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Cumulative Adversity Sensitizes Neural Response to Acute Stress: Association with Health Symptoms

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Cumulative adversity (CA) increases stress sensitivity and risk of adverse health outcomes. However, neural mechanisms underlying these associations in humans remain unclear. To understand neural responses underlying the link between CA and adverse health symptoms, the current study assessed brain activity during stress and neutral-relaxing states in 75 demographically matched, healthy individuals with high, mid, and low CA (25 in each group), and their health symptoms using the Cornell Medical Index. CA was significantly associated with greater adverse health symptoms ($P = 0.01$) in all participants. Functional magnetic resonance imaging results indicated significant associations between CA scores and increased stress-induced activity in the lateral prefrontal cortex, insula, striatum, right amygdala, hippocampus, and temporal regions in all 75 participants ($p < 0.05$, whole-brain corrected). In addition to these regions, the high *vs* low CA group comparison revealed decreased stress-induced activity in the medial orbitofrontal cortex (OFC) in the high CA group ($p < 0.01$, whole-brain corrected). Specifically, hypoactive medial OFC and hyperactive right hippocampus responses to stress were each significantly associated with greater adverse health symptoms ($p < 0.01$). Furthermore, an inverse correlation was found between activity in the medial OFC and right hippocampus ($p = 0.01$). These results indicate that high CA sensitizes limbic–striatal responses to acute stress and also identifies an important role for stress-related medial OFC and hippocampus responses in the effects of CA on increasing vulnerability to adverse health consequences.

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INTRODUCTION

Cumulative adversity (CA) refers to repeated, adverse social and environmental events that an individual experiences throughout a lifetime (Thoits, 2010; Turner *et al*, 1995). Accumulation of these adverse exposures throughout one's lifetime are conceptualized as independent of subjective perception of stress and, thus, may represent an 'allostatic' load, which may increase subsequent vulnerability to mental and physical disorders (Thoits, 2010). Stress and adversity increases risk for morbidity and mortality (Seeman *et al*, 2004), and a strong link between psychosocial stress and biological dysregulation in various physical domains is well documented (McEwen and Stellar, 1993; Seeman *et al*, 2004). For example, individuals with a history of trauma and environmental adversity are at a greater risk of psychiatric and other chronic diseases (Lloyd and Turner, 2008; Thoits, 2010). Thus, a significant literature has accrued identifying the biological factors influenced by high CA, including progressive alterations in neuroendocrine, autonomic, cardiovascular, and inflammatory responses (McEwen, 1998; McEwen and Stellar, 1993; Seeman *et al*, 2004). However, brain mechanisms underlying the link between high CA and its associated health risk in humans remain unclear.

Multiple studies have shown adverse effects of repeated and high chronic stress on the prefrontal cortex (PFC) and limbic–striatal function. Animal studies have shown repeated stress-related compromised function in the prefrontal–limbic–striatal regions, including dendritic damage in the medial PFC (Radley *et al*, 2006b), reduced GABA-stimulated chloride uptake in the amygdala (Martijena *et al*, 2002), altered synaptic structure in the hippocampus (Karst and Joels, 2003), and upregulated striatal function (Rossi *et al*, 2008). Specifically, the PFC, a crucial region for stress and emotion regulation (Li and Sinha, 2008), is found to be hypoactive during post stress cognitive manipulation (Liston *et al*, 2006; Ossewaarde *et al*, 2011). Disrupted prefrontal attentional control during acute stress was also found in healthy individuals (Liston *et al*, 2009).

In relation to physical health, the prefrontal–limbic–striatal region is involved in the modulation of physiological pain (Borsook *et al*, 2007), autonomic balance (Thayer *et al*, 2012), endocrine arousal (Diorio *et al*, 1993; Figueiredo *et al*, 2003), and immune responses (Phillips *et al*, 1999), suggesting that dysregulation of this circuit could have adverse health effects in vulnerable individuals even

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without physical or psychological disorders. These studies emphasize the need to investigate the functional role of this circuit in response to acute stress in the association between high CA and health problems in humans. To understand the neural responses that modulate CA and adverse health symptoms, the current study utilized functional magnetic resonance imaging (fMRI) to assess brain responses to acute stress *vs* non-stressful, neutral-relaxing scenarios in 75 healthy individuals representing a full range of CA scores. For the CA assessment, we used the Cumulative Adversity Interview (CAI; Turner *et al*, 1995), a well-validated measure shown to be predictive of physical and mental health problems in large population-based studies (Ansell *et al*, 2012a,b; Lloyd and Turner, 2008; Turner and Gil, 2002; Turner and Lloyd, 2004). To specifically identify neural substrates pertaining to CA-related vulnerability for adverse health consequences, we utilized extreme group comparisons by contrasting the high *vs* low CA groups, based on the previous evidence that such designs increase statistical power and show sensitivity to detect risk patterns with greater predictive validity (Abrahams and Alf, 1978; Angold *et al*, 2002; Fowler, 1992; Henshall and Goddard, 1999; Romens *et al*, 2009). In addition, healthy individuals with the intermediate range of CA were included to ensure a full range of continuous CA scores in the sample. The high, mid and low CA groups were demographically matched and were a representative subset of a large community cohort assessed on CA. Current adverse physical and psychological health symptoms were measured using the Cornell Medical Index (CMI; Abramson, 1966). We also utilized the well-established, individualized script-driven imagery method for a brief induction of stress and neutral-relaxing experiences (Sinha, 2009) to account for individual variation in stress responses (Stroud *et al*, 2002). On the basis of previous research cited above, we expected that CA would be associated with brain response to acute stress in the prefrontal–limbic–striatal circuitry. We also hypothesized that individuals with high CA would show decreased prefrontal regulatory function and increased stress-induced reactivity in limbic–striatal regions, and this pattern of response would be associated with current health symptoms as measured by the CMI.

MATERIALS AND METHODS

Participants

Participants were derived from a sample of 419 community individuals recruited via local newspaper and advertisements for research participation (see Supplementary Methods, for details). All subjects completed the CAI structured interview and were further screened for handedness and interest in participating in a neuroimaging study. A sample of 75, right-handed healthy individuals with high (top 30%), medium (middle 30–70%), or low (bottom 30%) total scores on the CA (25 in each group, demographically matched) participated in an fMRI session. Participants also completed psychiatric, cognitive, demographic, and health assessments, including the CMI and medical evaluations to ensure good health status (see Supplementary Information). All research staff interacting with study participants (including the administration of CAI and CMI) were blinded to study aims and hypotheses. All participants were free of any mental disorders verified by the Structured Clinical Interview for DSM-IV, and no participants were on any medications at the time of fMRI testing. All study procedures were approved by the Human Investigation Committee at the Yale University School of Medicine, and all participants signed an informed consent prior to study participation.

Cumulative Adversity Interview

The CAI is a semistructured interview that assesses 140 events encompassing childhood and adult trauma, recent or major adverse life events, and chronic stress including events pertaining to violence, death and loss of loved ones, natural disasters, work/job, school and education, finance/income, relationship and marital status, and living environment (Turner *et al*, 1995). The CAI consists of four subscales: three subscales related to the objective count of CA (major life events, recent life events, and life trauma) resulting from both external forces and subjects' own behaviors and a chronic stress subscale pertaining to subjective response to stress and adversity (for details, see Supplementary Methods). In order to detect neural activity only related to objective CA events, the current study excluded the chronic stress measure and focused only on the CA scores, which are derived from the sum count of number of adverse life events from recent life events, major life events, and trauma that have occurred over the lifespan (see Supplementary Table S1). Recent evidence from our laboratory indicated high CA scores were associated with lower gray matter volume in the medial PFC, insula, and striatal regions (Ansell *et al*, 2012b). The CAI has also been shown to be predictive of mental health disorders and physical health conditions in large population-based studies (Brown and Turner, 2010; Gayman *et al*, 2008; Russell *et al*, 2009; Scott *et al*, 2008; Turner and Lloyd, 2004).

Cornell Medical Index

The CMI captures current physical and psychological health symptoms presented in 195 questions in 18 sections (Abramson, 1966; see Supplementary Methods and Supplementary Table S2) and has been validated as a good indication of general health in many studies (Costa and McCrae, 1985; Perlmutter and Nyquist, 1990).

Individualized Imagery Method and Efficacy of Imagery Manipulation

The script-driven individualized imagery method is a well-established validated method for brief provocation of emotions, acute stress, and anxiety states in laboratory and neuroimaging studies (Britton *et al*, 2005; Jastreboff *et al*, 2013; Orr *et al*, 1993; Potenza *et al*, 2012; Seo *et al*, 2013; Sinha *et al*, 2004; see Supplementary Methods for further description).

Before the fMRI session, individually customized imagery scripts were developed based on participants' reports of two stressful and two neutral-relaxing experiences using the standardized Scene Construction Questionnaires (for detailed method see Sinha, 2009). For stress scripts, participants described a situation that made them sad, mad, and

upset that could not be changed in the moment (eg, being fired, family discord, or relationship conflict). The severity of the situations were rated by the participants on a 10-point Likert scale (1 = not at all stressful and 10 = the most stressful), using only events rated as 8 or above for stimulus provocation and script development. Neutral-relaxing scripts pertained to personal experiences of neutral-relaxing situations (eg, reading in a park or watching the waves at the beach). Each script was standardized across conditions and subjects in terms of script style, content format, and length, while preserving the individual stimulus and response descriptors, as described previously (Sinha, 2009), and then audiotaped for script presentation. Each 2-min audiotaped script was presented in random order during the scanning session.

In order to ensure efficacy of the imagery manipulation, imagery ability of participants were assessed using the Questionnaire on Mental Imagery (QMI; Sheehan, 1967). There was no statistical difference in QMI scores and in post-imagery ratings of vividness among the high, mid, and low CA groups, suggesting equivalent levels of imagery ability and task performance (see Supplementary Methods for detailed results and description). Before the scanning session, a standardized relaxation and imagery training procedure (Sinha, 2009) was implemented in all participants to minimize variability in imagery ability.

fMRI Acquisition, Task, and Physiological and Behavioral Anxiety Measures

A 3-T Siemens Trio MRI system with a single-channel standard quadrature head coil was utilized to acquire MRI imaging data using a T2*-sensitive gradient-recalled single-shot echo-planar pulse sequence (see Supplementary Information for fMRI parameters). Four fMRI trials were acquired, each lasting 5 min. It consists of a 1.5-min silent baseline followed by a 2.5-min imagery (2 min of read-imagery and 0.5 min of quiet-imagery) and a 1-min silent recovery period. Baseline period consisted of lying still with no mental activity, and the recovery period entailed stop imagining and staying still in the scanner. Across subjects, order of script condition was counterbalanced to control for order effects and then the condition order was randomly assigned to each subject. Each script was presented only once without the same condition presented consecutively. Before and after each fMRI trial, behavioral ratings for anxiety were collected using a 10-point verbal scale (1 = not at all and 10 = extremely high). Participants were instructed to rate how tense, anxious, and/or jittery they felt at that moment. Continuous measures of pulse during each trial were obtained using a pulse oximeter placed on the subject's non-dominant forefinger. All subjects participated in a 2-min progressive relaxation between fMRI trials to stabilize any residual anxiety and arousal from prior trials.

fMRI Analysis

fMRI data were converted from Digital Imaging and Communication in Medicine format to Analyze format using XMedCon (Nolfe, 2003). To reach a steady-state equilibrium between radio-frequency pulsing and relaxation, the first 10 images were removed from each functional run. The recovery period (1 min) was excluded due to potential carryover effects from the imagery period. Using MATLAB and Statistical Parametric Mapping (SPM5), fMRI data were preprocessed with slice time correction and motion correction for three translational and three rotational directions, discarding any trial with linear motion >1.5 mm and a rotation exceeding 2°. General linear model (GLM) was used for individual level analysis on each voxel in the entire brain volume with a regressor that compares time during imagery to the baseline for each trial per condition (stress–baseline and neutral–baseline) using BioImageSuite (Duncan *et al*, 2004). In order to account for any variability in baseline fMRI signal, drift correction was implemented in the GLM and drift regressors were used to remove the mean time course, linear, quadratic, and cubic trends for each functional run. Each trial was then spatially smoothed using a 6-mm Gaussian kernel and individually normalized to generate β-maps ($3.44\,\text{mm} \times 3.44\,\text{mm} \times 4\,\text{mm}$). To account for individual anatomical differences, three sequential registrations were applied to the individual normalized β-maps using BioImageSuite (Duncan *et al*, 2004): (1) linear registration between the individual subjects' functional image to the T1 structural image (within subject), (2) linear registration between the T1 structural image and the 3D MPRAGE image ($1 \times 1 \times 1$ mm), and (3) non-linear registration to a reference 3D image. The reference image was the Colin27 Brain (Holmes *et al*, 1998), a high-definition anatomical image registered to the Montreal Neurological Institute space.

The second-level group analysis was conducted with BioImageSuite and Analysis of Functional NeuroImages software (AFNI) utilizing random mixed effects models. In order to examine the association between CA and brain activity in all 75 individuals, a whole-brain correlational analysis was implemented using BioImageSuite. For the high *vs* low CA group comparison, a 2×2 ANOVA (group by condition) was carried out with condition (neutral/stress) as the within-subjects fixed-effect factor, group (high/low CA) as the between-subjects factor, and subject as the random-effect factor. To correct for multiple comparisons, we used cluster-wise control of family-wise errors; the *t*-value and correlation maps were cluster corrected at $p<0.05$ and $p<0.01$ ($p<0.05$ voxel-wise threshold and $3537\,\text{mm}^3$ at a cluster-level significance $\alpha<0.05$ through Monte Carlo simulation; $p<0.01$ voxel-wise threshold and $1134\,\text{mm}^3$ at a cluster-level significance $\alpha<0.01$; two-tailed). The minimum cluster size was determined by Monte Carlo simulation (Xiong *et al*, 1995) using the AFNI AlphaSim program on voxels within the gray matter ($58\,6710\,\text{mm}^3$). To best identify relevant neural substrates and be appropriately conservative, a threshold of 0.05 was used for whole-brain correlation analyses and 0.01 was applied for group difference maps.

RESULTS

Sample Description

Table 1 summarizes the demographic characteristics and CAI scores for the sample. There were no significant differences in age, education, gender, race, employment, economic status, and scores of State-Trait Anxiety Inventory (Spielberger *et al*, 1983) among the three CA groups,

Table 1 Demographics and Health Characteristics.

Subject variable	High CA N = 25	Mid CA N = 25	Low CA N = 25
Demographics			
Age (years)	28.84 (8.9)	26.56 (7.9)	27.44 (7.6)
Gender, female (%)	9 (36.0)	5 (20.0)	9 (36.0)
Smoker (%)	4 (16.0)	3 (12.0)	4 (16.0)
Race, Caucasian (%)	17 (68.0)	17 (68.0)	17 (68.0)
Education	15.24 (1.9)	15.24 (1.9)	15.16 (2.5)
Body mass index	28.31 (5.9)	26.33 (4.7)	27.52 (4.5)
Employment status[a], employed (%)	22 (88)	25 (100)	23 (92)
Average income, past 30 days	1211.6 (1774.6)	925.1 (1655.2)	1125 (1279.1)
STAI scores			
Total score	22.64 (5.6)	22.80 (5.5)	20.96 (4.1)
STAI state anxiety	31.08 (8.2)	32.32 (8.5)	30.68 (7.5)
STAI trait anxiety	32.0 (8.6)	32.28 (8.0)	32.52 (10.4)
CAI scores[b]			
CA life event***	14.84 (3.0)	7.64 (1.5)	3.36 (1.5)
Major life events***	2.64 (1.4)	1.64 (1.3)	0.44 (0.7)
Recent life events***	3.88 (1.9)	2.4 (1.8)	0.48 (0.7)
Trauma***	8.32 (3.4)	3.6 (2.0)	2.44 (1.3)
CMI scores[c]			
Total*	13.44 (11.6)	10.8 (8.3)	6.2 (4.8)
Physical health**	10.04 (8.2)	7.5 (4.9)	4.6 (3.3)
Psychological health	3.4 (4.6)	3.3 (5.0)	1.6 (2.3)

Abbreviations: BMI, body mass index; CA, cumulative adversity; CAI, Cumulative Adversity Interview; CMI, Cornell Medical Index; STAI, State-Trait Anxiety Inventory.

Mean values (SD) are denoted for age, education years, BMI, income, STAI, CAI, and CMI scores. All other measures reported in frequency (percents). There is no group difference in demographics and STAI anxiety scores. Asterisks show significant group differences. ***$p<0.001$; **$p<0.01$; *$p<0.05$.

[a]Individuals with full-time/regular part-time employment and students.

[b]See Supplementary Table S1 for the list of the CAI adverse life events.

[c]See Supplementary Table S2 for CMI health symptoms and the frequency with which each was endorsed.

except for CAI and CMI scores. The score of CA life event was positively correlated with CMI health problems ($r=0.29$, $p=0.01$), with no outliers in all 75 individuals.

Experimental Manipulations: Anxiety and Heart Rate

Behavioral data analysis was conducted to examine the effects of experimental manipulation and their associations with CA. Given the continuous nature of CA levels in 75 individuals, heart rate and anxiety ratings were examined using Student's *t*-tests between stress and neutral conditions in all subjects for an experimental manipulation check (Figure 1). Next, correlation analyses were conducted to examine the associations between CA and these measures. During stress exposure, both anxiety ($t=9.37$, $p<0.0001$) and heart rate ($t=5.13$, $p<0.0001$) were significantly elevated during stress exposure compared with the neutral condition, indicating successful stress induction. Heart rate response was positively correlated with CA scores during both the neutral ($r=0.24$, $p<0.05$) and stress ($r=0.25$, $p<0.05$) conditions in all 75 individuals with no outliers. Self-reported anxiety ratings were not correlated with CA.

fMRI Results

Correlation with CA. Whole-brain correlation analyses indicate significant positive correlations between stress-induced brain activity (stress–baseline) and CA scores in the prefrontal–limbic–striatal circuit in all 75 participants, including bilateral PFC, insula, striatum, right amygdala, hippocampus, temporal gyrus, right thalamus, and cerebellum (Figure 2 and Supplementary Table S3; whole-brain corrected at $p<0.05$). During neutral trials (neutral–baseline), one cluster involving the left parietal lobe (precuneus and inferior parietal lobe (IPL)), was found to be associated with CA (Supplementary Table S3). There were no outliers in any of these associations. The scatter plot in Figure 2 illustrates positively correlated patterns in areas of the lateral PFC, right amygdala, and striatum during stress.

High and low CA groups on stress/neutral responses. Significant group difference was also found between high and low CA individuals in brain response to stress in prefrontal–limbic–striatal regions (Figure 3 and Supplementary Table S4; whole-brain FWE corrected at $p<0.01$). During stress exposure (stress–baseline), high CA individuals showed increased activity in the lateral PFC, insula, right amygdala and hippocampus, striatum (putamen and ventral striatum), midbrain, posterior cingulate cortex, and temporal and parietal lobe, but decreased activity in the medial orbitofrontal cortex (OFC) compared with low CA individuals. No group differences during the neutral condition survived correction for multiple comparisons.

Neural link between CA and CMI health symptoms. To identify CA-related neural responses that may be associated with CMI health symptoms, mean signal changes in significant brain activation regions involved in CA (correlation and group difference maps) were independently correlated with the CMI scores. Among these regions of interests, only the medial OFC and right hippocampus from the high *vs* low CA group difference map (Figure 3) was significantly associated with CMI health problems (Figure 4; also see Supplementary Figure S1 for OFC and right hippocampus response in high, mid, and low CA groups). In 50 high/low CA individuals, medial OFC activity was negatively correlated with CMI total scores ($r=-0.42$, $p<0.01$), as well as with subscale scores of physical ($r=-0.38$, $p<0.01$) and psychological ($r=-0.39$, $p<0.01$) health symptoms. Right hippocampal activity was positively correlated with CMI total scores ($r=0.37$, $p<0.01$), as well as scores of physical ($r=0.31$, $p<0.05$) and psychological health ($r=0.39$, $p<0.01$) symptoms. Furthermore, medial OFC activity was inversely correlated with the right hippocampal activity ($r=-0.36$, $p=0.01$). No outliers were found in any of these associations. When

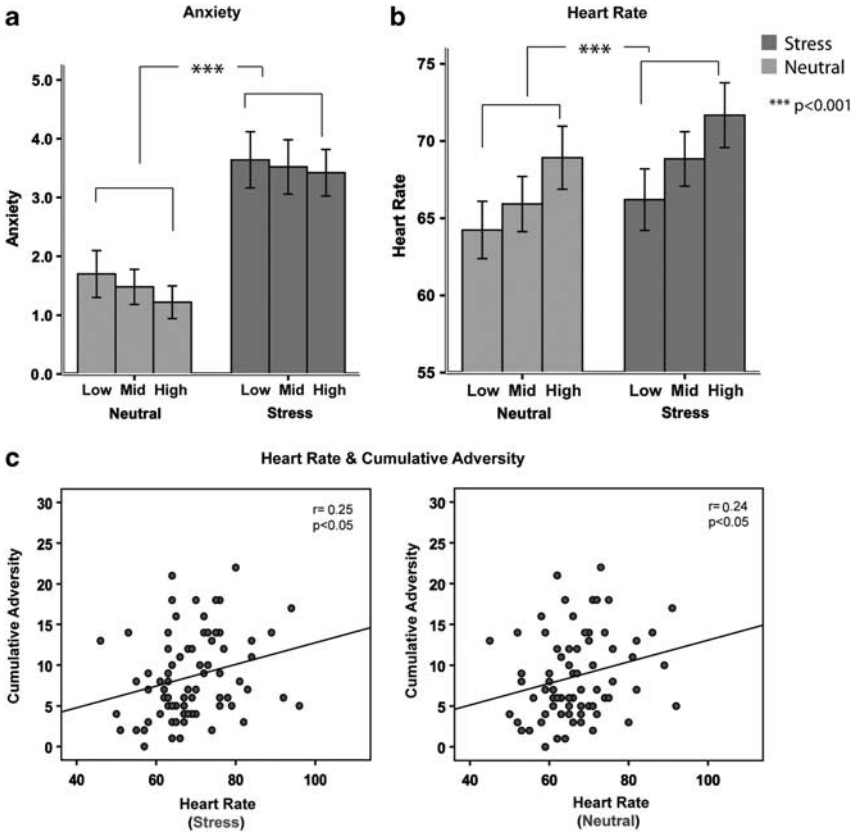

Figure 1 (a) Anxiety ratings; (b) heart rate responses during stress and neutral conditions; and (c) scatterplots showing significant correlations between cumulative adversity (CA) and heart rate during stress and neutral conditions in all 75 participants. During stress exposure, (a) anxiety ratings ($t = 9.37$, $p < 0.0001$) and (b) heart rate ($t = 5.13$, $p < 0.0001$) were significantly elevated relative to the neutral condition in all participants. (c) Heart rate response was correlated with CA score in both neutral ($r = 0.24$, $p < 0.05$) and stress ($r = 0.25$, $p < 0.05$) conditions. No correlation was found between anxiety ratings and CA. Error bar indicates SEM. ***$P < 0.0001$.

these two ROIs were applied to all 75 subjects, the associations with CMI health problems remained significant in both medial OFC ($r = -0.28$, $p = 0.016$) and right hippocampus ($r = 0.25$, $p < 0.05$) activity with a trend-level inverse relationship between the medial OFC and right hippocampus ($r = -0.21$, $p < 0.07$) in all 75 subjects.

DISCUSSION

We investigated neural responses to acute stress and its association with CA and health symptoms in a community sample of individuals, without psychiatric or physical diseases. In all participants, CA was significantly associated with increased activity in regions of the lateral PFC, insula, striatum, right amygdala, and hippocampus during stress exposure, and in the left parietal lobe during the neutral condition. When the high CA was directly compared with the low CA group, significant differences were also found in these key prefrontal–limbic–striatal regions during stress exposure. In addition, decreased activity in the medial OFC was specifically revealed in the high *vs* low CA group comparison. Among these CA-related brain activation regions, medial OFC and right hippocampal activity during stress exposure were inversely correlated and found to be associated with CMI-assessed health problems.

In subcortical response to acute emotional stress, significant associations with CA were mainly found in limbic–striatal regions, including the putamen, amygdala, hippocampus, and the insula cortex, indicative of a sensitized neural response to acute stress in individuals with high CA. Limbic–striatal activation has been associated with emotions and stressful experiences. During the experience of stress or aversive emotion, the amygdala is activated to initiate the stress response, including the release of corticotropin-releasing hormone and norepinephrine (Panksepp *et al*, 1997; Sinha, 2008), and interacts with the hippocampus for accessing emotional memory (Phelps, 2004). Limbic activity was predominantly found in the right hemisphere, consistent with the role of right-lateralized limbic activity in the processing of negative emotions (Lanteaume *et al*, 2007; Morris *et al*, 1999), suggesting sensitized right limbic responses during emotional stress in individuals with high CA. Along with amygdala–hippocampal regions, increased activity in the insula and striatum was evident. The insula is involved in emotional arousal and interoceptive awareness (Craig, 2009). The striatum has been associated with habitual responses to emotional stimuli (Schultz, 2006) and modulation of impulsive behaviors (Vink *et al*, 2005). Activation in the insula and striatum during acute stress has been reported in healthy individuals (Seo *et al*, 2011). In rats, chronic stress exposure upregulates and compromises striatal function (Rossi *et al*, 2008), and influences prefrontal executive function by altering dorsal striatal

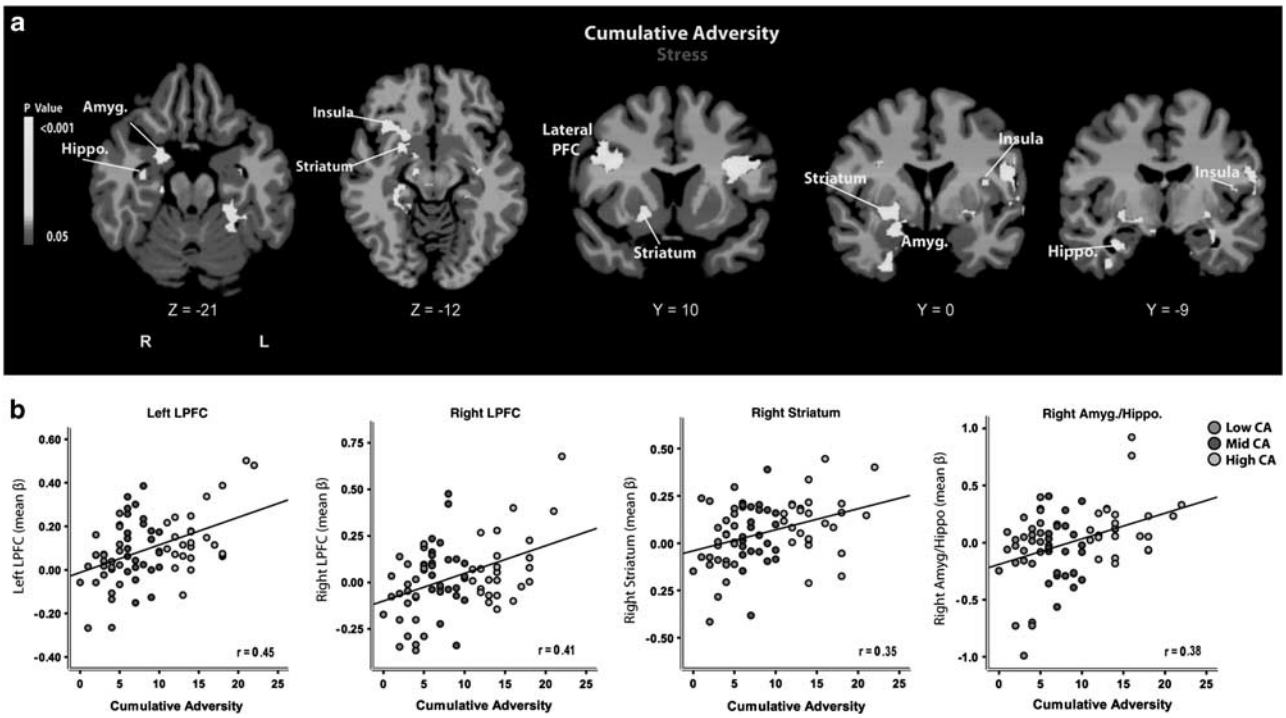

Figure 2 Results of whole-brain voxel-based correlation analysis showing associations between stress-induced brain activity and cumulative adversity (CA) scores. In all 75 healthy individuals, (a) CA was positively correlated with activity in the bilateral prefrontal cortex (PFC), insula, striatum, right amygdala, and hippocampus during stress exposure (whole-brain FWE-corrected, $p<0.05$). (b) Scatterplots further illustrate the correlated pattern in these regions with no outliers. L, left; R, right. Montreal Neurological Institute (MNI) coordinates were used.

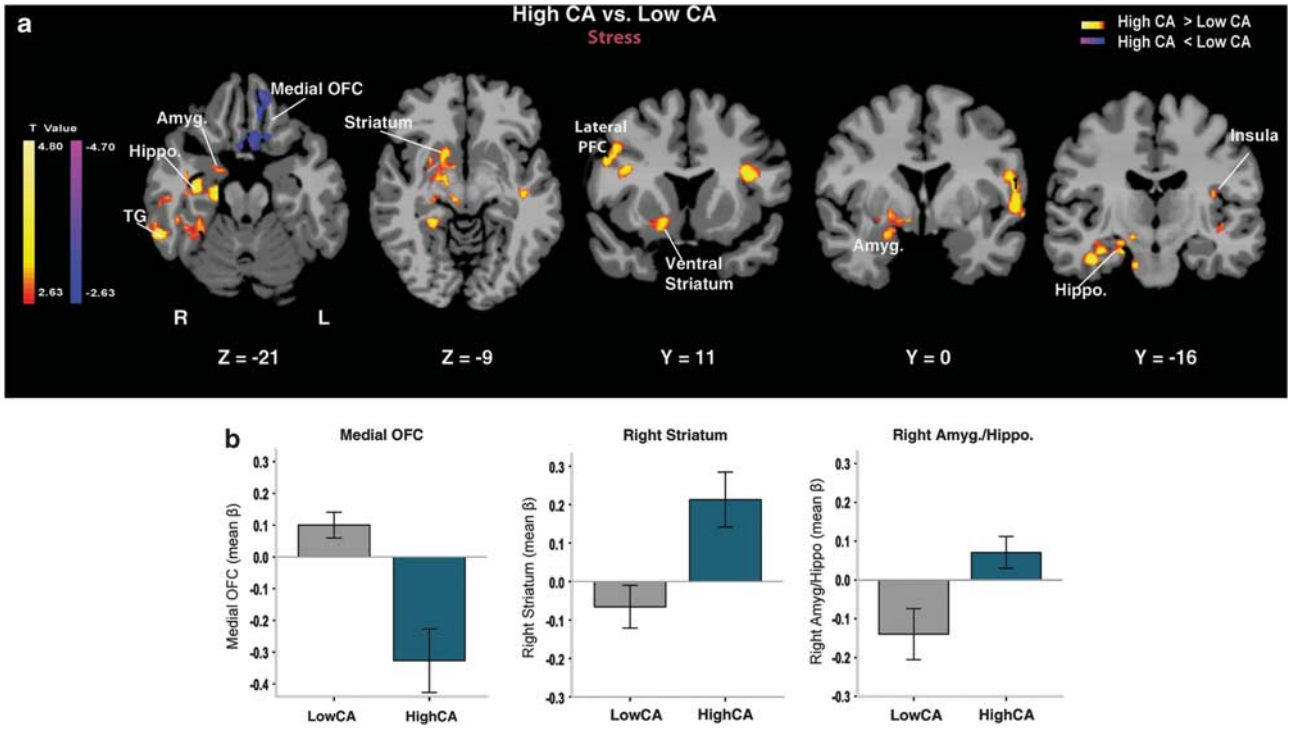

Figure 3 Group differences in stress-induced brain activity in high cumulative adversity (CA) *vs* low CA group. (a) During stress exposure, brain activity in the high CA group was elevated in the lateral prefrontal cortex (PFC), striatum, amygdala, hippocampus, insula, and superior and inferior temporal lobe, but decreased in the medial orbitofrontal cortex (OFC) relative to the low CA group (whole-brain FWE-corrected, $p<0.01$). (b) The bar graphs illustrate group differences in blood oxygenation level-dependent (BOLD) responses in the medial OFC, striatum, and amygdala/hippocampus. L, left; R, right; Amyg., amygdala; Hippo., hippocampus; TG, temporal gyrus. Montreal Neurological Institute (MNI) coordinates were used.

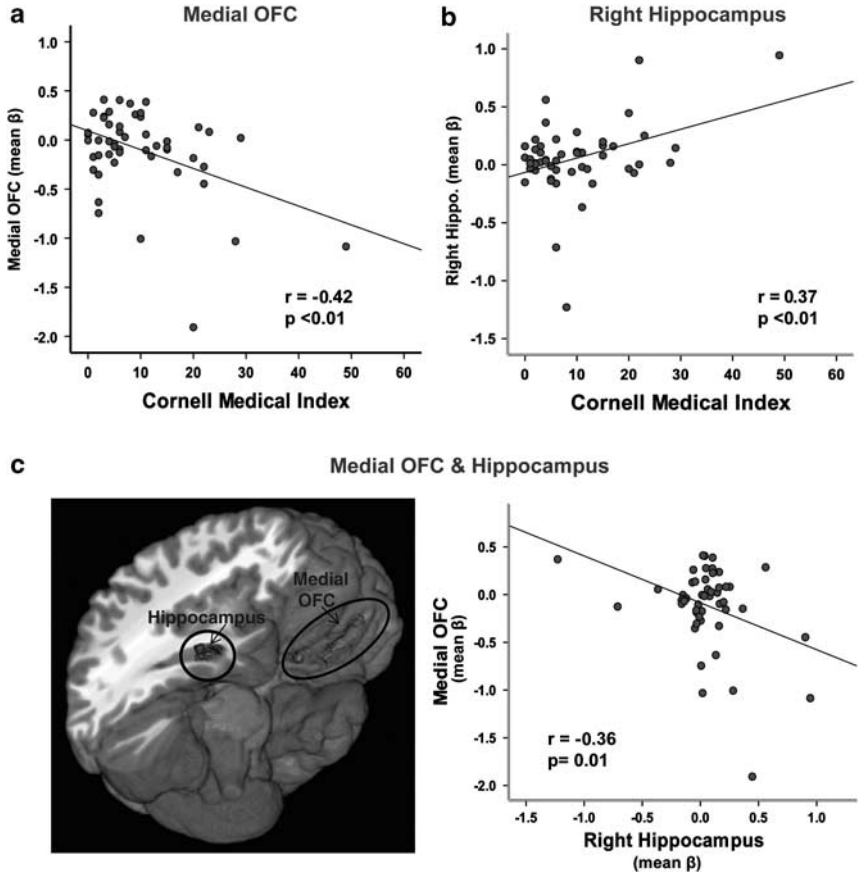

Figure 4 Neural substrates underlying the association between cumulative adversity (CA) and Cornell Medical Index (CMI) health problems in high and low CA individuals. During stress exposure, CMI health problems were (a) negatively correlated with medial orbitofrontal cortex (OFC) activity, but (b) positively correlated with right hippocampal activity. (c) Medial OFC activity was also inversely correlated with right hippocampal activity, suggesting that decreased regulatory function in the medial OFC and disinhibited right hippocampal activity contributes to the link between CA and greater CMI health symptoms. Decreased medial OFC activity and increased right hippocampal activity in high CA individuals relative to low CA individuals identified with whole-brain correction at $p<0.01$ are shown as a color overlay on the three-dimensional surface map.

projections to the frontal cortex (Dias-Ferreira *et al*, 2009). These results indicate that stress-induced hyperactivity in limbic–striatal regions may reflect sensitized responses to emotional distress in healthy individuals with high CA. During neutral-relaxing imagery, an active control condition reflecting general imagery processing, increased activity in the left parietal lobe (IPL, precuneus) was associated with CA in all subjects, but not in the high/low CA comparison, suggesting a general association pattern with mental imagery in healthy individuals. The left IPL and precuneus are involved in arousal and self-conscious mental process (Cavanna and Trimble, 2006; Singh-Curry and Husain, 2009), suggesting that increased activity in these regions may reflect greater levels of arousal and self-consciousness during neutral-relaxing imagery in individuals with high CA. It should be noted that altered response in the hypothesized PFC–limbic–striatal circuit was only found during stress exposure, suggesting that the altered PFC–limbic–striatal response is stress-specific and not related to general imagery processing.

In prefrontal regions, increased stress-induced activity in the lateral PFC was significantly associated with CA. Sensory information from limbic–striatal regions are transferred and represented in the lateral PFC (Miller and Cohen, 2001). The lateral PFC integrates this information and engages in prompt cognitive recognition of stress experiences (Miller and Cohen, 2001). It processes cognitive and emotional aspects of the experience and communicates with the medial PFC, a region involved in endogenous physiological and emotional regulation (Diorio *et al*, 1993; Spencer *et al*, 2005; Thayer *et al*, 2012; Urry *et al*, 2006). Current findings of hyperactive lateral PFC associated with high CA may reflect heightened sensitivity to cognitive processing and detection of stress experiences in these individuals. When high CA individuals were compared with low CA individuals, decreased medial OFC activity was additionally revealed during stress exposure, suggesting a specific neural response to acute stress among those with high levels of CA (see Figure 3; Supplementary Figure S1). The medial OFC has abundant anatomical connections to the amygdala and hippocampus (Carmichael and Price, 1995), allowing top–down regulation of emotional and physiological arousal, including cardiovascular activity and HPA axis responses (Figueiredo *et al*, 2003; Radley *et al*, 2006a). Hypofunction in the medial OFC has been associated with emotion regulatory difficulties such as greater anxiety, negative emotion, and impulsivity (Milad and Rauch, 2007; Seo *et al*, 2008). Taken together, these studies indicate that in high CA individuals, endogenous regulatory control in the medial OFC over emotional and physiological arousal

during stress may not be effectively implemented, suggesting a greater risk for stress-related health problems.

In our study, greater CMI health problems were associated with hypoactive medial OFC and hyperactive right hippocampal responses to stress, with an inverse relationship between these two regions, indicating a functional interaction between the lower medial OFC and higher right hippocampus during stress in modulating adverse health symptoms. The hippocampus is a key region involved in emotion, memory, and learning (Goosens, 2011). It is highly vulnerable to chronic stress (McEwen, 2002), including sensitivity to sustained glucocorticoid release and damage to the plasticity of the hippocampal nerve cells under prolonged stress exposure (McEwen, 2001). Individuals with long-term trauma and life stress typically show hippocampal volume reduction (Hull, 2002), especially in the right hemisphere (Gianaros *et al*, 2007). Multiple preclinical studies also demonstrate that repeated chronic stress compromises hippocampal function and hippocampus-associated learning (Nishimura *et al*, 1999).

The medial PFC, an important region for emotion regulation, has also been found to be impaired following stress exposure. Animal studies showed that repeated restraint stress impairs medial PFC function via dendritic spine loss in rats (Radley *et al*, 2006b). In humans, the medial PFC was shown to be hypoactive in response to acute stress (Ossewaarde *et al*, 2011), and a recent study with healthy individuals also showed that high CA and adversity was associated with lower gray matter volume in the medial PFC (Ansell *et al*, 2012b). The medial OFC has dense anatomical connections with the hippocampus (Carmichael and Price, 1995) and modulates firing of hippocampal neurons (Hyman *et al*, 2005). It has been suggested that altered medial PFC is involved in the behavioral disruptions associated with hippocampal damage in rats (O'Donnell *et al*, 2002). Hippocampal damage can also adversely impact medial PFC functions, including neonatal hippocampal lesion associated with decreased dendritic spine density of the PFC pyramidal neurons (Lipska *et al*, 2001), suggesting a close interaction between the hippocampus and medial PFC.

The medial OFC and hippocampus may influence physical health via their involvement in autonomic, neuroendocrine, and immune functions. For example, the ventromedial PFC (including the medial OFC) modulates stress-related physiological changes via its influence on the hypothalamic paraventricular nucleus, which regulates autonomic nervous system (ANS) and HPA activity (Radley *et al*, 2006a; Spencer *et al*, 2005). Specifically, the interaction between the medial PFC and amygdala–hippocampal complex has an important role in modulating stress-related ANS activity, including cardiovascular modulation (Thayer *et al*, 2012). The medial OFC and hippocampus are also likely to modulate immune responses via the HPA axis (Dhabhar, 2003). The medial PFC is a major regulator of the HPA axis (Figueiredo *et al*, 2003), and the hippocampus has a role in negative feedback of the HPA axis (Sapolsky, 1994) and in the modulation of immune function (Phillips *et al*, 1999). Additional evidence supports a role for the hippocampus and medial OFC in modulating physical health, such as hippocampal involvement in chronic pain (McEwen, 2001) and interleukin-6 inflammatory function (Marsland *et al*, 2008), hippocampal damage during cardiac arrest (Sadowski *et al*, 1999), and decreased OFC blood flow in patients with chronic pain syndrome (Honda *et al*, 2007). Taken together, current findings indicate a significant role of the medial OFC and hippocampus in regulation of stress and health symptoms, presumably via their effects on the autonomic, neuroendocrine, and immune function involved in homeostasis, supporting the notion that CA-related altered brain responses to stress in these regions may negatively affect physical and psychological health.

It is important to note that CA was retrospectively reported in a structured interview and may have been susceptible to reporting bias and over-reliance on memory. Nonetheless, the CAI has demonstrated validity in large epidemiologic studies that prospectively assessed prediction of psychiatric disorders and physical health conditions (Brown and Turner, 2010; Gayman *et al*, 2008; Russell *et al*, 2009; Scott *et al*, 2008; Turner and Lloyd, 2004). Furthermore, in our data no association was found between CA and STAI anxiety or in-scanner anxiety ratings. Given that anxious tendencies have been closely associated with negative emotional bias (Watson *et al*, 1988), these results suggest that the objective count of CA is independent of subjective perception or negative emotional bias. Supporting this, we also did not find correlations between CA-associated brain activity in the prefrontal–limbic–striatal regions and scores of a chronic stress subscale specifically designed to capture subjective reactions to adverse events, indicating that current findings are not likely to be driven by subjective response to those events. Furthermore, as significant associations with CA were mainly found in brain response and cardiac autonomic activity measured by increased heart rate, these data suggest that CA significantly influences neural and physiological responses rather than subjective responses in healthy individuals, as suggested in previous work (Ansell *et al*, 2012b; Thoits, 2010).

In conclusion, the current study identifies CA-related, sensitized prefrontal–limbic–striatal responses to acute stress in non-diseased individuals, and suggests that cumulative experience of adversity affects health problems via altered medial OFC and right hippocampus function during stress. It should be noted that our participants are healthy, community individuals who experienced traumatic or adverse life events, but without current or lifetime PTSD, other psychiatric disorders, or physical illnesses. Although it is possible that the observed stress-induced brain response reflects a protective neural pattern in healthy individuals, the significant associations between activity in the medial OFC/hippocampus and CMI health problems (including both psychological and physiological health symptoms) suggest that altered neural responses in high CA participants may more probably represent the prodromal pattern along a continuum of stress-related pathophysiology that promotes disease risk. To further clarify the exact nature of brain activity association with CA, future research would benefit from including individuals with clinical disorders and also examining whether sensitized responses to stress in vulnerable individuals is predictive of future health problems and development of stress-related diseases. Nonetheless, the current findings have important clinical implications. In the absence of resilience factors and

in the face of future stressful events, individuals with high CA may be at risk of developing stress-related psychiatric or physical disorders. The findings also suggest the need to further explore the utility of targeting individuals with high CA and low resilience for developing stress-related primary prevention strategies to reverse the identified neural pathophysiology and stress-related health symptoms, and proactively address their high risk for the development of stress-related disorders.

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