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 \([1^{11}}C\)AFM.

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

Results: \([1^{11}}C\)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 \((p = .027; 16.3\% \text{ reduction})\), which was largely driven by the between-group difference in the left amygdala \((p = .008; 20.5\% \text{ reduction})\). Furthermore, amygdala \([1^{11}}C\)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-HTTLPR) increases the vulnerability to develop PTSD (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 \([1^{11}}C\)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-
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) |
| Age (years)                             | 32.9 ± 9.8    | 30.1 ± 10.0     |
| Range                                   | 21—51         | 18—49           |
| Sex                                     | 9 M, 6 F      | 10 M, 5 F       |
| Ethnicity                               | 4 C, 5 AA, 5 H| 10 C, 4 AA      |
| BMI                                     | 29.7 ± 5.2    | 26.5 ± 4.6      |
| Smoking Status (yes/no)                 | 2/15          | 1/15            |
| Index Trauma Type (Combat/Noncombat*)    | 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       |
| MADRS Total Score                       | 26.5 ± 8.6    | 4.2 ± 4.4       |
| Injected Dose (MBQ)                     | 710 ± 38      | 699 ± 53        |
| Specific Activity (MBQ/nmol)            | 203 ± 144     | 219 ± 124       |
| Injected Mass (µg)                      | 1.57 ± 1.04   | 1.34 ± .85      |

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.

*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

\([11C]AFM BP_{ND}\) within the combined bilateral amygdala ROI was significantly reduced in the PTSD group compared with the HC group (HC: 3.38 ± 0.63, PTSD: 2.83 ± 0.64, \(df = 28, t = 2.33, p = .027; 16.3\%\) reduction). This finding was driven by the between-group \([11C]AFM BP_{ND}\) difference in the left amygdala (HC: 3.61 ± 0.69, PTSD: 2.87 ± 0.73, \(df = 28, t = 2.9, p = .008; 20.5\%\) reduction). The between-group difference in the right amygdala did not reach statistical significance (HC: 3.17 ± 0.63, PTSD: 2.80 ± 0.59, \(df = 28, t = 1.65, p = .11; 11.7\%\) reduction; Figure 1).

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

Exploratory analyses of \([11C]AFM BP_{ND}\) 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 \([11C]AFM BP_{ND}\) in patients with PTSD compared with HC participants. \([11C]AFM BP_{ND}\) 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...
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ture drives increased anxiety and vulnerability to the effects of

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the lesser expressing short allele of the 5-HTTLRP (1,5,11,13,44,45)

(2,31,32). reductions in 5-HTT would be associated with increased fear

in patients with PTSD is in line with prior animal studies that predict

stimuli in PTSD (20–22,25,26,28). The reduced availability of 5-HTT

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 vul-

nerability to stress, and altered morphology of basolateral

amygdala (BLA) in 5-HTT knockout (KO) mice (2,3). Conversely, over-

expression 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 ex-

tinction paradigm. This selective deficit 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 ex-

tinction recall in PTSD 24 hours following the conditioning–extinc-

tion protocol as indexed by skin conductance response (SCR),

whereas there was no difference in the acquisition or early extinct-

tion 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 acquisi-

tion, consistent with the hypothesized role of enhanced amygdala

activity driving the psychophysioologic substrates of the vulnerabil-

ty to PTSD (26). This finding replicated an earlier PET study of

cerebral blood flow in women with childhood sexual abuse demon-

strating 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 emo-

tional salient information with a fear-provoking component (51,52)

and vulnerability to PTSD (1,5,11,13,44,45) provide compelling sup-

port for this model. However, stress and other environmental fac-

tors 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 alterna-

tive models.

There are several limitations to this study. It focused on the

amygdala given its significance for PTSD and only secondarily ex-

plored [11C]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 [11C]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-

 acknowledge.

| Table 2. Regional [11C]AFM BP_{ND} in PTSD and Healthy Control Study Groups Outside of Amygdala A Priori Region of Interest |
|---|---|---|---|---|---|
| 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 |
| Raphé | 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 [11C]AFM binding potential (BP_{ND}) values between PTSD and health control subjects. Data presented in mean ± SD.

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

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ity in [11C]AFM BP_A. 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 [11C]AFM BP_A and specific PTSD symptoms as measured by CAPS score, and in contrast the association between lower [11C]AFM BP_A 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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