



Impact of chronic hypercortisolemia on affective processing[☆]

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ABSTRACT

Cushing syndrome (CS) is the classic condition of cortisol dysregulation, and cortisol dysregulation is the prototypic finding in Major Depressive Disorder (MDD). We hypothesized that subjects with active CS would show dysfunction in frontal and limbic structures relevant to affective networks, and also manifest poorer facial affect identification accuracy, a finding reported in MDD. Twenty-one patients with confirmed CS (20 ACTH-dependent and 1 ACTH-independent) were compared to 21 healthy control subjects. Identification of affective facial expressions (Facial Emotion Perception Test) was conducted in a 3 Tesla GE fMRI scanner using BOLD fMRI signal. The impact of disease (illness duration, current hormone elevation and degree of disruption of circadian rhythm), performance, and comorbid conditions secondary to hypercortisolemia were evaluated. CS patients made more errors in categorizing facial expressions and had less activation in left anterior superior temporal gyrus, a region important in emotion processing. CS patients showed higher activation in frontal, medial, and subcortical regions relative to controls. Two regions of elevated activation in CS, left middle frontal and lateral posterior/pulvinar areas, were positively correlated with accuracy in emotion identification in the CS group, reflecting compensatory recruitment. In addition, within the CS group, greater activation in left dorsal anterior cingulate was related to greater severity of hormone dysregulation. In conclusion, cortisol dysregulation in CS patients is associated with problems in accuracy of affective discrimination and altered activation of brain structures relevant to emotion perception, processing and regulation, similar to the performance decrements and brain regions shown to be dysfunctional in MDD.

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1. Introduction

Excessive, chronic, exposure to high levels of glucocorticoids (GC) has multiple adverse effects on brain biology in animals and humans (Abercrombie et al., 2011; Akil et al., 1993; Axelson et al., 1993; Erickson et al., 2003; Lupien et al., 1998; Starkman et al., 1992; Tessner et al., 2007), most clearly in animal studies (Magarinos and McEwen, 1995; Roozendaal et al., 2009). Specifically, GC administration and/or threat challenges that increase GC

concentrations in animals result in increased depressive and anxiety-like symptoms as well as enhancement of aversive/avoidance memories linked to limbic function (McEwen, 1997; Mitra and Sapolsky, 2008; Mitra et al., 2006; Vyas et al., 2003). These animal studies strengthen the hypothesis that GC exposure results in morphologic/functional changes in brain structures supporting memory and affective processing.

There is increasing interest in possible effects of chronic GC exposure on cognitive and affective processing in humans (Brown et al., 2007; Sapolsky, 2000; Seeman et al., 1997). While the animal studies are highly informative, there are difficulties in creating parallels between animal behaviors and human medical and psychiatric illnesses. Behaviors elicited in animals are not the same as those observed in humans, nor can they be clarified absent self-report of symptoms (Starkman et al., 1981). Investigation of

[☆] Precise: The present study shows for the first time a link between chronic HPA dysregulation, dysfunctional emotion processing, and activation abnormalities.

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in vivo brain changes in humans secondary to chronic GC exposure are needed in order to clarify translation of animal studies to humans and to better understand cognitive and affective outcomes in humans.

A useful human illustration of the pathophysiologic effects of chronic, excessive GC exposure is Cushing's Syndrome (CS). In CS, chronic, stress-level concentrations of cortisol lead to depressed mood in over 60% of patients (Starkman et al., 1981), vegetative symptoms, abnormal sleep profiles (Shipley et al., 1992) and cognitive dysfunction, especially in memory (Forget et al., 2000; Starkman et al., 2001, 1986a). In addition, there is evidence of reduced regional brain volumes in the hippocampus, as well as decreased glucose utilization during active hypercortisolism (Khat et al., 1999; Starkman et al., 1992).

With normalization in cortisol levels following treatment, we have shown reductions in mood and anxiety symptoms (Starkman et al., 1986b), increase in memory and hippocampal volume (Starkman et al., 1999, 2003), and improvements in fluency and processing speed (Hook et al., 2007). We have also observed a post-treatment decrease in depressed/anxious mood related to an increase in caudate head, but not hippocampal volume in this study (Starkman et al., 2007). In summary, the human work with CS, as well as the animal work with GC administration or manipulation suggest that chronic GC exposure has direct effects upon cognitive and affective functioning and supportive brain regions in medial temporal, limbic, and frontal areas.

Affective functioning and its neural correlates is an important, yet understudied area in humans that is also related to medial temporal function and disruption secondary to GC exposure. In human studies, a few functional neuroimaging studies of emotion processing and regulation in volunteers using observational, normal levels of GC have been conducted. Using naturalistic measurement of normal-range cortisol levels in healthy adults during fMRI, these studies demonstrate positive relationships of medial temporal and frontal activation with GC concentrations (Pruessner et al., 2008; Tessner et al., 2007; Urry et al., 2006; van Stegeren et al., 2007). In a PET study with a mixed bipolar and major depression (MDD) group, there was a positive association between left amygdala glucose metabolism and plasma cortisol concentrations (Drevets et al., 2002). In contrast, the study of acute GC administration in humans is now being explored more extensively with current imaging technologies, including in psychiatric groups (Abercrombie et al., 2011; Scheel et al., 2009).

In the present study, we extend our investigations of the impact of chronic GC exposure to sensitive brain regions with high GR/MR receptor concentrations. We expand our prior work with mood and cognition in CS to now use an affective identification task during fMRI. The goal of the present study was to examine the relationship between excessive GC exposure and disruption of affective networks and processing, by studying individuals with CS prior to treatment. We tested the following hypotheses: 1) CS patients would demonstrate decreased ability to identify facial expressions of emotion; 2) CS patients would exhibit dysfunction in frontal and limbic regions, regions that also mediate successful and efficient identification of facial emotional expressions; and 3) Decrements in emotional identification ability and markers of severity of HPA axis dysfunction and duration of hypercortisolism would be related to abnormal activation in regions within the affective processing circuits.

2. Materials and methods

2.1. Participants

Twenty-one patients with CS and 21 healthy control subjects participated in the study after giving informed consent. The study was approved by the University of

Michigan Institutional Review Board for Medical Experimentation, with protocols consistent with the Declaration of Helsinki. Demographic and select clinical data are reported in Table 1.

Patients with CS were recruited after the diagnosis was confirmed using diagnostic criteria which involved confirmation of elevated serum and urine free cortisol, absence of circadian rhythm and abnormal suppression with 1 mg dexamethasone (Scheingart, 1989). Twenty patients with CS were ACTH-dependent and had pituitary microadenomas confirmed by positive MRI, inferior petrosal sinus sampling, and/or transphenoidal surgery. One additional patient had pituitary-ACTH-independent CS due to adrenal cortical adenoma. This person was included in all analyses (except posthoc analyses with ACTH), as the imaging results were similar to patients who had pituitary-dependent disease. Three CS patients completed practice testing only. These patients had truncal obesity that exceeded the bore diameter for the 3 Tesla GE fMRI scanner and were unable to comfortably lie in the scanner to complete the protocol. One patient with CS had a history of longstanding seizure disorder. The results were in identical foci, but with larger extent of activation and Z values without inclusion. For sake of completeness given the small sample size, this subject was retained. The fMRI analyses compared the remaining 18 CS patients with the 21 control subjects.

The control subjects were recruited through advertisements in the Medical Center and surrounding community. The control group was screened using a semi-structured screening interview based upon neurological conditions, fMRI safety, and the Structured Clinical Interview for DSM-IV(SCID-I) non-patient edition (First et al., 1995; Landfield, 1987; Langenecker and Nielson, 2003). All control subjects were found to be free of any past or current psychiatric or neurologic disorder (self or first degree family members), including alcohol and substance abuse or dependence. They did not have any clinical manifestations of hypercortisolism, though urinary or serum cortisol measurements were not collected. Exclusion criteria for all participants included use of antipsychotic (last six months), hypnotic (48 h) or benzodiazepine (48 h) drugs. The patient with longstanding seizure disorder was prescribed Dilantin. Results were similar with and without inclusion of this subject in the data analyses.

In the patients with CS, samples for plasma cortisol were collected every two hours for 24 h for assessment of circadian rhythm (in Fig. 1, for CS participants only due to funding limitations). Urine for free cortisol was also collected over a 24-hour period during an inpatient stay at the University of Michigan Clinical Research Unit, typically either the day prior to or after the fMRI study. Cortisol levels were measured using a Diagnostic Products Corporation (DPC) Coat-A-Count radioimmunoassay kit and plasma ACTH by the Nichols Allegro radioimmunoassay. In some of the more recent patients, cortisol and ACTH were measured by automated methods that had a high degree of correlation with the other methods. Serum cortisol and plasma ACTH results are calculated as the mean of 12 values. Decrease in cortisol and ACTH from am peak (8, 10 and 12 measurements) to afternoon nadir (4, 6, and 8 pm measurements) was also calculated (Debono et al., 2009), weighing in the effect of the loss of circadian rhythm in patients with CS $-(\text{Peak-Nadir})/\text{Peak}$ (% decline in cortisol). A negative percentage would indicate a relatively normative circadian decline over this time period. Cortisol and ACTH percent change in peak-nadir measurements were highly correlated ($r = .79$, $P < .05$, see Table 2). Length of hypercortisolism was estimated in months by DES based upon prior procedures (Scheingart, 1989). These clinical endocrine values are also reported in Table 1 and correlations of endocrine, clinical, and performance variables are reported in Table 2.

CS is a disorder with a direct cause from GC, which has significant effects in CNS but also impact upon peripheral organs and functioning. As such, a number of covariates of interest are considered in the areas of interest to increase confidence in the direct effects of chronic GC exposure. In particular, diagnosis, treatment, and/or peripheral markers for depression/anxiety, hypertension, and hyperglycemia are evaluated in regions with between group differences to rule out these factors as

Table 1
Demographic and clinical data for CS and control participants.

Variable	Control		CS	
	M	SD	M	SD
Age	30.5	12.0	34.4	14.9
Education	14.6	1.9	13.3	2.7
Gender	13 F, 8 M		17 F, 4 M	
Estimated illness duration †			32.4	23.7
Plasma ACTH			67.0	34.7
ACTH, Peak-Nadir			−2.2	26.1
Plasma Cortisol			20.4	7.8
Cortisol, Peak-Nadir			2.8	6.1
Urinary Free Cortisol			451.5	497.9
BDI-II ^a	1.8	2.1	18.0	11.1

^a Significantly different at $P < .05$, with significantly higher Beck Depression Inventory-II (BDI) score in the CS (Cushing's Syndrome) group relative to the control group. † Estimated based upon first presentation of weight gain, irritability, and/or facial fullness (by DES).

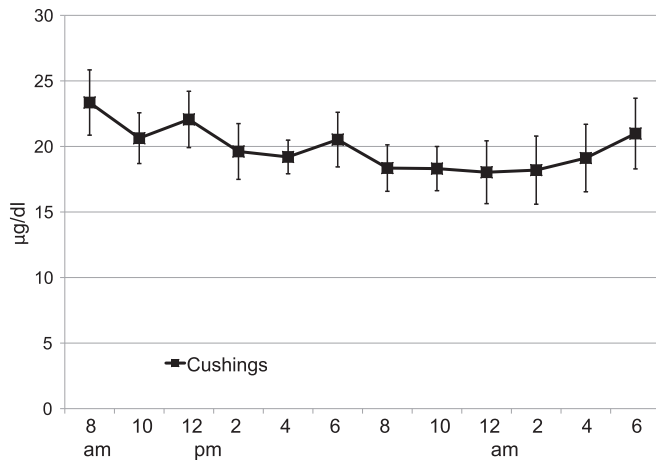


Fig. 1. Illustrates average cortisol levels across the measurement period (8 am to the following day at 6 am) in the patients with CS. Healthy control subjects did not have cortisol measurements for the study due to funding limitations. The cortisol levels were collected within one day of the fMRI scan, typically the following day when they were admitted to the GCRC for the clinical and research evaluation.

secondarily causative of between group differences. In addition, medication effects are also evaluated in this light. Because of the relatively small sample size of CS patients who completed imaging ($n = 18$), special care was taken to assess whether these secondary conditions/markers were the source of between group differences, and interpretations were adjusted accordingly.

2.2. Facial emotion perception task (FEPT)

The FEPT was used to assess accuracy and speed of identification of facial expressions, with categorization of animals used as a control for visual processing ability and fine motor speed (Ekman and Friesen, 1976; Langenecker et al., 2005, 2007b; Rapport et al., 2002; Tottenham et al., 2002). Briefly, for this task participants were asked to categorize faces into one of four possible categories (happy, sad, fearful, and angry) and animals from each of four categories (dogs, cats, primates, and birds), described elsewhere (Langenecker et al., 2005, 2007b). The FEPT version used outside the scanner included the Ekman faces, and the in-scanner version used the MACBrain Foundation faces (Ekman and Friesen, 1976; Langenecker et al., 2005, 2007b; Rapport et al., 2002; Tottenham et al., 2002).

Each event began with a briefly presented orienting cross (500 ms), followed by a brief presentation of the stimulus (300 ms), a visual mask (100 ms), and a response window (2600 ms). The outside-scanner version entailed one 7-minute run and included 12 animal trials and 54 face trials, with six neutral trials, used in prior studies of MDD and related disorders (Ekman and Friesen, 1976; Langenecker et al., 2005, 2007b; Rapport et al., 2002; Tottenham et al., 2002). The in-scanner version entailed 3.5-minute runs and included 56 animals and 148 faces, with emotion type counterbalanced in presentation to the second order (e.g., Angry-Fearful, Angry-Happy sequences, etc. were equal in number and randomized in position within a run). Dependent variables were accuracy in categorization of emotional expressions, as well as average response time. Foci of activation in this study included fusiform gyrus, amygdala, hippocampus, dorsal and subgenual anterior cingulate

gyrus, and bilateral ventro-lateral and dorso-lateral prefrontal cortex, consistent with those areas high in GC receptor concentrations (Diorio et al., 1993; Lopez et al., 1998; Patel et al., 2000; Swanson et al., 1983), and those regions reported in healthy control and psychiatric illness studies using protocols of this type.

2.3. Scanning procedures

Participants completed the computer version of the FEPT task before entering the fMRI scanner and completed the task while in the scanner. The fMRI scanning protocol with alternative tasks and populations reported previously (Hsu et al., 2010; Langenecker et al., 2007a).

2.4. MRI acquisition

Whole brain imaging was performed using a GE Signa 3T scanner (release VH3). fMRI series consisted of 30 contiguous oblique-axial sections acquired using a forward-reverse spiral sequence, which provides excellent fMRI sensitivity (Glover and Thomas, 2004). The image matrix was 64×64 over a 24 cm field of view for a $3.75 \times 3.75 \times 4$ mm voxel. The 30-slice volume was acquired serially at 1750 ms temporal resolution (TR) for a total of 590 time points for FEPT. One hundred six high-resolution Fast SPGR IR axial anatomic images [TE = 3.4 ms; TR (repetition time) = 10.5 ms, 27 degree flip angle, NEX (number of excitations) = 1, slice thickness = 1.5 mm, FOV (field of view) = 24 cm, matrix size = 256×256] were obtained for each participant for co-registration and normalization purposes.

2.5. MRI processing

Processing of images was conducted using SPM2, including realignment, slice timing correction, co-registration, normalization to the MNI world space, and smoothing with a 5 FWHM filter. Contrast images were derived based upon the face processing minus animal processing blocks subtractions (FP – AP). These were computed by using the Blood Oxygenation Level Dependent (BOLD) signal for all face processing blocks and subtracting similar BOLD signal changes for animal processing blocks for each individual in a first level analysis. The SPM2 hemodynamic response function (hrf) model was used to model the BOLD response. Two group, random effects analyses were conducted using whole brain analyses from the individual group contrasts between the CS and control groups in SPM5.

2.6. Statistical analyses

For behavioral data, repeated measures analyses of covariance for accuracy and response time were performed, with practice and in-scanner performance entered as within-subject variables, group entered as the between-subjects variable, and age and sex entered as covariates. For fMRI data, second level analyses were conducted with ANCOVAs within SPM5. The ANCOVA compared CS and control subjects using activation in the faces – animals contrast, with performance accuracy in classifying faces and sex as covariates. Statistical significance for between group comparisons was set at $P < .003$, with cluster minimum of 344 mm^3 . Based upon 1000 Monte Carlo simulations with AlphaSim inside the whole brain search region, a whole brain corrected alpha of .05 is achieved with this combined height by extent threshold strategy. Based upon *a priori* hypotheses, amygdala and hippocampus were used as regions of interest, with uncorrected $P < .05$ and extent threshold of 24 mm^3 . Posthoc analyses used extracted data from regions identified in whole-brain corrected, between group differences to evaluate the impact of disease and performance parameters.

3. Results

3.1. Decreased accuracy in classification of emotions in faces for CS compared to controls

The healthy control group outperformed the CS group in accuracy of classification of emotion in human faces in a repeated measures ANCOVA (with age and sex as covariates) including both practice and in-scanner performance ($F(1,35) = 7.67, P < .05$). There was no difference between performance inside the scanner and practice ($F(1,35) = 0.79, P > .05$) and the interaction between setting (practice versus in-scanner) and group was not significant ($F(1,35) = 1.77, P > .05$).

In a similar repeated measures ANCOVA for response time, there was no main effect of group ($F(1,35) = 2.51, P > .05$). There was a main effect of setting for response time, with slower response times inside the scanner ($F(1,35) = 11.91, P < .05$). The interaction between setting and group was significant ($F(1,35) = 5.59, P < .05$).

Table 2

Correlations of clinical variables of interest and with facial emotion identification performance.

	2	3	4	5	6	7	8
1. log 10 UFC	−0.04	.67**	0.16	0.11	0.39	0.27	−0.15
2. Illness duration	−.50*	−0.38	−0.35	−0.48	−0.25	−0.26	
3. Avg 24 h Cortisol			0.34	0.03	0.07	0.24	−0.02
4. Avg 24 h ACTH				−0.23	−0.15	0.03	−0.18
5. −(Peak-Nadir)/Peak Cortisol					.79**	0.09	0.39
6. −(Peak-Nadir)/Peak ACTH						0.17	0.44
7. % Correct emotion identification							0.01
8. Beck depression inventory							

** $P < .01$, * $P < .05$, rho correlations exclude the participant with ACTH-independent CS.

The control group responded faster than the CS group when initially classifying faces in practice, but not when performing the task inside the scanner. For classification of animals (the control condition), there was no difference between groups in accuracy or response time (P values $> .05$).

3.2. Bilateral ventral frontal activation for emotion identification for each group separately, with more extensive activation for CS

Activation for healthy control and CS groups separately is illustrated in Fig. 2. Both groups exhibited bilateral ventrolateral prefrontal cortex and anterior insula activation. In addition, the CS group foci of activation included more extensive regions than those in healthy control foci, to include contiguous dorsolateral prefrontal cortex, plus medial prefrontal cortex including rostral and dorsal anterior cingulate, globus pallidus, hippocampus, thalamus and amygdala (left only). The control group exhibited activation in superior temporal gyrus, which was not present in the CS group.

3.3. Hyperactivation in the CS group compared to control group in BOLD activation for emotion classification for CS

There were seven foci with significantly greater activity in the CS group compared to the healthy control group as initially hypothesized, in primarily medial and left frontal regions (Table 3, Fig. 3). In addition to the right rostral anterior cingulate, the left lateralized foci were middle frontal gyrus, dorsal anterior cingulate, caudate body, lateral posterior thalamus, substantia nigra, and superior parietal lobule. The control group exhibited greater activation relative to the CS group in left anterior superior/middle temporal gyrus (also Table 3, Fig. 3). Activation for the Fear-Neutral contrast, which might be expected to be most closely related to the effects of CS, is reported in Table 4 for comparative purposes. The results are largely similar to the faces-animals between group contrast, the focus of the paper.

Table 3

Foci of greater FP-AP activation in CS participants compared to control participants in identification of facial emotional expressions.

Lobe	BA	x	y	z	mm ³	Z
<i>CS greater than control</i>						
M. Frontal	6/8	−27	18	48	736	3.62
Dorsal anterior cingulate	32	−10	12	39	744	3.69
Rostral anterior cingulate	24/32	4	35	16	1920	3.84
Superior parietal	7	−20	−69	45	488	3.62
Caudate body		−17	−29	21	496	3.47
Lat. post./pulvinar		−17	−21	7	424	3.88
Substantia Nigra		−10	−23	−7	440	3.89
<i>Control greater than CS</i>						
Superior/middle temporal	21/38	−45	−3	−15	352	3.56

In addition to the whole brain analyses described above, we also investigated predefined regions of interest in the amygdala and hippocampus. The CS group exhibited greater activation than the HC group in left anterior hippocampus ($-27, -19, -19, Z = 1.83, P < .05, \text{mm}^3 = 48$). The healthy control group exhibited greater activation than the CS group in right middle hippocampus ($26, -25, -10, Z = 1.78, P < .05, \text{mm}^3 = 80$).

3.4. Posthoc analyses investigating the whole brain differences between healthy control and CS groups

Exploratory, posthoc correlations were performed with activation in the eight regions with between group differences in whole brain comparisons (from Table 3). In the CS group, we explored the role of estimated illness duration, degree of HPA dysfunction as measured by current mean elevation in hormone concentrations of ACTH and cortisol, and percent change in these measures from peak to nadir. We then evaluated the relationship of activation upon measures of performance accuracy in the CS group. Due to the relatively small sample size and total number of correlations, these are considered exploratory for hypothesis generation.

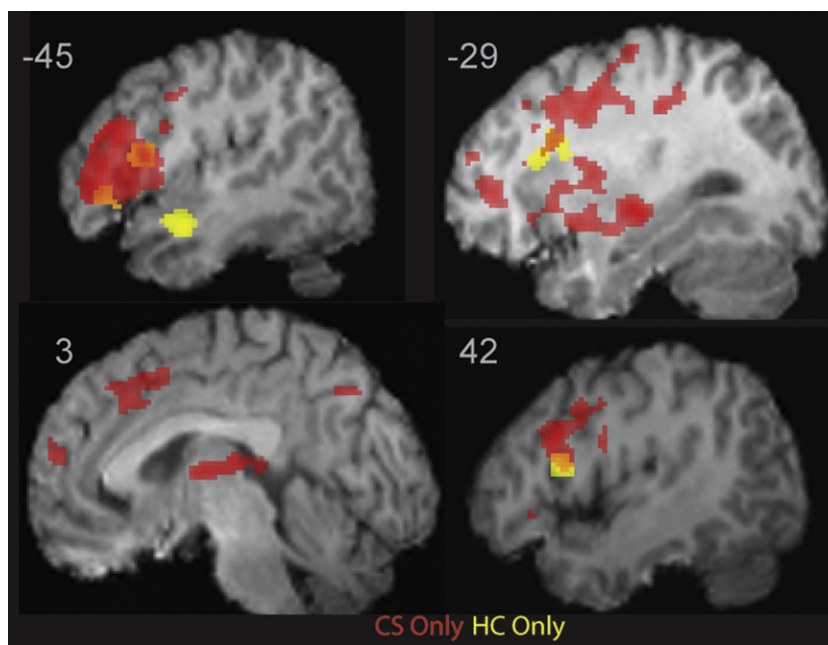


Fig. 2. Significant activation, after whole-brain correction using combined height and extent threshold with AlphaSim, for healthy control (yellow) and CS (red) groups separately. X (left to right) coordinates (Talairach) are listed for sagittal planes (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

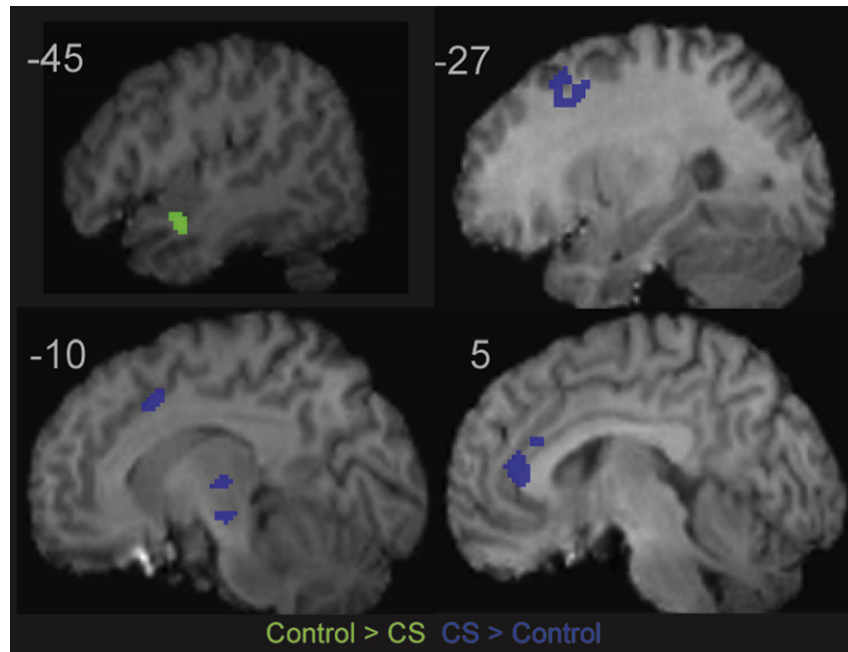


Fig. 3. Differences in emotion processing (FP – AP) between the CS and the healthy control group. Blue represents greater activation in CS relative to healthy controls. Healthy control activation that is greater than the CS group is illustrated in green. Clusters depicted are of significant activation between groups, after whole-brain correction ($p < .05$) using combined height and extent threshold with AlphaSim.

3.5. Left dorsal cingulate hyperactivation in CS correlates with disrupted circadian rhythm

The results of the hormone analyses revealed that activation in left dorsal anterior cingulate (DAC) was correlated with percent decline in ACTH from peak to nadir ($\rho = .51$, $P < .05$) and marginally so for percent decline in cortisol from peak to nadir ($\rho = .49$, $P = .055$), with both relationships illustrated in Fig. 4a. A flat or increasing pattern of ACTH/cortisol throughout the day, which is the greatest deviation from healthy hormone levels, was associated with the greatest activation in left DAC. Similarly, two clusters, left superior temporal gyrus ($\rho = .45$, $P = .08$) and left superior parietal lobule ($\rho = .48$, $P = .06$), showed marginally significant positive correlations between ACTH percent decline and activation. Percent decline in ACTH and cortisol Peak-Nadir was not associated with accuracy in emotion classification ($\rho = .17$, $P > .05$, $\rho = .14$, $P > .05$).

3.6. Left middle frontal and thalamic hyperactivation in CS correlates with accuracy in facial emotion identification

In contrast, accuracy in classification of emotions was associated with performance in other regions from amongst the eight regions of whole brain differences between groups. For CS, activation in two of the eight clusters, left lateral posterior/pulvinar nuclei of the thalamus ($\rho = .56$, $P < .05$) and left middle frontal gyrus

($\rho = .50$, $P < .05$), was positively associated with accuracy in emotion identification, with the latter relationship illustrated in Fig. 4b. A marginally significant correlation was also observed in left superior parietal lobule ($\rho = .43$, $P = .08$). Those individuals with CS who exhibited greater activation compared to the healthy control subjects in these three regions were able to attain good performance on the task.

3.7. Hyperactive regions in CS are not explained by comorbid conditions secondary to hypercortisolemia or by medication effects

We performed additional posthoc correlations on these eight regions from whole brain differences between groups to rule out the impact of comorbid conditions that occur secondarily to hypercortisolemia (hypertension, type II diabetes, depression). None of the seven CS > control or the one control > CS foci differed in the CS groups based upon the presence or absence of depression (p values $> .19$), type II diabetes (P values $> .05$), or presence of medications that might effect BOLD fMRI or affect processing (p values $> .05$), primarily focusing on antihypertensives and antidepressants). There were no differences between CS groups with and without hypertension in the seven clusters that were greater in CS relative to the control group. The CS group with hypertension did have lower activation in the left superior/middle temporal cluster that was observed in the control minus CS comparison ($t(17) = 2.69$, $P < .05$). In essence, comorbid conditions and medications did not play a significant role in the areas of hyperactivity in CS relative to healthy control subjects.

4. Discussion

The present results extend our previous observations linking a dysregulated HPA axis with alterations in central nervous system structure and cognitive performance, especially memory, and depressed mood (Hook et al., 2007; Starkman et al., 1992, 2003, 2007; Starkman et al., 1981, 1986b). The present study is the first

Table 4
Greater activation in CS compared to healthy control subjects in the fear-neutral contrast.

Lobe	BA	x	y	z	mm ³	Z
CS greater than control						
S. Frontal	9	13	51	25	376	3.97
Middle/inferior frontal	9/46	36	22	27	280	3.28
Precuneus	7	–6	–69	35	320	3.25
Cuneus, lingual	18/19	20	–81	–1	328	3.4

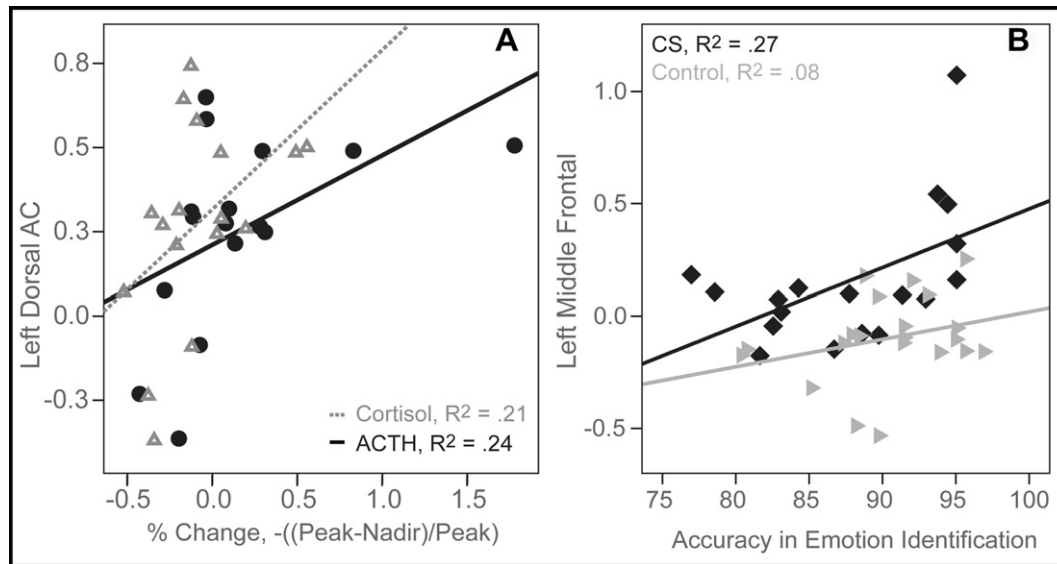


Fig. 4. a. Illustrates activation within the left dorsal anterior cingulate as it relates to change in cortisol (white) and ACTH (red) from peak to nadir. The value is inverted, so that a negative percent change reflects a decrease that would be closer to a normal circadian pattern. b. The illustration shows how activation in left middle frontal gyrus was positively associated with accuracy in emotional identification, to a greater extent in CS participants. Both the left dorsal anterior cingulate and middle frontal gyri activation are also shown in Fig. 3.

report of alterations in emotion perception and processing as measured by fMRI in adult patients with untreated CS. Chronic hypercortisolemia affects the functioning of medial temporal and frontal circuitry, regions where MR and GR receptors are most heavily represented (Wellman, 2001). Disruption of these regions, which is critical for efficient and accurate emotional processing, is reflected in the poorer performance within the CS group. As such, the key findings of the present study are likely to reflect disruption of critical foci within circuitry for emotion processing, and in those brains where successful adaptation has occurred, compensatory recruitment. The results also shed light on the importance of understanding the behavioral and biological correlates of differences in activation between CS and healthy controls, considerations that are also relevant for study of other conditions like MDD. Hyperactivation for CS in some regions (i.e., left dorsal anterior cingulate) was reflective of the severity of the disease process, vis-à-vis the complete disruption in a daily circadian pattern of ACTH and cortisol. Yet hyperactivation in other regions (i.e., left lateral posterior/pulvinar nuclei of the thalamus and left middle frontal gyrus) for CS was indicative of compensation by recruitment; additional brain regions were recruited to attain a level of performance equivalent to healthy controls.

The main findings from the present study were of hyperactivation in the CS compared to the healthy control group in medial frontal and left lateralized regions, and also included subcortical regions of interest. The specific areas that demonstrated effects of chronic hypercortisolism, irrespective of secondary comorbidities or other symptoms, were consistent with areas affected in acute stress paradigms in humans, including dorsal anterior cingulate and medial prefrontal cortex (Pruessner et al., 2008). When exposed to emotionally evocative stimuli, groups with psychiatric illness have also demonstrated increased activation relative to controls in these same regions (Chen et al., 2007; Fairhall and Ishai, 2007; Grimm et al., 2008; Hamilton et al., 2008). Activation in the right rostral anterior cingulate cluster was not related to performance accuracy or to current or chronic measures of disease impact or burden. It has been reported that levels of resting cortisol and early childhood trauma are related to cingulate volume in a mixed group of controls and depressed

subjects, supporting the sensitivity of this region to elevated hormones secondary to HPA axis dysfunction (Treadway et al., 2009).

The hyperactive regions most pertinent to the task are located where MR/GR receptors are most heavily represented, within rostral and dorsal anterior cingulate and superior temporal gyrus (Lopez et al., 1998; Patel et al., 2000; Wellman, 2001). It is also important to recognize, though, that with chronic GC administration, effects may be observed outside of regions with heavy receptor representation, including cascading effects of dendritic shrinkage and adjustment of these and related neuronal circuits. For example, animal and human studies of GC administration or stress/stimulation demonstrate a greater impact for chronic exposure in sensitive regions, including hippocampus, amygdala, caudate, and prefrontal cortex (Kole et al., 2004; Leverenz et al., 1999; Ohi et al., 2000; Scheel et al., 2009; Starkman et al., 1992, 2007; van der Beek et al., 2004; Wellman, 2001). There may also be restructuring or plasticity in the functional organization of these and related circuits to adjust to the changed endocrine milieu present in chronic hypercortisolemia or dysregulated cortisol, though this has not yet been considered. There is substantial work on chronic GC administration in animals showing changes in dendritic structuring of hippocampus and amygdala, yet little is known about how these local changes in very complex foci would impact structures within the same and related circuits (Magarinos and McEwen, 1995; Roozendaal et al., 2009). Preplanned region of interest analyses showed that even within these small comparison groups there was hypoactivation in right posterior hippocampus and hyperactivation in left anterior hippocampus in the CS group. Of course, in vivo measurements in humans rarely allow us to answer questions of mechanism or purpose of these activation changes, but correlation can be used to begin to dissect the processes that might be supporting or underlying the changes in question.

The relationship between a decrease or increase in ACTH from peak to nadir was reflective of normal, flattened, and inverted circadian rhythmicity in CS. There is not yet a clear understanding of how these dorsal and rostral cingulate regions of hyperactivation in CS might assist in regulation of mood or emotion in chronic

stress, although some studies have begun to investigate these links (Treadway et al., 2009; Wellman, 2001). For example, is it a phasic response that is modulated acutely? It may be that these regions are mediating the reactivity to the emotional stimuli in light of a cognitive/emotional identification objective. Or does chronic hypercortisolemia increase the amplitude of response to emotional stimuli in these regions? In addition, whereas chronic GC administration occurs in some medical populations, the long term cognitive and affective consequences are still poorly understood.

The decreased activation in left anterior superior temporal gyrus in the CS group relative to the control group is an exciting new lead. This region is important in emotion processing (Fusar-Poli et al., 2009) and also has high levels of MR and GR receptors (Diorio et al., 1993; Lopez et al., 1998; Patel et al., 2000). It may be a particularly vulnerable region to chronic stress and/or hypercortisolemia, and it deserves further study.

There were two areas of hyperactivation in CS that were associated with preserved capacity for emotion classification accuracy. The left thalamus and middle frontal gyrus have well described relationships with emotion processing and regulation (Fusar-Poli et al., 2009; Phan et al., 2002). Increased activation that is associated with preserved performance on a given task is often interpreted in one of two ways. First, it can be considered additional, or compensatory activation. This interpretation is based upon the idea that for a weaker circuit, increased resources are needed to attain similar levels of performance (Deckersbach et al., 2006; Langenecker and Nielson, 2003; Reuter-Lorenz et al., 1999; Woodard et al., 1998). A second interpretation is that of reorganization, assuming a lack of activation in important regions for task performance and increased activation in areas that are of distinct, but similar or perhaps supportive function (Bontempi et al., 1999; Madden et al., 1997; Stern et al., 2005). Unfortunately, as in this case, imaging studies are often underpowered for the task of addressing more nuanced interpretations such as reorganization, and results of this type are often viewed as compensatory.

There are a number of caveats to consider in interpreting the results. First, with regard to subjects, the CS sample is modest in number, although this is a reasonable sample for such a rare condition and for an fMRI investigation. In addition, in this recent sample of consecutively recruited CS subjects, cortisol levels were not as highly elevated as in our previous samples, and the subjects were relatively younger. Cortisol dysregulation has more deleterious effects with increasing age (Hook et al., 2007), rendering our study more conservatively biased. In addition, many of the CS subjects were taking medications, particularly including those for hypertension and depression. Post-hoc analyses indicated that the presence or absence of comorbid conditions did not influence the results, with the exception of hypertension for the left superior temporal gyrus. Those with hypertension were more likely to exhibit decreased activation in this region compared to those without hypertension and compared to healthy controls. Also, in previous work from our group examining cognitive decrements in 48 untreated CS subjects, when the potential confounding effect of medications was examined, there were no significant differences either between patients taking no medication versus those taking any medications, or among patients taking no drugs, antihypertensives only, antihypertensives plus other medications, and other medications only (Starkman et al., 2001). Finally, the uncorrected threshold within the amygdala/hippocampal ROI was also provided in the interests of comparison with previous ROI studies of emotion processing in mood disorders in these regions. It is less likely that these amygdala/hippocampal effects would be replicable.

To our knowledge, the present study is the first in adult humans to provide evidence for the strong relationship between emotion processing difficulties and hyperactivity in frontal, and subcortical

regions during hypercortisolemia, irrespective of comorbid conditions and symptoms. The present results add support to our overarching hypothesis that there is a direct impact of dysregulated HPA axis products upon the CNS. In this case, continuous exposure to elevated cortisol and related HPA axis products can result in altered processing and regulation of emotion. The present results showing relationships among chronically elevated cortisol, disrupted brain activation patterns in key areas, and decrements in facial emotion processing can be tested further in subjects with mood disorders and those at risk for developing mood disorders.

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Disclosure statement

The authors have nothing to disclose pertinent to the investigation conducted here.

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