Archival Report

Biological Psychiatry

Amygdala Reactivity and Anterior Cingulate Habituation Predict Posttraumatic Stress Disorder Symptom Maintenance After Acute Civilian Trauma

Jennifer S. Stevens, Ye Ji Kim, Isaac R. Galatzer-Levy, Renuka Reddy, Timothy D. Ely, Charles B. Nemeroff, Lauren A. Hudak, Tanja Jovanovic, Barbara O. Rothbaum, and Kerry J. Ressler

ABSTRACT

BACKGROUND: Studies suggest that exaggerated amygdala reactivity is a vulnerability factor for posttraumatic stress disorder (PTSD); however, our 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 after trauma. Growth curve modeling was used to estimate symptom recovery trajectories. Thirty-one individuals participated in functional magnetic resonance imaging 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 to 12 weeks after trauma was positively associated with the PTSD symptom intercept and predicted symptoms at 12 months after trauma. Habituation in the ventral anterior cingulate cortex was positively associated with the slope of PTSD symptoms, such that decreases in ventral anterior cingulate cortex activation over repeated presentations of fearful stimuli predicted increasing symptoms.

CONCLUSIONS: Findings point to neural signatures of risk for maintaining PTSD symptoms after trauma exposure. Specifically, chronic symptoms were predicted by amygdala hyperreactivity, and poor recovery was predicted by a failure to maintain ventral anterior cingulate cortex activation in response to fearful stimuli. The importance of identifying patients at risk after trauma exposure is discussed.

Keywords: Amygdala, Arousal, Fear, fMRI, Prospective, Trauma

http://dx.doi.org/10.1016/j.biopsych.2016.11.015

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

Findings from studies of chronic PTSD consistently show an association between symptoms and hyperreactivity 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 underactivity 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 hyperarousal symptoms in PTSD and to impairments in top-down emotion regulation and fear extinction (3,8,9,12). However, most 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 postcombat exposure (13,14),

SEE COMMENTARY ON PAGE e85

ISSN: 0006-3223

and that predeployment amygdala reactivity to emotionally arousing or risk-related stimuli positively predicted postdeployment PTSD symptoms (14,15). Perhaps because these studies recruited individuals from highly trained military samples, participants did not show significant levels of PTSD severity after 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 (ED) visit, and showed that default mode connectivity between the amygdala and posterior cingulate cortex 6 weeks after trauma was positively related to PTSD symptoms 12 weeks after 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 functional magnetic resonance imaging (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). In addition, 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 hyperreactivity 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 after acute trauma, measuring brain function using fMRI at an early timepoint before 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 ED within 24 hours of trauma and assessed for symptoms at 1, 3, 6, and 12 months after trauma. The fMRI scan took place within 2 to 3 weeks of the 1-month visit (timeline of visits shown in Figure 1B), with a mean (SD) of 57 (14) days after 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.

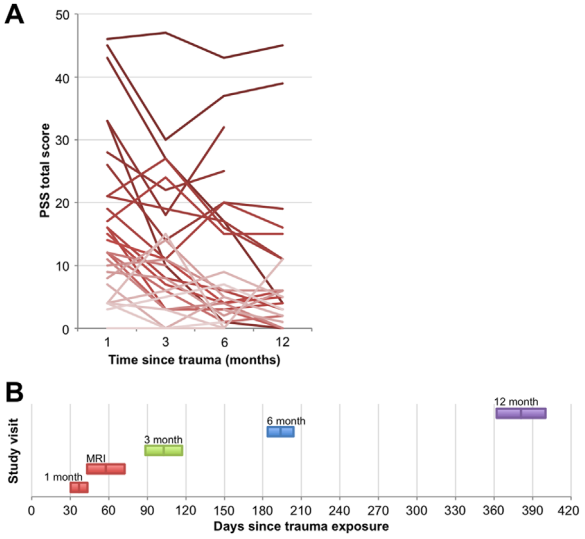

Figure 1. Posttraumatic stress disorder symptom trajectories. **(A)** Line graph shows each participant's posttraumatic stress disorder symptom severity scores (PTSD Symptom Scale total scores) across the four follow-up visits. Line shading (darker to lighter) indicates more severe to less severe symptoms at the 1-month visit. **(B)** Timeline of study visits. Colored bars show mean ± SD in the visit delay, relative to study enrollment in the emergency department. MRI, magnetic resonance imaging.

METHODS AND MATERIALS

Participants

Thirty-eight 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 ED of Grady Memorial Hospital in Atlanta, GA, who had experienced a traumatic event within the last 24 hours. Participants were included if they spoke English, were 18 to 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 fMRI data collection, seven participants were excluded from further analysis because of anatomical abnormalities, such as falx calcification ($n = 3$), head motion >3 mm ($n = 3$), and stimulus presentation malfunction ($n = 1$). Of the final sample of 31 individuals, 22 were in motor vehicle crashes, 4 were pedestrians who were hit by a vehicle, 3 were in motorcycle or bicycle accidents, and 2 were victims of sexual assault.

After the trauma, several participants sought therapy or mental health counseling (unrelated to the current study): 6 within the first month, 4 between 1 and 3 months, 2 between 3 and 6 months, and 2 between 6 and 12 months after trauma. Additional sample characteristics are summarized in Table 1. Supplemental Table S1 lists prescription medication use and comorbid diagnoses identified on a Mini-International Neuropsychiatric Schedule (22) administered during the ED 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.

ED Assessment at the Time of Trauma Exposure

Demographic information and information about the index trauma was gathered using the Standardized Trauma Interview, a 41-item clinician-administered interview gathering information

Table 1. Sample Characteristics

Characteristic	Mean (SD) or %
Age, Years	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 degree, some college	55
High school degree	29
Some high school	10
Household Monthly Income (%)	
$0–249	10
$250–499	10
$500–999	13
$1000–1999	20
≥$2000	47
Similar Previous Trauma? (%)	42

CTQ, Childhood Trauma Questionnaire.

on relevant aspects of the trauma at baseline and demographic information (23). To assess previous trauma history and baseline PTSD symptoms related to previous trauma, we administered the Posttraumatic Diagnostic Scale, a 49-item self-report measure (24). Depression symptoms in the 2 weeks before the ED visit were assessed using the Beck Depression Inventory, a 21-item self-report measure (25). Childhood trauma history was assessed using the Childhood Trauma Questionnaire, a 25-item instrument assessing physical, sexual, and emotional abuse, and physical and emotional neglect before 18 years of age, 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, 3, 6, and 12 months after the ED visit. PTSD symptom severity in response to the index trauma was measured using the PTSD Symptom Scale (27), a 17-item scale measuring symptom severity assessing DSM-IV-TR criteria for PTSD (21). The PTSD Symptom Scale items assess the same 17 symptoms assessed by the Posttraumatic Diagnostic Scale at the baseline ED visit for previous trauma, using a similar 0 to 3 scale for frequency. Depression symptoms were measured using the Beck Depression Inventory, a 21-item scale measuring symptom severity (28).

fMRI Study Procedure

Participants completed the fMRI session within 3 weeks (mean [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 eight 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 3.0T Magnetom Trio TIM MRI scanners (Siemens, Malvern, PA) using a 12-channel head coil. Twenty participants were scanned on the first scanner, and 11 on the second scanner. Functional images were acquired using the Z-SAGA pulse sequence (29) to minimize signal loss caused by susceptibility artifacts. Volumes contained 30 axially acquired 4-mm-thick images with an in-plane resolution of 3.44×3.44 mm^2 using a pulse repetition time = 3000 ms, echo time 1/2 = 30/67 ms, and flip angle = 90°. Structural images were acquired using a gradient-echo, T1-weighted pulse sequence (repetition time = 2300 ms; echo time = 2.78 ms; voxel size = $1.2 \times 1.3 \times 1.3$ mm).

Preprocessing and statistical analysis was conducted in SPM8 software (IBM Corp., Armonk, NY), and details can be found in Kilaru *et al.* (30). Briefly, spike and motion artifacts were corrected using ArtRepair software (available from the Center for Interdisciplinary Brain Sciences Research) (31). Images were corrected for slice timing, and spatial realignment was applied. Participants with head motion >3 mm over the entire session were excluded from further analyses. Images were normalized with unified segmentation, and smoothed with an 8-mm 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 (available at www.fmri.wfubmc.edu). A bilateral amygdala ROI was defined anatomically using the Automated Anatomical Labeling Toolbox (available at http://www.gin.cnrs.fr/spip-php-article217) (34). To anatomically define the dorsal and ventral aspects of the ACC, we selected primate analogs of rodent prelimbic and infralimbic cortex, because the rodent literature provides clear examples of functional differentiation among medial prefrontal cortical areas (35,36). The dACC (prelimbic) was defined using Brodmann area 32, and the vACC (infralimbic) was defined using Broadmann area 25 (37). To examine regions outside the ROIs, whole-brain analyses were conducted with SPM's cluster-based false discovery rate thresholding and an initial threshold of $p < .005$.

Data Analytic Strategy

A linear growth curve was estimated in the MPlus (version 7; available at http://www.statmodel.com/) environment using PTSD Symptom Scale scores from 1, 3, 6, and 12 months after ED admission. Two parameters were estimated, including the intercept (initial PTSD symptom severity) and slope (change over

Table 2. Group Summary of Symptoms at the Posttrauma Assessment Visits[a]

	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)
Hyperarousal	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)

BDI, Beck Depression Inventory; ED, emergency department; PDS, Posttraumatic Diagnostic Scale; PSS, PTSD Symptom Scale; PTSD, posttraumatic stress disorder.

[a]All values shown as mean (SD).

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 after trauma. The growth curve estimates demonstrated an initial intercept that was significantly > 0 (estimate [SE] = 14.92 [0.67]; p < .001) and a significant negative slope (–0.53 [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 after trauma. However, it is notable that mean symptoms were still moderate at 12 months (mean = 9.8).

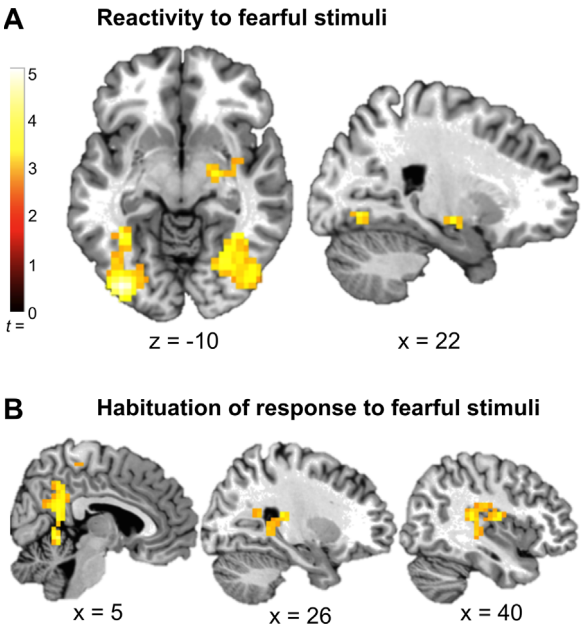

Figure 2. Task-related activation for the full sample. **(A)** The right amygdala (46, 0, –30; Z = 3.48; k = 124) and bilateral occipital cortex (right: 38, –88, 2; Z = 4.33; k = 345; left: = –38, –88, –18; Z = 4.55; k = 218) were significantly activated in the fearful > neutral contrast (p < .05, corrected). **(B)** A cluster overlapping the 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 five < last five fearful blocks) (p < .05, corrected).

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 five greater than the last five 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 the 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 (estimate [SE] = 3.44 [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 28 participants without PTSD related to previous trauma (3.43 [1.50]; p < .05). Reactivity in the dACC and vACC ROIs were not associated with the intercept, and neither was habituation of responses to fearful or neutral stimuli in any of the ROIs, p values > .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; p values > .05). Habituation to fearful stimuli in the vACC ROI demonstrated a positive relationship with the slope parameter (estimate [SE] = 0.94 [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 28 participants without PTSD related to

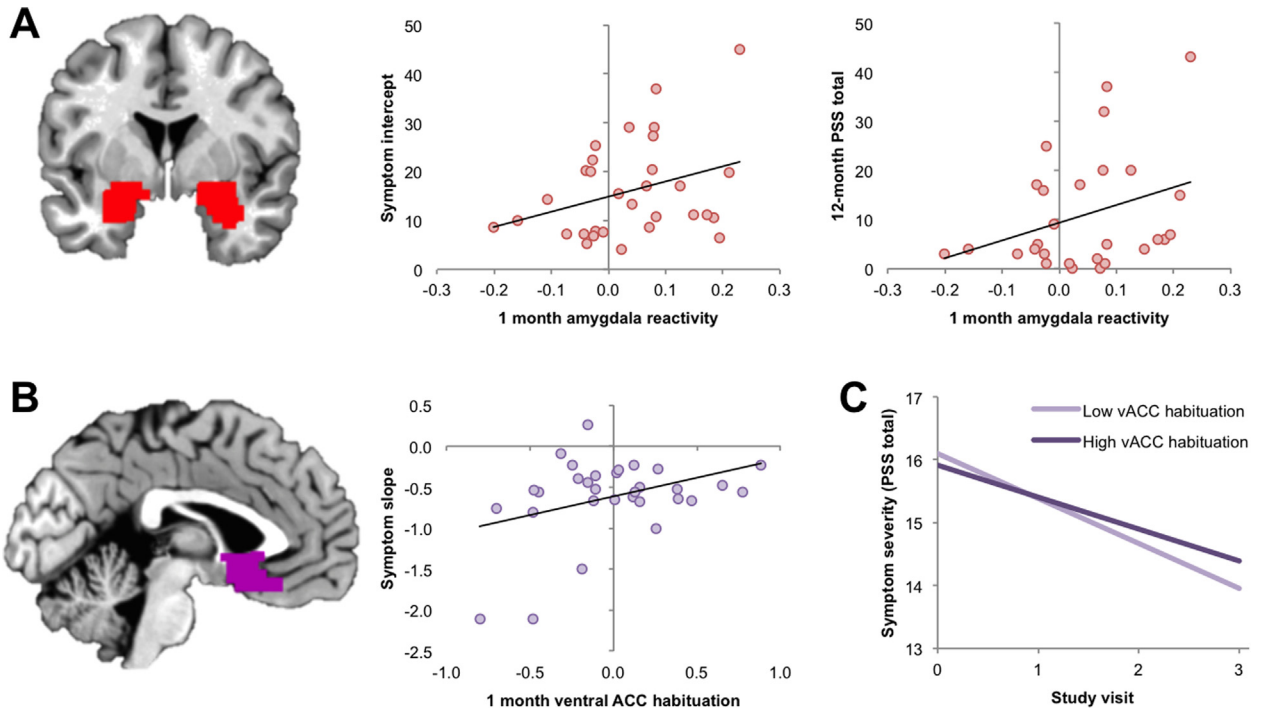

Figure 3. Measures of functional magnetic resonance imaging reactivity and habituation that were predictive of later posttraumatic stress disorder (PTSD) symptoms. **(A)** Reactivity to fearful faces (fearful > neutral) in the amygdala region of interest (ROI) positively predicted PTSD symptom severity as reflected by the intercept parameter (middle panel) and total symptom severity 12 months after trauma (right panel). Amygdala ROI is overlaid on a representative single-subject brain in Montreal Neurological Institute space. **(B)** Habituation to fearful faces (first five > last five fearful blocks) in the ventral aspects of the anterior cingulate cortex (vACC) ROI positively predicted PTSD symptom slope over the 12 months after trauma, such that more ACC habituation predicted flatter symptom trajectories. ACC ROI is overlaid on a representative single-subject brain in Montreal Neurological Institute space. **(C)** Slope and intercept of PTSD symptoms over the four study visits (0 = 1 month after trauma, 1 = 3 months, 2 = 6 months, and 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. The graph shows that higher vACC habituation is associated with a flatter symptom slope over time. PSS, PTSD Symptom Scale.

previous trauma (–2.51 [0.32]; $p < .001$). Habituation to fearful stimuli in the amygdala and dACC ROIs was not associated with slope (p values $> .10$), nor was habituation to neutral stimuli in any of the ROIs (p values $> .30$). 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 PTSD Symptom Scale severity scores (estimate [SE] = –0.50 [0.25]; $p \leq .05$), while vACC and dACC reactivity were not (vACC = 0.11 [0.23], $p = .63$; dACC = –0.23 [0.30], $p = .45$). Habituation of the amygdala, vACC, and dACC were not associated with 12-month severity scores (2.00 [1.26], $p = .11$; 1.49 [0.97], $p = .13$; and –0.24 [1.33], $p = .07$, respectively).

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 12 months after 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 *et al.* (14,15) found that greater amygdala reactivity before combat deployment predicted greater PTSD symptoms after deployment. In addition, Van Wingen *et al.* (13) found that amygdala reactivity increased from pre- to postdeployment, and this was interpreted as an effect of combat stress. Extending these findings, we found that those individuals with the highest amygdala reactivity after trauma exposure had the highest overall PTSD symptom levels and were most likely to maintain PTSD symptoms as many 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 after trauma exposure. An interesting question for future research will be to

investigate risk factors before 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 (30,41,42), and their interaction (43).

Few 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 (44–47) 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 Broadmann area 25, positively predicted the slope of PTSD symptom trajectories from 1 to 12 months after 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 after 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 (48,49) and impairments in fear extinction (6,50), processes that are mediated by both neurons within the vACC (infralimbic cortex in rodents) and their connections with the amygdala (3,35,36,51). 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-town 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 before and after trauma, the timepoint of 6 to 9 weeks after 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 a fMRI scan, and the timepoint is before the diagnosis of chronic PTSD at 3 months after 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 hyperreactivity and vACC habituation to threat predicted later PTSD symptoms in the aftermath of an index trauma. These markers of neural function in the peritraumatic 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.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by National Institute of Mental Health Grant Nos. R01 MH094757 (to KJR, BOR, CBN), R21 MH106902 (to TJ), F32 MH101976 (to JSS), K01 MH102415 (to IRG-L), and U01 MH110925 (to KJR, TJ).

We thank Debra Houry, M.D., and Abigail Hankin-Wei, M.D., for their generous collaborative efforts on this study. We also thank Vasiliki Michopoulous, Alex O. Rothbaum, Thomas Crow, Heather Grinstead, Rebecca C. Roffman, Jessica Maples, Lydia Odenat, Loren M. Post, Liza C. Zwiebach, Devika Fiorillo, Kathryn Breazeale, Jessica Morgan, Natasha Mehta, Elicia D. Skelton, Taleesha S. Booker, and Jonathan Zebrowski for their tireless work in the emergency department recruiting and assessing participants.

The authors report no biomedical financial interests or potential conflicts of interest.

ARTICLE INFORMATION

From the Department of Psychiatry and Behavioral Sciences (JSS, YJK, RR, TDE, TJ, BOR, KJR), and the Department of Emergency Medicine (LAH), Emory University School of Medicine, Atlanta, Georgia; Department of Psychiatry (IRG-L), New York University School of Medicine, New York, New York; Department of Psychiatry and Behavioral Sciences (CBN), University of Miami Leonard M. Miller School of Medicine, Miami, Florida; Department of Psychiatry (KJR), Harvard Medical School, Cambridge, Massachusetts; and the McLean Hospital (KJR), Belmont, Massachusetts.

Address correspondence to Jennifer S. Stevens, Ph.D., Emory University School of Medicine, Psychiatry and Behavioral Sciences, 954 Gatewood Drive, Suite 320, Atlanta, GA 30329; E-mail: jennifer.stevens@emory.edu.

Received May 14, 2016; revised Nov 16, 2016; accepted Nov 16, 2016.

Supplemental material cited in this article is available online at http://dx.doi.org/10.1016/j.biopsych.2016.11.015.

REFERENCES

1. Kessler RC, Sonnega A, Bromet E, Hughes M, Nelson CB (1995): Posttraumatic stress disorder in the National Comorbidity Survey. Arch Gen Psychiatry 52:1048–1060.
2. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE (2005): Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry 62:593–602.
3. Stevens JS, Jovanovic T, Fani N, Ely TD, Glover EM, Bradley B, *et al.* (2013): Disrupted amygdala-prefrontal functional connectivity in civilian women with posttraumatic stress disorder. J Psychiatr Res 47: 1469–1478.
4. Fonzo GA, Simmons AN, Thorp SR, Norman SB, Paulus MP, Stein MB (2010): Exaggerated and disconnected insular-amygdalar blood oxygenation level-dependent response to threat-related emotional faces in women with intimate-partner violence posttraumatic stress disorder. Biol Psychiatry 68:433–441.
5. Rauch SL, Whalen PJ, Shin LM, McInerney SC, Macklin ML, Lasko NB, *et al.* (2000): Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: A functional MRI study. Biol Psychiatry 47:769–776.
6. Milad MR, Pitman RK, Ellis CB, Gold AL, Shin LM, Lasko NB, *et al.* (2009): Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. Biol Psychiatry 66:1075–1082.
7. Ramage AE, Laird AR, Eickhoff SB, Acheson A, Peterson AL, Williamson DE, *et al.* (2013): A coordinate-based meta-analytic model of trauma processing in posttraumatic stress disorder. Hum Brain Mapp 34:3392–3399.
8. Etkin A, Wager TD (2007): Functional neuroimaging of anxiety: A meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. Am J Psychiatry 164:1476–1488.
9. Jovanovic T, Ely T, Fani N, Glover EM, Gutman D, Tone EB, *et al.* (2013): Reduced neural activation during an inhibition task is associated with impaired fear inhibition in a traumatized civilian sample. Cortex 49:1884–1891.

10. Shin LM, Wright CI, Cannistraro PA, Wedig MM, McMullin K, Martis B, *et al*. (2005): A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. Arch Gen Psychiatry 62: 273–281.
11. Sripada RK, King AP, Garfinkel SN, Wang X, Sripada CS, Welsh RC, *et al*. (2012): Altered resting-state amygdala functional connectivity in men with posttraumatic stress disorder. J Psychiatr Neurosci 37: 241–249.
12. Admon R, Milad MR, Hendler T (2013): A causal model of post-traumatic stress disorder: disentangling predisposed from acquired neural abnormalities. Trends Cogn Sci 17:337–347.
13. van Wingen GA, Geuze E, Vermetten E, Fernandez G (2011): Perceived threat predicts the neural sequelae of combat stress. Mol Psychiatry 16:664–671.
14. Admon R, Lubin G, Stern O, Rosenberg K, Sela L, Ben-Ami H, *et al*. (2009): Human vulnerability to stress depends on amygdala's predisposition and hippocampal plasticity. Proc Natl Acad Sci U S A 106: 14120–14125.
15. Admon R, Lubin G, Rosenblatt JD, Stern O, Kahn I, Assaf M, *et al*. (2013): Imbalanced neural responsivity to risk and reward indicates stress vulnerability in humans. Cereb Cortex 23:28–35.
16. Lanius RA, Bluhm RL, Coupland NJ, Hegadoren KM, Rowe B, Theberge J, *et al*. (2010): Default mode network connectivity as a predictor of post-traumatic stress disorder symptom severity in acutely traumatized subjects. Acta Psychiat Scand 121:33–40.
17. Gee DG, McEwen SC, Forsyth JK, Haut KM, Bearden CE, Addington J, *et al*. (2015): Reliability of an fMRI paradigm for emotional processing in a multisite longitudinal study. Hum Brain Mapp 36: 2558–2579.
18. Plichta MM, Grimm O, Morgen K, Mier D, Sauer C, Haddad L, *et al*. (2014): Amygdala habituation: A reliable fMRI phenotype. Neuroimage 103:383–390.
19. Johnstone T, Somerville LH, Alexander AL, Oakes TR, Davidson RJ, Kalin NH, *et al*. (2005): Stability of amygdala BOLD response to fearful faces over multiple scan sessions. Neuroimage 25:1112–1123.
20. Protopopescu X, Pan H, Tuescher O, Cloitre M, Goldstein M, Engelien W, *et al*. (2005): Differential time courses and specificity of amygdala activity in posttraumatic stress disorder subjects and normal control subjects. Biol Psychiatry 57:464–473.
21. American Psychiatric Association (2000): Diagnostic and Statistical Manual-Text Revision (DSM-IV-TRim). Washington, DC: American Psychiatric Association.
22. Pinninti NR, Madison H, Musser E, Rissmiller D (2003): MINI International Neuropsychiatric Schedule: Clinical utility and patient acceptance. Eur Psychiatry 18:361–364.
23. Foa EB, Rothbaum BO (2001): Treating the trauma of rape: Cognitive-behavioral therapy for PTSD. New York: Guilford Press.
24. Foa EB, Cashman L, Jaycox L, Perry K (1997): The validation of a self-report measure of posttraumatic stress disorder: The Posttraumatic Diagnostic Scale. Psychol Assess 9:445–451.
25. Beck AT, Steer RA, Brown GK (1996): Beck Depression Inventory, 2nd ed. San Antonio, TX: The Psychological Corporation.
26. Bernstein DP, Fink L, Handelsman L, Foote J, Lovejoy M, Wenzel K, *et al*. (1994): Initial reliability and validity of a new retrospective measure of child abuse and neglect. Am J Psychiatry 151:1132–1136.
27. Foa EB, Tolin DF (2000): Comparison of the PTSD Symptom Scale-Interview Version and the Clinician-Administered PTSD scale. J Trauma Stress 13:181–191.
28. Beck AT, Steer RA, Carbin MG (1988): Psychometric properties of the Beck Depression Inventory: Twenty-five years of evaluation. Clin Psychol Rev 8:77–100.
29. Heberlein KA, Hu X (2004): Simultaneous acquisition of gradient-echo and asymmetric spin-echo for single-shot z-shim: Z-SAGA. Magn Reson Med 51:212–216.
30. Kilaru V, Iyer S, Almli L, Stevens J, Lori A, Jovanovic T, *et al*. (2016): Genome-wide gene-based analysis suggests an association between Neuroligin 1 (NLGN1) and post-traumatic stress disorder. Transl Psychiatry 6:e820.
31. Mazaika P, Whitfield-Gabrieli S, Reiss A, (2007): Artifact repair for fMRI data from high motion clinical subjects. Presented at the Human Brain Mapping Conference, June 10–14, Chicago, Illinois.
32. Breiter HC, Etcoff NL, Whalen PJ, Kennedy WA, Rauch SL, Buckner RL, *et al*. (1996): Response and habituation of the human amygdala during visual processing of facial expression. Neuron 17:875–887.
33. Blackford JU, Allen AH, Cowan RL, Avery SN (2012): Amygdala and hippocampus fail to habituate to faces in individuals with an inhibited temperament. Soc Cogn Affect Neurosci 8:143–150.
34. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, *et al*. (2002): Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage 15:273–289.
35. Milad MR, Quirk GJ (2002): Neurons in medial prefrontal cortex signal memory for fear extinction. Nature 420:70–74.
36. Sierra-Mercado D, Padilla-Coreano N, Quirk GJ (2011): Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. Neuropsychopharmacol 36:529–538.
37. Heilbronner SR, Rodriguez-Romaguera J, Quirk GJ, Groenewegen HJ, Haber SN (2016): Circuit-based corticostriatal homologies between rat and primate. Biol Psychiatry 80:509–521.
38. Dannlowski U, Stuhrmann A, Beutelmann V, Zwanzger P, Lenzen T, Grotegerd D, *et al*. (2012): Limbic scars: Long-term consequences of childhood maltreatment revealed by functional and structural magnetic resonance imaging. Biol Psychiatry 71:286–293.
39. Grant MM, Cannistraci C, Hollon SD, Gore J, Shelton R (2011): Childhood trauma history differentiates amygdala response to sad faces within MDD. J Psychiatr Res 45:886–895.
40. Tottenham N, Hare TA, Millner A, Gilhooly T, Zevin JD, Casey BJ (2011): Elevated amygdala response to faces following early deprivation. Dev Sci 14:190–204.
41. Stevens JS, Almli LM, Fani N, Gutman DA, Bradley B, Norrholm SD, *et al*. (2014): PACAP receptor gene polymorphism impacts fear responses in the amygdala and hippocampus. Proc Natl Acad Sci U S A 111:3158–3163.
42. Hariri AR, Mattay VS, Tessitore A, Kolachana B, Fera F, Goldman D, *et al*. (2002): Serotonin transporter genetic variation and the response of the human amygdala. Science 297:400–403.
43. White MG, Bogdan R, Fisher PM, Munoz KE, Williamson DE, Hariri AR (2012): FKBP5 and emotional neglect interact to predict individual differences in amygdala reactivity. Genes Brain Behav 11:869–878.
44. Shin LM, Rauch SL, Pitman RK (2006): Amygdala, medial prefrontal cortex, and hippocampal function in PTSD. Ann N Y Acad Sci 1071:67–79.
45. Norrholm SD, Glover EM, Stevens JS, Fani N, Galatzer-Levy IR, Bradley B, *et al*. (2014): Fear load: The psychophysiological over-expression of fear as an intermediate phenotype associated with trauma reactions. Int J Psychophysiol 98(2 pt 2):270–275.
46. Nemeroff CB, Bremner JD, Foa EB, Mayberg HS, North CS, Stein MB (2006): Posttraumatic stress disorder: A state-of-the-science review. J Psychiatr Res 40:1–21.
47. Keane TM, Kolb LC, Kaloupek DG, Orr SP, Blanchard EB, Thomas RG, *et al*. (1998): Utility of psychophysiology measurement in the diagnosis of posttraumatic stress disorder: Results from a Department of Veteran's Affairs cooperative study. J Consult Clin Psychol 66:914–923.
48. American Psychiatric Association (2013): Diagnostic and Statistical Manual of Mental Disorders, 5th edition Washington, DC: American Psychiatric Association.
49. Jovanovic T, Norrholm SD, Blanding NQ, Davis M, Duncan E, Bradley B, *et al*. (2010): Impaired fear inhibition is a biomarker of PTSD but not depression. Depress Anxiety 27:244–251.
50. Norrholm SD, Jovanovic T, Olin IW, Sands LA, Karapanou I, Bradley B, *et al*. (2011): Fear extinction in traumatized civilians with post-traumatic stress disorder: Relation to symptom severity. Biol Psychiatry 69:556–563.
51. Fani N, King TZ, Brewster R, Srivastava A, Stevens JS, Glover EM, *et al*. (2015): Fear-potentiated startle during extinction is associated with white matter microstructure and functional connectivity. Cortex 64:249–259.