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Stress Reactivity and Corticolimbic Response to Emotional Faces in Adolescents

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Abstract

Objective—Adolescence is a critical period in the development of lifelong patterns of responding to stress. Understanding underpinnings of variations in stress-reactivity in adolescents is important, as adolescents with altered stress-reactivity are vulnerable to negative risk-taking behaviors including substance use, and have increased lifelong risk for psychopathology. While both endocrinological and corticolimbic neural system mechanisms are implicated in the development of stress-reactivity patterns, the roles of these systems and interactions between the systems in reactivity to social stimuli in adolescents are not clear. We investigated the relationship between cortisol response to a lab-based social stressor and regional brain responses to emotional face stimuli in adolescents.

Method—Changes in cortisol levels following the Trier Social Stress Test-Child version (TSST-C) were measured in twenty-three disadvantaged and chronically stressed adolescents who also participated in functional magnetic resonance imaging during processing of emotional faces and structural magnetic resonance imaging. The relationships between changes in cortisol following the TSST-C with regional brain activation during face processing, as well as with regional brain morphology, were assessed.

Results—Cortisol change on the TSST-C showed a significant inverse relationship with left hippocampus to fearful faces ($p < 0.05$, corrected); significant associations to volume were not observed.

Conclusions—Increased cortisol response to the Trier social stressor was associated with diminished response of the left hippocampus to faces depicting fear. This suggests HPA-corticolimbic system mechanisms may underlie vulnerability to maladaptive responses to stress in adolescents that may contribute to development of stress-related disorders.

Keywords

adolescent; Psychological Stress; cortisol; hippocampus; Magnetic Resonance Imaging

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INTRODUCTION

Adolescence is a critical period in the development of lifelong patterns of responding to stress.¹ While both endocrinological and corticolimbic neural system mechanisms are implicated, the roles of these systems and interactions between the systems in reactivity to social stimuli in adolescents are not clear. Understanding the underpinnings of these variations in stress-reactivity in adolescents is important, as adolescents with altered stress-reactivity may be vulnerable to a range of negative risk-taking behaviors including substance use, as well as increases in lifelong risk for developing psychopathology.

Converging evidence supports important interactions between a frontal-mesial temporal system that subserves responses to emotional stimuli and responses of the hypothalamic pituitary adrenal (HPA) axis to stress. The prefrontal cortex (PFC), and mesial temporal hippocampus and amygdala, have each shown modulatory effects on the HPA-axis,²⁻⁵ affecting levels of cortisol secretion in response to stress. Rodent and non-human primate models support neuroendocrine relationships that are phylogenetically conserved across species with important roles for the PFC and hippocampus in inhibition of, and for amygdala stimulation of, cortisol secretion.²⁻⁹ Neuroimaging studies largely support similarities in neuroendocrine stress responses in humans for the hippocampus and PFC; however, discrepant findings have been observed, particularly for amygdala for which increases or decreases paired with hippocampus and PFC decreases were observed in response to cortisol elevations.¹⁰⁻¹²

Cortisol excesses have been shown to have deleterious effects on the structure and function of frontotemporal structures, in turn altering their regulation of the HPA axis. For example, rodent and non-human primate studies show that chronic cortisol increases lead to decreases in dendritic spines and volume in PFC and hippocampus.^{8,9} This suggests cortisol increases may lead to gray matter decreases in humans; however, this relationship has received little study. This self-regulatory stress system develops during puberty.³ The frontotemporal corticolimbic brain structures mature during adolescence.¹³ In particular, PFC matures in adolescence, increasing its regulation of limbic structures to provide more mature and adaptive control over responses to emotional stimuli.¹³ This suggests alterations in the development of the corticolimbic-HPA system may contribute to variation in stress responsivity in adolescents.

There has been little previous study in adolescents to investigate the relationship between endocrinological responses to social stressors and corticolimbic neural system responses to social emotional stimuli. There have been several recent studies in adults. One functional magnetic resonance imaging (fMRI) study of adults demonstrated associations between cortisol responses to fear-related images and responses in PFC, amygdala and hippocampus.¹² A second study used a modified version of the Trier Social Stress Test (TSST) as a social emotional stressor to study adults.¹⁴ In the TSST, individuals perform a speech and arithmetic in front of judges, which elicit robust changes in the HPA-axis.¹⁵⁻¹⁷ The imaging study revealed cortisol increases following the TSST task associated with decreased responses of rostral medial PFC.¹⁴ A third study found an inverse association between cortisol responses and PFC and hippocampus responses to a modification of the TSST arithmetic component.¹¹

We previously reported increased HPA axis responses to the TSST-Child version (TSST-C), adapted to adolescents.¹⁸ Being evaluated in a social setting, on speech and math tasks by two unfamiliar adult “judges,” can be especially stressful for adolescents, given the heightened sensitivity to social evaluation experienced during adolescence,^{15,19} and simulation of common school experiences of the speech and math tasks.¹⁶⁻¹⁸ Recent

research showed the TSST-C to be robust in stimulating physiological aspects of stress in adolescents, in the HPA axis and sympathetic nervous system, and emotional aspects of stress.^{20,21}

This study included a sample from a population that has received little previous similar study, from an urban inner city environment facing economic hardship and psychosocial stressors that can be associated with it including maltreatment. The children are at high-risk for developing substance use, mood and anxiety disorders. We studied children who did not meet criteria for these disorders, as it is important investigate mechanisms that may underlie risk. The association between cortisol responses on the TSST-C and regional brain responses to emotional stimuli was assessed. We hypothesized heightened cortisol response to the TSST-C stressor would be associated with altered frontotemporal corticolimbic responses to fearful face stimuli, anticipating inverse associations with PFC and hippocampus responses and positive associations with amygdala responses. Given the putative neurotoxic effects of cortisol, the relationship between cortisol responses and regional gray matter volume was also assessed to investigate whether cortisol response might also be inversely associated with volumes in these corticolimbic structures.

METHOD

Subjects

Participants were twenty-three healthy adolescents (ages 14–17years, mean 15.3±SD 1.0years, 10 females, 18 African American, 2 Caucasian, 1 Hispanic, 1 Native American and African American, 1 African American and Caucasian) recruited from a sample of disadvantaged children from families living at or below the federal poverty line, followed longitudinally by us since birth. These children were at risk for chronic exposure to high levels of stress from multiple sources, although the sample varied in exposures to serious stressors of different types. For example, on the Childhood Trauma Questionnaire (CTQ),²² 18% of adolescents denied any maltreatment. The remainder endorsed one or more item describing some form of past maltreatment, with mean subscale scores (each from 5–25 points) of 6.0 (physical abuse), 5.4 (sexual abuse), 7.1 (emotional abuse), 6.0 (physical neglect), and 9.5 (emotional neglect). Twenty-nine percent of their mothers had less than a high school education, 71% graduated high school. All subjects were attending school from eighth to twelfth grade. The Family History Screen for Epidemiologic Studies was administered to a parent or guardian.²³ This screen was positive for biological parents of 14 children (61%), including alcohol and/or substance abuse for 13, 6 of whom also had anxiety and/or depressive symptoms, and anxiety and depressive symptoms for one. Subjects were without history of medical or neurological illness or head trauma. The presence or absence of DSM-IV Axis I diagnoses were confirmed by semi-structured interviews of the revised Schedule for Affective Disorders and Schizophrenia: Present and Lifetime version (KSAD-PL),²⁴ of the child and her/his guardian, performed by highly experienced, reliable research personnel as well as psychiatrists or psychologists expert in assessment of both adults and children. Subjects were excluded for psychotic, mood and anxiety disorders and did not meet diagnostic criteria for these or other major Axis I Disorders with the exception of one subject with a history of cannabis dependence in remission for more than 1.5 years. Pubertal maturation was assessed by self-report on the Pederson Pubertal Development Scale.²⁵ Two girls were postpubertal. The remaining subjects were in early to mid puberty. Written informed consent was provided by a guardian, and written informed assent was obtained for the subject, in accordance with a University Institutional Review Board.

Trier Social Stress Test

The TSST-C laboratory session began at 4:15pm. For the first 40min, the adolescents adapted to the environment, including 5min of guided muscle relaxation followed by instructions to continue practicing relaxation. At 4:55pm, pre-stress salivary cortisol samples were obtained. Adolescents then participated in the TSST-C, following procedures by Buske-Kirschbaum et al²⁶ adapted slightly for adolescents.¹⁸ In brief, at 5pm, two unfamiliar adult “judges” entered the room and told adolescents they will have to finish writing a story and to “make the story as exciting as possible” because they will be “competing against other teenagers.” The adolescents had 5min to prepare a story. The judges then asked the adolescents to stand and recite the story for 5min in front of the judges while audio- and video-taped. Then, the adolescents were asked to perform an arithmetic task for 5min.

Salivary cortisol samples were collected pre-stressor, just prior to story preparation, immediately after the stressor, and then at 15min, 30min, 45min, and 60min post-stress. Samples were placed immediately on dry ice and stored at -20°C . Assays for cortisol were performed in duplicate in accordance with standard procedures for the radioimmunoassay kits (Coat-A-Count Cortisol Kit, Diagnostic Products Corporation, Los Angeles, CA) at a university laboratory. The measure used for analyses was “cortisol change”, i.e. cortisol levels at pre-stress subtracted from cortisol levels at the timepoint of the typical peak for salivary cortisol at 15min post-stress.²⁷ This difference score approach is commonly used when interested in looking at associations between various outcomes with stress response variables in youth.²⁸ We also calculated Area under the Curve (AUC) with respect to cortisol increases²⁹ and explored this measure in analyses. At 15min post-stress, adolescents also completed ratings to assess their subjective evaluation of the stressfulness of the TSST-C. A story-related item (“How stressful was it for you to tell the story?”) and a math-related item (“How difficult/stressful was it to do the math problem?”) were each assessed on an 11-point scale (0 least and 10 most stressful, a score of >5 considered stressful).²⁶

MRI Data Acquisition and Emotional Face Paradigm

MRI data were obtained using a 3-Tesla Siemens Trio MR scanner (Siemens Erlangen, Germany). fMRI data were acquired with a sing-shot echo planar imaging sequence. Thirty-two slices were acquired in alignment with the anterior commissure-posterior commissure plane with parameters TR=2000ms, TE=25ms, matrix=64×64, field of view=240mm², flip angle=80°, thickness=3mm without gaps. In the same imaging session, high-resolution T1-weighted MRI images were acquired in the sagittal plane with a three-dimensional magnetization prepared rapid acquisition gradient echo T1-weighted sequence with parameters TR=1500ms, TE=2.83ms, FOV=256mm², slice thickness=1.0 mm without gaps.

An event-related emotional faces paradigm was conducted as described previously.^{30,31} In brief, subjects viewed fearful, happy, and neutral human faces from the Ekman series.³² Each face stimulus was displayed for 2s during which participants were instructed to make a gender judgment using a two-response button box. Between face stimuli subjects viewed a fixation cross-hair for intervals that varied between 4s, 8s or 12s. Each of 4 runs lasted 4min 50s and included 30 face stimuli. The order of face stimuli was counterbalanced for the actor, facial expression, sex, and inter-stimulus interval length.

The mean time interval between TSST and fMRI was 3.7 months (SD=3.0 months). The two tests were not performed the same day to minimize carry-over effects between stressful tasks; the time interval was feasible in this population.

FMRI Data Processing

Images were processed using Statistical Parametric Mapping (SPM5) software (<http://www.fil.ion.ucl.ac.uk/spm>). The fMRI data were first corrected for slice timing and movement, then spatially coregistered to the standard EPI template from Montreal Neurological Institute (MNI) using a 12-parameter affine transformation followed by a nonlinear warping.³³ The fMRI data were resampled to 3mm³ during the normalization. Finally, images were spatially smoothed with a Gaussian filter of 8mm full-width-at-half-maximum (FWHM). Low-frequency noise was removed via a high pass filter (128s).

FMRI Data Analyses

SPM5 was used for model specification and estimation. At the individual subject level, event-related response amplitudes were estimated by using the general linear model for each of the three event types: fearful, happy and neutral expressions as compared to baseline fixation. In the second level analyses, whole brain analyses included data for all adolescents using random-effects simple regression analyses in SPM5, with the TSST-C cortisol changes as continuous independent variables.

Consistent with previous publications, findings in hypothesized frontal and mesial temporal regions were considered as significant for $p < 0.001$ (uncorrected) and clusters > 20 voxels.^{34,35} In order to adjust for multiple comparisons, we also performed small volume correction (SVC) in SPM5 to confirm the findings [$p < 0.05$, corrected for multiple comparisons using false discovery rate (FDR)] in a priori hypothesized regions.³⁶ The frontal and medial temporal regions of interest (ROIs) were defined with the WFU PickAtlas Tool (<http://www.fmri.wfubmc.edu/download.htm>). Specifically, the frontal ROI included Brodmann areas (BAs) 9–12, 24/25/32, 44–47 and medial temporal ROIs included hippocampus and amygdala. For remaining brain regions, for associations between cortisol measure and brain activation to emotional faces, findings were considered as significant with $p < 0.05$ corrected for FDR and a 20-voxel extent threshold.

Structural Data Processing and Analysis

High-resolution structural images were processed with SPM5. Protocol details are described in our previous publications.^{30,37} Briefly, the SPM5 segmentation function was implemented for bias correction, spatial normalization and segmentation of the original structural images in the same model.³⁸ Bias correction produced images that had more uniform intensities. SPM5 tissue probability maps (voxel size 2mm³) were used to guide the normalization and segmentation. During the spatial normalization, a “modulation” step was used to ensure that the overall amount of each tissue class was not altered.³⁹ Finally, the segmented, normalized and modulated gray matter images were smoothed with an 8mm FWHM isotropic Gaussian kernel. Whole-brain linear regression analysis in SPM5 was implemented with the TSST-C changes in cortisol as the continuous independent variables. Analogous significant levels were set for the structural data analysis.

RESULTS

Consistent with previous studies, adolescents found the TSST-C task stressful. After the task, mean scores were elevated for items assessing story and the math task stressfulness with mean scores of 7.0 (SD=2.99) and 7.0 (SD=2.82), respectively. Mean cortisol levels were 0.147ug/dL (SD=0.069) prior to the TSST-C and 0.202 ug/dL (SD= 0.185) 15min after the TSST-C stressor. There were no significant effects of menstrual cycle stage in the girls.

Corticolimbic regions that subserve emotional processing were robustly activated in this subject group by the fearful, happy and neutral face task conditions, including the amygdala,

hippocampus and PFC, surviving SVC using FDR. Cortisol change scores were negatively correlated with responses to faces depicting fear in the left hippocampus (maximum in MNI coordinates of $x=-24\text{mm}$, $y=-7\text{mm}$, $z=-23\text{mm}$, cluster=41 voxels, $T=5.69$) and in left rostral PFC (BA 10) ($x=-15\text{mm}$, $y=56\text{mm}$, $z=22\text{mm}$, cluster=29 voxels, $T=4.16$) (Figure 1). The finding in hippocampus survived SVC. On inspection of individual data (Figure 2), one subject had values $>3\text{SD}$ above mean values. After removal of that subject from analyses, correlations remained significant for parametric (Pearson) and non-parametric (Spearman) analyses for hippocampus, however, not for PFC. Findings for AUC were similar to cortisol change findings. The relationships above were not detected for happy or neutral face conditions for change or AUC measures. There were no significant effects of gender on the findings.

Whole brain analysis identified two additional brain regions with significant inverse correlations between fear-related brain activation and cortisol change scores: left inferior parietal lobule (maximum $x=-63\text{mm}$, $y=-43\text{mm}$, $z=28\text{mm}$, cluster size=46 voxels, $T=5.98$) and left precentral gyrus ($x=-60\text{mm}$, $y=-4\text{mm}$, $z=34\text{mm}$, cluster size=28 voxels, $T=6.77$).

There were no significant associations between cortisol change scores and regional brain volume. Analyses were also performed with gender, pubertal stage and age as covariates; however, no significant results were detected.

DISCUSSION

The findings indicate an inverse association between cortisol responses to a social stressor and corticolimbic responses to faces depicting fear in adolescents. Specifically, the more elevated the cortisol responses to the stress, the more reduced the responses in the left hippocampus and rostral PFC to fear stimuli. Only hippocampus findings survived SVC. There were no associations detected between cortisol responses to stress and neural responses to faces of positive or neutral valence, or regional gray matter volume.

Extensive evidence supports a central role of the hippocampus, and role of the PFC, in the negative feedback regulation of the HPA axis system's glucocorticoid response to stress and deleterious effects of excessive glucocorticoids have been shown in the hippocampus and PFC.^{3,40} The hippocampus and PFC provide inhibitory innervation to the paraventricular nucleus of hypothalamus (PVN) that in turn regulates the pituitary and its control of the release of cortisol by the adrenal glands to stress. Activation of the hippocampus and PFC lead to inhibition of the PVN, curtailing the HPA-axis response to stress.^{2,41} The hippocampus and PFC contain abundant glucocorticoid receptors³ and excessive glucocorticoids have been shown by multiple research groups to exert deleterious effect on the hippocampus and PFC.^{6,42} Acute rises in glucocorticoids have been associated with functional problems in the hippocampus and PFC,^{43,44} and prolonged glucocorticoid exposure with frontotemporal neuron loss and proliferation decreases.^{4,7} Thus, cortisol excesses and decreased response of the hippocampus and PFC could lead to a self-reinforcing cycle and the development in adolescents of altered responses to socially stressful and fear stimuli. Although findings in the hippocampus were robust, findings in PFC did not survive SVC and were influenced by a subject with high values. It will be important to follow the adolescents, especially during the critical adolescent period of PFC maturation, to assess whether the PFC alterations progress.

Decreases in ability to respond adaptively to fear stimuli, especially during critical developmental periods, could have serious implications for youths. We speculate that these could lead to difficulties in processing and learning from fearful experiences and abnormalities in the development of ability to regulate and cope with negative emotional

situations. Alterations in processing emotional stimuli are implicated in the development of anxiety and mood disorders. Cortisol dysregulation and hippocampus and PFC structural and functional abnormalities have been linked to psychiatric disorders.^{45–50} For example, hippocampus changes in structure and function following stress have been associated with increased vulnerability to PTSD.⁵¹ Elevated cortisol levels have been found in some individuals with depression⁵² and HPA-axis hyperactivity with smaller hippocampal volume has been reported in adolescent girls with high familial risk for depression.^{51,53} Increased stress reactivity has been associated with both substance initiation and progression to addiction and relapse.⁴⁷ Thus, we speculate that adolescents who are more highly reactive to stress and who show decreased hippocampal and PFC response to stress may be more vulnerable to the development of these disorders; however, longitudinal studies are needed to fully address this question.

As a caveat, it must be noted that elevated stress reactivity may be adaptive for some individuals⁵⁴ and the diminished hippocampus and PFC responses associated with high cortisol response to stress may represent adaptive compensatory mechanisms that may enhance resilience. Future longitudinal research examining links between HPA axis stress response, hippocampal and PFC function, and the development of social functioning and psychopathology are needed to fully examine this.

Our findings show some consistencies with studies in adults, which show an inverse association between cortisol reactivity with hippocampus and rostral PFC responses,^{11,14} suggesting some patterns may have been established by adolescence especially in hippocampus and others may continue to progress such as in PFC as above. Adult neuroendocrine-neuroimaging studies of stress have also shown increases or decreases in amygdala;^{11,12} however we did not find associations for the amygdala. This could relate to methodological differences between studies. The responses of the amygdala on this task were quite robust. It may be that there was a ceiling effect and that future studies that use face stimuli with differing levels of intensity of emotional expressions may facilitate detection of amygdala effects. Conversely, a study that did elicit amygdala differences used emotional stimuli highly salient to the subjects (images of World Trade Center attacks) that may have enhanced ability to detect differences.¹² Of note, this sample was largely non-Caucasian, however, the Ekman faces are all Caucasian, and previous studies have found differences in neural responses, especially in amygdala, depending on whether faces were of the same race as study participants.^{55,56} It is also possible that there are age-dependent regional effects. For example, preclinical studies suggest that in adolescence cortisol excesses decrease cortisol receptors in hippocampus but not in the amygdala.⁵⁷ However, the absence of a finding in amygdala could also result from decreased sensitivity given the small size (Type II error).

Notably, the neuroendocrine-fMRI correlations were observed only during processing of fearful stimulus; not during happy or neutral stimulus processing. This was anticipated as cortisol increases particularly in response to threatening situations, and the cortisol change measure was obtained following a stressful social situation and so was expected to be most closely associated to neural responses to faces depicting fear. The findings were observed in the anterior region of the hippocampus, related to the ventral hippocampus in rodent models, which has extensive hypothalamic projections and important roles in fear responses.^{58,59} The rostral PFC has roles in appraisal and conditioned responses to fearful stimuli, attending to the emotions of one's self and others and in guiding adaptive behavioral responses.^{60,61} The PFC appears to be especially sensitive to effects of stress⁶² and associated disruptions in adaptive behavioral control. An inverse relationship was also found between cortisol response and response in both left inferior parietal lobule and precentral gyrus also implicated in emotional processing and fear conditioning.^{63,64} Future studies are warranted

to examine behavioral dysfunction mediated by the corticolimbic differences observed, including examination of fear conditioning and ability to regulate responses in the face of negative social stimuli.

A significant relationship between cortisol reactivity on the TSST-C and corticolimbic volume was not observed, suggesting associations are at the level of function and not gross structure in adolescents. It is possible that we could not detect the latter owing to limitations in the resolution of our structural imaging methods; however, structural differences may accumulate with increasing age and longer duration of the self-reinforcing cortisol-corticolimbic pattern, and with the accumulation of exposure to highly stressful situations. It is also possible there are interactions with age and structural differences are not found in this adolescents age group. Corticolimbic sensitivities to cortisol do change over the course of development.¹

There are limitations to this initial study and findings should therefore be considered preliminary. For example, the sample size is modest and the adolescents chronically stressed by multiple environmental stressors. The latter may limit generalizability to populations with differing types of high stress exposures and to populations with lower stress exposure. Studies with larger samples sizes are needed, as well as studies that investigate interactions with specific types of stressors that occurred at specific times in development and ones that compare chronically-stressed groups to lower risk groups exposed to less stress. Although we did not detect effects of pubertal stage or menstrual cycle phase, our ability to do so was limited by the sample size. The data are cross-sectional. In order to understand the impact of HPA-corticolimbic system alterations on development, longitudinal studies are needed. It is also unclear whether the HPA or the corticolimbic effects are primary. Studies that include younger subjects are needed.

In summary, the study provides preliminary evidence for an association between higher HPA-axis response to social stress and decreased corticolimbic responses to negative emotional stimuli in adolescents. These findings suggest chronic disturbances in corticolimbic-HPA system responses to stress may alter development leading to altered ability to adaptively respond to negative emotional stimuli in adolescence. Future longitudinal studies, with increased sample sizes, are needed to identify causal associations between cortisol increases and corticolimbic response decreases. Identification of corticolimbic-HPA system alterations underlying higher stress reactivity could contribute to understanding of mechanisms that make highly reactive adolescents vulnerable to developing stress-related psychopathology. Longitudinal follow-up may also identify mechanisms underlying resilience. Such studies could inform the development of methods for early identification and prevention programs with potential to reduce risk for developing stress-related substance use disorders and other psychopathology.

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Figure 1.

Inverse Association between Cortisol and Hippocampus and Prefrontal Cortex Responses.

Note: The sagittal images display the regions of left hippocampus (left image, x plane -24mm in the Montreal Neurological Institute system) and left rostral prefrontal cortex (right image, $x=-14\text{mm}$) in which response to fearful face stimuli showed an inverse relationship with cortisol level changes on the Trier Social Stress Test. Results are displayed at a threshold of $p<0.001$ and extent of 20 voxels. The color bar shows the T values. The hippocampus finding survived small volume correction.

Figure 2.

Association between Cortisol Change and Activation during Fearful Face Processing. Note: The scatterplot shows the inverse relationship between cortisol change on the Trier Social Stress Test and the parameter estimate of mean signal change to fearful faces in the left hippocampus ($r=-0.79$, $p<0.001$).