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Limbic response to stress linking life trauma and hypothalamus-pituitary-adrenal axis function

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ABSTRACT

Trauma alters neuroendocrine responses to stress and increases vulnerability to stress-related disorders. Yet, relationships among trauma, stress-induced neural changes and hypothalamic–pituitary–adrenal (HPA) axis activity have not been determined. The present study used functional magnetic resonance imaging to investigate the impact of life trauma on basal cortisol levels and neural responses to acute stress in 73 healthy individuals during brief stress and neutral-relaxing imagery using a well-established, individualized imagery method. We hypothesized that trauma experience would have a negative impact on brain function, resulting in altered basal cortisol levels via dysregulated neural control over the HPA axis system. Results showed that higher life trauma exposure was significantly associated with lower basal cortisol levels. Neuroimaging results indicated that both higher life trauma and low morning cortisol levels were associated with increased response to acute stress in limbic-medial temporal lobe (MTL) regions including the amygdala and hippocampus. A mediation analysis showed that increased limbic-MTL response to stress mediated the relationship between life trauma and low cortisol levels. Findings revealed a significant impact of lifetime trauma on neural responses to acute stress and HPA axis activity. Life trauma may sensitize limbic-MTL regions and its related peripheral systems, which could compromise stress regulation and HPA axis function, and increase risk for negative stress-related health outcomes.

1. Introduction

Lifetime trauma exposure has been shown to significantly impact physical and psychological well-being, increasing risk for stress-related disorders and negative health outcomes (Bevans et al., 2008; McEwen, 2002; Sinha, 2008). Studies have identified the neurobiological underpinnings of trauma exposure; this line of studies has largely found altered function associated with trauma in two stress-related pathways, the hypothalamic–pituitary–adrenal (HPA) axis system (Fries et al., 2005) and limbic brain regions (McEwen, 2001; Williams et al., 2006).

More specifically, neuroendocrine studies have indicated that HPA axis response is impacted as a result of chronic stress exposure (McEwen, 2002; Sinha, 2008). For example, cortisol typically increases under stressful conditions. However, studies have indicated a negative association between basal cortisol levels and traumatic experience, indicative of a state of hypocortisolism, including in individuals with early trauma (Meinlschmidt and Heim, 2005), repeated trauma exposures (Bevans et al., 2008; Kolassa et al., 2007), and in those with various stress-related disorders (Fries et al., 2005). Researchers have suggested that under chronic stress, a decrease in basal cortisol levels occurs due to overcompensation as accompanied by a highly sensitized HPA axis system (Edwards et al., 2011). Additional evidence suggests that corticotropin-release-factor (CRF) down-regulation in the hypothalamus generates this response pattern via negative feedback inhibition resulting from excessive cortisol response during repeated stress (Heim et al., 2000).

Previous neuroimaging studies have demonstrated the impact of prolonged stress and trauma on limbic brain function in regions such as the amygdala (Williams et al., 2006) and hippocampus (McEwen, 2001). For example, a study incorporating both functional and structural imaging showed that childhood maltreatment was associated with increased amygdalar activity in response to threatening faces and reduced gray matter volumes in the hippocampus (Dannlowski et al., 2012). This is also consistent with the results of preclinical studies. In rats, an uncontrollable stressor diminished GABA-stimulated chloride uptake in the amygdala (Martijena et al., 2002), and chronic stress altered synaptic activity in the hippocampus (Karst and Joels, 2003).

These studies provide insights into the neurobiology underlying

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trauma experience. However, endocrine and neuroimaging studies have been conducted separately, and the impact of repeated trauma on HPA axis alterations and neural responses to stress has not yet been addressed. Recurring trauma exposure may adversely affect neural functioning (e.g., in limbic regions), thereby altering HPA axis function. For example, there is evidence that the amygdala modulates the HPA axis via the hypothalamus (Price et al., 1987). This suggests that an altered limbic response (e.g., amygdala) might underlie the relationship between trauma and HPA axis activity.

Therefore, to thoroughly understand the neurobiology underlying trauma, the current study sought to identify the link between lifetime trauma exposure, HPA axis activity and neural responses to acute stress using functional magnetic resonance imaging (fMRI). We assessed basal morning cortisol levels and brain activity during acute stress via a well-established and validated, individualized script-driven guided imagery method involving stress *vs.* neutral-relaxing cues in 73 healthy individuals. Trauma exposure was measured using the Life Trauma subscale of the Cumulative Adversity Interview (CAI; adopted from (Turner et al., 1995)), a widely used instrument which has been shown to predict psychophysical consequences in a variety of populations (see Supplementary information). The fMRI procedure involved the use of a well-validated, individualized script method to provoke stressful and neutral-relaxing situations (see the published manual (Sinha and Tuit, 2012) and a review that summarizes previous evidence supporting the method in Sinha (2009)).

Based on prior research, we hypothesized that higher life trauma would be associated with lower basal morning cortisol. We also predicted that greater life trauma and lower cortisol levels would correlate with increased neural activity during stress in limbic regions including the amygdala and hippocampus. Further we expected to find a negative association between hypothalamus activity and cortisol levels, given the crucial role of the hypothalamus in regulating HPA axis function (Edwards et al., 2011). Finally, because HPA axis regulation at the level of the hypothalamus occurs via afferent limbic projections (Smith and Vale, 2006), we hypothesized that stress-induced limbic response would play a mediating role in the relationship between trauma and basal cortisol levels.

2. Material and methods

Seventy-three healthy individuals (right-handed) between the ages of 18 and 50 participated in this study (see Table 1). Participants from the community were recruited via local newspaper advertisements and flyers posted for research participation. All participants completed assessments during 2–3 sessions pertaining to cognitive, demographic, and health status as well as HPA axis function prior to an fMRI scan. Exclusion criteria included history of head trauma, current or past substance use disorder, presence of any mental disorders (as determined by the Structured Clinical Interview for DSM-IV), use of any prescription medications at the time of fMRI testing, pregnancy, and claustrophobia. Women underwent the fMRI session only during the follicular or luteal phases of their menstrual cycle and not the ovulatory or pre-menstrual/menstrual phase in order to avoid any potential influence of steroid hormonal increases on the stress response. Assessment of menstrual cycle was based on self-report. Among 24 women, there were 13 women in the follicular phase (54%) and 11 women in the luteal phase (46%). Prior to study participation, all participants provided informed written consent. All study procedures were approved by the Human Investigation Committee at the Yale University School of Medicine.

Table 1
Demographics, basal cortisol levels and life trauma scores.

Subject Variable	Total N = 73
Demographics	
Age	27.5 (7.8)
Gender – % female	24 (33%)
Race – % Caucasian	52 (71%)
Education	15.2 (2.1)
Body Mass Index (BMI)	27 (5.0)
Morning Fasting Cortisol (ug/dL)	14.8 (5.8)
Life Trauma scores[a]	4.6 (3.4)

Note: Mean values (standard deviations) are denoted.
For gender and race, frequency (percents) are reported in parenthesis.
[a] Life trauma scores from the Cumulative Adversity Interview.

2.1. Lifetime trauma

Lifetime trauma was measured using the life trauma subscale of the Cumulative Adversity Interview (adapted from Turner et al. (1995)), a 140- item interview that addresses stressful life experiences and has been well-validated in predicting psychiatric disorders and health conditions (see Supplementary information). The life trauma scale comprises 34 items and primarily measures traumatic life experience. Trained interviewers inquired about the occurrence and frequency of traumatic events during participants' lifetime, as previously described. Examples of items comprising the life trauma scale included exposure to violence, witnessing or experiencing serious accident, death or injury, personal trauma involving force or coercion and physical, emotional and /or sexual abuse or assault. Witnessed violence encompasses items that involve being present in dangerous or distressing situations, such as seeing someone get shot or attacked with a weapon. In addition, some items are related to traumatic news involving someone important being killed, injured or abused.

2.2. Hormonal evaluation

The current study collected basal morning cortisol under fasting conditions, which is regarded as a reliable biological marker of HPA axis pathology (e.g., (Hagg et al., 1987)). Cortisol samples were collected and analyzed as described previously (Chao et al., 2017). On a separate day prior to the fMRI session, participants were asked to visit the Yale Stress Center, for an early morning laboratory session at 7:30 am after fasting overnight. Four repeated samples of cortisol were obtained at 15-minutes intervals for an hour. Cortisol samples were collected in heparinized plasma collection tubes, centrifuged at 4 °C within 30 min of drawing, aliquoted, and subsequently stored at − 80 °C. Then, radio-immuno-assays for cortisol were conducted by Yale Center for Clinical Investigation Core Laboratories. Basal cortisol was calculated as the mean plasma cortisol value taken from the repeated measurements over the hour. An MRI session was conducted within a week of this laboratory session.

2.3. Individualized imagery method

We used a brief guided imagery of individualized stress and neutral-relaxing situations (Sinha and Tuit, 2012; Sinha, 2009), which is a well-validated method in laboratory and neuroimaging studies using emotion and stress induction (see Supplementary information). Before the fMRI session, imagery scripts were developed for each participant based on the participant's accounts of two stressful and two neutral-relaxing experiences that occurred in the past year using the standardized Scene Construction Questionnaires (Sinha, 2009). The stress scripts were developed based on participants' description of a non-traumatic stressful experience that occurred in the past year (e.g., losing a job, family discontent, or relationship troubles). More specifically, they were developed based on participants' description of a stressful experience that made them sad, mad, and upset in a manner that could not be changed in the moment (e.g., losing a job, family discontent, or relationship

troubles). The participants rated the intensity of the distress situations on a 10-point Likert scale (1 = not at all stressful and 10 = most stressful), and only events rated 8 or above were considered for inclusion in the script. In doing so, we were able to control for the self-reported stressfulness of the situation across subjects. This stress provocation method has been shown to have predictive validity of clinical and health symptoms (see Supplementary information). Participants were also asked to describe experiences evoking neutral-relaxing scenarios (e.g., reading a book or relaxing on the beach) for the development of the neutral-relaxing scripts. These neutral-relaxing scripts were used to compare an individuals' stress imagery and response to their own non-stress imagery control. Due to the individualized nature of the script, script content was specific to each participant's experience. However, the script format, style, and length were standardized across conditions and subjects, as detailed in previously published guidelines (Sinha, 2009).

A trained master's level research associate was audiotaped reading the scripts which were each 2.5 min in length and had a tone and valence matching the content of the script. The pre-recorded scripts were then audio played to participants during the fMRI session while they had their eyes closed. The order of scripts was presented in the counterbalanced manner. In order to ensure equal ability to evoke mental images during the imagery process, participants underwent standardized imagery and relaxation training, as described previously (Sinha, 2009). Sample scripts are presented in Table S3 (see Supplementary information).

2.4. fMRI task and acquisition

Each fMRI trial lasted 5 min, consisting of a 1.5-min baseline period followed by a 2.5-min imagery period (2 min of read imagery and 0.5 min of silent imagery recall) and a 1-min quiet recovery period. There were two trials per condition (2 stress, 2 neutral) for each participant, and the order of script presentation (stress or neutral) was counterbalanced across subjects. Each script was presented only once without the same condition presented consecutively. Participants were instructed to rate their anxiety prior to and after each trial based on how anxious or nervous they felt on a 10-point Likert scale (1 = not at all and 10 = extremely high). Following each imagery trial, participants rated the vividness of imagery on a 10-point Likert scale (1 = cannot visualize the image and 10 = extremely clear, 'as if' it were happening right now). Heart rate was measured throughout the task using a pulse oximeter placed on the non-dominant forefinger. In between each trial, participants listened to a 2- min progressive relaxation recording to eliminate any residual effects of previous trials.

MRI data were collected via a 3-T Siemens Trio MRI system with a standard quadrature head coil using a T2*-sensitive gradient-recalled single-shot echo-planar pulse sequence. Anatomical MRI data were collected with spin echo imaging in the axial plane parallel to the AC-PC line (TR = 300 msec, TE = 2.5 msec, bandwidth = 300 Hz/pixel, flip angle = 60°, field of view = 220 × 220 mm, matrix = 256 × 256) and 32 slices with slice thickness = 4 mm with no gap. Functional MRI data were acquired with a single-shot gradient echo planar imaging sequence with thirty-two axial slices parallel to the AC-PC line covering the whole brain (TR = 2000 msec, TE = 25 msec, bandwidth = 2004 Hz/pixel, flip angle = 85°, field of view = 220 × 220 mm, matrix = 64 × 64, 32 slices with slice thickness = 4 mm and no gap). Sagittal anatomical images were obtained for multi-subject registration using a high-resolution 3D Magnetization Prepared Rapid Gradient Echo sequence (TR = 2530 ms; echo time (TE) = 3.34 ms; bandwidth = 180 Hz/pixel; flip angle (FA) = 7°; slice thickness = 1 mm; field of view = 256 × 256 mm; matrix = 256 × 256).

2.5. fMRI analysis

Functional MRI data were converted from Digital Imaging and Communication in Medicine format to Analyze format using XMedCon (Nolfe, 2003). The first 10 images were discarded from each functional run to reach a steady-state equilibrium between radio-frequency pulsing and relaxation. Due to potential carryover effects from the imagery period, the 1-min recovery period was eliminated from data analysis. fMRI data were preprocessed using MATLAB and Statistical Parametric Mapping (SPM5) and slice time and motion corrected for three translational and three rotational directions. Any trial with linear motion > 1.5 mm and a rotation larger than 2° was removed.

For each trial per condition, a general linear model (GLM) was used for individual-level analysis with a regressor comparing time during imagery to baseline (stress–baseline and neutral–baseline) using BioImageSuite (www.bioimagesuite.org., (Duncan et al., 2004)). Drift correction was also applied in the GLM, such that drift regressors were utilized to remove the mean time course, linear, quadratic, and cubic trends for each run. Each trial was spatially smoothed using a 6-mm Gaussian kernel and normalized to generate β-maps (3.44 mm × 3.44 mm × 4 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 × 1 × 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.

For group level analysis, a *t*-test comparing stress and neutral conditions was conducted using BioImageSuite. In addition, to examine the associations between life trauma, basal cortisol levels, and brain activity, whole-brain correlational analyses were conducted using BioImageSuite. To correct for multiple comparisons, cluster-wise correction of family-wise errors was implemented for all analyses. The cluster size was determined by AFNI's 3dClustSim program (https://afni.nimh.nih.gov (Cox, 1996), version 16.0.09) using Monte Carlo simulation (Xiong et al., 1995). To best identify specific neural correlates and be appropriately conservative, thresholds were determined based on the type of data analysis using whole brain correction for multiple comparisons; a threshold of 0.05 was used for whole-brain correlation analysis and 0.01 was used for fMRI task results.

2.5.1. Functional connectivity

To examine connectivity patterns between the hypothalamus (*a priori* region) and other brain regions, a functional connectivity analysis was implemented using BioImageSuite. Using the inverse transform from the GLM, a reference region was inversely transformed into individual subject space. The time-course of the transformed reference region was calculated for each subject as the average time-course across all pixels within the reference region. Then, the time-course was correlated with the time-courses of all the other voxels in the brain using a whole brain voxel-wise Pearson correlation, fisher transformed to z-values, averaged across runs and then spatially smoothed with a 6 mm Gaussian filter.

2.6. Mediation analysis

To examine whether brain activity mediates the relationship between trauma scores and basal cortisol levels, we conducted mediation analyses. We tested a, b, c and c′ pathways using the ordinary least squares (OLS) for trauma scores (independent variable) with brain correlates as the mediating variable and basal cortisol levels as the dependent variable. For the mediated effect (a *x* b) of brain activity in associations between trauma scores and basal cortisol levels, we employed the approach via the SPSS Process macro (Hayes, 2013). Bootstrapping was used to estimate the significance of the indirect effects, as indirect effects do not meet normality assumptions. Bias corrected and accelerated 95% confidence intervals (CI) of the mediated effects were

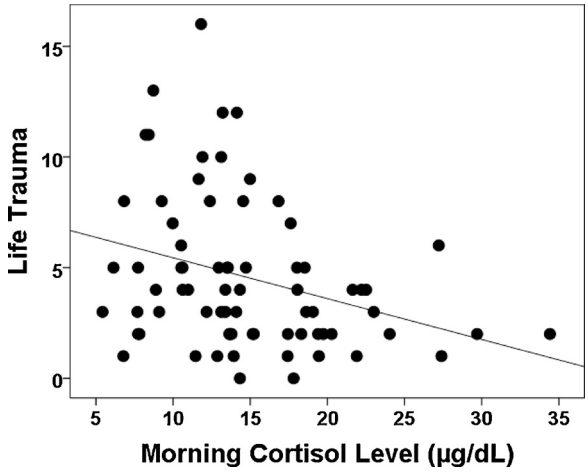

Fig. 1. The relationship between life trauma and basal cortisol level. Higher life trauma scores were associated with decreased basal morning cortisol levels ($r = -.31$, $p = 0.007$).

generated using 10,000 bootstrapped re-samples for each indirect effect point estimate. CIs that do not include a zero value indicate a significant indirect effect, as the effect is significantly different from zero at $p < 0.05$ (two-tailed). For consistency with the literature, unstandardized OLS coefficients are reported (MacKinnon and Dwyer, 1993).

3. Results

3.1. Demographics and baseline information

Table 1 presents the demographic characteristics, basal cortisol levels and life trauma scores of all 73 participants. Life trauma scores were negatively correlated with fasting morning cortisol levels in our sample, such that higher levels of trauma were associated with lower cortisol levels ($r = -.31$, $p = 0.007$; see Fig. 1).

3.2. Stress versus neutral imagery task effects

Task-related effects were evident in ratings, heart rate, and brain activity, such that stress exposure elicited greater response in anxiety ratings and heart rate, as well as increased activity in the cortico-striatal regions compared to the neutral condition (Fig. 2).

3.2.1. Ratings and heart rate

Task-related significance in ratings and heart rate response were tested using *t*-test to examine the effects of stress induction. Anxiety ratings in the stress imagery condition ($M = 3.6$, $SD = 2.1$) were significantly elevated compared to those in the neutral-relaxing imagery condition ($M = 1.4$, $SD = 1.5$) ($t = 9.7$, $p < 0.0001$) (see Fig. 2A). Heart rate response was greater for the stress condition ($M = 68.5$, $SD = 9.6$) than the neutral-relaxing condition ($M = 65.8$, $SD = 9.1$) ($t = 5.2$, $p < 0.0001$) (see Fig. 2B). The means of vividness ratings were 8.2 (1.2) for the stress condition and 7.9 (1.3) for the neutral condition with higher ratings found in the stress compared to the neutral condition ($t = 2.1$, $p < 0.05$).

3.2.2. Task-related brain activity

The results of the *t*-test comparing stress vs. neutral conditions showed significant task effects in brain regions including the medial PFC, precuneus, anterior and posterior cingulate cortex (ACC/PCC), left lateral prefrontal cortex (LPFC), left anterior insula, superior/middle temporal gyrus, thalamus, striatum, right amygdala, mid-brain and cerebellum ($p < 0.01$, whole-brain FWE corrected; see Fig. 2C). Brain activity in these areas was greater in the stress condition than in the neutral condition. There was no region that was more active in the neutral condition compared to the stress condition.

3.3. Correlates of life trauma and fMRI responses to stress

To understand the associations between lifetime trauma scores and brain activity, a whole-brain correlation analysis was conducted separately for the stress and neutral conditions (See Fig. 3, Table S1). There was no brain response during the neutral-relaxing condition that was significantly correlated with life trauma. During the stress condition (stress-baseline), the results showed that high life trauma scores were

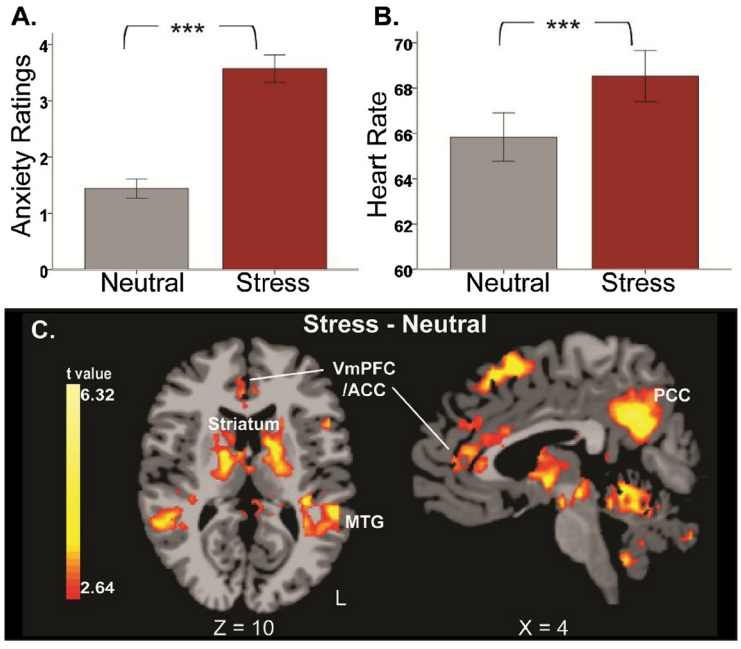

Fig. 2. Task-related effects. During stress exposure, (A) anxiety ratings and (B) heart rate response were significantly increased compared to the neutral condition. *** $p < 0.0001$. (C) Task-related activity during stress exposure relative to the neutral condition. A whole-brain voxel-based analysis showed that activity in cortico-striatal regions was significantly increased during stress relative to the neutral condition. These areas included the medial prefrontal cortex (PFC), anterior/posterior cingulate cortex (ACC/PCC), superior/middle temporal gyrus, precuneus, striatum, thalamus, mid-brain, and cerebellum ($p < 0.01$, whole-brain FWE corrected). VmPFC = ventromedial prefrontal cortex; ACC = anterior cingulate cortex; PCC = posterior cingulate cortex; MTG = middle temporal gyrus; L, left. Coordinates are given in Montreal Neurological Institute (MNI) space.

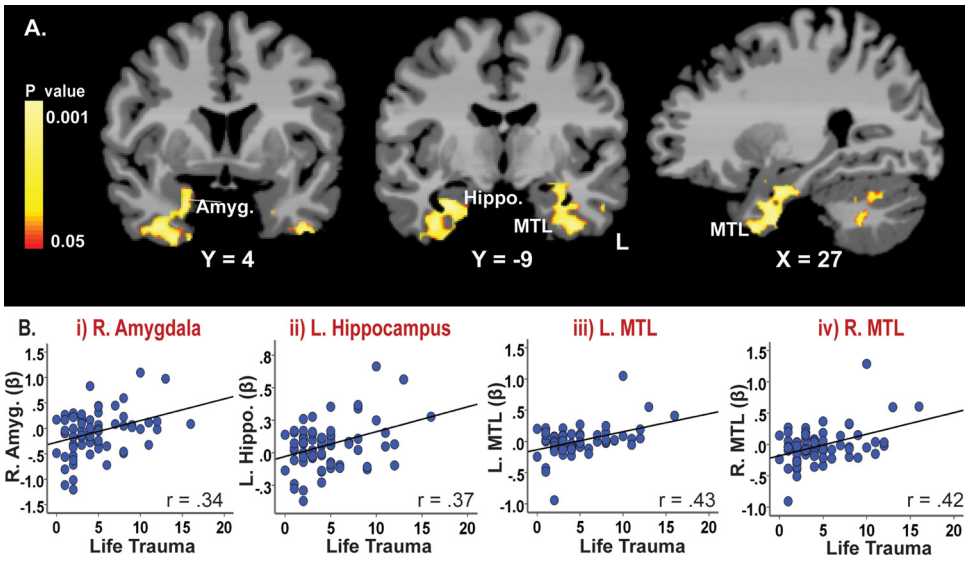

Fig. 3. Neural correlates of lifetime trauma during stress exposure (Stress-Baseline). Results of a whole brain correlation analysis showed that (A) life trauma scores were positively correlated with neural response to stress in limbic-medial temporal regions including the right amygdala, hippocampus, medial temporal lobe and parts of the brain stem/cerebellum ($p < 0.05$, whole-brain corrected). Yellow/red colors = positive correlations. Amyg = amygdala; Hippo. = hippocampus; MTL = medial temporal lobe, L = left. MNI coordinates were used. (B) No outliers were found in associations between life trauma and brain activity, as illustrated by the scatterplots demonstrating correlated patterns in the i) R. amygdala, ii) L. hippocampus, iii) L. medial temporal lobe and iv) R. medial temporal lobe. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

significantly associated with increased neural responses to stress in limbic-medial temporal lobe (MTL) regions including right amygdala, bilateral hippocampus, MTL, and parts of brain stem and cerebellum ($p < 0.05$, whole-brain FWE corrected). Fig. 3 shows (A) the whole-brain correlation map with life trauma and (B) corresponding scatterplots showing no outliers in associations with the i) amygdala, ii) hippocampus, iii) left medial temporal lobe and iv) right medial temporal lobe. A whole-brain correlation result between life trauma and brain activity during the Stress-Neutral contrast is presented in the Supplemental information (Fig. S1). The results show similar findings to Fig. 3, suggesting that the stress condition drives the observed effects.

3.4. Correlates of basal cortisol levels and fMRI response to stress

Whole-brain correlation results with basal cortisol levels showed significant negative correlations with brain activity during stress (stress-baseline; see Fig. 4, Table S2). The associated regions showed a substantial overlap with regions found in neural correlates of life trauma (described above), which were localized in limbic-MTL regions including the amygdala, hippocampus, and medial temporal lobe and parts of the brain stem and cerebellum. In addition, increased stress-induced activity in the hypothalamus, mid-cingulate gyrus, and precuneus was associated with lower basal cortisol levels ($p < 0.05$, whole-brain FWE corrected). Scatterplots show no outliers in these associations (Fig. 4B).

3.5. Functional connectivity with the hypothalamus

Based on substantial literature indicating the critical role of the hypothalamus in regulating the HPA axis function (e.g., (Sinha, 2008; Smith and Vale, 2006)), hypothalamus activity was defined as *a priori* region to further examine the relationship between the hypothalamus and other brain regions during stress. To understand a functionally connected network influencing hypothalamus activity associated with low basal cortisol levels, a reference region of the hypothalamus was functionally defined from a cortisol-correlation map (Fig. 4). Then a

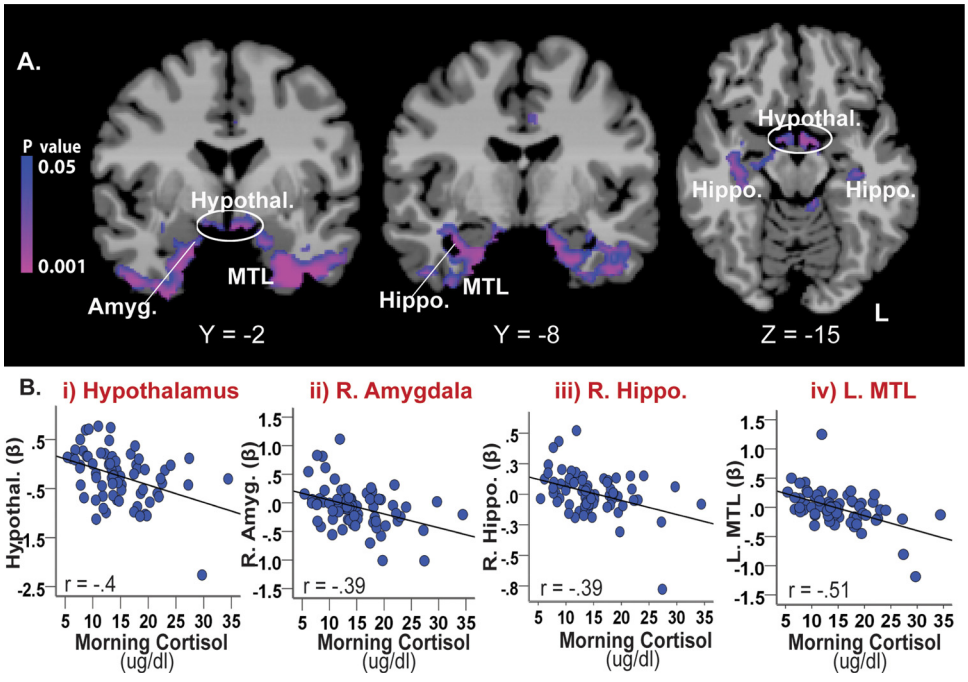

Fig. 4. The association between basal cortisol and stress-related neural activity (Stress-Baseline). (A) Results of a whole brain correlation analysis showed that basal cortisol levels were negatively correlated with stress-related activity in limbic-MTL regions including the amygdala, hippocampus, and medial temporal lobe as well as the hypothalamus (whole-brain FWE corrected, $p < 0.05$). (B) The scatterplots show negative correlated patterns with no outliers between basal cortisol levels and stress-induced activity in the i) hypothalamus, ii) R. amygdala, iii) R. hippocampus, and iv) L. medial temporal lobe. Amyg. = amygdala; Hypothal. = hypothalamus; Hippo. = hippocampus, MTL = medial temporal lobe; R = right; L = left. Blue/purple colors = negative correlations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

connectivity analysis was conducted with the hypothalamus as a seed region using BioImageSuite. The results showed that the hypothalamus has increased connectivity with the amygdala, ventral striatum and the medial orbitofrontal cortex (See Fig. S2; $p < 0.001$, whole-brain corrected).

3.6. Mediation analysis

The mediation analysis was conducted to examine whether neural correlates of life trauma (limbic-MTL regions; Fig. 3) mediates the relationship between trauma and basal cortisol. The results showed that stress-induced activity in the bilateral limbic and left MTL regions mediated the relationship between trauma scores and basal cortisol levels (see Fig. 5, for description of a, b, c, and c′ pathways). The right MTL was not shown to mediate the relationship. Results showed that trauma scores were positively associated with brain activity during stress in bilateral limbic (left, $t = 4.3$, $\beta = .03$, $p = .0001$; right, $t = 3.95$, $\beta = .037$, $p < .001$) and left MTL ($t = 3.99$, $\beta = .029$, $p < .001$) regions. The effects of the mediators, stress-induced activity in limbic (left, $t = -2.9$, $\beta = -.9.9$, $p < .01$; right, $t = -2.7$, $\beta = -6.2$, $p < .01$) and left MTL regions ($t = -2.8$, $\beta = -8.3$, $p < .01$), on cortisol levels were also significant, after controlling for trauma scores. The effect of trauma on cortisol levels was significant ($t = -2.8$, $\beta = -.53$, $p < .01$). However, this effect disappeared when the mediators, bilateral limbic (left, $t = -1.3$, $\beta = -.26$, n.s.; right, $t = -1.5$, $\beta = -.3$, n.s.) or left MTL activity ($t = -1.4$, $\beta = -.29$, n.s.), were included in the model. These results indicate that the effect of trauma on basal cortisol levels were mediated by stress-induced activity in limbic-left MTL regions. In addition, the mediation effect of trauma on basal cortisol levels via brain activity was significant for limbic (left $axb = -.27$, (95% CI = −.54, −.09); right, $axb = -.23$ (95% CI = −.48, −.07)) and left MTL ($axb = -.24$, (95% CI = −.51, −.04)) regions, further confirming statistical mediation effects of brain activity (Fig. 5).

3.7. Secondary analyses to assess imagery vividness and sex differences

Secondary analyses were conducted to examine the effect of imagery vividness on the associations between basal cortisol, trauma scores, and brain response to stress. The results of these analyses indicated that the main findings were not altered when imagery vividness was statistically controlled, suggesting that individual differences in imagery vividness did not contribute to the observed results. In terms of sex differences, no significant differences were found in our main findings including baseline cortisol levels, trauma scores, and correlations of life trauma and cortisol levels with task-related brain response displayed in Figs. 3 and 4. Furthermore, among 24 women, there were no differences between those with follicular vs. luteal phases in our main findings including basal cortisol, trauma scores, and their associations with brain response. This may be due to a small number of women included in our study or indicate that transient menstrual cycle may not affect our findings based on chronic, repeated trauma and associated neurobiological characteristics.

4. Discussion

The present study examined relationships among life trauma, HPA axis activity, and brain response to stress in healthy community individuals. Our findings showed that greater trauma experience was associated with lower morning cortisol levels, both of which were associated with increased limbic-MTL responses to stress. Decreased cortisol levels were additionally associated with an enhanced response to stress in the hypothalamus, a modulator of the HPA axis system (Smith and Vale, 2006). Functional connectivity was found between the hypothalamus and limbic regions. In addition, a subsequent mediation analysis showed that limbic-MTL activity mediated the relationship between trauma and basal cortisol levels. These findings suggest that repeated trauma exposure sensitizes limbic-MTL regions and their

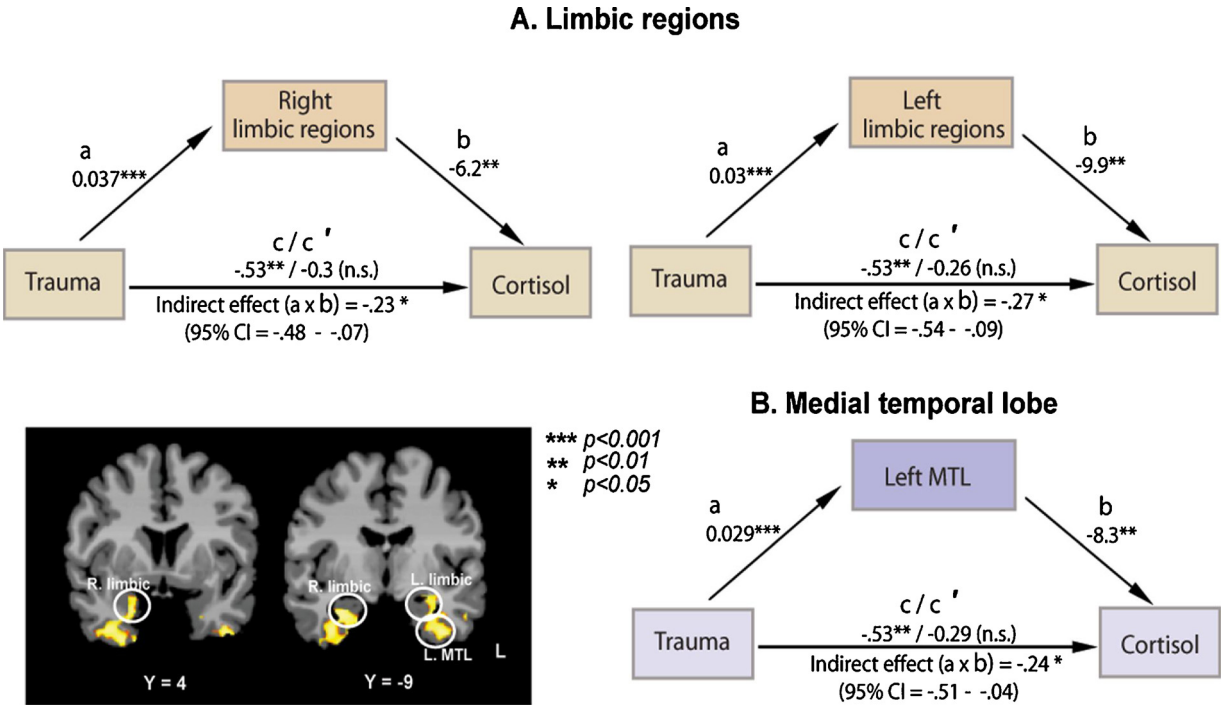

Fig. 5. A path diagram showing the mediating effect of brain activity during stress on the relation between trauma and basal cortisol levels. Mediator(s) = beta(s) in limbic-MTL regions during stress (Stress-Baseline); **a** = Effect of trauma on the mediator; **b** = Effect of the mediator on basal cortisol levels, controlling for trauma score; **c** = Effect of the trauma on cortisol levels without brain activity in the model; **c′** = Direct effect of trauma on cortisol levels with the effects of brain activity controlled; **a *x* b** = Indirect effects of trauma on cortisol levels via the effects of bilateral limbic and left MTL activity; CI = Confidence Interval. The effects of trauma on cortisol levels are not significant when controlling for brain activity, suggesting significant mediating effects of stress-induced limbic-left MTL activity on the relationship between trauma and basal cortisol levels. Note: * $p < 0.05$, **$p < .01$, *** $p < 0.001$, n.s. = not significant.

functionally connected network, which may negatively impact the HPA axis system.

4.1. Trauma and basal cortisol levels

Our results showed an inverse relationship between life trauma and basal morning cortisol levels. This is consistent with previous studies demonstrating associations between life trauma and reduced basal morning cortisol levels (Bevans et al., 2008). Another study found decreased baseline saliva cortisol levels in trauma-exposed individuals in the absence of psychopathology (Klaassens et al., 2010). Our findings along with these studies support the idea that reduced basal cortisol, or a state of hypocortisolism (Fries et al., 2005), is indicative of altered HPA axis activity in individuals with traumatic experiences. It should be noted that we found no sex differences in baseline morning cortisol levels. While it is widely accepted that there are sex differences in HPA axis reactivity, especially in the cortisol response following a stressor (Handa et al., 1994), there has been some discrepancy in reports of sex differences in baseline morning cortisol levels. Our results are consistent with studies demonstrating similar null findings of sex differences in baseline cortisol levels (e.g., (Barra et al., 2015)).

A major role of the HPA axis system is to modulate adaptive physiological responses and maintain homeostasis via the regulation of cortisol release (Smith and Vale, 2006). Stress exposure generates corticotropin-releasing factor (CRF) in the hypothalamus which activates the stress response system, initiating the release of cortisol (McEwen, 1998). Prolonged and repeated exposure to high amounts of stress results in an increased 'allostatic load', which has deleterious effects on HPA axis function (McEwen, 2002) resulting in long-term biochemical changes leading to decreased basal cortisol response (Clow et al., 2010). Further, altered cortisol response has been regarded as a symptomatic marker reflecting poor stress and emotion regulation (Compton et al., 2013), especially in people who have experienced trauma. Together, our findings of low basal cortisol associated with trauma may be indicative of altered HPA-axis activity and associated stress dysregulation in individuals with trauma experiences.

4.2. Life trauma and neural response to acute stress

A significant association between life trauma and brain response to acute stress was mainly found in limbic-MTL regions and a portion of the brain stem and cerebellum.

Limbic regions including the amygdala and hippocampus have been implicated in emotional processing, acute stress (Roozendaal et al., 2009), aversive learning (LeDoux, 2000), and trauma exposure (Williams et al., 2006). Heightened responses in the amygdala and hippocampus were also found in individuals with high cumulative adversity (Seo et al., 2014), suggesting a crucial role of these regions in adaptive stress responses. In terms of emotional reactivity, the amygdala has been associated with negative emotion, fear, and enhanced vigilance during acute stress (Davis and Whalen, 2001). Specifically, the right amygdala is known to be more involved in aversive aspects of emotional processing (Morris et al., 1999). Thus, increased activity in the right amygdala, as demonstrated in our study, may be indicative of emotional distress and sensitized limbic function associated with life trauma. The MTL plays a crucial role in emotional memory and consolidation (Dolcos et al., 2005). The hippocampus is a limbic region located in MTL structure that is critically involved in memory encoding and storage. The hippocampus is known to be vulnerable to damage from chronic stress (McEwen, 2001), and compromised MTL function was also found following stress induction via cortisone administration (de Quervain et al., 2003).

The limbic and MTL regions are closely associated with each other and play a key role in stress adaptation, as the amygdala interacts with the MTL memory system during stress (Dolcos et al., 2005; Roozendaal et al., 2009). It is known that learning and memory is negatively impacted under stress in both quantitative and qualitative ways via stress pathways including the amygdala (Schwabe et al., 2010). These studies along with our results suggest that stressful and traumatic events may sensitize limbic-MTL regions, which might compromise their cognitive and emotional adaptive functions and result in maladaptive coping with stress.

4.3. Neural circuits of basal cortisol

Similar patterns of stress-induced sensitization were observed in brain regions associated with basal cortisol levels. That is, lowered cortisol levels were correlated with greater stress-induced activity in limbic-MTL regions along with the hypothalamus. The association between HPA axis activity and limbic brain regions has been well documented in prior studies showing associations of cortisol levels with amygdala activity during emotion regulation (Urry et al., 2006), amygdala metabolism (Drevets et al., 2002) as well as the role of the hippocampus in HPA axis function during stress (Mizoguchi et al., 2003). It has been suggested that interactions between limbic and HPA axis activity play a crucial role in mediating stress-related adaptive behaviors (Cunningham-Bussel et al., 2009). Specifically, repeated stress exposure increases vulnerability to allostatic load by adversely impacting limbic-HPA axis regulation of cortisol that is required for stress-related adaptation (McEwen, 2002).

In addition to limbic regions, lowered morning cortisol levels were associated with an increased response to stress in the hypothalamus. In the brain, the paraventricular nucleus of the hypothalamus produces corticotropin-releasing hormone (CRH) that has regulatory control over cortisol release in the adrenal cortex (Dickerson and Kemeny, 2004). It follows that the association between the hypothalamus and low basal cortisol levels observed in our study may reflect disrupted hypothalamic control over HPA axis functioning resulting from trauma-related sensitization.

4.4. Limbic mediation between trauma and basal cortisol

Limbic-MTL response to stress was associated with both life trauma and lower basal cortisol levels, suggesting that this circuit may play a mediating role. Consistent with this, a subsequent analysis showed that increased limbic-MTL response to stress mediated the relationship between trauma and low basal cortisol levels. These results suggest that trauma may sensitize limbic-MTL response to stress, which could further compromise HPA axis functions governed by the hypothalamus.

It should be noted that our study did not find direct associations between life trauma and hypothalamic activity, a modulator of the HPA axis system (Smith and Vale, 2006). One possible explanation is that trauma may indirectly impact the hypothalamus via other brain regions, such as the amygdala. There is evidence that the amygdala is involved in the regulation of the hypothalamus and governs adaptive HPA axis response to stress (Herman et al., 2005). Corroborating this, our connectivity analysis results showed that hypothalamus activity during stress exhibited increased functional connectivity with the medial amygdala. The medial amygdala is known to relay sensory information to the hypothalamus (Keshavarzi et al., 2014), suggesting the influence of the amygdala on HPA axis activity via the hypothalamus. Consistent with this, we showed that the amygdala mediated the relationship between trauma and decreased cortisol levels. This is in line with findings of preclinical literature. The central nucleus of the amygdala innervates the paraventricular nucleus of the hypothalamus, which allows amygdalar modulation of HPA axis response to stress (Gray et al., 1989). In addition to limbic activity, other mechanisms involving more specific pathways within the limbic system may also have contributed to this mediation. For example, repeated stress exposure increases CRF and γ-aminobutyric acid (GABA) release in the amygdala, followed by increased CRF secretion in the paraventricular nucleus (Cook, 2004). Over the long term, hypothalamic CRF increase

results in low basal cortisol levels (Fries et al., 2005) via negative feedback inhibition that decreases adrenocorticotropic hormone (ACTH) response to CRF stimulation by down-regulating CRF receptors in the pituitary gland (Edwards et al., 2011; Heim et al., 2000). These studies suggest that the amygdalar mediation of the relationship between trauma and low basal cortisol in our study may reflect trauma-related, sensitized pathways (e.g., CRF) between the central nucleus of the amygdala and the paraventricular nucleus of the hypothalamus leading to alteration in the HPA axis system. This evidence emphasizes the need to further investigate more specific mechanisms underlying the role of limbic-HPA axis system in trauma-related sensitization.

5. Conclusion and clinical implications

To summarize, the current study identifies neural correlates of life trauma and associated HPA axis function localized in limbic-MTL regions. It is notable that there is a substantial overlap in brain regions associated with both life trauma and basal cortisol levels. To our knowledge, this is the first study demonstrating similar neural circuits underlying trauma and HPA axis disruption. In particular, the current study shows that the limbic system plays a crucial role in mediating the relationship between life trauma and low cortisol levels, potentially via functional connectivity with the hypothalamus. These results suggest that repeated life trauma may sensitize limbic-MTL circuits and its connected network (e.g., hypothalamus), resulting in compromised HPA axis function in the periphery.

It should be noted that our findings of trauma-related limbic sensitization were observed in healthy community individuals. Similar patterns of brain sensitization were also found in psychiatric samples, such as limbic dysfunction observed in patients with post-traumatic stress disorder (Francati et al., 2007). These patterns of brain sensitization in healthy individuals may reflect prodromal patterns or susceptibility of these individuals to stress-related clinical disorders. Such a chronic state may lead to increased neurobiological sensitivity to stress, thereby adversely affecting adaptive responses required for regulating high levels of stress. Over time, these alterations may continue to impair adaptive physiological functioning and increase vulnerability to stress-related disorders. However, due to the nature of our sample, caution should be taken when generalizing our findings, and further studies are needed to verify these findings in clinical samples.

Despite the significance of our findings for trauma-related vulnerability, it is important to explore alternative interpretations of our results. Due to the retrospective and self-reported nature of life trauma measurements, it is plausible that individuals with higher limbic-MTL activation may recall past traumas more vividly, resulting in greater self-reports of life trauma experience. However, since vividness ratings were not associated with trauma scores, nor with activity in limbic-MTL regions, it is unlikely that the vividness of stress imagery was a contributing factor in our main findings. Additionally, while life trauma events are reported retrospectively, the imaging data are not based on traumatic situations but rather non-traumatic stressful situations that were reported during script development. Therefore, it is unlikely that an individual's ability to recall past traumas can explain the observed increases in limbic-MTL activation.

In a similar vein, our study focused only on traumatic life stressors as opposed to more chronic stressors such as neglect or poverty. Therefore, it is unclear whether our results generalize to other types of life stressors. Future studies could address this by exploring how different types of trauma influence HPA axis functioning and brain response to stress.

An additional limitation to our study is the use of morning basal cortisol in interpreting HPA dysfunction related to trauma. We did not collect cortisol samples during the day, nor was it collected during the neuroimaging protocol. While the decision to collect only morning basal cortisol was based on previous research demonstrating its reliability as biological marker of HPA axis condition (e.g., (Hagg et al., 1987)), it is unclear if diurnal changes in cortisol would affect our results. In order to develop a more complete picture of the effect that trauma has on HPA axis function, future studies should utilize cortisol levels at fixed times over a 24-hour period and during stress challenges. Another direction of research may also involve examining neural correlates of concurrent cortisol response to acute stress, along with morning cortisol levels. To achieve this, research using multimodal neuroimaging techniques (e.g., simultaneous collection of brain and cortisol response to acute stress) is necessary to further clarify neural substrates underlying brain and HPA axis response to acute stress in individuals with trauma. Taken together, future studies should continue to explore whether the limbic-MTL circuit could serve as a neural marker of lifetime trauma and associated HPA disruption, and a target for developing appropriate prevention and treatment strategies.

Conflict of interest

The authors declare that they have no conflict of interest pertaining to the aims and results of this study.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2018.08.023.

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