Title

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Journal

Neuropsychopharmacology, 39(11)

ISSN

0893-133X

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Publication Date

2014-10-01

DOI

10.1038/npp.2014.110

Copyright Information

Peer reviewed

Neuropsychopharmacology (2014) 39, 2519–2528
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Cannabinoid Type 1 Receptor Availability in the Amygdala Mediates Threat Processing in Trauma Survivors

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Attentional bias to threat is a key endophenotype that contributes to the chronicity of trauma-related psychopathology. However, little is known about the neurobiology of this endophenotype and no known *in vivo* molecular imaging study has been conducted to evaluate candidate receptor systems that may be implicated in this endophenotype or the phenotypic expression of trauma-related psychopathology that comprises threat (ie, re-experiencing, avoidance, and hyperarousal) and loss (ie, emotional numbing, depression/dysphoria, generalized anxiety) symptomatology. Using the radioligand [^{11}C]OMAR and positron emission tomography (PET), we evaluated the relationship between *in vivo* cannabinoid receptor type 1 (CB_1) receptor availability in the amygdala, and performance on a dot-probe measure of attentional bias to threat, and clinician interview-based measures of trauma-related psychopathology. The sample comprised adults presenting with a broad spectrum of trauma-related psychopathology, ranging from nontrauma-exposed, psychiatrically healthy adults to trauma-exposed adults with severe trauma-related psychopathology. Results revealed that increased CB_1 receptor availability in the amygdala was associated with increased attentional bias to threat, as well as increased severity of threat, but not loss, symptomatology; greater peripheral anandamide levels were associated with decreased attentional bias to threat. A mediation analysis further suggested that attentional bias to threat mediated the relationship between CB_1 receptor availability in the amygdala and severity of threat symptomatology. These data substantiate a key role for compromised endocannabinoid function in mediating both the endophenotypic and phenotypic expression of threat symptomatology in humans. They further suggest that novel pharmacotherapies that target the CB_1 system may provide a more focused, mechanism-based approach to mitigating this core aspect of trauma-related psychopathology.

Neuropsychopharmacology (2014) **39,** 2519–2528; doi:10.1038/npp.2014.110; published online 11 June 2014

INTRODUCTION

Studies of how neurobiological systems are linked to the transdiagnostic endophenotypic and phenotypic expression of psychopathology (Cuthbert, 2014) are particularly relevant to trauma-related psychopathology, as three of the most common trauma-related disorders—posttraumatic stress disorder (PTSD), major depressive disorder (MDD), and generalized anxiety disorder (GAD)—are highly comorbid and share common transdiagnostic dimensions of threat and loss (ie, dysphoria) symptomatology (Forbes *et al*, 2011; Forbes *et al*, 2010; Grant *et al*, 2008; Zoellner *et al*, 2014). Trauma-related threat symptomatology includes intrusive thoughts and memories, and hyperarousal symptoms such as sleep disturbance and hypervigilance, whereas trauma-related loss (ie, dysphoria) symptomatology includes emotional numbing and depressive/dysphoric and generalized anxiety symptoms. Elucidation of neurobiological systems implicated in trauma-related endophenotypes can inform etiologic models of trauma-related psychopathology, as well as the development of more targeted, mechanism-based prevention and treatment strategies.

Attentional bias to threat is one of the core endophenotypic characteristics of trauma-related psychopathology (Fani *et al*, 2012b). Attentional biases to threatening information, such as faces and words, which are often assessed using a dot-probe paradigm, have been found to contribute to and maintain the persistence of trauma-related threat symptomatology, even months to years after trauma exposure (Fani *et al*, 2012b; Lindstrom *et al*, 2011; Sveen *et al*, 2009). Greater attentional bias to threat is also associated with exaggerated fear expression and impaired extinction in individuals with PTSD (Fani *et al*, 2012b).

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Received 19 March 2014; revised 5 May 2014; accepted 6 May 2014; accepted article preview online 13 May 2014

Hyperarousal symptoms, such as exaggerated startle response during fear learning, in particular, have been found to contribute to attentional bias to threat in symptomatic trauma survivors (Fani *et al*, 2012b). Recent functional neuroimaging work has implicated increased amygdala activation in relation to attentional bias to threat among individuals with PTSD (Fani *et al*, 2012a), suggesting that the amygdala modulates the orientation of attention toward and processing of threatening information in this population.

Although cannabinoid type 1 (CB_1) receptors are widely distributed in the human brain (Glass *et al*, 1997a; Herkenham, 1991), they are found in particularly high concentrations in the amygdala, and have been associated with the processing and storage of threat-related memories, as well as the coordination of threat-related behaviors (LeDoux, 2000). Recently, we reported *in vivo* evidence of abnormal CB_1 receptor-mediated endocannabinoid signaling in individuals with PTSD (Neumeister *et al*, 2013) and suggested that increased CB_1 receptor availability may be a molecular adaptation to reduced endocannabinoid availability. In addition to this work, a large body of preclinical studies has found strong support for a major role of the endocannabinoid anandamide and CB_1 receptor signaling in the amygdala in modulating stress-induced threat behaviors (for review, see Gunduz-Cinar *et al*, 2013a). Understanding how key neuroreceptor systems such as CB_1 relate to intermediate endophenotypic (ie, attentional bias to threat) and phenotypic expression of trauma-related psychopathology (ie, threat symptomatology) may thus provide insight into molecular targets that could inform the development of mechanism-based treatment approaches. To date, however, human data evaluating this possibility are lacking.

In the current study, we aimed to address this gap in the literature by using the CB_1 receptor antagonist radiotracer [^{11}C]OMAR, which measures volume of distribution (V_T) linearly related to CB_1 receptor availability, to evaluate the relationship between CB_1 receptor availability in the amygdala, and objectively assessed attentional bias to threat, and the transdiagnostic and dimensional expression of trauma-related threat and loss symptomatology. To obtain a sample that encompassed the full-dimensional range of study measures (Cuthbert, 2014), we employed an inclusive sampling approach by recruiting a sample of individuals who represented a broad transdiagnostic and dimensional spectrum of trauma-related psychopathology, ranging from healthy, nontrauma-exposed individuals to trauma-exposed individuals with severe trauma-related psychopathology. On the basis of prior work linking CB_1 receptor availability in the amygdala to threat processing (Gunduz-Cinar *et al*, 2013a; LeDoux, 2000; Rodrigues *et al*, 2004; Rogan *et al*, 1997) and threat symptomatology (ie, hyperarousal) to attentional bias to threat (Fani *et al*, 2012b), we hypothesized that greater CB_1 receptor availability in the amygdala would be associated with greater attentional bias to threat, as well as increased severity of threat symptomatology, particularly hyperarousal. We then evaluated a mediational model to examine whether attentional bias to threat mediated the relationship between CB_1 receptor availability in the amygdala and trauma-related psychopathology.

MATERIALS AND METHODS

Participants

A total of 20 participants were recruited from the Molecular Imaging Program for Mood and Anxiety Disorders at NYU Langone Medical Center. Trauma-exposed participants were referred from NYU-affiliated outpatient psychiatry clinics ($n = 16$) and psychiatrically healthy, nontrauma-exposed participants ($n = 4$) were recruited from the community using public advertisements. Scores on clinician-administered measures of threat and loss symptomatology (see *Assessments* below) in the sample represented a broad transdiagnostic and dimensional spectrum of trauma-related psychopathology (see Table 1). This sample is thus representative of the broader population of individuals in the community (ie, unaffected individuals), as well as those who present for treatment at an outpatient mood and anxiety disorders clinic (ie, mild-to-severe symptomatology).

Table 1 Demographic, Trauma, and Clinical Characteristics of Sample ($n = 20$)

Demographic characteristics	Mean (SD) or *n* (%)	Range
Age	33.3 (8.8)	21–50
Female sex (%)	11 (55.0%)	
Race/ethnicity		
Caucasian	10 (50.0%)	
Hispanic	7 (35.0%)	
African-American	2 (10.0%)	
Mixed	1 (5.0%)	
Years of education	15.4 (2.3)	12–20
Trauma characteristics[a]		
Age of first trauma	13.4 (7.5)	3–28
Number of lifetime traumas	3.8 (4.7)	1–20
Index trauma		
Sexual assault	8 (50.0%)	
Witnessed death	3 (18.8%)	
Physical assault	3 (18.8%)	
Motor vehicle accident	2 (12.4%)	
Clinical characteristics		
CAPS total score	46.7 (37.5)	0–110
HAM-A total score	12.2 (9.9)	0–34
HAM-D total score	10.2 (8.3)	0–29
Categorical classification		
Nontrauma exposed and healthy	4 (20.0%)	
Trauma-exposed and healthy	4 (20.0%)	
Trauma-exposed with PTSD	12 (60.0%)	
Attentional bias to threat (ms)	9.4 (15.0)	– 19 to 41

[a] Assessed only among trauma-exposed individuals ($n = 16$).

The New York University Institutional Review Board, Yale University School of Medicine Human Investigation Committee, Yale University Magnetic Resonance Research Center, and Yale–New Haven Hospital Radiation Safety Committee approved this study. All participants provided written informed consent.

Assessments

Lifetime traumatic events were assessed using the Traumatic Life Events Questionnaire (TLEQ) and psychiatric diagnoses were established using DSM-IV-TR criteria and the Structured Clinical Interview for DSM-IV (SCID) that was administered by an experienced master- or doctoral-level psychiatric clinician. Only traumatic events that met criteria A1 and A2 for a DSM-IV-TR-based diagnosis of PTSD were counted toward participants' trauma histories in this study. Nontrauma-exposed healthy adults did not report any trauma exposures on the TLEQ and did not have any lifetime psychiatric diagnosis, including substance abuse or dependence or nicotine dependence. Severity of trauma-related threat and loss symptomatology was assessed using the Clinician-Administered PTSD Scale for DSM-IV (CAPS); the Hamilton Rating Scale for Depression (HAM-D) to assess depressive symptoms; and the Hamilton Rating Scale for Anxiety (HAM-A) to assess nonspecific anxiety symptoms. Scores on these structured clinician-administered measures of trauma-related psychopathology represented a transdiagnostic and dimensional spectrum of trauma-related psychopathology, ranging from nontrauma-exposed asymptomatic adults to trauma-exposed adults with severe trauma-related psychopathology (see Table 1).

All participants were evaluated by physical examination, electrocardiogram, standard blood chemistry, hematology laboratory testing, toxicology testing, and urinalysis. All but two participants were psychotropic medication naive, and two took antidepressants for less than a week before the study but were medication free for at least 6 months before the study. Participants with significant medical or neurologic conditions, with substance abuse within 12 months of the scan, lifetime history of intravenous substance dependence, or with history of head injury that involved loss of consciousness were excluded from the study. Lifetime cannabis abuse/dependence was an exclusion criterion, and occasional cannabis users were eligible to participate but not if they had used cannabis within 12 months of the scan. The absence of substance use was determined by self-report and confirmed by the results of urine toxicology and breathalyzer tests at screening and on the days when magnetic resonance imaging (MRI) and positron emission tomography (PET) scans were conducted. The medical and psychiatric evaluation was followed by MRI and a resting-state PET scan on a High Resolution Research Tomograph (HRRT) PET scanner (Siemens/CTI, Knoxville, TN) with the CB_1-selective radioligand [^{11}C]OMAR (Horti *et al*, 2006). To obtain plasma anandamide levels, blood samples were collected at the time of tracer injection and processed immediately after collection in the laboratory that is adjacent to the scan room and frozen at $-80\,^{\circ}C$ until analyzed, as previously described (Neumeister *et al*, 2013).

PET and MRI Acquisition and Modeling

[^{11}C]OMAR was prepared in high specific activity (111 ± 63 MBq/nmol at end of synthesis). The radiotracer (injected dose: 492 ± 155 MBq, injected mass: 0.03 ± 0.03 μg/kg) was infused over 1 min through the antecubital vein. The radioactivity concentration in blood from the radial artery was measured continuously using an automated system (PBS101, Veenstra Instruments, Joure, The Netherlands) for the first 7 min after radiotracer administration and manually drawn and counted thereafter. Discrete samples were acquired at selected times and measured on a gamma counter (Wizard 1480, Perkin-Elmer, Waltham, MA) to determine radioactivity concentration in whole blood and plasma. Five discrete blood samples (5, 15, 30, 60 and 90 min) were analyzed for the fraction of unchanged [^{11}C]OMAR and its radiometabolites using a column-switching high-pressure liquid chromatography method (Hilton *et al*, 2000). The fraction of tracer unbound to plasma proteins was determined in triplicate by ultrafiltration. Listmode emission data were collected for 120 min after radiotracer administration using the HRRT (Siemens Medical Systems, Knoxville, TN), a dedicated brain PET scanner with spatial resolution better than 3 mm. Head motion was measured using the Polaris Vicra optical tracking system (Northern Digital, Waterloo, ON, Canada) and incorporated into PET image reconstruction with all corrections (Carson *et al*, 2003). The dynamic image sequences had 33 frames with the following number and duration: 6×30 s, 3×1 min, 2×2 min, and 22×5 min. The PET images were registered to subject-specific T1-weighted magnetic resonance images ($256 \times 256 \times 176$ grid of 1 mm isotropic voxels) acquired on a 3 Tesla Trio imaging system (Siemens Medical Systems, Erlangen, Germany). Anatomical MR images were in turn nonlinearly registered to an MR template where regions of interest (ROIs) were defined (Tzourio-Mazoyer *et al*, 2002). Regional time activity curves (TACs) were extracted from the dynamic PET data and analyzed using the multilinear analysis method (Ichise *et al*, 2002) with metabolite-corrected arterial input functions and cutoff time $t^* = 30$ min. The kinetic analysis yielded regional estimates of total volume of distribution (V_T), the equilibrium ratio of radioligand concentration in tissue relative to arterial plasma (Innis *et al*, 2007) that is directly proportional to CB_1 receptor availability. Additional details regarding PET and MRI acquisition and modeling are provided elsewhere (Neumeister *et al*, 2013). Although [^{11}C]OMAR V_T values in the amygdala were of primary interest in this study based on prior research (Gunduz-Cinar *et al*, 2013a; LeDoux, 2000; Rodrigues *et al*, 2004; Rogan *et al*, 1997); (Fani *et al*, 2012b), we also explored how [^{11}C]OMAR V_T values in other brain regions, including the caudate, putamen, pallidum, cerebellum, semiovale, hippocampus, hypothalamus, insula, anterior and posterior cingulate cortex, and occipital, parietal, temporal, and frontal cortices, were related to attentional bias to threat.

Dot-Probe Task

The dot-probe task (Bar-Haim *et al*, 2007; MacLeod *et al*, 1986) measures attentional biases toward or away from threatening stimuli. The dot-probe task was composed of 160 trials. Each trial started with a fixation cross ('+') presented in the center of the screen for 500 ms. When the fixation cross disappeared, two words in 12-pt Arial font immediately appeared in the center of the screen for 500 ms, one above and one below the location of the fixation cross, separated by 1.5 cm. Following the presentation of the words, a target probe (either a letter E or F) appeared in the location previously occupied by one of the words. The probe remained on the screen until participants responded, after which the next trial started. Participants were instructed to focus their attention on the fixation cross at the start of each trial, and when a probe appeared they were to identify the probe letter (E or F) using a designated mouse button, as quickly as possible. Given the heterogeneity of trauma histories in our sample, the stimuli used were 32 trauma-related and 64 neutral words that were selected from a larger list developed by MacLeod *et al* (2002). Word pairs were chosen for salience to the experience of traumatic life events (eg, 'harm', 'suffer'). Word pairs were matched in terms of first letter, number of letters, and frequency of usage in the English language, as suggested by MacLeod *et al* (2002), and were presented in random order. Stimuli presentation and data collection used E-Prime software (Psychology Software Tools, Pittsburgh, PA). To reduce the effect of anticipatory responding and outliers, response times (RTs) <200 ms and >3 SD above the mean for each trial were discarded (Salemink *et al*, 2007). Attentional bias to threat was calculated as the difference between average RT to targets at neutral word locations and average RT to targets at threat word locations. Negative scores indicate attentional bias away from threat, whereas positive scores indicate attentional bias toward threat.

Data Analysis

Simple descriptive statistics were computed to summarize demographic, trauma-related, and clinical variables for the sample. To reduce symptom clusters into composite measures of trauma-related threat and loss symptomatology based on prior work (Forbes *et al*, 2010, 2011; Grant *et al*, 2008), we conducted two principal components analyses (PCAs): the first contained CAPS measures of re-experiencing and hyperarousal symptoms (ie, threat symptomatology), and the second contained CAPS measure of avoidance/numbing symptoms and HAM-D and HAM-A measures of major depressive and anxiety symptoms. Pearson or Spearman correlations, as appropriate based on data distributions, were then computed to evaluate associations between [^{11}C]OMAR V_T values in the amygdala, attentional bias to threat, and composite measures of trauma-related threat and loss symptomatology. If significant associations were observed, exploratory *post hoc* analyses were conducted to evaluate associations between component aspects of composite measures; exploratory *post hoc* analyses were also conducted to evaluate associations between [^{11}C]OMAR V_T values in brain regions other than the amygdala in relation to attentional bias to threat; α was set to 0.01 for all of these analyses to reduce the likelihood of type I error. To evaluate whether attentional bias to threat mediated the relation between CB_1 receptor availability in the amygdala and the phenotypic expression of trauma-related psychopathology, we conducted a bootstrapped mediation analysis with 10 000 replicates using Mplus version 7.11. Model fit was assessed using χ^2, comparative fit index (CFI), and standardized root mean square residual (SRMR) fit statistics; by convention, nonsignificant χ^2 values, CFI values ≥ 0.90, and SRMR values <0.05 indicate a good fit to the data (Kline, 2010).

RESULTS

PCAs of Trauma-Related Threat and Loss Symptomatology

Two PCAs were conducted to compute *a priori* composite measures of trauma-related threat and loss symptomatology (Forbes *et al*, 2010, 2011Grant *et al*, 2008). The first PCA, which included CAPS measures of re-experiencing and hyperarousal symptoms yielded a 1-factor solution (eigenvalue $=1.93$; 96.4% of variance explained). Factor loadings were 0.982 for both component measures. The second PCA, which included CAPS measures of avoidance/numbing symptoms, and HAM-D and HAM-A measures of major depressive and anxiety symptoms, also yielded a 1-factor solution (eigenvalue $=2.91$; 97.1% of variance explained). Factor loadings were very high for all component measures: 0.982 for CAPS total scores; 0.984 for HAM-A total scores; and 0.990 for HAM-D-total scores. Composite measures of trauma-related threat and loss symptomatology were correlated ($r=0.86$, $p<0.001$).

Sample Characteristics

Table 1 shows demographic, trauma-related, and clinical characteristics of the sample. On average, the sample was 33.3 years of age, had slightly more female participants (55.0%), was equally Caucasian and non-Caucasian, and had a mean 15.4 years of education. Among the 16 trauma-exposed individuals, the mean age of first trauma was 13.4, mean number of lifetime traumas was 3.8, and the most commonly endorsed index trauma (ie, worst traumatic event according to the participant) was sexual abuse (50.0%).

Correlates of Attentional Bias to Threat, and Trauma-Related Threat and Loss Psychopathology

Table 2 shows correlations between independent variables, attentional bias to threat, and scores on composite measures of trauma-related threat and loss psychopathology. Results of these analyses revealed that [^{11}C]OMAR V_T values in the amygdala and severity of trauma-related threat and loss psychopathology were significantly positively related to attentional bias to threat. Furthermore, [^{11}C]OMAR V_T values in the amygdala were significantly positively related to severity of trauma-related threat symptomatology. Exploratory *post hoc* analyses revealed that this association was significant at the $p<0.01$ level for hyperarousal symptoms ($r=0.59$, $p=0.006$); the correlation for re-experiencing symptoms was 0.52, $p=0.020$. Exploratory *post hoc* analyses of [^{11}C]OMAR V_T values in brain regions other than the amygdala revealed that [^{11}C]OMAR V_T values in the posterior cingulate cortex were associated

Table 2 Correlations Between Independent Variables and Attentional Bias to Threat and Trauma-Related Threat and Loss Symptomatology

	Attentional bias to threat		**Threat symptomatology**		**Loss symptomatology**	
	r	***p***	***r***	***p***	***r***	***p***
Age	0.03	0.90	0.09	0.69	0.07	0.77
Female sex (%)	0.10	0.69	0.30	0.20	0.19	0.42
Non-Caucasian race/ethnicity	0.05	0.83	0.19	0.42	0.23	0.32
Years of education	−0.01	0.96	−0.04	0.87	−0.05	0.84
Trauma characteristics[a]						
Age of first trauma	−0.22	0.41	−0.36	0.17	−0.43	0.10
Number of lifetime traumas	0.41	0.11	0.35	0.18	0.35	0.19
Sexual assault (*vs* nonsexual) index trauma	0.40	0.12	0.20	0.46	0.11	0.69
[^{11}C]OMAR V_T values in amygdala	0.54*	0.018	0.48*	0.031	0.39	0.091
Threat symptomatology	0.60**	0.005	—	—	—	—
Loss symptomatology	0.58**	0.007	—	—	—	—

Significant association: *$p<0.05$, **$p<0.01$.
[a] Assessed only among trauma-exposed individuals ($n=16$).

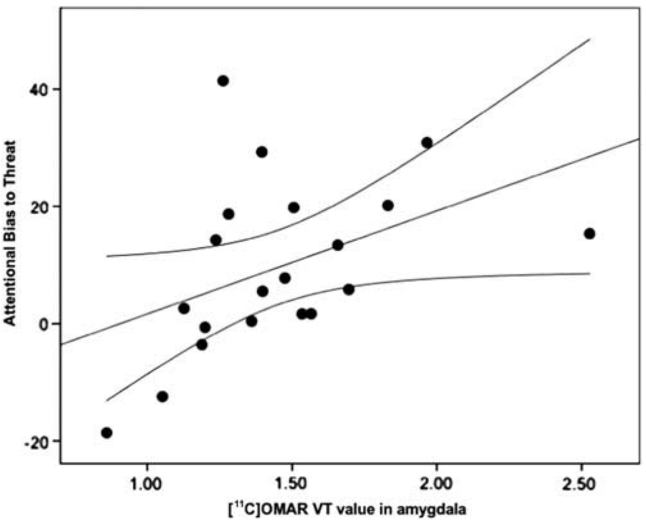

Figure 1 Scatterplot of the relation between [^{11}C]OMAR volume of distribution (V_T) values in the amygdala and performance on the dot-probe task. Note that higher attentional bias to threat scores are related to higher amygdala [^{11}C]OMAR VT values.

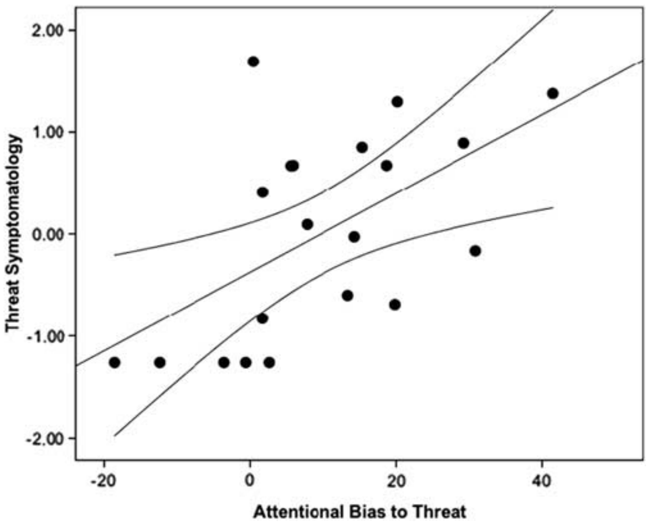

Figure 2 Scatterplot of relation between performance on the dot-probe task and severity of threat symptomatology. Note that higher attentional bias to threat scores are related to higher threat symptoms.

with attentional bias to threat ($r=0.56$, $p=0.011$), but this association was not significant at the $p<0.01$ level. None of the other brain regions were significant (all r's <0.36, all p's >0.12).

Figure 1 shows a scatterplot of the relation between [^{11}C]OMAR V_T values in amygdala and attentional bias to threat as assessed by the dot-probe task. Figure 2 shows a scatterplot of the relation between attentional bias to threat and severity of trauma-related threat symptomatology.

Correlation of [^{11}C]OMAR V_T Values in the Amygdala, Plasma Anandamide Levels, and Attentional Bias to Threat

The [^{11}C]OMAR V_T values in the amygdala were also significantly negatively associated with plasma anandamide levels ($r=-0.46$, $p=0.041$; Figure 3), suggesting that greater CB_1 receptor availability in the amygdala was associated with lower plasma levels of anandamide. Plasma anandamide levels were also significantly negatively associated with attentional bias to threat ($r=-0.53$, $p=0.017$; Figure 4).

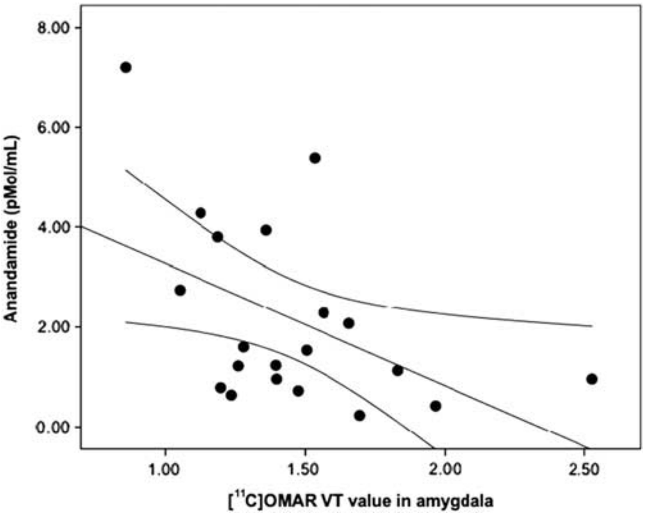

Figure 3 Scatterplot of relation between [^{11}C]OMAR volume of distribution (V_T) values in the amygdala and anandamide levels.

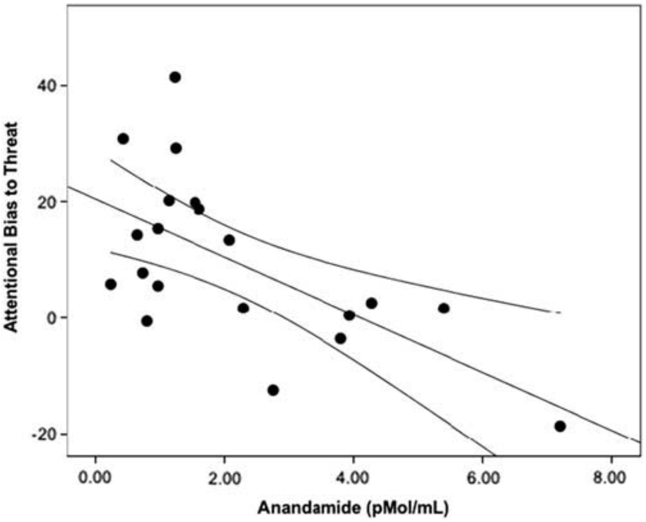

Figure 4 Scatterplot of relation between anandamide levels and performance on the dot-probe task. Note that higher attentional bias to threat scores are related to lower plasma anandamide levels.

Mediation Analysis

Figure 5 shows results of a mediation analysis that evaluated the role of attentional bias to threat in mediating the relation between CB_1 receptor availability in the amygdala and severity of trauma-related threat symptomatology. Although greater CB_1 receptor availability in the amygdala was associated with greater severity of trauma-related threat symptomatology in a bivariate analysis ($\beta = 0.41$, $p = 0.040$), this association was no longer significant when attentional bias to threat was incorporated into the model ($\beta = 0.16$, $p = 0.51$). In the final model, which provided a good fit to the data ($\chi^2(1) = 0.51$, $p = 0.48$; CFI $= 1.00$; SRMR $= 0.038$), greater CB_1 receptor availability was associated with increased attentional bias to threat that was in turn associated with increased severity of trauma-related threat symptomatology.

Figure 5 Path model of associations between CB_1 receptor availability measured with [^{11}C]OMAR and positron emission tomography in the amygdala, attentional bias to threat, and threat symptomatology. **Significant association, $p<0.01$. The r^2 for negative attentional bias $= 0.28$; r^2 for threat symptomatology $= 0.31$. The dashed line indicates a nonsignificant association. The 95% confidence intervals for CB_1 → attentional bias to threat $= 0.01$–0.95; for attentional bias to threat → threat symptomatology $= 0.16$–0.85.

DISCUSSION

Using the CB_1 receptor radiotracer [^{11}C]OMAR, we found that greater CB_1 receptor availability in the amygdala was associated with increased attentional bias to threat, as well as increased severity of trauma-related threat symptomatology (ie, hyperarousal) in humans presenting with a broad dimensional spectrum of trauma-related psychopathology. We also found that attentional bias to threat mediated the relation between CB_1 receptor availability in the amygdala and severity of threat symptomatology. These results extend a growing body of research demonstrating an association between trauma-related disorders such as PTSD, MDD, and GAD, and attentional bias to threat (Fani *et al*, 2012b; Lindstrom *et al*, 2011; Sveen *et al*, 2009) by implicating the CB_1 receptor system as a key neurobiological underpinning of this endophenotype and its concomitant phenotypic expression of trauma-related threat symptomatology, particularly hyperarousal symptoms. They further suggest that attentional bias to threat may mediate the association between CB_1 receptor availability in the amygdala and threat symptomatology, with greater CB_1 receptor availability being linked to greater attentional bias to threat that is in turn linked to greater severity of threat symptomatology.

Results of the current study build on extant neurobiological studies that have implicated the endocannabinoid system in the amygdala as an important modulator of anxiety (Mackie, 2005; Ramikie *et al*, 2014), as well as functional activation of the amygdala in mediating attentional bias to threat among individuals with PTSD (El Khoury-Malhame *et al*, 2011). Specifically, results of this study suggest that CB_1 receptor availability in the amygdala may directly mediate this endophenotype and its associated phenotypic expression of trauma-related threat symptomatology. Preclinical work suggests that the activation of membrane glucocorticoid receptors appears to engage a G-protein-mediated cascade through the activation of G_s proteins (Di *et al*, 2003) that, in turn, increases the activity of cAMP and protein kinase A. This increase in protein kinase A appears to induce the rapid synthesis of an endocannabinoid signal through an as yet unknown mechanism that may be an increase in intracellular calcium signaling (Cadas *et al*, 1996; Malcher-Lopes *et al*, 2006; Vellani *et al*, 2008) that is then released from principal neurons in the amygdala and activates CB_1 receptors localized on the terminals of GABAergic neurons in the amygdala. It should be noted, however, that other mechanisms than CB_1 receptor stimulation by anandamide could contribute to the etiology of attentional bias to threat and threat symptomatology. First, the two endocannabinoids anandamide and 2-arachidonoylglycerol have differential roles in endocannabinoid (Ahn *et al*, 2008) and have distinctly different metabolic pathways (fatty acid amide hydrolase (FAAH) for anandamide and monoacylglycerollipase (MAGL) for 2-arachidonoylglycerol; (Ahn *et al*, 2008; Long *et al*, 2009). To date, the relative contribution of these two endocannabinoids and their pathways in the modulation of anxiety remains unclear. Furthermore, recent evidence suggests that CB_1 receptor signaling varies across brain regions (Bosier *et al*, 2010), and that diverse effects of anandamide–CB_1 receptor signaling mechanisms are evident even within the extended amygdala (Puente *et al*, 2011). Finally, the actions of anandamide are not restricted to CB_1 receptors, as endocannabinoids also act on CB_2 receptors (Mechoulam *et al*, 1995), GPR55 (Ryberg *et al*, 2007), transient receptor potential vanilloid type 1 channels (Melck *et al*, 1999; Smart *et al*, 2000; Zygmunt *et al*, 1999), and other G-protein subtypes (Glass and Felder, 1997b; Howlett, 2004).

Although additional research is needed to further evaluate how the endocannabinoid system mediates attentional bias to threat, the results of this study suggest that greater CB_1 receptor availability in the amygdala, as well as lower levels of peripheral anandamide, are associated with a greater attentional bias to threat in trauma-exposed individuals. However, we acknowledge, that no human studies that we are aware of have found that anandamide concentrations directly influence CB_1 receptor availability, and hence additional work is needed to ascertain how these variables are causally related. Nevertheless, the present data extend prior work linking attentional bias to threat to hyperarousal symptoms (Fani *et al*, 2012b) to suggest that the CB_1 receptor system in the amygdala is implicated in modulating attentional bias to threat that is in turn linked to the transdiagnostic and dimensional phenotypic expression of trauma-related threat symptomatology. Further research will be useful in further elucidating molecular mechanisms that account for the observed association between CB_1 receptor availability and the endophenotypic and phenotypic expression of threat processing in humans.

An important question to be addressed in future work is whether pharmacotherapies that act on catabolic enzymes for endocannabinoids may be useful in the prevention and treatment of endophenotypic and phenotypic aspects of trauma-related threat symptomatology. Emerging evidence supports the potential utility of such targets, suggesting that variation in the FAAH gene is linked to reduced expression of FAAH that consequently results in elevations in circulating levels of anadamide (Chiang *et al*, 2004; Sipe *et al*, 2010), as well as decreased amygdala response to threat (Hariri *et al*, 2009) and more rapid habituation of the amygdala to repeated threat (Gunduz-Cinar *et al*, 2013b). Notably, elevating anandamide levels via FAAH inhibition appear to provide a more circumscribed spectrum of behavioral effects than blocking MAGL (Blankman and Cravatt, 2013) that could potentially result in a more beneficial side effect profile, as anandamide is less prone to CB_1 receptor desensitization and resultant behavioral tolerance (Lichtman *et al*, 2002; Schlosburg *et al*, 2010). These classes of compounds are currently being investigated for their potential efficacy in treating mood and anxiety disorders. Given that core aspects of threat symptomatology such as hyperarousal are key drivers of more disabling aspects of the trauma-related phenotype such as emotional numbing (Marshall *et al*, 2006; Pietrzak *et al*, 2013; Schell *et al*, 2004; Solomon *et al*, 2009), pharmacotherapeutic targeting of threat symptomatology in symptomatic trauma survivors may have utility in reducing the chronicity and morbidity of trauma-related psychiatric disorders such as PTSD, MDD, and GAD.

Methodological limitations of this study must be noted. First, we studied a cohort of individuals with heterogeneous trauma histories. Although this is typical for most PTSD studies and we endeavored to recruit individuals who represented a broad and representative spectrum of trauma-related psychopathology, additional studies of samples with noncivilian trauma histories will be useful in extending these results. Second, 95% confidence intervals for coefficients in the mediation analysis were markedly wide, and hence additional studies in larger samples will be useful in ascertaining magnitudes of the observed associations. Third, we observed a high correlation between threat and loss symptomatology that may call into question the extent to which these symptom clusters reflect separable components of trauma-related psychopathology that are uniquely related to CB_1 receptor availability in the amygdala and attentional bias to threat. Nevertheless, high correlations among symptom clusters of trauma-related psychopathology are not uncommon, with confirmatory factor analytic studies of substantially larger samples often observing intercorrelations among symptom clusters >0.80 (Forbes *et al*, 2011; Grant *et al*, 2008; Pietrzak *et al*, 2010; Wang *et al*, 2011). Furthermore, the finding that CB_1 receptor availability in the amygdala was associated only with threat, but not loss symptomatology, suggests greater specificity of association that accords with prior work (Gunduz-Cinar *et al*, 2013a). Fourth, it is important to recognize that our outcome measure in this study, V_T, represents specific plus nondisplaceable binding. Because of the lack of a suitable reference region devoid of CB_1, we and others using different CB_1 receptor ligands (Ceccarini *et al*, 2014; Neumeister *et al*, 2012; 2013; Tsujikawa *et al*, 2014) cannot directly calculate binding potential (BP_{ND}), a measure of specific binding. Thus, an implicit assumption in the interpretation of our results is that there are no group differences in V_{ND}, the distribution volume of nondisplaceable tracer uptake. An alternative assumption would be that the magnitude of nondisplaceable binding is small compared with the total binding. To definitively address this issue would require a blocking study in humans to estimate V_{ND}. To the best of our knowledge, such data are not currently available because of the lack of suitable selective CB_1 antagonist drugs approved for human use. Blocking data with the CB_1 receptor antagonist rimonabant (1 mg/kg) in baboons (Horti *et al*, 2006), however, did show a large reduction in tracer uptake, suggesting that a substantial fraction of V_T can be attributed to specific binding.

Notwithstanding these limitations, the results of this study provide the first known *in vivo* molecular evidence of how a candidate neuroreceptor system—CB$_1$—relates to attentional bias to threat and the dimensional expression of trauma-related psychopathology. Results revealed that greater CB$_1$ receptor availability in the amygdala is associated with increased attentional bias to threat, as well as the phenotypic expression of threat-related symptomatology, particularly hyperarousal symptoms. Given that these results were based on a relatively small sample, further research in larger, transdiagnostic cohorts with elevated threat symptomatology will be useful in evaluating the generalizability of these results, as well as in examining the efficacy of candidate pharmacotherapies that target the anandamide–CB$_1$ receptor system in mitigating both the endophenotypic and phenotypic expression of threat symptomatology in symptomatic trauma survivors.

FUNDING AND DISCLOSURE

Dr Neumeister has received consulting fees from Pfizer. Dr Pietrzak is a scientific consultant to Cogstate. Dr Potenza has consulted for Ironwood, Lundbeck, Boehringer Ingelheim, Somaxon, gambling businesses and organizations, law offices, and the federal defender's office in issues regarding impulse control disorders. He has received research support from the Mohegan Sun Casino, the National Center for Responsible Gaming, and the following pharmaceutical companies: Psyadon, Forest Laboratories, Ortho-McNeil, Oy-Control/Biotie, and GlaxoSmithKline. These activities are unrelated to the present report. The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We acknowledge the excellent work of the staff of the Yale PET Center and the nursing support from Brenda Breault, Cynthia D'Amico, Michelle San Pedro, Jamie Cyr, and Deborah Campbell for their contributions with patient care during the PET scans. This project was supported by the National Institutes of Health (NIH) through the following awards: R21MH096105, R21MH085627, RO1MH096876, and RO1MH102566. This publication was also made possible by CTSA Grant Number UL1 RR024139 from the National Center for Research Resources (NCRR) and the National Center for Advancing Translational Science (NCATS), components of the National Institutes of Health (NIH), NIH Roadmap for Medical Research, and Clinical Neurosciences Division of the United States Department of Veterans Affairs National Center for Posttraumatic Stress Disorder. Dr Neumeister has received grant support from Lilly. The work of Drs Neumeister, Huang, Potenza, and Carson was funded by the NIH.

The NIH or VA played no role in the design of the study, collection and analysis of the data, and decision to publish. Contents of this publication are solely the responsibility of the authors and do not necessarily represent the official view of NIH or VA.

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