Reduced Amygdala Serotonin Transporter Binding in Posttraumatic Stress Disorder

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Background: The amygdala is a key site where alterations in the regulation of the serotonin transporter (5-HTT) may alter stress response. Deficient 5-HTT function and abnormal amygdala activity have been hypothesized to contribute to the pathophysiology of posttraumatic stress disorder (PTSD), but no study has evaluated the 5-HTT in humans with PTSD. On the basis of translational models, we hypothesized that patients diagnosed with PTSD would exhibit reduced amygdala 5-HTT expression as measured with positron emission tomography and the recently developed 5-HTT-selective radiotracer [^{11}C]AFM.

Methods: Fifteen participants with PTSD and 15 healthy control (HC) subjects without trauma history underwent a resting-state positron emission tomography scan.

Results: [^{11}C]AFM binding potential (BP_{ND}) within the combined bilateral amygdala region of interest was significantly reduced in the PTSD group compared with the HC group ($p = .027$; 16.3% reduction), which was largely driven by the between-group difference in the left amygdala ($p = .008$; 20.5% reduction). Furthermore, amygdala [^{11}C]AFM BP_{ND} was inversely correlated with both Hamilton Rating Scale for Anxiety scores ($r = -.55$, $p = .035$) and Montgomery–Åsberg Depression Rating Scale scores ($r = -.56$, $p = .029$).

Conclusions: Our findings of abnormally reduced amygdala 5-HTT binding in PTSD and its association with higher anxiety and depression symptoms in PTSD patients support a translational neurobiological model of PTSD directly implicating dysregulated 5-HTT signaling within neural systems underlying threat detection and fear learning.

Key Words: Amygdala, neuroimaging, positron emission tomography, posttraumatic stress disorder, serotonin, serotonin transporter

Brain serotonin (5-HT) systems have been linked to the neurobiology of posttraumatic stress disorder (PTSD) based on evidence from both preclinical and clinical studies (1–6). In humans, the 5-HT agonist m-chlorophenylpiperazine (mCPP) was found to transiently evoke panic attacks and trauma-related flashbacks in patients with PTSD (7) that were not observed when mCPP was administered to patients with other psychiatric disorders (8–10). Moreover, the 5-HT transporter protein (5-HTT) is the target of the two U.S. Food and Drug Administration–approved pharmacotherapies for PTSD. The most direct evidence for the importance of 5-HTT function in PTSD can be inferred from recent human genetic studies showing that the short allele of the common repeat polymorphism in the promoter region of the gene coding for the 5-HTT (5-HTTLRP) increases the vulnerability to develop PTSD (1,5,11,12) and may predict poor treatment outcome (13). However, to date no study has directly examined brain 5-HTT in patients with PTSD.

Fear conditioning experiments highlight the role of the amygdala as a key brain structure responsible for processing and storing fear-related memories and for coordinating fear-related behaviors (14–16), leading to the hypothesis that PTSD may be characterized by amygdala overactivity or hyperresponsiveness to threatening stimuli in humans (17–19). Indeed, a convergence of findings from functional neuroimaging investigations in clinical populations supports a neurocircuitry model of PTSD characterized by abnormally elevated amygdala activity coupled with deficient regulation by prefrontal cortical structures (20–27). Studies specifically suggest that amygdala function may be enhanced during the acquisition of conditioned fear in PTSD (26,28), potentially leading to deficient fear extinction hypothesized to play a role in PTSD (19,29). Despite an emerging neurocircuitry model of PTSD, the neurochemical regulation of this circuitry remains incompletely understood.

The amygdala is a major forebrain target of 5-HT neurons arising from the dorsal raphe (30), and 5-HT signaling within the amygdala regulates normal fear and threat responsiveness (2,31,32), supporting the hypothesis that abnormal 5-HTT function within the amygdala specifically may be an important mechanism in the pathophysiology of PTSD. In support of this hypothesis, common genetic variants that lead to differential expression of 5-HTT are associated with differences in the acquisition of a conditioned fear response and altered startle response in humans (33,34). In aggregate, these data suggest a model through which altered 5-HTT function influences amygdala activity to enhance the acquisition of conditioned fear and/or decrease fear extinction, which in turn mediates a vulnerability to PTSD.

Positron emission tomography (PET) imaging is the most direct, sensitive, and straightforward means of probing the functional neurochemistry of human subjects and assessing molecular targets in the brain in vivo, provided the proper tracer is available. In the current study, we used PET and the selective 5-HTT radioligand [^{11}C]AFM (35,36) to characterize 5-HTT receptor binding in patients with PTSD and matched healthy control (HC) subjects. Given that the low-expressing 5-HTT genotype (the short allele of the 5-HT-

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TLRP) is associated with elevated risk for PTSD and the important role of the amygdala in fear-related neurocircuitry, we hypothesized reduced 5-HTT binding in the amygdala in patients with PTSD.

Methods and Materials

Subjects

Fifteen participants with PTSD and 15 age- and sex-matched HC participants without trauma history were recruited through public advertisement. After giving informed consent, participants were screened and diagnosed using DSM-IV criteria and the Structured Clinical Interview for DSM-IV (37,38). PTSD participants suffered both combat and noncombat trauma exposures. Noncombat trauma exposure consisted of physical or sexual assault, domestic violence, or natural disaster. PTSD participants were free of comorbid psychiatric disorders, with the exception of major depressive disorder if the primary diagnosis was determined to be PTSD, which was defined by PTSD being the dominant clinical syndrome and the onset of major depressive disorder occurred after the onset of PTSD. PTSD symptom severity was measured using the Clinician-Administered PTSD Scale for DSM-IV (CAPS) (39), and trauma history was quantified with the Traumatic Life Events Questionnaire (40). Depression and anxiety severity was assessed using the Montgomery–Åsberg Depression Rating Scale (MADRS) and the Hamilton Rating Scale for Anxiety, respectively (41,42). All participants were evaluated by physical examination, electrocardiogram, standard laboratory tests, urine analysis, and toxicology and were free of significant medical or neurological conditions. None of the participants were receiving psychotherapy or psychotropic medication for at least 4 weeks before scanning. The protocol was approved by the Yale University School of Medicine Human Investigation Committee, the Human Subjects Subcommittee of the Veterans Affairs Connecticut Healthcare System, the Magnetic Resonance Research Center, and the Yale New Haven Hospital Radiation Safety Committee.

Scanning and Imaging Procedures

Magnetic resonance imaging (MRI) was obtained for each subject on a Siemens 3T Trio system (Siemens Medical Solutions, Knoxville, Tennessee) to exclude individuals with anatomic abnormalities and for coregistration. Participants subsequently underwent a resting PET scan with 20 mCi of [^{11}C]AFM (35,36). PET scans were done on a High Resolution Research Tomograph (HRRT) (Siemens Medical Solutions), which acquires 207 slices (1.2-mm slice separation) with a reconstructed image resolution of approximately 3 mm. Images were reconstructed with corrections for motion, attenuation, scatter, randoms, and dead time. A summed image (0- to 10-min postinjection) was created from the motion-corrected PET data and registered to the subjects' MRIs, which, in turn, were registered (12-parameter affine transformation) to an MR template (Montreal Neurological Institute space). The cerebellum region of interest (ROI) was taken from the template for SPM2 (Anatomical Automatic Labeling) and applied to the PET data to produce time-activity curves for the reference region (43). [^{11}C]AFM, pixelwise BP_{ND} images were created by SRTM2 (Simplified Reference Tissue Model). Amygdalar BP_{ND} values were extracted from the parametric images using the template. Results from test–retest studies using [^{11}C]AFM BP_{ND} from the amygdala ROI demonstrate approximately 3% mean difference, indicating very good reliability (W. Williams, M.D., unpublished data; August 1, 2011).

Statistical Analysis

Independent sample *t* tests were used to compare continuous clinical, demographic variables and [^{11}C]AFM BP_{ND} values between PTSD and HC subjects. Data were normally distributed as determined by visual inspection and the Kolmogorov–Smirnov *D* test. Chi-square was used in the case of dichotomous variables. Tests of association between continuous variables were performed using Pearson's Product–Moment correlations. All tests were performed two-tailed, with results considered significant at $p < .05$. Means and standard deviations are reported. All statistical analyses were conducted using SPSS version 16.0 (SPSS, Chicago, Illinois).

Results

Demographics and Clinical Characteristics

Participants in the PTSD and HC groups were matched for age and gender frequency. Participants in the PTSD group had a history

Table 1. Demographic, Clinical, and Positron Emission Tomography Procedural Characteristics

	PTSD ($n = 15$)	Healthy Control ($n = 15$)	*p* Value
Age (years)	32.9 ± 9.8	30.1 ± 10.0	.45
Range	21−51	18−49	—
Sex	9 M, 6 F	10 M, 5 F	.71
Ethnicity	4 C, 5 AA, 5 H	10 C, 4 AA	—
BMI	29.7 ± 5.2	26.5 ± 4.6	.09
Smoking Status (yes/no)	2/15	1/15	.54
Index Trauma Type (Combat/Noncombat[a])	5/10	—	—
No. of Lifetime Criteria A Traumas	5.4 ± 3.0	—	
CAPS Total Score	68.4 ± 15.8	—	—
CAPS Re-Experiencing Subscore	18.8 ± 5.6	—	—
CAPS Avoidance Subscore	27.5 ± 8.1	—	—
CAPS Hyperarousal Subscore	22.1 ± 6.2	—	—
HAM-A Total Score	18.5 ± 6.9	2.5 ± 5.1	<.001
MADRS Total Score	26.5 ± 8.6	4.2 ± 4.4	<.001
Injected Dose (MBQ)	710 ± 38	699 ± 53	.53
Specific Activity (MBQ/nmol)	203 ± 144	219 ± 124	.75
Injected Mass (μg)	1.57 ± 1.04	1.34 ± .85	.52

Data presented in mean ± standard deviation, unless otherwise indicated. P values determined by independent sample t-tests for continuous variables or by chi-square for dichotomous variables.

AA, African-American; BMI, body mass index; C, Caucasian; CAPS, Clinician-Administered PTSD Scale for DSM-IV; F, female; H, Hispanic; HAM-A, Hamilton Anxiety Scale; M, male; MBQ, megabecquerel; MADRS, Montgomery–Åsberg Depression Rating Scale; PTSD, posttraumatic stress disorder.

[a]Noncombat trauma exposure consisted of physical or sexual assault, domestic violence or natural disaster.

of combat ($n = 5$) or noncombat ($n = 10$) index trauma exposure. The mean age of onset of the first criteria A trauma was 15.6 ± 5.1 years (range 8–25), and participants suffered from 5.4 ± 3.0 lifetime Criteria A traumas (range 1–25). Participants in the PTSD group experienced moderate to severe PTSD symptom severity as well as significant levels of depression and anxiety symptoms at the time of the PET scan (see Table 1).

Neuroreceptor Imaging and Behavioral Correlations

[^{11}C]AFM BP_{ND} within the combined bilateral amygdala ROI was significantly reduced in the PTSD group compared with the HC group (HC: 3.38 ± .63, PTSD: 2.83 ± .64, $df = 28$, $t = 2.33$, $p = .027$; 16.3% reduction). This finding was driven by the between-group [^{11}C]AFM BP_{ND} difference in the left amygdala (HC: 3.61 ± .69, PTSD: 2.87 ± .73, $df = 28$, $t = 2.9$, $p = .008$; 20.5% reduction). The between-group difference in the right amygdala did not reach statistical significance (HC: 3.17 ± .63, PTSD: 2.80 ± .59, $df = 28$, $t = 1.65$, $p = .11$; 11.7% reduction; Figure 1).

In the PTSD group, amygdala [^{11}C]AFM BP_{ND} was inversely correlated with both HAM-A scores ($r = -.55$, $p = .035$) and MARDS scores ($r = -.56$, $p = .029$; Figure 2). There was no correlation between [^{11}C]AFM BP_{ND} and total CAPS score ($r = -.21$, $p = .45$) or subscore. There were no associations between [^{11}C]AFM BP_{ND} and age, gender or BMI in either group and no associations between [^{11}C]AFM BP_{ND} and number or age of traumatization in the PTSD group.

Exploratory analyses of [^{11}C]AFM BP_{ND} in brain regions outside of the amygdala did not reveal between-group differences any region (see Table 2).

Discussion

In this study we demonstrate in vivo reductions in 5-HTT availability in the amygdala with [^{11}C]AFM BP_{ND} in patients with PTSD compared with HC participants. [^{11}C]AFM BP_{ND} reductions are associated with characteristic features of the PTSD phenotype such that lower levels of ligand binding were associated with higher levels of both anxiety and depression. These results support the link between 5-HT regulation and the role of the amygdala in the pathophysiology of PTSD and are consistent with prior studies describing

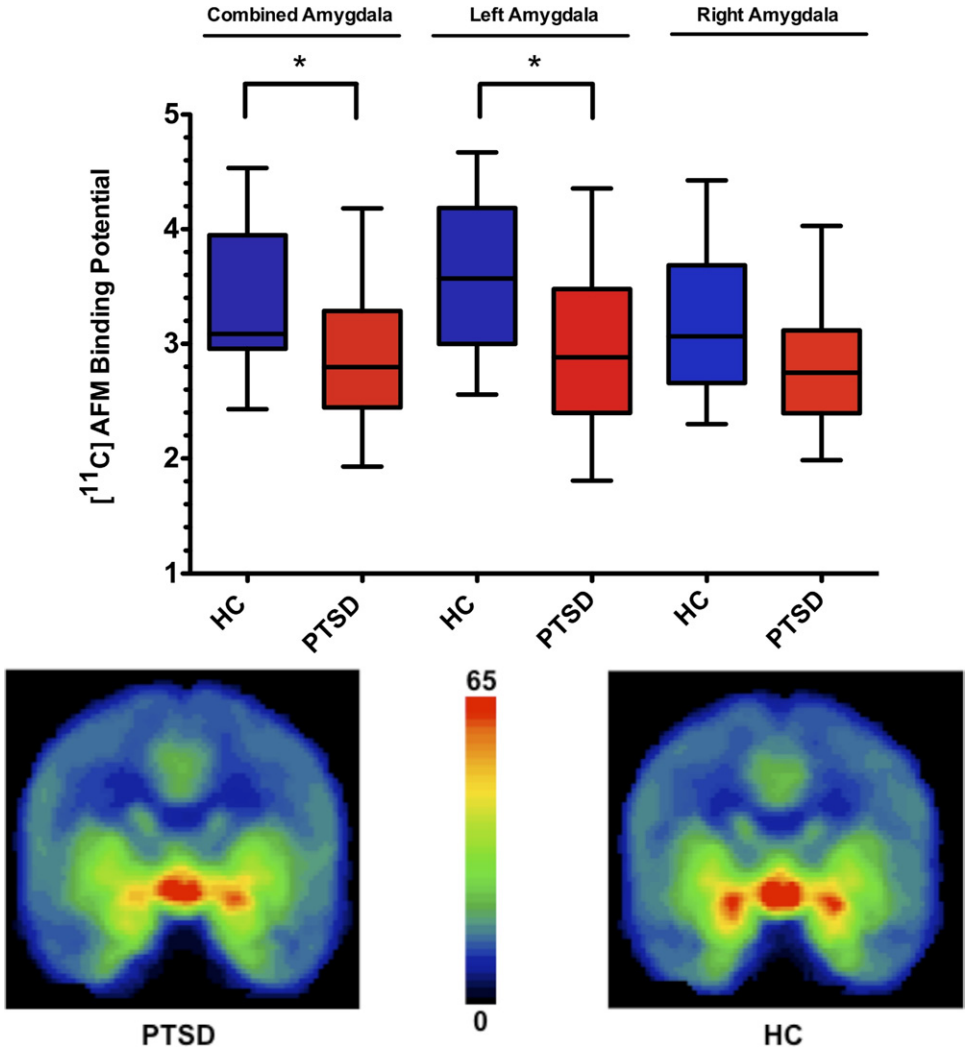

Figure 1. Reduced amygdala [^{11}C]AFM binding potential (BP_{ND}) in patients with posttraumatic stress disorder (PTSD) compared with healthy control (HC) subjects. Upper panel: plot showing [^{11}C]AFM BP_{ND} differences in the combined bilateral amygdala region of interest (ROI) and in both left and right amygdala ROI between patients with PTSD and HC subjects. *$p < .05$, two-tailed. Lower panel: Averaged [^{11}C]AFM PET images (coronal view) illustrate reduced amygdala distribution volume in PTSD (left) relative to HC (right).

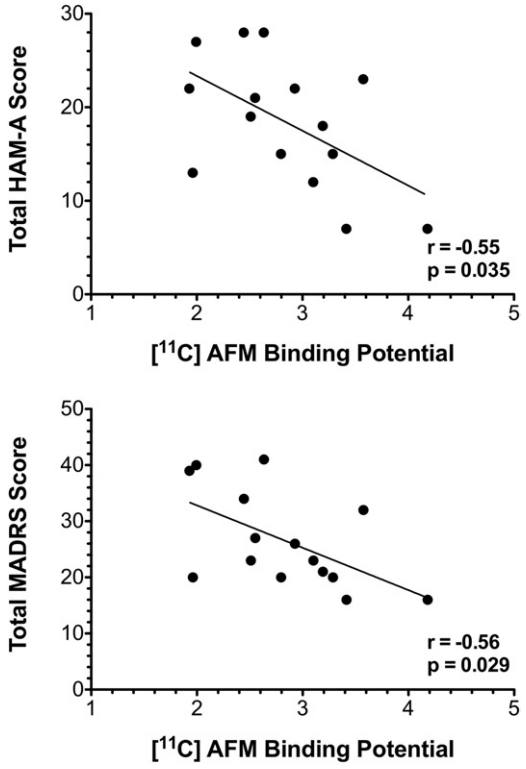

Figure 2. Correlation between [^{11}C]AFM binding potential and levels of anxiety and depression in individuals with posttraumatic stress disorder. In the posttraumatic stress disorder group ($n = 15$), amygdala [^{11}C]AFM binding potential was inversely correlated with both Hamilton Rating Scale for Anxiety (HAM-A) scores ($r = -.55$, $p = .035$) and Montgomery–Åsberg Depression Rating Scale (MADRS) scores ($r = -.56$, $p = .029$). Tests of association between continuous variables were performed using Pearson's Product–Moment correlations.

amygdala hyperactivity upon exposure to trauma- or fear-related stimuli in PTSD (20–22,25,26,28). The reduced availability of 5-HTT in patients with PTSD is in line with prior animal studies that predict reductions in 5-HTT would be associated with increased fear (2,31,32).

The results of our study are consistent with a model of PTSD in which reduced functioning of 5-HTT, resulting from inheritance of the lesser expressing short allele of the 5-HTTLRP (1,5,11,13,44,45) or other mechanism, leads to altered amygdala functioning that in turn drives increased anxiety and vulnerability to the effects of stress and trauma. Support for this model comes from preclinical studies showing deficient extinction recall, enhance behavioral vulnerability to stress, and altered morphology of basolateral amygdala (BLA) in 5-HTT knockout (KO) mice (2,3). Conversely, overexpression of the human 5-HTT gene in transgenic mice resulted in a low-anxiety phenotype (46).

It is notable that in the study by Wellman *et al.* (2), 5-HTT KO mice showed a selective deficit in the recall of an extinguished fear memory 24 hours following a standard fear conditioning and extinction paradigm. This selective deficient in extinction mirrors the findings in human PTSD populations of persistently elevated fear responses often in the face of normal fear acquisition (29,47–49). Furthermore, a recent fear conditioning–extinction functional MRI study comparing PTSD to healthy volunteers found impaired extinction recall in PTSD 24 hours following the conditioning–extinction protocol as indexed by skin conductance response (SCR), whereas there was no difference in the acquisition or early extinction phase in congruence with the selective effects of 5-HTT KO on extinction recall reported Wellman *et al.* (26). The authors also found greater amygdala activation in the PTSD during fear acquisition, consistent with the hypothesized role of enhanced amygdala activity driving the psychophysiologic substrates of the vulnerability to PTSD (26). This finding replicated an earlier PET study of cerebral blood flow in women with childhood sexual abuse demonstrating elevated amygdala blood flow during fear acquisition in a conditioned fear paradigm (28).

Although our model suggests that altered 5-HTT function is a risk factor for PTSD, this study was not designed to determine the causal relationship between ligand binding to 5-HTT and PTSD. Genetic studies linking reduced 5-HTT gene expression (50) with both increased amygdala activation during processing of emotional salient information with a fear-provoking component (51,52) and vulnerability to PTSD (1,5,11,13,44,45) provide compelling support for this model. However, stress and other environmental factors also may lower 5-HTT gene expression (53). Thus, reductions in 5-HTT may in fact predispose or be a consequence of extreme stress exposure and future studies will be necessary to test these alternative models.

There are several limitations to this study. It focused on the amygdala given its significance for PTSD and only secondarily explored [^{11}C]AFM BP_{ND} in other regions outside our a priori ROI. Although we did not find evidence for abnormal binding outside of the amygdala, the sample size of the study may have limited our power to detect smaller differences between groups. Our findings of reduced amygdala [^{11}C]AFM BP_{ND} in PTSD appeared to be driven by the left amygdala (20.5%), whereas reductions in the right amygdala (11.7%) did not reach statistical significance. The reason for the laterality of our finding is not clear, although it is possible that the lack of significance in the right amygdala represents a Type II error given the modest sample size and the within-group variabil-

Table 2. Regional [^{11}C]-AFM BP_{ND} in PTSD and Healthy Control Study Groups Outside of Amygdala A Priori Region of Interest

	PTSD ($n = 49$)	Healthy Control ($n = 27$)	*df*	*t* Value	*p* Value
ACC	.97 ± .20	1.02 ± .15	28	.74	.47
Caudate	2.11 ± .60	2.11 ± .31	28	.007	.99
Hippocampus	1.21 ± .27	1.3 ± .32	28	.69	.49
Occipital Cortex	.41 ± .09	.46 ± .09	28	1.55	.13
Pallidum	3.36 ± .66	3.22 ± .76	28	.51	.61
Raphe	6.78 ± 1.06	7.34 ± .80	28	1.60	.12
Thalamus	3.06 ± .46	2.9 ± .72	28	.70	.49

Independent sample *t* tests were used to compare [^{11}C]AFM binding potention (BP_{ND}) values between PTSD and health control subjects. Data presented in mean ± SD.

ACC, anterior cingulate cortex; PTSD, posttraumatic stress disorder.

ity in [^{11}C]AFM BP_{ND}. Our study did not include a trauma-control group, leaving open the possibility that our findings reflect trauma exposure per se. Future studies using a three-group design will be required to explore this possibility further. Whether our observation of low 5-HTT availability represents a state or trait finding is not addressed by the current cross-sectional study, and future longitudinal studies are necessary to address this important question. The absence of an association between [^{11}C]AFM BP_{ND} and specific PTSD symptoms as measured by CAPS score, and in contrast the association between lower[^{11}C]AFM BP_{ND} and both higher depression and anxiety symptoms suggests that 5-HTT reductions contribute to but do not fully explain the complex phenotype of PTSD. Finally, we did not collect genetic data, which may have contributed to explaining the observed 5-HTT reductions in the PTSD cohort given the findings of human genetic studies.

Together, our findings support a translational neurobiological model implicating dysregulated amygdala 5-HTT signaling in the neurobiology of PTSD. Whether reduced 5-HTT binding is a preexisting condition enhancing the vulnerability to develop PTSD after trauma or is alternatively a consequence of trauma exposure, remains to be determined.

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