Serotonin Transporter Binding after Recovery from Bulimia Nervosa

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**ABSTRACT**  

**Objective:** Physiological and pharmacological studies indicate that altered brain serotonin (5-HT) activity could contribute to a susceptibility to develop appetitive and behavioral alterations that are characteristic of bulimia nervosa (BN).  

**Method:** Eight individuals recovered from BN (REC BN) and eight healthy control women were scanned with [11C]DASB and positron emission tomography imaging of the 5-HT transporter (5-HTT). Logan graphical analysis was applied, and parametric binding potential (BP<sub><sup>n</sup>ondisplaceable</sub>) images were generated. Voxel-by-voxel <i>t</i>-tests and a region of interest (ROI) analysis were conducted.  

**Results:** REC BN had significantly lower [11C]DASB BP<sub><sup>n</sup>ondisplaceable</sub> in midbrain, superior and inferior cingulate and significantly higher [11C]DASB BP<sub><sup>n</sup>ondisplaceable</sub> in anterior cingulate and superior temporal gyrus in the voxel-based analysis. ROI analysis indicated lower [11C]DASB BP<sub><sup>n</sup>ondisplaceable</sub> in midbrain (<i>p</i> = .07), containing the dorsal raphe, in REC BN, consistent with our earlier studies.  

**Discussion:** These preliminary findings of a small-scale study confirm and extend previous data suggesting that ill and recovered BN have altered 5-HTT measures, which potentially contribute to BN symptomatology and/or differential responses to medication. © 2011 by Wiley Periodicals, Inc.

**Keywords:** bulimia nervosa; serotonin; transporter; positron emission tomography; imaging; midbrain

**Introduction**  

Bulimia nervosa (BN) is a disorder of unknown etiology that tends to have an onset during adolescence in women. It is characterized by restrictive eating alternating with binge eating and purging, as well as concomitant body image distortions. Most individuals maintain a normal body weight. BN is commonly associated with mood disturbances, obsessionality, and poor impulse control.¹–³

Physiological and pharmacological data support the possibility that altered central nervous system serotonin (5-HT) neurotransmitter activity could contribute to a susceptibility to develop appetitive and behavioral alterations in BN.²,⁴ Altered 5-HT activity in BN could be a consequence of pathological dietary behaviors that occur during the ill (symptomatic) state. However, people who have recovered (REC) from BN continue to have 5-HT alterations, as well as behavioral symptoms consistent with a dysregulation of 5-HT neuronal pathways,⁵,⁶ raising the possibility that such alterations are trait-related and contribute to the pathogenesis of this disorder.

There has been much interest in determining whether individuals with BN have alterations of the 5-HT transporter (5-HTT). The 5-HTT is located presynaptically on the axon terminals of 5-HT neurons and on the cell bodies of serotonin neurons.⁷ A main function of the 5-HTT is to transport serotonin from the synapse back into the presynaptic neuron for storage and future release. This contributes to modulating 5-HT neurotransmission and intrasynaptic 5-HT availability. In fact, imaging studies have found reduced 5-HTT levels in symptomatic individuals who binge compared with controls. Tauscher et al.,⁸ using single-photon emission...
computed tomography (SPECT), found reduced \(^{123}\text{I}\)\(\beta\)-CIT binding in the thalamus and striatum in 10 individuals who were ill with BN. Reduced \(^{123}\text{I}\)\(\beta\)-CIT binding values in the brainstem have also been found using SPECT in participants ill with binge eating disorder.\(^9\) We previously evaluated midbrain (dorsal raphe), striatum, thalamus, and subgenual cingulate in subgroups of patients REC from eating disorders using positron emission tomography (PET) and \(^{11}\text{C}\)McN5652, an older radioligand for the 5-HTT, and showed lower dorsal raphe values in REC BN than in control women (CW). MANOVA confirmed global group differences and univariate ANOVA confirmed group differences for dorsal raphe and antero-ventral striatum although individual group contrasts for CW versus REC BN did not reach statistical significance.\(^10\) In addition, Steiger et al.\(^11\) showed that individuals REC from BN have reduced platelet \(3\text{H}\)paroxetine binding when compared with CW.

This is the first study to use PET and \(^{11}\text{C}\)-3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile (\([11\text{C}]\text{DASB}\)), a newer ligand, to investigate 5-HTT binding in REC BN individuals. \([11\text{C}]\text{DASB}\) is superior to \(^{123}\text{I}\)\(\beta\)-carbomethoxy-3 beta-(4-iodophenyl) tropone (\([123\text{I}]\beta\)-CIT) or trans-1,2,3,5,6,10-\(\beta\)-hexahydro-6-[4-(methylthio)phenyl] pyrrolo-[2,1-a]-isoquinoline (\([11\text{C}]\text{McN5652}\)) because of higher specific-to-nonspecific ratios.\(^12\) Previous data have shown that \([11\text{C}]\text{DASB}\) can reliably assess 5-HTT binding in brain regions including the midbrain, thalamus, striatum, medial temporal lobe, and anterior cingulate, as described by Frankle et al.\(^13\) On the basis of previous findings, we predicted that REC BN would have diminished \([11\text{C}]\text{DASB}\) \(\text{BP}_{\text{ND}}\) in midbrain and striatal regions compared to healthy CW.

**Method**

**Participants**

Eight control women who had REC from BN were compared to eight control women (CW). REC BN must have met a lifetime diagnosis of BN with no history of AN. The individuals were recruited as previously described for earlier independent samples.\(^14,15\) All individuals, both REC BN and CW, underwent four levels of screening: (1) a brief phone screening, (2) an intensive screening assessing psychiatric history, lifetime weight, binge eating and methods of weight loss/control, and menstrual cycle history as well as eating pattern for the past 12 months, (3) a comprehensive assessment using structured and semi-structured psychiatric interviews conducted by phone or in person, and (4) a face-to-face interview and physical examination with a psychiatrist. To be considered “recovered,” participants had to (1) maintain a weight above 85% average body weight, (2) have regular menstrual cycles, and (3) have not binged, purged, or engaged in significant restrictive eating patterns for at least 1 year before the study. Restrictive eating patterns were defined as regularly occurring behaviors, such as restricting food intake, restricting high-caloric food, counting calories, and dieting. In addition, participants must not have used psychoactive medication such as antidepressants or met criteria for alcohol or drug abuse or dependence, major depressive disorder, or severe anxiety disorder within 3 months of the study. Normal volunteer women were recruited through local advertisements and did not have any stigmata suggestive of an eating disorder (ED). They had maintained an average body weight between 90% and 120% since menarche, had normal menstrual cycles, and had never binged, purged, or engaged in significant restrictive eating patterns suggestive of an ED. Their lab tests, medical and psychiatric histories, and physical and neurological examinations indicated no current or past psychiatric, medical, or neurological illness.

This study was conducted according to the institutional review board regulations of the University of California, San Diego, and all participants gave written informed consent.

The Structured Clinical Interview for DSM-IV Axis I Disorders (SCID I)\(^16\) was used to assess the lifetime prevalence of Axis I psychiatric disorders. The eating disorder diagnosis was made by a modified version of module H of the SCID I. Current psychopathology was assessed with a battery of standardized instruments including the Beck Depression Inventory,\(^17\) the Spielberger State-Trait Anxiety Inventory,\(^18\) the Eating Disorders Inventory,\(^19\) the Yale-Brown Obsessive Compulsive Scale,\(^20,21\) the Barratt Impulsiveness Scale,\(^22\) and the Temperament and Character Inventory\(^23\) for assessment of harm avoidance, novelty seeking, and reward dependence.

**\([11\text{C}]\text{DASB}\) Radiochemistry Preparation**

\([11\text{C}]\text{DASB}\) was prepared at the UCSD Center for Molecular Imaging, PET Radiochemistry Laboratory. Radioactive \([11\text{C}]\text{CO}_2\) was produced by the \(14\text{N}(p,\gamma)^{11}\text{C}\) nuclear reaction at the CTI RDS 111 cyclotron operated by PETNET. The \([11\text{C}]\text{CO}_2\) was converted into \([11\text{C}]\text{CH}_3\text{I}\) or sequentially swept into a silver triflate oven to produce \([11\text{C}]\text{methyl triflate}\). Desmethyl-DASB precursor was reacted using the loop injection port procedure. Then, the contents of the loop were quantitatively injected into a semi-preparative liquid chromatography column. The \([11\text{C}]\text{DASB}\) fraction was isolated, passed through a 0.22-\(\mu\)m sterile Millex-GV membrane filter, and collected in a 30-ml sterile vial containing 10 ml of 0.9% sterile saline.
For [11C]DASB, the radiochemical yield was 18–22% at the end of synthesis. The total synthesis time was 50 ± 5 min. The average specific activity at the end of the analysis averaged 13 ± 2 Ci/μmol. The studies were carried out under IND 74, 254 for [11C]DASB.

**Image Acquisition**

Participants were scanned at around 1 pm depending on [11C]DASB radiochemistry preparation and were asked to eat their usual breakfast in the morning after they got up. The breakfast was not standardized. Participants were positioned in a Siemens/CTI ECAT HR+ scanner (Siemens Medical Systems, 810 Innovation Dr., Knoxville, TN) with the head oriented such that the lowest imaging plane was approximately 1 cm above and parallel to the cantho-meatal line. A vacuum head holder attached to the PET scanner bed minimized head movement during the scan acquisition. A 20-min transmission scan with integral germanium rods was performed for scatter and attenuation correction before radiopharmaceutical injection. [11C]DASB (10 mCi) was injected and dynamic frame-based emission scanning was initiated: 6 s/frame × 15 frames, 10 s/frame × 3 frames, 30 s/frame × 12 frames, one frame at 120 s, 300 s/frame × 2 frames, 600 s/frame × 7 frames, giving a total of 40 frames and 90 min of imaging. A calibration was performed between the dose calibrator, gamma well counter, and scanner (Siemens Medical Systems, 810 Innovation Dr., Knoxville, TN) with the head oriented such that the lowest imaging plane was approximately 1 cm above and parallel to the cantho-meatal line. A vacuum head holder attached to the PET scanner bed minimized head movement during the scan acquisition. A 20-min transmission scan with integral germanium rods was performed for scatter and attenuation correction before radiopharmaceutical injection. [11C]DASB (10 mCi) was injected and dynamic frame-based emission scanning was initiated: 6 s/frame × 15 frames, 10 s/frame × 3 frames, 30 s/frame × 12 frames, one frame at 120 s, 300 s/frame × 2 frames, 600 s/frame × 7 frames, giving a total of 40 frames and 90 min of imaging. A calibration was performed between the dose calibrator, gamma well counter, and scanner using an F-18 cylinder calibration on the same day of the dose calibrator, gamma well counter, and scanner. 

**Computation of Binding Potential**

Logan graphical analysis was applied to the [11C]DASB images, and parametric images were generated on a voxel-by-voxel basis using the cerebellar time-activity curve as the input function. The Logan graphical analysis is a technique that allows the estimation of local tissue distribution volumes \( V_T \) of reversibly bound radiopharmaceuticals by generating a plot in which the y-axis consists of ratios of integrated and measured tissue time-activity data and the x-axis typically contains the ratio of integrated plasma activity and measured tissue activity. The slope of this plot represents \( V_T \). A modification of the Logan analysis, which incorporates an assumed constant average tissue to plasma clearance of the cerebellum as the reference tissue region, was used. The binding potential (BP) was calculated with the formula:

\[
\text{BP}_{\text{nondisplaceable}} = \left( \frac{V_T}{V_{\text{ND}}} \right) - 1
\]

This assumption was based on the negligible density of 5-HTT in the cerebellum. The cerebellar radioactivity detected on the images was due to free unbound blood pool activity.

The resulting parametric images have cerebellar values of approximately zero (unity) because the hand-drawn regions of interest in the cerebellum (similar to those used elsewhere) were used as the plasma input function.

Image Analysis

[11C]DASB parametric images had the skull removed with the Brain Extraction Tool (BET), were coregistered, and were standardized to the Montreal Neurological Institute (MNI) brain using the Linear Image Registration Tool (FLIRT) of the Functional Magnetic Resonance Imaging of the Brain [FMRIB] Software Library (FSL). Voxel-by-voxel t-tests comparing the two participant groups were computed with AFNI 3dttest using a white matter mask and compared using the Brainflow visualizer (Fig. 1).

Statistical Analysis

On the basis of earlier studies in eating disorders, t-test maps are presented with a \( p < .05 \) threshold for analysis which confirm other previously published results and a color bar extending up to .005 for exploratory analyses where the result has not been published before.

In addition, a region of interest (ROI) analysis was performed. The following ROIs from AFNI were used to match our previously used ROIs for the assessment of...
FIGURE 1. Mean [11C]DASB \( V_T/V_{ND} \) in REC BN and CW. Top Row: Images of [11C]DASB \( V_T/V_{ND} \) in sagittal section. Left: Mean [11C]DASB \( V_T/V_{ND} \) in eight CW standardized to the MNI anatomical brain; color bar at right edge shows cerebellar values at reference level of approximately 1.0 (1.0 not subtracted). Right: [11C]DASB \( V_T/V_{ND} \) in REC BN on same color scale. Bottom Row: Left: Regions with [11C]DASB \( V_T/V_{ND} \) lower than CW are shown in parasagittal slice \((y = -9)\) located at maximum \(Z\) value for largest cluster (#1) in superior cingulate, Brodmann area 23. Cluster numbers correspond to Table 2. Threshold for one-tailed test, \( t = -1.76, p < .05 \) is the right edge of the color bar under left sagittal image and levels 0.025, 0.01 are marked. The left edge of the color bar is the \( p < .005 \) threshold, and all \( t \) values \( > 2.97 \) are the color of the left edge. Using this threshold combined with a full \( p\)-value color bar (rather than merely a threshold) the reader can either compare the data with our earlier findings, the current ROI MANOVA findings, or our directional hypotheses, or alternately view all analyses as exploratory. Note this is a parasagittal slice so that the medial frontal cortex is present for region 1 (Talairach daemon, see Table 2). Bottom row: Right: Regions with [11C]DASB \( V_T/V_{ND} \) higher than CW. Sagittal slices at \( x \) centers of largest clusters. Vertical color bar at right edge of illustration shows one-tailed \( p \)-values for both positive and negative values with low threshold light orange \( p < .05 \), one-tailed for \( t > 1.76, df = 14 \) (marked with black triangle) and high threshold \( t = 2.97 \) for \( p > .005 \) (marked with red-orange triangle); \( t \)-values lower than 1.76 are not presented as colored areas. Each cluster number corresponds to Table 2 entries giving the \( xyz \) location of the cluster center, \( Z \) maximum value, volume of each voxel cluster, and anatomical area as indicated by Talairach Daemon. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

5-HTT binding: dorsal raphe, a modification of the “red nucleus” AFNI region, by moving it to the midline and extending it dorsoventrally; anterior ventral striatum, anterior half of the AFNI caudate region; thalamus; dorsal caudate; and subgenual cingulate (Brodmann Area 25/11). Since we have previously reported significant group differences with MANOVA and follow-up ANOVA, we have presented univariate follow-up statistics in replication with one-tailed tests.

Results

Demographics and Assessments

The REC BN and CW groups did not differ significantly in age, body-mass index (BMI), or assessments (Table 1). Lifetime comorbidity in REC BN included history of major depressive disorder \((n = 4)\), depressive disorder, not otherwise specified \((n = 2)\), obsessive-compulsive disorder \((n = 3)\), alcohol dependence \((n = 2)\), cocaine dependence \((n = 1)\), and any anxiety disorder \((n = 3)\). All eight REC BN had at least one lifetime comorbid psychiatric disorder.

Maps of [11C]DASB \( V_T/V_{ND} \) and Differences between Recovered BN versus CW

[11C]DASB \( V_T/V_{ND} \) values showed highest levels in midbrain regions, consistent with previous reports. Mean values were somewhat lower than individual peak values, reflecting the effects of image averaging. The cingulate appeared to have the highest cortical values. Quantitative images with color bar are shown in Figure 1. REC BN had higher values than CW in superior temporal cortex and anterior cingulate (Fig. 1, Table 2). Lower
[11C]DASB $V_T/V_{ND}$ values were observed in midbrain regions as well as superior and inferior cingulate (Fig. 1, Table 2).

**Region of Interest Analysis**

An overall MANOVA showed a significant group by region difference ($F = 8.41$ $df = 4,11$ $p = .0023$; Wilks’ lambda = 0.246) (Table 3), with REC BN showing lower [11C]DASB $BP_{ND}$ in the dorsal raphe at a level approaching significance ($p = .07$).

**Discussion**

This study, which is the first to use PET and [11C]DASB in REC BN participants, lends further support to the notion that BN have altered 5-HTT functional activity after recovery. Two methods of analysis were used in this study: a voxel-by-voxel analysis and a ROI-based analysis. The voxel-based analysis showed that REC BN had significantly diminished 5-HTT measures in the midbrain, most likely involving the dorsal raphe, which contains the cell bodies of serotonin neurons. Importantly, the voxel-by-voxel-based data also showed that REC BN have diminished [11C]DASB binding in the superior and inferior cingulate, as well as increased binding in the superior temporal cortex and anterior cingulate (subgenual cingulate). These preliminary data reflecting increased and decreased binding will require confirmation in future larger scale studies. Subgenual cingulate and mesial temporal regions are part of the ventral limbic system and thus play a pivotal role in modulating emotionality and integration of cognition and mood. It is possi-
Table 3. Regional [11C]DASB BPND between groups

<table>
<thead>
<tr>
<th></th>
<th>CW (n = 8)</th>
<th>RECN (n = 8)</th>
<th>ANOVA Uncorrected p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal raphe</td>
<td>0.88 ± 0.15</td>
<td>0.71 ± 0.18</td>
<td>.07</td>
</tr>
<tr>
<td>Anterior ventral striatum</td>
<td>0.51 ± 0.16</td>
<td>0.46 ± 0.08</td>
<td>.41</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.51 ± 0.14</td>
<td>0.45 ± 0.06</td>
<td>.24</td>
</tr>
<tr>
<td>Dorsal caudate</td>
<td>0.38 ± 0.12</td>
<td>0.35 ± 0.12</td>
<td>.44</td>
</tr>
<tr>
<td>Subgenual cingulate</td>
<td>0.05 ± 0.07</td>
<td>0.10 ± 0.09</td>
<td>.24</td>
</tr>
</tbody>
</table>

CW, healthy control women; RECN, recovered; BN, bulimia nervosa; SD, standard deviation. Overall MANOVA showed a significant group by region difference (see text, f = 8.41 df = 4.11 p = 0.0023; Wilks’ lambda = 0.246).

Possible that aberrant function of these circuits might contribute to extremes of behavioral disinhibition, disorganization, and loss of self-control in BN.

The “a priori” choice of regions for the ROI analysis in this study was based on two factors. First, 5-HTT has a large range of region-specific functional activity throughout the brain. Thus, for the ROI-based analysis, we have chosen ROIs with moderate to high binding, including the midbrain (dorsal raphe), striatum (anterior ventral striatum and dorsal caudate), thalamus, and subgenual cingulate. Second, these ROIs were previously selected for the assessment of 5-HTT binding in REC EDs in our [11C]McN5652 study. That study used a ROI-based analysis with [11C]McN5652 and showed that nine REC BN had a nonsignificantly lower midbrain (dorsal raphe) binding compared to 10 CW (t = 0.90, p = ns, effect size (group difference/group SD) = 0.41).

Because of limited funding, we were only able to study eight REC BN and eight CW with [11C]DASB. Although the MANOVA ROI analysis showed a significant group by region difference, post-hoc individual ROI analysis showed a group difference in the dorsal raphe which approached significance (p = .07, uncorrected). PET studies with radioligands are an expensive and difficult technology. Consequently, studies to date in REC BN participants have been underpowered. Still, these data support the need for larger scale studies that are adequately powered to confirm these findings.

A better understanding of altered 5-HTT function may have important clinical relevance for BN. First, selective serotonin reuptake inhibitors (SSRIs) and other antidepressants are commonly used in BN. However, many individuals have partial to poor response, or response diminishes over time. Second, 5-HTT function has been associated with extremes of impulse control in BN. The binding of the 5-HTT on PET presumably reflects 5-HTT density and/or affinity. [11C]DASB is not displaced from 5-HTT sites by physiologically relevant serotonin concentrations. One model proposes a clearance effect of 5-HTT, with less functioning 5-HTT associated with greater extracellular 5-HT. This is consistent with our studies’ findings of elevated cerebrospinal fluid measures of the major metabolite of 5-HT, 5-hydroxyindoleacetic acid. One possibility, although conjectural, is that those individuals with reduced 5-HTT binding may have increased extracellular 5-HT, which in turn will result in altered 5-HT receptor activation, and thus may contribute to behavioral symptoms. It is worth noting that REC BN individuals tend to have higher binding of 5-HT1A postsynaptic receptors and autoreceptors. The cause and effect relationship between altered 5-HT1A and 5-HTT function in REC BN remains to be determined. Still, these data raise questions as to whether individuals with aberrant 5-HTT function may respond differently to SSRIs, or may be prone to dysregulated impulse control. Such insights may allow for the development of new strategies that could result in improved treatments.

This small-scale study suggests that [11C]DASB is superior to [11C]McN5652 in terms of revealing differences of 5-HTT binding between REC BN and CW. Because of its higher specific-to-nonspecific binding ratios, [11C]DASB showed binding differences in regions, such as cingulate and temporal cortices, that were not found with [11C]McN5652. However, this study has certain limitations. For instance, we were only able to compare the ROI-based analysis between the current and the previous study, as we did not perform a voxel-by-voxel analysis in the [11C]McN5652 study. In addition, our dorsal raphe assessment was based on the MNI brain, and not hand drawn. Therefore, variations in the exact position of the raphe, as well as the other assessed ROIs, may have created random error, which limited our power to detect further differences in the ROI-based analysis. Moreover, though persistent alterations in monoamine activity after recovery raise the question of whether this is a premorbid vulnerability for developing ED symptoms, it is also possible that chronic disturbances of nutrition during the ill state might contribute to a persistent “scar,” caused by chronic malnutrition, in recovered individuals. Patients were off medication (e.g., SSRIs) for greater than 3 months, so it is unlikely that persistent effects of 5-HT active medication accounted for the results. Lastly, our findings are limited by the variable of seasonality, as demonstrated previously. To evaluate this, we grouped individual scans for each diagnostic group (REC BN, CW) according to season of scan with the equinoxes as the cut-off between the fall and winter season and the spring.
and summer season: for fall and winter, the scan dates were September 22 to March 20 (1 CW, 3 REC BN), and for spring and summer, the scan dates were March 21 to September 21 (7 CW, 5 REC BN). Thus, we have a too small sample to carry out statistical analysis. However, we note that Kalbitzer et al.39 showed no seasonal effect for the midbrain, our area of strongest finding. Praschak-Rieder et al.38 found higher binding in fall and winter, but since the majority of both our CW and REC BN were studied in summer it would be difficult to attribute our results entirely to seasonal bias.

The findings of this study support the hypothesis of altered 5-HTT binding in individuals recovered from BN, specifically in the midbrain and temporal and cingulate cortices. Although the necessity for larger scale studies remains, insight gained from this and previous studies may prove invaluable to our improved understanding of BN and to the development of superior pharmacological treatment strategies for the disease.

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