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Amygdala reactivity and anterior cingulate habituation predict PTSD symptom maintenance after acute civilian trauma

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Abstract

Background—Studies suggest that exaggerated amygdala reactivity is a vulnerability factor for post-traumatic stress disorder (PTSD), however understanding is limited by a paucity of prospective, longitudinal studies. Recent studies in healthy samples indicate that, relative to reactivity, habituation is a more reliable biomarker of individual differences in amygdala function. We investigated reactivity of the amygdala and cortical areas to repeated threat presentations in a prospective study of PTSD.

Methods—Participants were recruited from the emergency department of a large level-I trauma center within 24 hours of trauma. PTSD symptoms were assessed at baseline, and approximately 1, 3, 6, and 12 months post-trauma. Growth curve modeling was used to estimate symptom recovery trajectories. $N=31$ participated in fMRI around the 1 month assessment, passively viewing fearful and neutral face stimuli. Reactivity (fearful>neutral) and habituation to fearful faces was examined.

Results—Amygdala reactivity, but not habituation, 5–12 weeks post-trauma was positively associated with the PTSD symptom intercept and predicted symptoms at 12 months post-trauma.

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Habituation in the ventral anterior cingulate cortex (vACC) was positively associated with the slope of PTSD symptoms, such that decreases in vACC activation over repeated presentations of fearful stimuli predicted increasing symptoms.

Conclusions—Findings point to neural signatures of risk for maintaining PTSD symptoms following trauma exposure. Specifically, chronic symptoms were predicted by amygdala hyper-reactivity and poor recovery was predicted by a failure to maintain vACC activation in response to fearful stimuli. The importance of identifying patients at risk following trauma exposure is discussed.

Keywords

trauma; fear; arousal; fMRI; prospective; amygdala

Introduction

Early identification of risk factors that predispose individuals to trauma-related psychopathology, such as post-traumatic stress disorder (PTSD), could help providers prevent or limit symptoms before a disorder develops. Such risk assessment in the peri-traumatic period could benefit a significant proportion of the general population; it has been estimated that 50–60% experience a potentially traumatizing event (1), and 6–8% develop PTSD following trauma exposure (1, 2). Markers of brain function may be particularly powerful biomarkers of risk, as they can provide insight into the mechanisms leading to maladaptive responses to trauma, and potential targets for treatment. Furthermore, understanding the intermediate phenotypes of brain function which underlie PTSD development may lead to novel therapeutic approaches.

Findings from studies of chronic PTSD consistently show an association between symptoms and hyper-reactivity of the amygdala and dorsal aspects of the anterior cingulate cortex (dACC), key brain regions for emotional expression and appraisal (3–7). In addition, PTSD is associated with under-activity and reduced functional connectivity among regions that regulate amygdala function including ventral aspects of the anterior cingulate cortex (vACC) (6, 8–11). This pattern of abnormalities is thought to contribute to hyper-arousal symptoms in PTSD, and impairments in top-down emotion regulation and fear extinction (3, 8, 9, 12). However, the majority of previous research has been conducted cross-sectionally in chronic PTSD, and cannot distinguish between risk factors or acquired features of the disorder.

Recent findings from prospective studies of trauma and PTSD implicate amygdala function as a potential predictor of PTSD. For example, studies of military service members before and after combat deployment showed that amygdala and vACC reactivity increased significantly from pre- to post-combat exposure (13, 14), and that pre-deployment amygdala reactivity to emotionally arousing or risk-related stimuli positively predicted post-deployment PTSD symptoms (14, 15). Perhaps because these studies recruited individuals from highly trained military samples, participants did not show significant levels of PTSD severity post-trauma, and additional studies are needed to determine whether findings generalize to the broader population. A pilot study in a civilian population (N=9) was conducted to assess responses to acute traumas that led to a hospital emergency department

visit, and showed that default-mode connectivity between the amygdala and posterior cingulate cortex 6 weeks post-trauma was positively related to PTSD symptoms 12 weeks post-trauma (16). However, amygdala reactivity has not been investigated as a PTSD predictor in a civilian cohort.

Ideal biomarkers of brain function are those that are reliable and minimally influenced by transient day-to-day changes. Test-retest reliability for fMRI measures of reactivity to emotional face stimuli in regions including the amygdala and ACC has been shown to be fair to excellent (17–19). Furthermore, recent studies indicate that amygdala habituation, or the change over time in the response to a repeated stimulus, shows greater test-retest stability within individuals than reactivity (17, 18). Interestingly, individuals with chronic PTSD show an increased initial amygdala response to trauma-related negative stimuli and altered patterns of amygdala habituation relative to controls (20). This heightened initial response, and differences in habituation, may contribute to previous findings of amygdala hyper-reactivity in PTSD. However, no prospective study of PTSD has examined habituation of responses to emotional stimuli.

In the current study, we conducted a prospective longitudinal investigation of PTSD symptoms following acute trauma, measuring brain function using functional magnetic resonance imaging (fMRI) at an early timepoint prior to PTSD diagnosis, approximately 1 month after the index trauma. We investigated neural reactivity and habituation to emotional stimuli as predictors of later PTSD symptom trajectories over the next year. Participants were recruited from an ongoing longitudinal study of biomarkers for PTSD in which individuals who experienced a traumatic event were approached in the emergency department within 24 hours of trauma and assessed for symptoms at 1, 3, 6, and 12 months post-trauma. The fMRI scan took place within 2–3 weeks of the 1-month visit (timeline of visits illustrated in Figure 1b), with $M(SD) = 57(14)$ days following trauma exposure. We hypothesized that heightened reactivity to negative stimuli in the amygdala and dACC, and blunted reactivity in the vACC, would predict later PTSD symptom severity, consistent with the idea that these brain phenotypes reflect vulnerability factors for PTSD. In addition, we assessed the exploratory hypothesis that reduced habituation of the amygdala response to repeated presentations of negative stimuli would predict later PTSD symptoms.

Methods and Materials

Participants

38 participants were recruited from a larger study of biomarkers for PTSD. Participants from the parent study who indicated interest in neuroimaging were approached to participate in the neuroimaging study. This add-on study was not designed to be representative of the larger study. Participants were patients in the Emergency Department (ED) of Grady Memorial Hospital in Atlanta, GA, who had experienced a traumatic event within the last 24 hours. Participants were included if they were English-speaking, 18–65 years of age, endorsed a criterion A trauma as defined by the DSM-IV-TR (21), and provided contact information for follow-up visits. Exclusion criteria included previous hospitalization for mental health reasons, current suicidal ideation, attempted suicide in the past 3 months, current intoxication, or altered mental status during the ED visit. After MRI data collection,

7 participants were excluded from further analysis due to anatomical abnormalities such as falx calcification (n=3), head motion > 3mm (n=3), and stimulus presentation malfunction (n=1). Of the final sample of 31, 22 participants were in motor vehicle crashes, 4 were pedestrians who were hit by a vehicle, 3 were motorcycle or bicycle accidents, and 2 were sexual assault victims.

Following the trauma, several participants sought therapy and/or mental health counseling (unrelated to the current study): 6 within the first month, 4 between 1–3 months, 2 between 3–6 months, and 2 between 6–12 months post-trauma. Additional sample characteristics are summarized in Table 1. Table S1 lists prescription medication use and co-morbid diagnoses identified on a Mini International Neuropsychiatric Schedule (MINI)(22) administered during the emergency department visit. Participants provided written informed consent for all parts of the study, and the Institutional Review Boards of Emory University and Grady Memorial Hospital approved the study procedures.

Emergency Department (ED) assessment at the time of trauma exposure

Demographic information and information about the index trauma was gathered using the Standardized Trauma Interview (STI), a 41-item clinician administered interview gathering information on relevant aspects of the trauma at baseline as well as demographic information (23). To assess prior trauma history and baseline PTSD symptoms related to prior trauma, we administered the Posttraumatic Stress Diagnostic Scale (PDS), a 49-item self-report measure (24). Depression symptoms in the two weeks prior to the ED visit were assessed using the Beck Depression Inventory (BDI), a 21-item self-report measure (25). Childhood trauma history was assessed using the Childhood Trauma Questionnaire (CTQ), a 25-item instrument assessing physical, sexual, and emotional abuse, and physical and emotional neglect before age 18, which has shown high reliability and validity relative to external measures of child abuse (26).

Follow-up assessments

PTSD and depression symptoms were assessed 1 month, 3 months, 6 months, and 12 months following the ED visit. PTSD symptom severity in response to the index trauma was measured using the (PSS)(27), a 17-item scale measuring symptom severity assessing DSM-IV-TR criteria for PTSD (21). The PSS items assess the same 17 symptoms assessed by the PDS at the baseline ED visit for prior trauma, using a similar 0–3 scale for frequency. Depression symptoms were measured using the Beck Depression Inventory (BDI), a 21-item scale measuring symptom severity (28).

FMRI study procedure

Participants completed the fMRI session within three weeks [$M(SD) = 21(3)$ days] of the 1 month follow-up assessment [delay relative to index trauma = 57(14) days]. Study procedures followed Stevens et al. (3). Participants passively viewed static fearful and neutral face stimuli, which were presented in blocks of 8 trials, with a total of 30 blocks (15 fearful, 15 neutral) that randomly alternated between the fearful and neutral conditions.

Brain imaging acquisition and analysis

Brain imaging data were acquired on two Siemens 3.0-Tesla Magnetom Trio TIM MRI scanners (Siemens, Malvern, PA) using a 12-channel head coil. N=20 participants were scanned on the first scanner, and N=11 on the second. Functional images were acquired using the Z-SAGA pulse sequence (29) to minimize signal loss due to susceptibility artifacts. Volumes contained 30 axially acquired 4mm thick images with an in-plane resolution of $3.44 \times 3.44 \text{ mm}^2$ utilizing the parameters: pulse repetition time 3000 ms, TE1/TE2= 30/67ms, FA= 90°. Structural images were acquired using a gradient-echo, T1-weighted pulse sequence (TR=2300ms, TE=2.78ms; 1.2mm \times 1.3mm \times 1.3mm voxel size).

Preprocessing and statistical analysis was conducted in SPM8 (Wellcome Department of Cognitive Neurology), and details can be found in (30). Briefly, spike and motion artifacts were corrected using ArtRepair software (31). Images were corrected for slice timing, and spatial realignment was applied. Participants with head motion > 3mm over the entire session were excluded from further analyses. Images were normalized with unified segmentation, and smoothed with an 8mm Gaussian kernel.

Blocks of fearful and neutral stimuli were modeled with a boxcar function representing the onset and 8000 ms duration of the block, convolved with a canonical hemodynamic response function. Participant-specific motion parameters were included as covariates. To assess reactivity to threat, contrast images for the fearful versus neutral conditions were entered into group-level random effects analyses. To assess habituation to threat, the first third of the fearful face blocks (5 blocks) were compared to the last third, following analytic strategies used in previous research with healthy samples (18, 32, 33). To investigate whether habituation effects were specific to threat stimuli, we also examined habituation to neutral faces (first third of neutral blocks – last third). Hypothesis-driven regions of interest (ROIs) were constructed in WFUPickAtlas 2.4 software (www.fmri.wfubmc.edu). A bilateral amygdala ROI was defined anatomically using the Automated Anatomical Labeling Toolbox (AAL)(34). To anatomically define the dorsal and ventral aspects of the ACC, we selected primate analogs of rodent prelimbic (PL) and infralimbic (IL) cortex, because the rodent literature provides clear examples of functional differentiation among medial prefrontal cortical areas (e.g., 35, 36). The dACC (PL) was defined using Brodmann Area (BA) 32, and the vACC (IL) was defined using BA 25 (37). To examine regions outside the ROIs, whole-brain analyses were conducted with SPM's cluster-based False Discovery Rate (FDR) thresholding and an initial threshold of $p < .005$.

Data analytic strategy

A linear growth curve was estimated in the MPlus (version 7) environment using PSS scores from 1, 3, 6 and 12 months' post emergency room admission. Two parameters were estimated including the intercept (initial PTSD symptom severity) and slope (change over time in symptoms) using maximum likelihood estimation. The slope and intercept parameters were separately regressed on amygdala, vACC, and dACC reactivity and habituation while controlling for age, gender, exposure to similar previous trauma, and scanner in the overall growth curve model. The intercept and slope values were then saved outside of the MPlus environment for use in additional whole-brain analyses, with age,

gender, and scanner as covariates. To improve estimation for saved values, the growth curve was estimated using the larger ED study sample ($N=355$). However, the only data reported are for the subset ($N=31$) with neuroimaging data.

Results

Figure 1a and Table 2 show PTSD symptoms related to the index trauma at each of the follow-up visits. Twenty-nine participants of the initial sample of 31 returned for the follow-up assessment at 3 months, 29 at 6 months, and 24 at 12 months post-trauma. The growth curve estimates demonstrated an initial intercept that was significantly greater than 0 ($Est=14.92$; $S.E.=0.67$; $p<.001$) and a significant negative slope ($Est=-0.53$; $S.E.=0.09$; $p<.001$) together indicating significant levels of PTSD symptom severity at 1 month, followed by a significant average decline in symptoms by 12 months post-trauma. However, it is notable that mean symptoms were still moderate at 12 months ($M=9.8$).

Reactivity and habituation to fearful face stimuli

Whole-brain analysis of task-related fMRI activation across the full sample showed significant activation in the right amygdala and bilateral occipital cortex activation for fearful > neutral stimuli, $p<.05$, corrected (Figure 2a). For the habituation contrast comparing the first 5 > last 5 fearful blocks, there was no significant habituation in any region. Instead there was significant sensitization (increased response from beginning to end of scan) in a large cluster overlapping posterior cingulate cortex, right posterior hippocampus, and right posterior insula, $p<.05$, corrected (Figure 2b).

Associations between fMRI activation and the intercept (initial symptom levels)

Emotional reactivity in the amygdala ROI was significantly positively associated with the intercept parameter ($Est=3.44$; $S.E.=1.46$; $p=.02$), indicating a positive relationship between amygdala reactivity and symptom levels (Figure 3a). This effect remained significant in follow-up analysis of the $N=28$ participants without PTSD related to prior trauma ($Est=3.43$; $S.E.=1.50$; $p<.05$). Reactivity in the dACC and vACC ROIs were not associated with the intercept, nor was habituation of responses to fearful or neutral stimuli in any of the ROIs, $ps>.20$. In whole-brain analyses of the emotional reactivity and habituation contrasts, no region showed a significant correlation with the intercept parameter.

Associations between fMRI activation and symptom trajectories

Emotional reactivity in the amygdala and ACC ROIs was not predictive of the slope (change over time in symptoms; $ps>.05$). Habituation to fearful stimuli in the vACC ROI demonstrated a positive relationship with the slope parameter ($Est=0.94$; $S.E.=0.40$; $p=.05$; Figure 3b), indicating that greater habituation was associated with a flatter symptom slope (Figure 3c). This effect was stronger in follow-up analysis of the $N=28$ participants without PTSD related to prior trauma ($Est=-2.51$; $S.E.=0.32$; $p<.001$). Habituation to fearful stimuli in the amygdala and dACC ROIs was not associated with slope, $ps>.10$, nor was habituation to neutral stimuli in any of the ROIs, $ps>.30$. In whole-brain analyses of the emotional reactivity and habituation contrasts, no region showed a significant correlation with symptom slope.

Contrast estimates for the reactivity and habituation contrasts in the amygdala, vACC, and dACC ROIs were then examined as predictors of 12 month scores as a time-variant covariate nested in the growth curve model. Amygdala reactivity was positively associated with 12 month PSS severity scores ($Est=-0.50$; $S.E.=0.25$; $p<0.05$) while vACC and dACC reactivity were not ($Est_{vACC}=0.11$; $S.E.=0.23$; $p=.63$; $Est_{dACC}=-0.23$; $S.E.=0.30$; $p=.45$). Habituation of the amygdala, vACC, and dACC were not associated with 12 month severity scores ($Est_{amygdala}=2.00$; $S.E.=1.26$; $p=.11$; $Est_{vACC}=1.49$; $S.E.=0.97$; $p=.13$; $Est_{dACC}=-2.4$; $S.E.=1.33$; $p=.07$).

Discussion

In the current study we examined relationships between emotional brain function and later PTSD symptoms in an acutely traumatized sample. The findings supported the hypothesis that amygdala reactivity would positively predict later symptoms; individuals with a greater amygdala response to fearful faces had greater initial symptom severity and greater severity months post-trauma. This pattern was not related to the amygdala's habituation to the fearful stimuli, which showed no relationship with current or later PTSD symptoms. In addition, greater vACC habituation to fearful stimuli positively predicted symptom change from 1 to 12 months. Individuals with greater vACC habituation showed a poorer recovery trajectory (flatter slope of recovery) over this time period.

The findings were consistent with previous studies in military samples. A pair of studies by Admon and colleagues found that greater amygdala reactivity before combat deployment predicted greater PTSD symptoms after deployment (14, 15). In addition, Van Wingen and colleagues found that amygdala reactivity increased from pre- to post-deployment, and this was interpreted as an effect of combat stress (13). Extending these findings, we found that those individuals with the highest amygdala reactivity following trauma exposure had the highest overall PTSD symptom levels and were most likely to maintain PTSD symptoms as long as 12 months later. It is possible that stress related to combat or other forms of trauma may increase amygdala reactivity to threatening stimuli, in turn increasing risk for high levels of PTSD symptoms following trauma exposure. An interesting question for future research will be to investigate risk factors prior to or during trauma exposure that explain these important individual differences in amygdala reactivity observed shortly after trauma exposure. This is likely multiply determined by risk factors previously shown to be associated with greater amygdala reactivity and PTSD risk, such as childhood maltreatment (38–40), genomic risk pathways (41–43), and their interaction (44).

Few previous studies have examined patterns of neural habituation as potential contributors to PTSD symptoms. However, theories that posit heightened and inflexible emotional and physiological arousal as a primary contributor to PTSD (45–48) might predict abnormalities in the habituation response to emotionally evocative or threatening stimuli. One previous study showed abnormal patterns of habituation in the amygdala response to trauma-related word stimuli among individuals with PTSD relative to healthy controls (20), but did not examine other brain regions. Here, however, we did not find any relationship between amygdala habituation and current or later PTSD symptoms. Instead, we observed that habituation in vACC, specifically BA 25, positively predicted the slope of PTSD symptom

trajectories from 1 to 12 months post-trauma. This indicated that individuals whose vACC response to the fearful stimuli decreased more sharply over the course of the scan showed a slower course of recovery over the year following trauma. There was no association between symptoms and vACC habituation to neutral stimuli, suggesting that the effect was specific to threat stimuli. This was not a finding that we hypothesized, but is interesting given that PTSD is associated with difficulties in regulating arousal (49, 50), and impairments in fear extinction (6, 51), processes that are both mediated by neurons within the vACC (infralimbic cortex in rodents) and their connections with the amygdala (3, 35, 36, 52). Although the current findings regarding ACC habituation are exploratory in nature, it is possible that faster habituation of ACC responses to fearful stimuli may reflect an inability to maintain top-down regulatory control of emotional responses to fearful stimuli, which is predictive of poor recovery trajectories. Additional neuroimaging research specifically probing individual differences in emotion regulation would be helpful in informing the role of vACC function in predicting PTSD recovery outcomes.

The primary limitation of the current study is that it did not capture brain function before trauma onset. However, given the practical difficulty of scanning individuals in the general population both prior to and after trauma, the timepoint of 6–9 weeks following trauma is a reasonable alternative because initial reactions to the traumatic event have subsided, major injuries have healed enough for most individuals to participate in an MRI scan, and the timepoint is before the diagnosis of chronic PTSD at 3 months post-trauma. In addition, this study was conducted in a small pilot sample, and PTSD symptoms were assessed using self-report measures. Additional replication in larger samples with additional varieties of traumas, and replication with clinician-administered interview measures of PTSD, is needed.

To summarize, amygdala hyper-reactivity and vACC habituation to threat predicted later PTSD symptoms in the aftermath of an index trauma. These markers of neural function in the peri-traumatic period suggest that amygdala and ACC function are key targets for early interventions such as psychotherapy or pharmacological treatments administered in the acute aftermath of trauma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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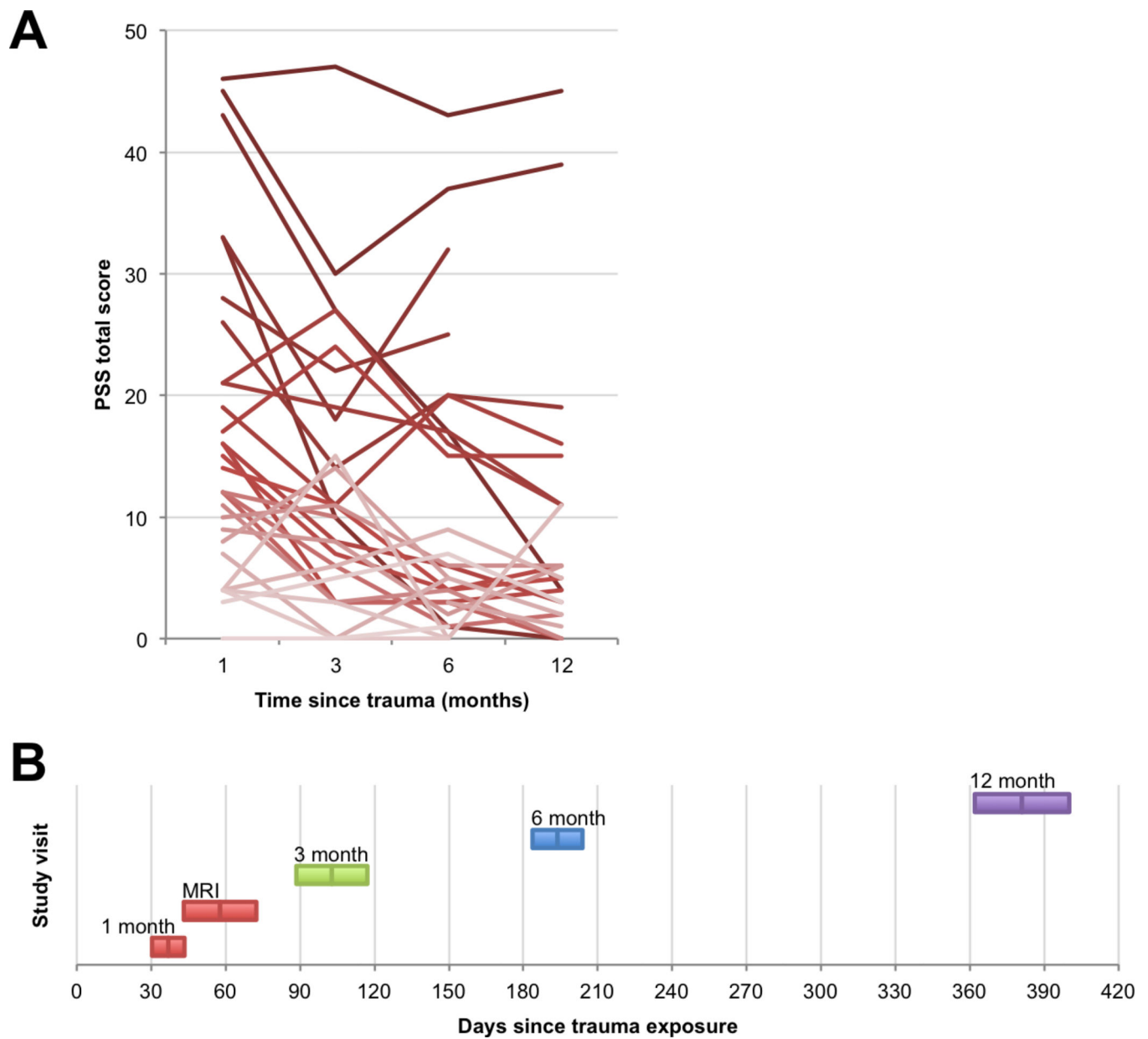


Figure 1. PTSD symptom trajectories. A: Line graph shows each participant's PTSD symptom severity scores (PSS total scores), across the four follow-up visits. Line shading (darker to lighter) indicates more severe to less severe symptoms at the 1-month visit. B: Timeline of study visits. Colored bars show $M \pm SD$ in the visit delay, relative to study enrollment in the Emergency Department.

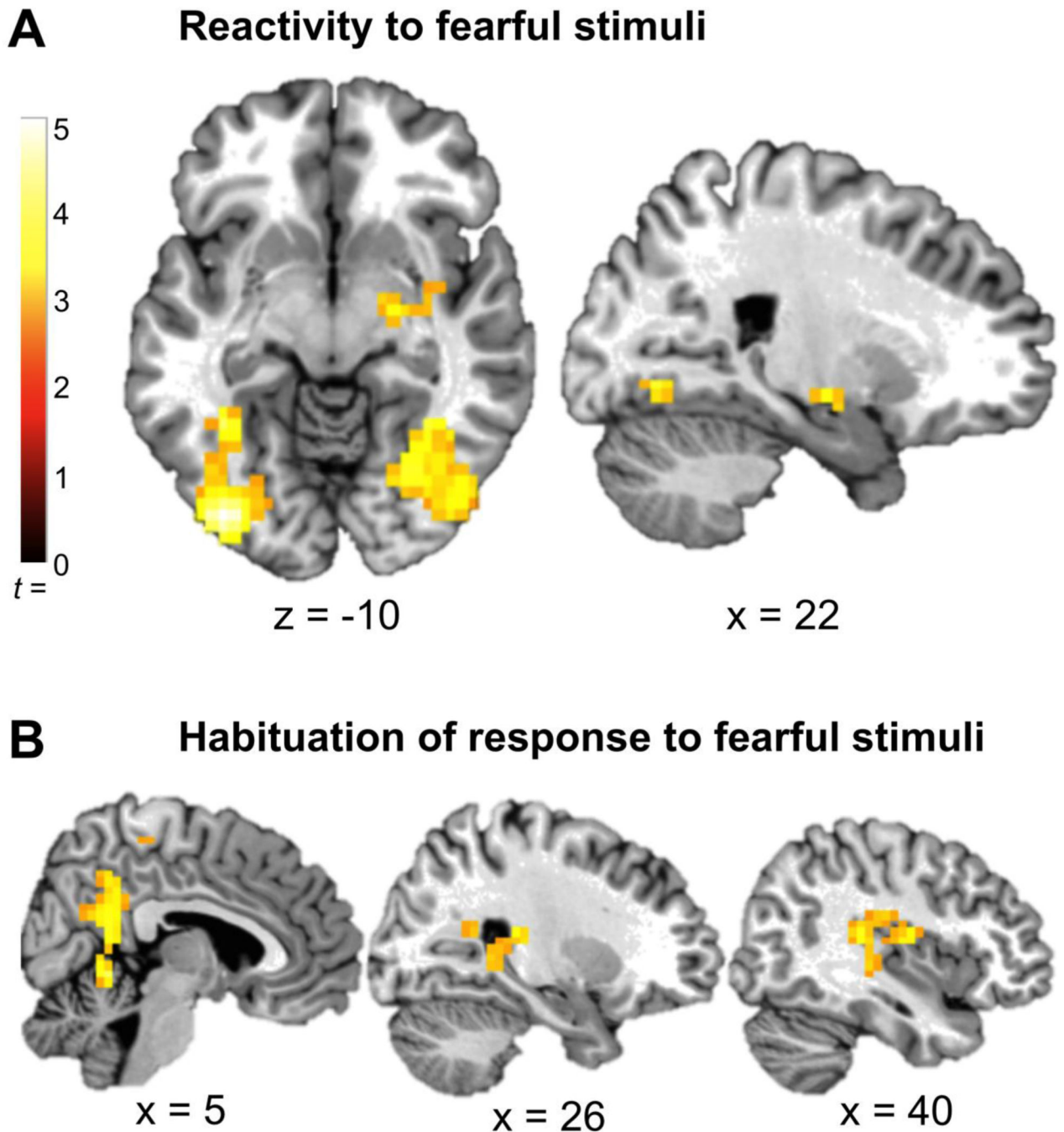


Figure 2. Task-related activation for the full sample. A: The right amygdala (46, 0, -30; $Z = 3.48$; $k = 124$) and bilateral occipital cortex (right: 38, -88, 2; $Z = 4.33$; $k = 345$; left: -38, -88, -18; $Z = 4.55$; $k = 218$) were significantly activated in the fearful>neutral contrast, $p < .05$, corrected. B: A cluster overlapping posterior cingulate, right posterior hippocampus, and right posterior insula (2, -56, -6; $Z = 3.84$; $k = 441$) showed a pattern of sensitization to the fearful stimuli (first 5 < last 5 fearful blocks), $p < .05$, corrected.

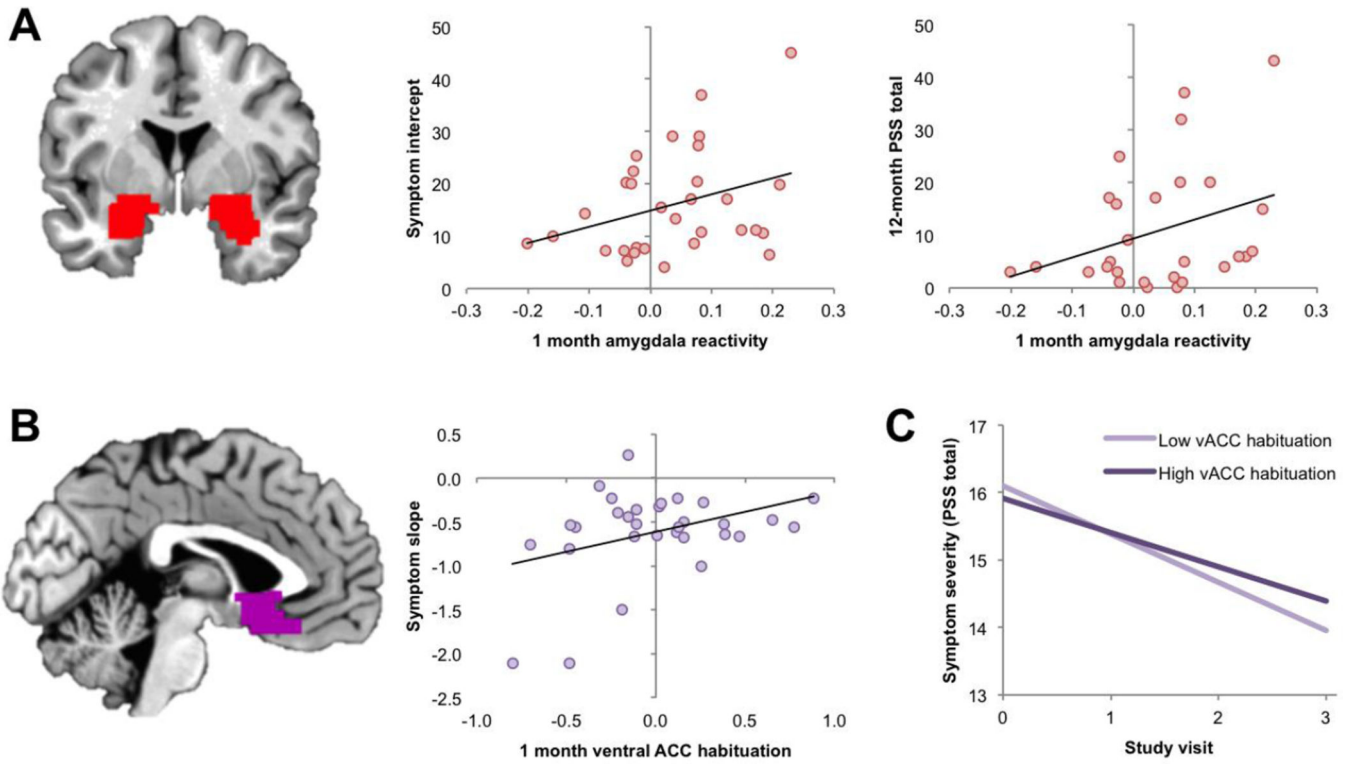


Figure 3.

Measures of fMRI reactivity and habituation that were predictive of later PTSD symptoms. A: Reactivity to fearful faces (fearful > neutral) in the amygdala ROI positively predicted PTSD symptom severity as reflected by the intercept parameter (middle panel) and total symptom severity 12 months post-trauma (right-most panel). Amygdala ROI is overlaid on a representative single-subject brain in MNI space. B: Habituation to fearful faces (first 5 > last 5 fearful blocks) in the ventral ACC ROI positively predicted PTSD symptom slope over the 12 months following trauma, such that more ACC habituation predicted flatter symptom trajectories. ACC ROI is overlaid on a representative single-subject brain in MNI space. C: Slope and intercept of PTSD symptoms over the 4 study visits (0 = 1 month post-trauma, 1 = 3 months, 2 = 6 months, 3 = 12 months), as a function of vACC habituation. For illustrative purposes, the sample was divided into low- and high-habituation groups based on a median split of vACC habituation. Graph shows that higher vACC habituation is associated with a flatter symptom slope over time.

Table 1

Sample characteristics

Characteristic	<i>M (SD) or %</i>
Age	31.9 (10.4)
Childhood trauma, CTQ	37.3 (13.0)
Gender, Female (%)	48%
Ethnicity, Hispanic (%)	10%
Race (%)	
Black	71 %
White	16%
Mixed	13%
Education (%)	
Master's degree	3%
Bachelor's degree	3%
Associate's, some college	55%
High School degree	29%
Some high school	10%
Household monthly income (%)	
\$0 – 249	10 %
\$250 – 499	10%
\$500 – 999	13%
\$1,000 – 1,999	20%
\$2,000 or more	47%
Similar prior trauma?	42%

Table 2

Group summary of symptoms at the post-trauma assessment visits [M(SD)]

	In ED (N=31)	1 month (N=31)	3 months (N=29)	6 months (N=29)	12 months (N=24)
PTSD symptoms (PSS or PDS)	4.7(8.2)	17.2(12.4)	12.9(10.8)	10.9(11.7)	9.8(11.6)
Re-experiencing	0.9(1.7)	4.7(4.2)	3.1(3.8)	2.6(3.4)	2.0(3.4)
Avoidance/Numbing	1.8(4.0)	6.2(5.5)	4.3(4.1)	3.7(5.0)	3.3(5.1)
Hyper-arousal	2.0(3.3)	6.3(4.0)	5.6(3.9)	4.6(4.2)	4.5(4.0)
Depression symptoms (BDI)	9.2(9.2)	13.5(10.4)	10.3(8.7)	9.5(10.2)	9.0(9.3)