges are not yet well studied. The present study investigated whether cortisol activity prior to, and during, an fMRI scan may be related to neural processing of emotional information. ***Methods:*** Twenty-six healthy individuals (10 female) completed a facial emotion perception test during 3-tesla fMRI. ***Results:*** Prescan cortisol was significantly correlated with enhanced amygdala, hippocampal, and subgenual cingulate reactivity for facial recognition. Cortisol change from pre- to postscanning predicted a greater activation in the precuneus for both fearful and angry faces. A negative relationship between overall face accuracy and activation in limbic regions was observed. ***Conclusion:*** Individual differences in response to the fMRI environment might lead to a greater heterogeneity of brain activation in control samples, decreasing the power to detect differences between clinical and comparison groups.

The hypothalamic-pituitary-adrenal (HPA) axis is the primary circuit of the neuroendocrine stress system and a critical route by which the brain influences physical and psychological processes following exposure to threatening stimuli [1]. The HPA axis response to stress is a relatively slow hormonal cascade resulting in the production of glucocorticoids (cortisol in humans and corticosterone in animals). One of the primary functions of the HPA axis is to mobilize resources for defense during stress and/or threat and subsequently for repair and healing [2]. Al-

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though fundamentally adaptive, the stress response system can become dysregulated under conditions of chronic or traumatic stress, resulting in increased susceptibility to physical and mental disorders [3]. Illustratively, exaggerated cortisol stress responses and elevated basal levels of endogenous cortisol have been implicated in the development and maintenance of depression and other mental disorders [4]. This relation may be partially mediated by enhanced activation and dendritic hypertrophy in the amygdala, as well as reduced dendritic arborization in the hippocampus [5].

Much remains to be learned about the relations between neurobiological and physiological responses to stress and associated emotions. Recent research on the effects of exogenous cortisol suggests that relations between cortisol and negative emotions are dependent on situational factors, including the novelty of the experimental environment and task familiarity [6]. Studies have also found that elevated levels of cortisol may improve accuracy on emotional memory tasks [7, 8]. Few investigations have examined anticipatory cortisol or cortisol change in adults undergoing a functional magnetic resonance imaging (fMRI) scan in the absence of a standardized stressor task prior to scanning. Thus, it is still unknown whether the actual assessment of neural activity in a novel, confined space itself induces a physiological stress response in healthy adults, or if this may accentuate individual differences in stress physiological response, either of which could have implications for interpreting neuroimaging data gathered during an emotional processing task.

Networks involved in cortisol production and modulation overlap with cognitive and affective networks that process emotion experience and regulation. Experimental and neuroimaging research on the neural correlates of cortisol has largely focused on the hippocampus, amygdala, and the prefrontal cortex (PFC) [5, 9]. Following stress, increased activity in the hippocampus facilitates downregulation of the HPA axis via inhibitory connections to the paraventricular nucleus of the hypothalamus [10], revealing an inverse relationship between hippocampal activation and the cortisol response to stress [11–13]. Conversely, increased activation in the amygdala is correlated with increased cortisol levels, suggesting that the amygdala is involved in the recruitment and ongoing excitation of the HPA axis [14, 15]. In addition, the insula is involved in interoceptive monitoring [16] and may respond to fluctuations in cortisol. Insula and amygdala involvement in saliency, emotion, and attention is well established [17], and hyperactivity within this network may underlie anxiety-related processes [5, 17, 18]. Anatomical connections to the hypothalamus, amygdala, nucleus accumbens, and additional limbic structures have led to the supposition that this network is involved in autonomic and visceral regulation [19]. Areas of the lateral and medial PFC participate in both the activation and regulation of the HPA axis [20]. For example, left and right lateral PFC activation has been associated with increased and decreased cortisol reactivity to psychosocial stress, respectively [11, 20–22], and activation of the medial PFC has been associated with decreased cortisol reactivity to stress [11]. Given its functional connectivity with the amygdala, the medial PFC likely participates in the downregulation of the stress response and might facilitate attenuation of cortisol levels [23–26].

fMRI is increasingly being used to examine the neural circuits implicated in the activation and modulation of the stress response system. However, it is unclear whether the fMRI scanner environment itself may elicit HPA activation or may be affected by individual differences in stress responses. The anticipation and experience of fMRI procedures can potentially evoke distress, anxiety, claustrophobia, and arousal of the sympathetic nervous system [27–30]. Low to moderate levels of stress from fMRI exposure may increase cortisol levels, thereby affecting neural functioning and task performance, even among healthy control subjects [31]. A few investigations have demonstrated that anticipation of and first-time exposure to fMRI procedures increases cortisol and heart rate [28, 31–33], whereas others have found that fMRI produces a more nuanced stress effect, impacting only some individuals or dissipating over the course of an fMRI scan [27, 34]. The lack of clarity on this important topic is notable given the substantial amount of research conducted using emotion regulation paradigms that operate on existing assumptions about cortisol reactivity for 'baseline' conditions. In short, these presumptions maintain that cortisol levels measured over the course of fMRI procedures do not differ from HPA activity as measured over typical daily activities [35, 36]. These assumptions may impede the appropriate use of emotion and stress challenge tasks in the fMRI environment and may threaten clear interpretation of results when these challenge tasks are used in concert with assessment of HPA reactivity.

In building a better understanding of the physiological response elicited by fMRI procedures, we tested the following hypotheses: (1) cortisol levels immediately prior

to the fMRI procedures would be higher compared to baseline day measurements from nonscan days; (2) individual differences in cortisol in anticipation of an fMRI scanning session (prescan cortisol) will predict greater subsequent activation in the limbic and regulatory regions of the brain during an emotional perception task; (3) individual differences in cortisol change from pre- to postscan during the emotional perception task will be related to greater activation in the limbic and regulatory regions of the brain, and (4) increased brain activation in the limbic and regulatory regions of the brain during the emotional perception task will be associated with poorer performance on the task.

Methods

Participants

Twenty-six participants (10 female) were recruited as healthy control comparison subjects for one of two studies, an investigation of emotion processing in Cushing's disease [5] and an investigation of emotion processing in major depressive disorder [37]. Participants for both studies were recruited through advertisements at the University of Michigan and in the surrounding communities. Healthy controls for the study of Cushing's disease were scanned from 2005 to 2008, and healthy controls for the major depressive disorder study were scanned from 2007 to 2012. In the current study, participants ranged in age from 18 to 65 years, with an average age of 37.2 years (SD 17.2). For both studies, individuals were nonsmokers and medication free, including hormonal contraceptives and hormone replacement therapy for female participants. The menstrual cycle phase on the day of scanning was not collected for female participants. Participants were excluded from scanning if their weight exceeded 220 pounds (body mass index >35).

Procedure

Upon arrival at the study laboratory, participants completed informed consent and were interviewed by a psychologist or a clinical psychology trainee at the advanced graduate level in accordance with the Structured Clinical Interview for DSM-IV (SCID-IV) nonpatient edition. Only participants who did not endorse any past or current psychiatric or neurologic disorder, including alcohol or other substance abuse or dependence (both for themselves and first-degree family members), were included. Participants were also screened using a structured interview to assess them for neurological conditions and for fMRI safety. At the time of the screening interview, participants were briefed on the scanning procedure. The fMRI data were collected on a separate day following the screening interview. All participants in the current study were run through an identical screening, diagnostic, neuroimaging, and cortisol protocol, including acquisition of images in the same 3-tesla Signa scanner. Upon arrival, participants' salivary cortisol (prescan cortisol) was measured (see procedure timeline, online suppl. fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000369027). Participants were then guided through a practice run of the study tasks while outside of the scanner in order to limit learning bias in the results. The study tasks, including the facial emotion perception test (FEPT), were then administered in the scanner, for a total time of approximately 70 min (including placement). After being removed from the scanner, participants were given a brief break before providing a final saliva sample. Finally, all participants were debriefed and compensated for their time.

Salivary Cortisol Collection and Assay

Participants provided saliva samples 15–30 min prior to entering the scanner and approximately 20–35 min following completion of the 25-min FEPT [38, 39], using Salivette Cortisol tubes (Sarstedt AG & Co.; online suppl. fig. 1). These cortisol measurements provide an assessment of participants' anticipatory stress related to the fMRI tasks/scanner, as indicated by prescan cortisol level, and of their response to the FEPT, as indicated by measuring the change between prescan and postscan cortisol levels. Scans were collected between 8 a.m. and 4 p.m., though the majority of the scans (62%) were collected in the morning. Scheduled arrival times for scanning assured that all participants were awake at least 1 h prior to scan to diminish the impact of the cortisol awakening response. Although participant wake time was not available, the time of scan was recorded and transformed into a 24-hour variable for use as a covariate in imaging regression analyses to account for circadian profile throughout the day. Due to concerns about prescan anticipatory stress with fMRI, we invited the last 13 subjects (5 female) to provide additional saliva samples throughout a nonscan weekday. These subjects consented to collecting saliva samples at 8 a.m., 12 p.m., 4 p.m. and 9 p.m. during the course of their normal weekday activities (Tuesday, Wednesday or Thursday) on a day different from the fMRI scan. These measurements allowed for a rough comparison of baseline cortisol to pre- and postscan levels assessed on the day of fMRI participation.

All cortisol samples were stored at –80°C until they were sent to the Clinical Ligand Assay Service Satellite (CLASS) Laboratory at the University of Michigan School of Public Health Department for analysis. The competitive immunoassay was on a Siemens Centaur automated analyzer, using chemiluminescent technology. The inter- and intra-assay coefficients of variation at 0.7 μg/dl were 12.4 and 3.6%, respectively [40]. Average prescan cortisol was 0.62 μl (SD 0.42) and postscan cortisol was 0.54 μl (SD 0.55). To allow for comparison to other studies, all cortisol values (μg/dl) were log transformed. The log-transformed and raw cortisol values were highly correlated ($r = 0.87$). One subject was excluded from prescan cortisol modeling and 1 subject was excluded from postscan cortisol modeling for having insufficient quantities of saliva for analysis. The 2 subjects missing one cortisol sample were excluded from analyses of cortisol change (pre- to postscan cortisol change), resulting in 24 subjects for this regression model. To assess cortisol change over the course of the experiment, a standardized residualized change score was computed by regressing the postscan cortisol sample on the prescan sample. A residualized change score is preferable to a difference score because it adjusts for individual differences in prescan cortisol. The residualized change score, in the majority of cases, will be negative, consistent with the typical decline in cortisol for any two consecutive measurements during the course of a day. Thus, individual differences in the presence and amount of decline, or the absence thereof, can be assessed as they relate to blood oxygenation level-dependent (BOLD) changes.

Facial Emotion Perception Task

The FEPT [5, 37, 41] was designed to assess both the accuracy and speed with which participants can identify facial expressions. As part of the task, participants also identify animals, in order to provide a way by which to control for visual processing ability and fine motor speed and isolate the face-specific performance. During the task, participants categorize faces (MACBrain Foundation) [42, 43] into one of four categories (fearful, angry, happy, or sad) and animals into one of four categories (dogs, cats, primates, or birds). To limit the bias introduced by learning, participants first complete a trial run with the same timing and instructions, outside the scanner, using the Ekman faces [38, 44]. The task was designed based upon prior work to detect biases in emotional identification, so, neutral faces are presented for some trials, yet neutral is not a choice available to participants.

Each trial began with a briefly presented orienting cross (500 ms) followed by presentation of the stimulus face or animal (300 ms), a visual mask (100 ms), and a response window (2,600 ms) during which participants select the category of choice using a 5-button response claw. The practice run, which was conducted outside of the scanner, included 12 animal trials and 43 face trials and ran for 7 min. The in-scanner version of the task was comprised of 56 animal and 147 face trials and ran for 25 min, with 21 face blocks and 8 animal blocks of 7 consecutive stimuli separated by 2 repetition times (TRs; 3,500 ms) across 5 runs. To control for any unanticipated effects on subsequent processing speed or accuracy, the emotions portrayed on the faces were counterbalanced to the second order, such that every emotion was equally likely to be followed by every other emotion. For example, there was an equal number of fear faces preceded by and followed by every other emotion. This allows for deconvolution of individual emotion events, as each event is separated by at least 2 TRs, and similar events are separated by at least 4 TRs. Dependent variables were the number of faces correctly categorized and the speed of response time for each emotion. Accuracy for correctly identified facial emotions (face accuracy) for this sample was 83%, with an average reaction time of 1,296.3 ms (SD 142.6), which is consistent with previous findings [38].

fMRI Acquisition and Processing

Whole-brain imaging was performed using a GE Signa 3-tesla scanner. fMRI series consisted of 30 contiguous oblique-axial sections acquired using a forward-reverse spiral sequence. The image matrix was 64 × 64 over a 24-cm field of view (FOV) for a 3.75 × 3.75 × 4 mm voxel. The 30-slice volume was acquired serially at 1,750 ms temporal resolution for a total of 590 time points for the FEPT. One hundred six to one hundred twenty-four high-resolution Fast SPGR IR axial anatomic images [TE = 3.4 ms, TR = 10.5 ms, 27° flip angle, number of excitations = 1, slice thickness = 1–1.2 mm, FOV = 24 cm, matrix size = 256 × 256] were obtained for each participant for co-registration and normalization purposes.

Processing of images was conducted using SPM8, including slice timing, realignment, motion correction, co-registration, DARTEL warping (using VBM8 toolbox), normalization to the MNI world space, and smoothing with a 5 FWHM filter. Contrast images were derived by subtracting the BOLD signal during the animal-processing blocks from the BOLD signal during the face-processing blocks ('faces – animals'). Images from the event-related models were created by subtracting the BOLD signal for emotional face events from neutral face events (e.g. 'fear – neutral'). Fearful and angry faces in particular were chosen for analysis given the ability of threatening stimuli to influence HPA axis and amygdala reactivity in both human and animal studies [9, 22, 45]. Sad and happy, in comparison to neutral, faces are included as online supplementary tables 1 and 2 for purposes of completeness.

Data Analysis

The SPM8 hemodynamic response function model was used to model the BOLD response. Multivariate linear regression analyses were conducted using whole-brain analyses from the individual group contrasts in SPM8. All coordinates for activation foci were translated from MNI to Talairach space. Statistical significance for regression analyses in SPM8 was set at $p < 0.005$, with a cluster minimum of 440 mm^3 (55 2-mm cubic voxels). Based upon 1,000 Monte Carlo simulations with AlphaSim inside the whole-brain search region, a whole-brain corrected α of 0.05 is achieved with this combined height by extent threshold strategy [46]. Based upon a priori hypotheses, the hypothalamus, amygdala, and hippocampus were used as regions of interest (ROI), with Bonferroni correction, as well as an extent threshold of 15 mm^3. Post hoc analyses used extracted data from regions identified in analyses to probe underlying performance correlates.

All regression analyses were completed with gender, age, percent of correctly identified faces (face accuracy), and time of scan (converted to 24-hour time variable) as covariates. Multivariate linear regression was used to determine if prescan cortisol levels were predictive of activation in the limbic and regulatory regions of the brain during the task and to determine if activation in the limbic and regulatory regions of the brain during the task were predictive of cortisol change across the task in those with pre- and postscan measurements. Multiple regression was also used to examine whether brain activation in the limbic and regulatory regions of the brain predicted poorer task performance. The dependent variables of interest were brain activation for 'faces – animals' (block), 'fear – neutral' (event-related model), 'anger – neutral' (event-related model). 'Sad – neutral' and 'happy – neutral' were included for completeness, although there is a weaker link between these emotions and stress reactivity. These data are available in online supplementary tables 1 and 2.

Results

fMRI Cortisol and Baseline Day Cortisol

To evaluate whether the anticipation and completion of fMRI alters cortisol change from baseline (hypothesis 1), weekday cortisol values were used to create an interpolated trend line representing average cortisol across the day for the subsample (n = 13). Time of scan was linearly related to cortisol level. The trend line is depicted in figure 1 and includes standard error bars with raw scores to evaluate fit. Pre- and postscan cortisol values were plotted for each subject (n = 26) against the interpolated trend line. There was an average of 16% increase

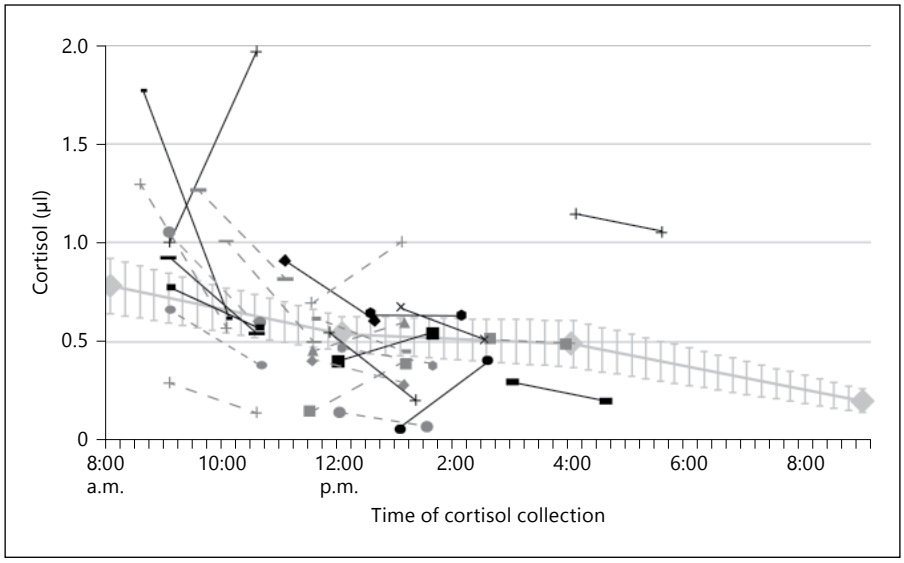

Fig. 1. Pre- to post-fMRI cortisol (raw values) by individual subject compared to baseline average. Blackened markers indicate subjects who contributed to baseline values (n = 13).

in cortisol for the prescan measurement compared to baseline assessments on a nonscanning day, a small effect size (d = 0.28). Sixteen individuals had values above the interpolated baseline and 10 had values at or below the baseline, adjusting for time of day. For a more specific analysis, to make certain that there were no undue influences of cortisol measurements from those who contributed baseline values, the analyses were repeated for only the 13 participants who contributed values at baseline and during the scanning day. There was a 20% increase of cortisol at baseline relative to the baseline day. Nine subjects exhibited an increase in cortisol, 3 subjects showed a decrease, and 1 subject had no change relative to the interpolated baseline from the same subjects. Age and gender were not related to cortisol values.

Individual Differences in Prescan Cortisol and BOLD Activation to Emotional Faces

To evaluate whether greater prescan cortisol was associated with increased activation in limbic regions (hypothesis 2), prescan cortisol levels were regressed onto activation for fearful faces ('fear – neutral' contrast) (table 1; fig. 2). Those with higher prescan cortisol had greater activation in the cingulate gyrus, anterior cingulate and postcentral regions for the 'fear – neutral' contrast (fig. 2a). There were no significant foci of activation in the 'anger – neutral' contrast in the regression with prescan cortisol. Activations for contrasts of 'sad' and 'happy' are reported in online supplementary table 1. Activation for the 'sad – neutral' contrast was not associated with prescan cortisol. For the 'happy – neutral' contrast, there was a positive association between prescan cortisol and activation in the right rostral cingulate and precuneus.

Differences in activation for the 'faces – animals' contrast are illustrated in figure 3 and reported in table 2. Whole-brain-corrected analyses showed activation in the subgenual cingulate gyrus (fig. 3a, b), which was significantly predicted by higher levels of prescan cortisol. ROIs indicated that higher prescan cortisol was also significantly related to greater activation in the right amygdala (fig. 3d) and left parahippocampal gyrus. Activation from the subgenual cingulate was significantly positively correlated with the right amygdala and hippocampus (extracted mean activation; fig. 3c). Post hoc regression analysis was computed for each emotion as a predictor of activation in the subgenual anterior cingulate and right amygdala. 'Fear' (B = 0.18, t = 2.49, p = 0.02*)* and 'happy' (B = 0.77, t = 7.51, p < 0.0001) significantly predicted subgenual anterior cingulate activation for 'faces – animals'. 'Fear' (B = 0.41, t = 2.64, p = 0.02*)* and 'anger' (B = 0.43, t = 3.34, p = 0.004*)* predicted significant overall 'faces – animals' activation in the right amygdala.

Individual Differences in Cortisol Change during Scan and BOLD Activation to Emotional Faces

We also examined the association between changes in cortisol from pre- to postscan and activation in response to the 'fear – neutral' contrast (hypothesis 3; fig. 2). Positive associations were observed between cortisol change and activation for 'fear – neutral' in the precuneus and lingual gyrus (fig. 2b). In the 'anger – neutral' contrast, activation in the inferior temporal gyrus, superior parietal

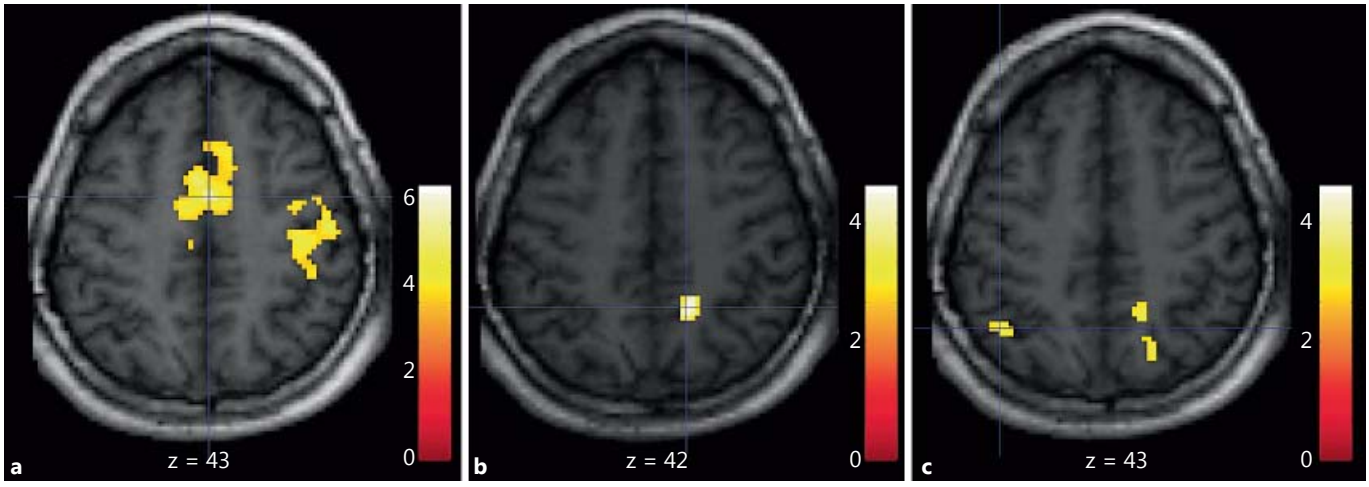

Fig. 2. Areas of significant activation regressing 'fear – neutral' and 'anger – neutral' on prescan cortisol (whole-brain corrected). **a** Activation in the mid-cingulate gyrus and anterior cingulate in response to fearful faces is associated with increased prescan cortisol. **b** Activation in the right precuneus in response to fearful faces is associated with a positive residualized change in cortisol. **c** Activation in the right precuneus and left superior parietal lobule in response to angry faces is associated with a positive residualized change in cortisol.

Table 1. Foci of significant activation for prescan cortisol in 'fear – neutral' event-related contrasts

Contrast/lobe	BA	Talairach coordinates			Z	mm^3
		x	y	z		
Fear – neutral						
Positive regressor						
Frontal						
Anterior cingulate	33	–3	6	18	4.48	2,528
	24	–3	22	11	3.20	480
Parietal						
Postcentral	3, 7	32	–29	44	4.25	4,040
Limbic						
Cingulate	6, 24, 32	–3	1	44	3.97	8,936
Posterior cingulate	31	8	–38	33	3.64	528
Temporal						
Middle temporal	21	47	3	–21	4.27	696
Insula	13	–41	–17	–6	3.57	632

BA = Brodmann area.

lobule, precuneus, and rectal gyrus was positively related to change in cortisol (fig. 2c). Activation in the cerebellar tonsil and inferior semi-lunar lobule for whole-brain-corrected analyses was negatively associated with cortisol change. Results of whole-brain corrected analyses for the 'fear – neutral' and 'anger – neutral' contrasts are listed in table 3. For the 'sad – neutral' contrast (online suppl. table 2), change in cortisol was positively associated with activation in the bilateral motor cortex, posterior cingulate, and right precuneus. For the 'happy – neutral' contrast, there was also a positive association between change in cortisol and activation in the right putamen and rostral cingulate, left posterior middle temporal gyrus and precentral gyrus, and bilateral precuneus, posterior cingulate,

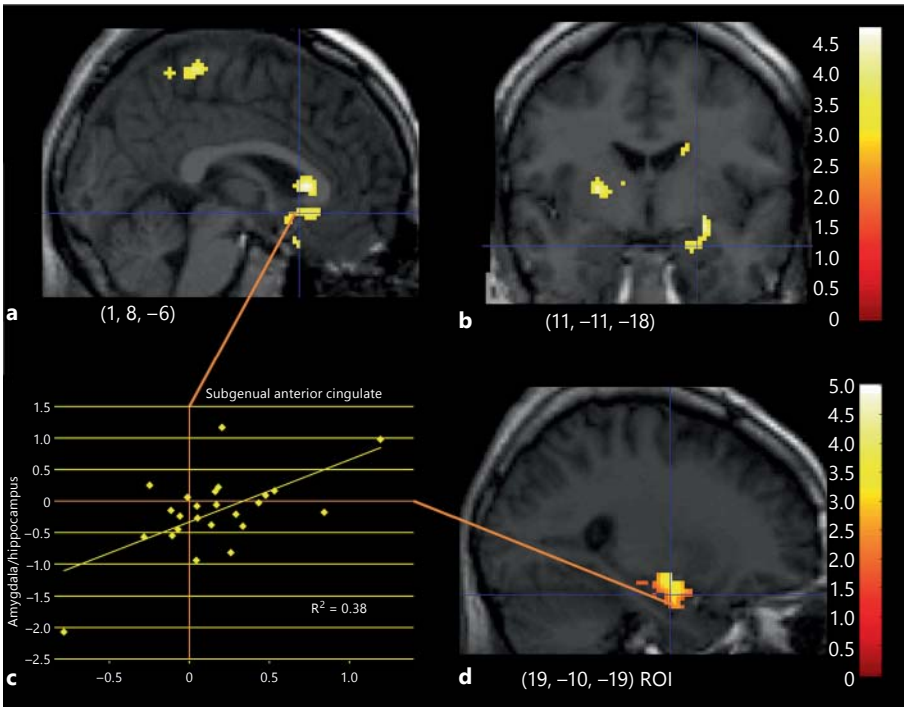

Fig. 3. Areas of significant activation regressing 'faces – animals' on prescan cortisol. **a**, **b** Activation in the subgenual anterior cingulate in response to emotional faces is predicted by increased prescan cortisol (whole-brain corrected). **c** Extracted mean activations between the right subgenual anterior cingulate and amygdala are positively correlated. **d** ROI analyses show that activation in the right amygdala (and hippocampus) is also predicted by higher levels of prescan cortisol.

Table 2. Foci of significant activation for prescan cortisol regression in 'faces – animals' contrast

Contrast/lobe	BA	Talairach coordinates			Z	mm^3
		x	y	z		
Positive regressor						
Frontal						
Paracentral	5	4	–36	54	3.18	640
Precentral	4	20	–19	53	3.78	488
Caudate/subgenual anterior cingulate	25	–3	14	4	3.75	2,936
Temporal						
Parahippocampal		25	–11	–10	3.41	1,208
Subcortical						
Red nucleus		–4	–21	–8	3.67	1,144
Caudate		15	1	19	3.28	896
Putamen		–22	–15	2	3.72	1,504
ROI-positive regressor						
Temporal						
Amygdala		24	–11	–13	3.30	720
		20	1	–16	2.52	24
Parahippocampal	28	–25	–21	–14	3.90	168
	28	26	–23	–10	2.66	48
	36	–30	–32	–13	2.72	32

BA = Brodmann area.

Table 3. Residual cortisol change (pre- to postscan) for 'fear – neutral' and 'anger – neutral' event-related contrasts

Contrast/lobe	BA	Talairach coordinates			Z	mm^3
		x	y	z		
Fear – neutral						
Positive residual regressor						
Parietal						
Precuneus	7	13	–52	38	3.64	1,376
	7	13	–42	57	3.2	464
Occipital						
Lingual	18	15	–77	3	3.29	1,680
Anger – neutral						
Positive residual regressor						
Frontal						
Rectal	11	3	28	–29	3.47	592
Parietal						
Superior parietal	7	–33	–60	44	3.35	872
Precuneus	7	13	–54	41	3.37	672
Temporal						
Inferior temporal	20	–45	–15	–31	4.91	2,200
Negative residual regressor						
Subcortical						
Cerebellar tonsil		–27	–42	–34	3.33	520
Inferior semilunar		–1	–62	–37	3.75	504

BA = Brodmann area.

Table 4. Residual cortisol change (pre- to postscan) for 'faces – animals' contrast

Contrast/lobe	BA	Talairach coordinates			Z	mm^3
		x	y	z		
Positive residual regressor						
Frontal						
Superior frontal	10	32	49	18	2.99	488

BA = Brodmann area.

middle cingulate, cerebellum, and inferior parietal lobule. For the 'faces – animals' contrast, activation in the left superior frontal gyrus (whole-brain) was positively associated with cortisol change from pre- to postscan (table 4).

Emotion Perception Activation Responses Related to Task Performance

Figure 4 (table 5) illustrates the areas of activation that were significantly negatively correlated with face accuracy when evaluated within the prescan cortisol regression models (hypothesis 4). Whole-brain-corrected analyses indicated an inverse relationship between activation in the superior temporal gyrus and insula and accuracy for identifying facial emotions. Prescan cortisol and change in cortisol were not related to face accuracy.

Discussion

The current study evaluated differences between cortisol on a nonscan day and fMRI-related cortisol (pre- and postscan) and examined whether anticipatory corti-

Table 5. Foci of significant activation inversely associated with face accuracy in the 'faces – animals' contrast

Contrast/lobe	BA	Talairach coordinates			Z	mm^3
		x	y	z		
Faces – animals						
Temporal						
Superior temporal	39	47	–54	25	3.16	448
Insula	13	40	4	–7	3.72	1,336
	13	–41	3	–4	3.16	648
Subcortical						
Cerebellum		22	–40	–23	3.82	1,688

BA = Brodmann area.

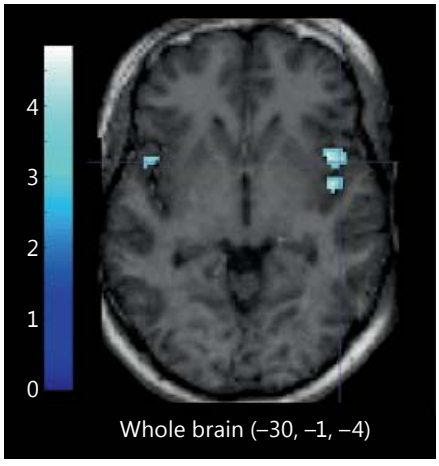

Fig. 4. Areas of significant inverse relationship between activation and face accuracy, regressed on prescan cortisol. Whole-brain-corrected analyses show that increased activation in the insula was negatively related to performance on a measure of overall face accuracy when evaluated within the prescan cortisol regression model.

sol levels immediately prior to a scan and change in cortisol level during an fMRI scan were related to brain activation and task performance in an emotional face paradigm. Pre-fMRI cortisol levels were higher in subjects relative to baseline averages derived from a subsample of participants on a nonscan day, suggesting potentially heightened levels of cortisol on a scan day. Moreover, individual differences in cortisol prior to the fMRI scan were related to activation in the amygdala, parahippocampal gyrus, subgenual anterior cingulate, and several other limbic regions during both face (vs. animal) and emotional face recognition.

Over 50% of subjects showed prescan cortisol levels above the group time-corrected average on a nonscan day. Individual differences in pre-fMRI cortisol may be capturing anticipatory reactivity [47], suggesting that anticipation of the fMRI environment may act as a mild stressor, regardless of scan time, even among adults with no personal or family history of mental illness. Although this effect could reflect individual differences, independent of the experience of the fMRI environment, this finding is consistent with previous research [27, 28, 31, 33, 34] and highlights the importance of considering how stress responses may change over the course of an fMRI experiment and/or impact the task performance. Capitalizing on these individual differences in elevation and temporal pattern of cortisol activity may help to reduce heterogeneity in studies of those with active illness (i.e. major depressive disorder and anxiety disorders). Alternatively, these patterns in healthy subjects may result in increased heterogeneity in control samples that weakens control and patient comparisons [11–13].

Our results support the hypothesis that anticipatory cortisol is associated with greater activation in limbic and regulatory regions during an emotion-processing task. These increases in limbic activation, along with heightened cortisol levels on the day of fMRI procedures compared to baseline in the subsample of participants, might be conceptualized as an index of individual differences in reactivity with exposure to salient emotional stimuli and the stress of the fMRI scanning environment. Interestingly, activation in the anterior parahippocampal gyrus, amygdala, and subgenual cingulate, key emotion-processing and regulation regions, were positively associated with change in cortisol. Be-

cause greater activation of emotion-processing and regulation regions during emotional face paradigms has been linked with psychopathology, this may obscure important differences between patient and control populations.

There was also evidence that stress responses may diminish performance when processing emotional stimuli, supporting hypothesis 4 [5]. Activation in regions such as the superior temporal gyrus and insula were related to poorer performance on face accuracy. It is possible that anticipatory stress prior to entering the fMRI environment affects the ability of subjects to perform at an optimal level during an emotion challenge [48]. However, consistent with other studies [49], cortisol was not found to relate to task performance. Future investigations can more explicitly challenge specific emotions (i.e. fear and anger) to assess the potential effects of cortisol change from pre- to postscan on performance in regions associated with emotional face processing.

We observed an interesting dissociation of links between emotion processing of specific emotions and change in cortisol across the scan. Although there were areas of robust correlation between fear-related activation and cortisol measurements, this was not the case for anger. Historically, fear has a stronger link to HPA function, and the present results are consistent with this area of work [50, 51]. Further investigation of the effect of the fMRI environment on cortisol reactivity and emotional task performance should include clinical populations, including those with higher levels of baseline cortisol, such as individuals diagnosed with major depressive disorder or anxiety disorders.

Several limitations to the current study should be considered when interpreting the results. Although the comparison between cortisol levels on a nonscan day and cortisol before and after the scan is a significant contribution to the growing research on this topic, the use of a small subgroup for the collection of 'baseline' cortisol data and the known individual differences in diurnal cortisol may have influenced results. In the future, researchers should attempt to overcome inherent difficulties in cortisol collection to obtain data that are reflective of an entire sample. Second, most of the subjects in the current study were scanned early in the day, a period when cortisol is typically descending from a peak shortly after waking. This is reflected in the number of negative change scores in our sample. Additional sample characteristics, such as menstrual cycle phase for female participants and subjects' wake time on the day of scanning, should also be collected in future studies, as these can also affect cortisol measurements. Another potential limitation arises from the fact that fMRI data collection was undertaken over a period of 7 years. Upgrades to fMRI equipment and software over time may affect signal quality, which may compromise the reliability of neuroimaging data. Furthermore, we did not evaluate subjective levels of stress prior to or after fMRI. Although relations between subjective stress and cortisol are not typically found [52], scores on self-reports such as the PANAS-X [53] or subscales of the Brief Symptom Inventory [54] related to anxiety or stress could be used to corroborate the findings of imaging results and pre-/postscan cortisol measurements. Finally, the wide age range observed here could obscure stronger relationships between cortisol measurements and activation, or even interactions between age and cortisol.

In conclusion, this study provides insights into the relationship between individual differences in cortisol activity and increased activation during an emotional face paradigm. It provides a context for understanding group and individual differences in activation contrasts with emotional content. This study also extends previous findings regarding the effects of fMRI-related stressors on brain activation and subjective task performance and suggests a number of directions for future research that will be important to clarifying the influence of stress and stress hormones related to the fMRI experience on brain activation and task performance during emotion-processing tasks.

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Disclosure Statement

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References

1 Tsigos C, Chrousos GP: Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. J Psychosom Res 2002;53:865–871.
2 Susman EJ: Psychobiology of persistent antisocial behavior: stress, early vulnerabilities and the attenuation hypothesis. Neurosci Biobehav Rev 2006;30:376–389.
3 McEwen BS, Wingfield JC: The concept of allostasis in biology and biomedicine. Horm Behav 2003;43:2–15.
4 Lopez-Duran NL, Kovacs M, George CJ: Hypothalamic-pituitary-adrenal axis dysregulation in depressed children and adolescents: a meta-analysis. Psychoneuroendocrinology 2009;34:1272–1283.
5 Langenecker S, Weisenbach S, Giordani B, Briceño E, Guidotti Breting L, Schallmo M, et al: Impact of chronic hypercortisolemia on affective processing. Neuropharmacology 2012; 62:217–225.
6 Wirth MM, Scherer SM, Hoks RM, Abercrombie HC: The effect of cortisol on emotional responses depends on order of cortisol and placebo administration in a within-subject design. Psychoneuroendocrinology 2011; 36:945–954.
7 Van Honk J, Kessels RPC, Putman P, Jager G, Koppeschaar HPF, Postma A: Attentionally modulated effects of cortisol and mood on memory for emotional faces in healthy young males. Psychoneuroendocrinology 2003;28: 941–948.
8 Buchanan TW, Lovallo WR: Enhanced memory for emotional material following stress-level cortisol treatment in humans. Psychoneuroendocrinology 2001;26:307–317.
9 Dedovic K, Duchesne A, Andrews J, Engert V, Pruessner JC: The brain and the stress axis: the neural correlates of cortisol regulation in response to stress. Neuroimage 2009;47:864–871.
10 Jankord R, Herman JP: Limbic regulation of hypothalamo-pituitary-adrenocortical function during acute and chronic stress. Ann NY Acad Sci 2008;1148:64–73.
11 Kern S, Oakes TR, Stone CK, McAuliff EM, Kirschbaum C, Davidson RJ: Glucose metabolic changes in the prefrontal cortex are associated with HPA axis response to a psychosocial stressor. Psychoneuroendocrinology 2008;33:517–529.
12 Liu J, Chaplin TM, Wang F, Sinha R, Mayes LC, Blumberg HP: Stress reactivity and corticolimbic response to emotional faces in adolescents. J Am Acad Child Adolesc Psychiatry 2012;51:304–312.
13 Pruessner JC, Dedovic K, Khalili-Mahani N, Engert V, Pruessner M, Buss C, et al: Deactivation of the limbic system during acute psychosocial stress: evidence from positron emission tomography and functional magnetic resonance imaging studies. Biol Psychiatry 2008;63:234–240.
14 Root JC, Tuescher O, Cunningham-Bussel A, Pan H, Epstein J, Altemus M, et al: Frontolimbic function and cortisol reactivity in response to emotional stimuli. Neuroreport 2009;20:429–434.
15 Van Stegeren A, Roozendaal B, Kindt M, Wolf O, Joëls M: Interacting noradrenergic and corticosteroid systems shift human brain activation patterns during encoding. Neurobiol Learn Mem 2010;93:56–65.
16 Damasio A, Carvalho GB: The nature of feelings: evolutionary and neurobiological origins. Nat Rev Neurosci 2013;14:143–152.
17 Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, et al: Dissociable intrinsic connectivity networks for salience processing and executive control. J Neurosci 2007;27:2349–2356.
18 Etkin A, Wager TD: Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. Am J Psychiatry 2007; 164:1476–1488.
19 Ongür D, Ferry AT, Price JL: Architectonic subdivision of the human orbital and medial prefrontal cortex. J Comp Neurol 2003;460: 425–449.
20 Sullivan RM, Gratton A: Prefrontal cortical regulation of hypothalamic-pituitary-adrenal function in the rat and implications for psychopathology: side matters. Psychoneuroendocrinology 2002;27:99–114.
21 Wang J, Rao H, Wetmore GS, Furlan PM, Korczykowski M, Dinges DF, et al: Perfusion functional MRI reveals cerebral blood flow pattern under psychological stress. Proc Natl Acad Sci U S A 2005;102:17804–17809.
22 Taylor SE, Burklund LJ, Eisenberger NI, Lehman BJ, Hilmert CJ, Lieberman MD: Neural bases of moderation of cortisol stress responses by psychosocial resources. J Pers Soc Psychol 2008;95:197–211.
23 Amodio DM, Frith CD: Meeting of minds: the medial frontal cortex and social cognition. Nat Rev Neurosci 2006;7:268–277.
24 Urry HL, van Reekum CM, Johnstone T, Kalin NH, Thurow ME, Schaefer HS, et al: Amygdala and ventromedial prefrontal cortex are inversely coupled during regulation of negative affect and predict the diurnal pattern of cortisol secretion among older adults. J Neurosci 2006;26:4415–4425.
25 Veer IM, Oei NY, Spinhoven P, van Buchem MA, Elzinga BM, Rombouts SA: Endogenous cortisol is associated with functional connectivity between the amygdala and medial prefrontal cortex. Psychoneuroendocrinology 2012;37:1039–1047.
26 Phelps EA: Human emotion and memory: interactions of the amygdala and hippocampal complex. Curr Opin Neurobiol 2004;14:198–202.
27 Lueken U, Muehlhan M, Evens R, Wittchen H-U, Kirschbaum C: Within and between session changes in subjective and neuroendocrine stress parameters during magnetic resonance imaging: a controlled scanner training study. Psychoneuroendocrinology 2012;37: 1299–1308.
28 Chapman H, Bernier D, Rusak B: MRI-related anxiety levels change within and between repeated scanning sessions. Psychiatry Res 2010;182:160–164.
29 Eatough EM, Shirtcliff EA, Hanson JL, Pollak SD: Hormonal reactivity to MRI scanning in adolescents. Psychoneuroendocrinology 2009;34:1242–1246.
30 Wolf OT: The influence of stress hormones on emotional memory: relevance for psychopathology. Acta Psychol (Amst) 2008;127:513–531.
31 Tessner KD, Walker EF, Hochman K, Hamann S: Cortisol responses of healthy volunteers undergoing magnetic resonance imaging. Hum Brain Mapp 2006;27:889–895.
32 Lueken U, Muehlhan M, Wittchen H-U, Kellermann T, Reinhardt I, Konrad C, et al: (Don't) panic in the scanner! How panic patients with agoraphobia experience a functional magnetic resonance imaging session. Eur Neuropsychopharmacol 2011;21:516–525.
33 Peters S, Cleare AJ, Papadopoulos A, Fu CHY: Cortisol responses to serial MRI scans in healthy adults and in depression. Psychoneuroendocrinology 2011;36:737–741.
34 Muehlhan M, Lueken U, Wittchen H-U, Kirschbaum C: The scanner as a stressor: evidence from subjective and neuroendocrine stress parameters in the time course of a functional magnetic resonance imaging session. Int J Psychophysiol 2011;79:118–126.
35 Lovallo W, Farag N, Vincent A: Use of a resting control day in measuring the cortisol response to mental stress: diurnal patterns, time of day, and gender effects. Psychoneuroendocrinology 2010;35:1253–1258.
36 Stone A, Schwartz J, Smyth J, Kirschbaum C, Cohen S, Hellhammer D, et al: Individual differences in the diurnal cycle of salivary free cortisol: a replication of flattened cycles for some individuals. Psychoneuroendocrinology 2001;26:295–306.
37 Briceno E, Weisenbach S, Rapport L, Hazlett K, Bieliauskas L, Haase B, et al: Shifted inferior frontal laterality in women with major depressive disorder is related to emotion-processing deficits. Psychol Med 2013;43:1433–1445.
38 Langenecker SA, Bieliauskas LA, Rapport LJ, Zubieta JK, Wilde EA, Berent S: Face emotion perception and executive functioning deficits in depression. J Clin Exp Neuropsychol 2005; 27:320–333.
39 Rapport LJ, Friedman SR, Tzelepis A, Van Voorhis A: Experienced emotion and affect recognition in adult attention-deficit hyperactivity disorder. Neuropsychology 2002;16:102–110.
40 Kamali M, Saunders EFH, Prossin AR, Brucksch CB, Harrington GJ, Langenecker SA, et al: Associations between suicide attempts and elevated bedtime salivary cortisol levels in bipolar disorder. J Affect Disord 2012;136:350–358.

41 Gur RC, Erwin RJ, Gur RE, Zwil AS, Heimberg C, Kraemer HC: Facial emotion depression discrimination: II. Behavioral findings in depression. Psychiatry Res 1992;42:241–251.
42 Tottenham N, Borscheid A, Ellersten K, Markus D, Nelson C: Categorization of facial expressions in children and adults: establishing a larger stimulus set; in Annual Meeting of the Cognitive Neuroscience Society. San Francisco, 2002.
43 Tottenham N, Tanaka JW, Leon AC, McCarry T, Nurse M, Hare TA, et al: The NimStim set of facial expressions: judgments from untrained research participants. Psychiatry Res 2009;168:242–249.
44 Ekman P, Friesen WV: Pictures of Facial Affect. Palo Alto, Consulting Psychologists Press, 1976.
45 Phillips ML, Drevets WC, Rauch SL, Lane R: Neurobiology of emotion perception I: the neural basis of normal emotion perception. Biol Psychiatry 2003;54:504–514.
46 Ward BD: Simultaneous Inference for FMRI Data. Biophysics Research Institute, Medical College of Wisconsin, 2000. http://afni.nimh.nih.gov/pub/dist/doc/manual/AlphaSim.pdf.
47 Engert V, Efanov SI, Duchesne A, Vogel S, Corbo V, Pruessner JC: Differentiating anticipatory from reactive cortisol responses to psychosocial stress. Psychoneuroendocrinology 2013;38:1328–1337.
48 Nagai M, Kishi K, Kato S: Insular cortex and neuropsychiatric disorders: a review of recent literature. Eur Psychiatry 2007;22:387–394.
49 Li S, Weerda R, Milde C, Wolf OT, Thiel CM: Effects of acute psychosocial stress on neural activity to emotional and neutral faces in a face recognition memory paradigm. Brain Imaging Behav 2014;8:598–610.
50 Merz CJ, Tabbert K, Schweckendiek J, Klucken T, Vaitl D, Stark R, et al: Investigating the impact of sex and cortisol on implicit fear conditioning with fMRI. Psychoneuroendocrinology 2010;35:33–46.
51 Wong ML, Kling MA, Munson PJ, Listwak S, Licinio J, Prolo P, et al: Pronounced and sustained central hypernoradrenergic function in major depression with melancholic features: relation to hypercortisolism and corticotropin-releasing hormone. Proc Natl Acad Sci U S A 2000;97:325–330.
52 Campbell J, Ehlert U: Acute psychosocial stress: does the emotional stress response correspond with physiological responses? Psychoneuroendocrinology 2012;37:1111–1134.
53 Watson D, Clark LA: Development and validation of brief measures of positive and negative affect: the PANAS scales. J Pers Soc Psychol 1988;54:1063–1070.
54 Derogatis LR, Melisaratos N: The Brief Symptom Inventory: an introductory report. Psychol Med 1983;13:595–605.