Positron Emission Tomography Quantification of Serotonin Transporter in Suicide Attempters with Major Depressive Disorder

Jeffrey M. Miller, Natalie Hesselgrave, R. Todd Ogden, Gregory M. Sullivan, Maria A. Oquendo, J. John Mann, and Ramin V. Parsey

Background: Several lines of evidence implicate abnormal serotonergic function in suicidal behavior and completed suicide, including low serotonin transporter binding in postmortem studies of completed suicide. We have also reported low in vivo serotonin transporter binding in major depressive disorder (MDD) during a major depressive episode using positron emission tomography (PET) with \[^{[1]}C\]McN5652. We quantified regional brain serotonin transporter binding in vivo in depressed suicide attempters, depressed nonattempters, and healthy controls using PET and a superior radiotracer, \[^{[1]}C\]DASB.

Methods: Fifty-one subjects with DSM-IV current MDD, 15 of whom were past suicide attempters, and 32 healthy control subjects underwent PET scanning with \[^{[1]}C\]DASB to quantify in vivo regional brain serotonin transporter binding. Metabolite-corrected arterial input functions and plasma free-fraction were acquired to improve quantification.

Results: Depressed suicide attempters had lower serotonin transporter binding in midbrain compared with depressed nonattempters (p = .031) and control subjects (p = .0093). There was no difference in serotonin transporter binding comparing all depressed subjects with healthy control subjects considering six a priori regions of interest simultaneously (p = .41).

Conclusions: Low midbrain serotonin transporter binding appears to be related to the pathophysiology of suicidal behavior rather than of major depressive disorder. This is consistent with postmortem work showing low midbrain serotonin transporter binding capacity in depressed suicides and may partially explain discrepant in vivo findings quantifying serotonin transporter in depression. Future studies should investigate midbrain serotonin transporter binding as a predictor of suicidal behavior in MDD and determine the cause of low binding.

Key Words: \[^{[1]}C\]DASB, depression, midbrain, pathophysiology, PET, serotonin transporter, suicide

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0006-3223/$36.00
http://dx.doi.org/10.1016/j.biopsych.2013.01.024

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binding between MDD subjects and healthy control subjects (15–17). One study found higher [11C]DASB binding in MDD subjects than healthy control subjects across a broad anatomic distribution (18), while two others reported lower [11C]DASB binding, one in the thalamus specifically (19) and another across a broad range of cortical and subcortical regions (20). These divergent findings may be partially explained by demographic and clinical differences in study populations, including rates of suicidal behavior, and by different PET outcome measures employed.

In addition to examining effects of diagnosis on binding, we previously examined the effect of a functional promoter polymorphism in the 5-HTT gene (SLC6A4, polymorphism: serotonin-transporter-linked polymorphic region [5-HTTLPR]) that regulates in vitro expression of 5-HTT (21,22). A gene-environment interaction between the 5-HTTLPR polymorphism and the severity of stressful life events may predict the presence and severity of subsequent depression, as well as the later occurrence of suicidal behavior (23–25). We found no effect of 5-HTTLPR on 5-HTT binding using [11C]McN5652 (26). In vivo findings from other studies are discordant [reviewed in (27)]. We also reported an effect of early life stress on 5-HTT binding using [11C]McN5652, with low 5-HTT binding in MDD subjects reporting childhood abuse (28), but the sample size was too small to examine gene-environment interactions.

In the current study, we used [11C]DASB in the largest MDD cohort examined to date to examine the relationship between depression and suicide attempt history on serotonin transporter binding in vivo. Our primary hypotheses were that MDD subjects with a history of prior suicide attempt would have low [11C]DASB binding compared with control subjects and MDD nonattempters in the regions identified from postmortem studies of suicide: ventral prefrontal cortex (vPFC), anterior cingulate (ACN), and midbrain (containing dorsal raphe nuclei, which cannot be reliably delineated on magnetic resonance imaging [MRI]); and [11C]DASB binding would be low in unmedicated current MDD subjects as compared with healthy control subjects across the six brain regions identified in our study using [11C]McN5652. We anticipated that 5-HTTLPR genotype would not be associated with [11C]DASB binding. In exploratory analyses, we examined the effects of reported childhood abuse and of a gene-environment interaction between 5-HTTLPR and reported childhood abuse on [11C]DASB binding.

Methods and Materials

Sample

Currently depressed participants (n = 51) with MDD and healthy control subjects (n = 32) were recruited prospectively through print and online advertisements. Eligibility was assessed by psychiatric and medical history, chart review, physical examination, routine blood tests, pregnancy test, and urine toxicology. Axis I diagnoses were based on the Structured Clinical Interview for DSM-IV (29), conducted by doctoral or masters’ level psychologists and reviewed in a consensus conference of research psychologists and psychiatrists. Inclusion criteria for MDD subjects included 1) current major depressive episode; 2) 17-item Hamilton Depression Rating Scale (HDRS) ≥16 at screening; 3) age 18 to 65 years; and 4) off all psychotropic and other types of drugs likely to interact with 5-HTT for a minimum of 14 days (off antipsychotics for ≥3 weeks). While this was the minimum duration according to inclusion criteria, 11 MDD subjects were antidepressant-naïve, and among the 40 MDD subjects with prior psychotropic medication exposure, the mean duration off psychotropic medication at time of scan was 122 weeks (median = 11.5 weeks, range = 14 days to 35.9 years). Short-acting benzodiazepines were allowed for distressing anxiety or insomnia up to 72 hours prior to PET scanning but were only used by six subjects. Exclusion criteria included 1) current or past psychotic illness or bipolar disorder, anorexia nervosa or bulimia nervosa in the past year, and drug or alcohol abuse within the past 2 months or dependence within 6 months; 2) first-degree family history of schizophrenia in subjects <33 years old to exclude individuals possibly presenting with the prodrome of schizophrenia [mean onset of schizophrenia = 21.4 in male subjects and 26.8 in female subjects (30)]; 3) significant active physical illness; 4) lack of capacity to consent to study participation; 5) pregnancy or lactation among women; 6) previous head injury with loss of consciousness; and 7) exposure to 3,4-methylenedioxymethamphetamine on more than two occasions.

For healthy control subjects, inclusion criteria included: 1) absence of current or past DSM-IV Axis I diagnosis, with the exception of specific phobia; 2) absence of cluster B personality diagnosis as assessed using the Structured Clinical Interview for DSM-IV Axis II Personality Disorders (31); and 3) age 18 to 65 years. Exclusion criteria included MDD exclusion criteria 3 through 7 above, as well as: 1) past or present alcohol/substance abuse or dependence; 2) first-degree relative with history of major depression, schizophrenia, schizoaffective disorder, or suicide attempt; and 3) two or more first-degree relatives with a history of substance dependence.

The Beck Depression Inventory (32) and HDRS (33) were used to assess depression severity and functional impairment. Lifetime history of aggression was measured by the Brown Goodwin Aggression History Scale (34). The Columbia Suicide History Form was used to assess suicide attempt history (35), and the Beck Medical Lethality Scale was used to rate the degree of medical damage caused by their most lethal attempt (36). The scale scores medical damage from 0 (no injury) to 8 (fatal), with anchor points dependent on the method of attempt. In a semistructured interview, participants were asked whether they experienced physical and/or sexual abuse over the course of their lifetime. If subjects endorsed a history of abuse, they were asked whether the abuse took place before age 15.

Genotyping

Genotyping of the triallelic 5-HTTLPR polymorphism (L_A, L_G, and S) was performed as previously described (21). The triallelic genotypes were classified by their reported level of in vitro expression as follows: L_A was reclassified as higher expressing L'; L_G and S were classified as lower expressing S'.

Radiochemistry and Input Function Measurement

Preparation of [11C]DASB and measurement of arterial input function, metabolites, and plasma free fraction (f_p) were performed as previously described (37,38). The chemical purity of [11C]DASB was ≥95%. Injected mass, injected dose, and f_p did not differ between MDD and control subjects or between MDD suicide attempters and nonattempters (Table 1).

PET Protocol

Details of the PET protocol are described elsewhere (38). Briefly, a venous catheter was used for radiotracer injection and an arterial catheter was used to obtain arterial samples for the input function. A polyurethane head holder system (Soule Medical, Tampa, Florida) was molded around the subject’s head.
for immobilization purposes. Positron emission tomography imaging was performed with the ECAT HR+ (Siemens/CTI, Knoxville, Tennessee). A 10-minute transmission scan was obtained before radiotracer injection. At the end of the transmission scan, [11C]DASB was administered intravenously as a bolus over 30 seconds (Table 1). Emission data were collected in three-dimensional mode for 100 minutes with 19 frames of increasing duration (38).

Magnetic Resonance Imaging

Acquisition of T1-weighted MRI images for co-registration of PET images and identification of ROIs was performed as previously described using a 1.5 T Signa Advantage or a 3 T Signa HDx system (General Electric Medical Systems, Milwaukee, Wisconsin) (39).

Image Analysis

To correct for subject motion, PET frames were registered to the eighth frame using the FMRI B image registration tool, FLIRT, version 5.0 (FMRIB Image Analysis Group, Oxford, United Kingdom). An automated algorithm identified ROIs (midbrain, vPFC, putamen, amygdala, thalamus, hippocampus, and ACN), as well as cerebellar gray matter, on individuals’ T1-weighted MRIs. Each subject’s mean PET image was co-registered to their MRI using FLIRT, optimized as previously described (41). Time activity curves were generated by plotting measured activity within ROIs over the time course of the PET acquisition.

Outcome Measure Estimation

As we previously demonstrated that no brain region is devoid of specific binding with [11C]DASB (42), we used an outcome measure that does not rely on a reference region (REF): $V_T/f_P$ (where $V_T$ = volume of distribution in the region of interest). This outcome measure has been used in several studies by different groups in cases where a reference region is not available (43–47). [11C]DASB regional $V_T$ values were derived using likelihood estimation in the graphical approach, which reduces the noise-dependent bias inherent in the graphical approach (48,49). Brain activity was corrected for the contribution of plasma activity, assuming a 5% blood volume in the regions of interest (50). For purposes of comparison with other [11C]DASB studies using different outcome measures, the following types of binding potential (BP) were also estimated [see (51) for more details]: $BP_E\ [V_T(ROI) - V_T(REF)]/f_P$; $BP_P\ [V_T(ROI) - V_T(REF)]$; and $BP_{ND}\ [V_T(ROI) - V_T(REF)]/V_T(REF)$, using cerebellar gray matter as reference region. As there is approximately 30% specific/displaceable [11C]DASB binding in the reference region (42), $V_T(REF)$ overestimates the distribution volume of the nondisplaceable compartment ($V_{ND}$) (51), leading to biases in estimates of these binding potential measures (52). Results with these alternate outcome measures are described concisely in the Results section and are presented in greater detail in Supplement 1.

Results

Demographics

Demographic and clinical variables are presented in Table 2. Among MDD participants, 36 (70.6%) had at least one comorbid Axis I diagnosis, including 8 (15.7%) with remitted alcohol or substance use disorder, 32 (62.8%) with current or past anxiety disorder, 4 (7.8%) with lifetime dysthymia, 3 (5.9%) with current attention-deficit/hyperactivity disorder, and one (2%) with remitted bulimia. Rates of these comorbidities did not differ between MDD attempters and nonattempters (remitted alcohol or substance use disorder: Fisher’s exact $p = .41$; comorbid anxiety: $p = .13$; Table 2). MDD attempters had an earlier age of onset than MDD nonattempters. While age differed between MDD subjects and control subjects, it did not differ between MDD attempters and MDD nonattempters (Table 2). 5-HTTLPR genotype did not differ between MDD subjects and control subjects or between MDD attempters and MDD nonattempters (Table 3).

Possible Covariates

Across the six ROIs, there was no effect of sex ($F = 1.55, df = 1.79, p = .22$) or prior antidepressant exposure ($F = .68, df = 1.79, p = .41$) on 5-HTT binding. Because the MDD and control groups

Table 1. [11C]DASB PET Scan Parameters of the Sample

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects ($n = 31$)</th>
<th>MDD ($n = 51$)</th>
<th>MDD Suicide Attempters ($n = 15$)</th>
<th>MDD Nonattempters ($n = 36$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injected Dose (mCi)</td>
<td>16.14 ± 2.36</td>
<td>16.28 ± 2.14</td>
<td>−.27,.79</td>
<td>16.34 ± 1.80</td>
</tr>
<tr>
<td>Corrected Mass (μg)</td>
<td>4.36 ± 2.31</td>
<td>4.49 ± 2.18</td>
<td>−.27,.71</td>
<td>5.07 ± 2.30</td>
</tr>
<tr>
<td>Free Fraction ($f_P$)</td>
<td>.12 ± .03</td>
<td>.11 ± .02</td>
<td>1.59,.12</td>
<td>.11 ± .03</td>
</tr>
</tbody>
</table>

MDD, major depressive disorder; PET, positron emission tomography.

Statistics

To borrow strength across all ROIs and properly account for correlation among ROIs measured on the same subject, we fit linear mixed-effects models to the ROI-level $V_T/f_P$ estimates with region and diagnostic group as fixed effects and subject as the random effect, and this approach was taken for all analyses involving more than one ROI. Other fixed effects considered in linear mixed-effects modeling include sex, age, antidepressant exposure, depression severity, and genetic and environmental factors. Data entered in linear mixed-effects models were first log transformed to remedy slight skewness of $V_T/f_P$ estimates (53–55), to stabilize the variance, and because our principal hypothesis of a difference between groups specifies that differences in each ROI are proportional to each ROI’s binding level. Log transformation has been used in numerous PET studies by our group and others to address these issues (12,26,28,55–67). As the natural log is a monotone transformation, demonstrating a difference in log($V_T/f_P$) is equivalent to demonstrating a difference (in the same direction) in $V_T/f_P$. Estimated $V_T/f_P$ values were weighted in the model according to standard errors computed using a bootstrap algorithm taking into account errors in metabolite, plasma, and brain data (68). Analyses on single regions were performed using linear models. SPSS Statistics 19 (IBM Corporation, New York, New York; http://www.spss.com/software/statistics/) was used to perform t tests. All other analyses were performed in R 2.10.0 (69).
Table 2. Clinical and Demographic Characteristics of the Sample

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects</th>
<th>MDD</th>
<th>Control vs. MDD (χ², p Value)</th>
<th>MDD Suicide Attempters</th>
<th>MDD Nonattempters</th>
<th>Attempter vs. Nonattempter (χ², p Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female Subjects</td>
<td>16 (51.6%)</td>
<td>28</td>
<td>(54.9%)</td>
<td>8 (53.3%)</td>
<td>20 (55.6%)</td>
<td>.021, .88</td>
</tr>
<tr>
<td>Inpatient</td>
<td>14 (27.5%)</td>
<td></td>
<td></td>
<td>6 (40.0%)</td>
<td>8 (22.2%)</td>
<td>2.13, .15</td>
</tr>
<tr>
<td>Number with Family History of MDD in First Degree Relatives</td>
<td>25 (49.0%)</td>
<td>5</td>
<td>(33.3%)</td>
<td>5 (33.3%)</td>
<td>20 (55.6%)</td>
<td>2.09, .15</td>
</tr>
<tr>
<td>Current or Past Comorbid Anxiety Disorder</td>
<td>32</td>
<td>7</td>
<td>(46.7%)</td>
<td>25 (69.4%)</td>
<td></td>
<td>2.35, 13</td>
</tr>
<tr>
<td>Axis II Comorbidity</td>
<td>21 (37.2%)</td>
<td></td>
<td></td>
<td>6 (40.0%)</td>
<td>13 (36.1%)</td>
<td>.069, .69</td>
</tr>
<tr>
<td>Race/Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>6 (19.4%)</td>
<td>3</td>
<td>(5.9%)</td>
<td>0 (0%)</td>
<td>3 (8.3%)</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>6 (19.4%)</td>
<td>5</td>
<td>(9.8%)</td>
<td>3 (20%)</td>
<td>2 (5.6%)</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>15 (48.4%)</td>
<td>33</td>
<td>(64.7%)</td>
<td>8 (53.3%)</td>
<td>26 (72%)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>2 (3.2%)</td>
<td>8</td>
<td>(15.7%)</td>
<td>2 (13.3%)</td>
<td>5 (13.9%)</td>
<td></td>
</tr>
<tr>
<td>&gt;1 race</td>
<td>1 (3.2%)</td>
<td>2</td>
<td>(3.9%)</td>
<td>2 (13.3%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (3.2%)</td>
<td>0</td>
<td>(0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Any Current Tobacco Use</td>
<td>2 (6.5%)</td>
<td>7</td>
<td>(13.7%)</td>
<td>1 (6.7%)</td>
<td>6 (16.7%)</td>
<td>.66</td>
</tr>
</tbody>
</table>

| Age (years)                  | 32.6 ± 11.3      | 40.3 ± 10.7 | −3.02, .004 | 38.5 ± 11.5 | 41.0 ± 10.5 | −76, .45 |
| Hamilton Depression Rating Scale (24-Item) | 1.7 ± 2.4 | 24.6 ± 6.4 | −19.01, <.001 | 26.5 ± 6.2 | 23.8 ± 6.4 | 1.40, .17 |
| Beck Depression Inventory    | 1.3 ± 1.7        | 25.7 ± 8.8  | −15.19, <.001 | 27.5 ± 9.2 | 24.9 ± 8.7 | .94, .35 |
| Median Number of Depressive Episodes | 4       | 5           | 2.5          | 1.70, .088b |                       |                                      |
| Median Length of Current Depressive Episode (Weeks)a | 52 | 52 | 52 | 1.15, .25b |                                      |                                      |
| Age at First Depressive Episode | 19.3 ± 10.8 | 14.7 ± 5.8  | 21.2 ± 11.9 | −2.03, .05 |                                      |                                      |
| Number of Suicide Attempters | 15 (29.4%) | 15 (100%) |                                      | | |                                      |
| Mean Number of Attempts       | 2.1 ± 1.6        | 2.3 ± 2.0   |                                      | | |                                      |
| Maximum Lethality of Attemptsd | 1.5 ± 1.6 | 2.3 ± 2.0  |                                      | | |                                      |

MDD, major depressive disorder.

aMann-Whitney test presented as (Z, p value), as data are not normally distributed.
bBest estimate from patient self-report.
cFrom the Beck Medical Lethality Scale (details in Methods and Materials).

dFisher’s exact p value as cells contain values too small to fulfill assumptions of χ² test.

dSerotonin transporter binding differed between control subjects, MDD attempters, and MDD nonattempters in midbrain (F = 3.77, df = 2.78, p = .027; Figure 1), with MDD attempters having lower midbrain binding than both MDD nonattempters (F = 5.88, df = 1.78, p = .031) and control subjects (F = 7.12, df = 1.78, p = .0093); midbrain binding did not differ significantly between MDD nonattempters and control subjects (F = 4.0, df = 1.78, p = .53). Low midbrain 5-HTT binding in MDD suicide attempters compared with MDD nonattempters was significant in all analyses with alternative PET outcome measures examined (BPj: F = 7.27, df = 1.78, p = .0086; BPm: F = 6.15, df = 1.78, p = .015; BPmid: F = 5.51, df = 1.78, p = .0076; Table S1 in Supplement 1). Serotonin transporter binding did not differ as a function of suicide attempt history in the two other regions examined, vPFC and ACN (vPFC: F = 8.7, df = 1.78, p = .35; ACN: F = .13, df = 1.78, p = .72).

Table 3. 5-HTTLP Triallelic Genotype Distribution

<table>
<thead>
<tr>
<th>Goldman Functional Genotype</th>
<th>SS'</th>
<th>LS'</th>
<th>LL'</th>
<th>Fisher’s Exact p Value</th>
<th>S’ Allelic Frequency</th>
<th>L’ Allelic Frequency</th>
<th>χ², p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Subjects (n = 31)</td>
<td>11</td>
<td>13</td>
<td>3</td>
<td>.32</td>
<td>35</td>
<td>19</td>
<td>.21</td>
</tr>
<tr>
<td>MDD (n = 51)</td>
<td>16</td>
<td>22</td>
<td>13</td>
<td></td>
<td>54</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>MDD Attempters (n = 15)</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>.74</td>
<td>18</td>
<td>12</td>
<td>.48</td>
</tr>
<tr>
<td>MDD Nonattempters (n = 36)</td>
<td>10</td>
<td>16</td>
<td>10</td>
<td></td>
<td>36</td>
<td>36</td>
<td></td>
</tr>
</tbody>
</table>

MDD, major depressive disorder.

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Diagnosis Effect

Considering six a priori ROIs simultaneously (dorsal putamen, amygdala, thalamus, hippocampus, midbrain, and anterior cingulate), 5-HTT binding did not differ between MDD and control subjects (F = .69, df = 1.79, p = .41; Table S2 in Supplement 1). This finding was consistent for all alternative outcome measures examined (BP\textsubscript{ND}; F = 1.53, df = 1.79, p = .22; BP\textsubscript{ND}; F = 3.49, df = 1.79, p = .066; BP\textsubscript{ND}; F = 1.79, df = 1.79, p = .19; Table S2 in Supplement 1). Within the MDD group, we did not observe a relationship between depression severity assessed by the HDRS and binding across the six ROIs (F = .009, df = 1.48, p = .93).

Genetic and Environmental Effects

Considering 5-HTTLPR, we did not observe a stepwise effect of the number of L alleles on 5-HTT binding across the six ROIs (F = .04, df = 1.74, p = .84). MDD subjects reporting childhood abuse had higher binding than nonabused MDD subjects across the six ROIs (F = 4.34, df = 1.48, p = .043). There was no gene-environment interaction detected between number of 5-HTTLPR L\textsubscript{a} alleles and childhood abuse status on binding in MDD (F = .65, df = 1.47, p = .42). Including childhood abuse as a covariate did not alter the significance of the contrast of midbrain 5-HTT binding between MDD suicide attempters and nonattempters (F = 6.31, df = 1.78, p = .014).

Discussion

Primary Findings and Comparison with Existing Literature

This study examined the effects of prior suicide attempt and diagnosis in the largest cohort to date of MDD subjects undergoing 5-HTT quantification using PET or single photon emission computed tomography. We observed lower 5-HTT binding in MDD attempters compared with both MDD nonattempters and control subjects in midbrain and no differences as a function of suicide attempt history in vPFC or ACN. In addition, we found no difference in 5-HTT binding between MDD and control groups in six a priori regions. Taken together, these findings suggest that regionally specific, lower 5-HTT binding in midbrain in MDD attempters may be related to the pathophysiology of suicidal behavior, rather than of MDD. The lack of a depression effect on 5-HTT binding is consistent with a series of previous \textsuperscript{11}C\textsuperscript{[DASB] studies using the outcome measure BP\textsubscript{ND} (15–17), although others have reported lower (19,20) and higher (18) \textsuperscript{11}C\textsuperscript{[DASB] binding in MDD using BP\textsubscript{p} and BP\textsubscript{ND}. It is notable that the current finding was replicated with all alternative PET outcome measures examined (BP\textsubscript{p}, BP\textsubscript{ND}, and BP\textsubscript{ND}).

Our data suggest that discrepant \textsuperscript{11}C\textsuperscript{[DASB] PET findings in MDD may be at least partly due to differences in the proportion of suicide attempters in previous samples. Four of six previous \textsuperscript{11}C\textsuperscript{[DASB] MDD studies did not report rates of suicide attempt history in their samples (15–17,19). Of the two \textsuperscript{11}C\textsuperscript{[DASB] studies reporting suicide attempt status, one found lower 5-HTT binding in anteroventral striatum in MDD attempters compared with MDD nonattempters in the same direction as our finding (18). The other study had only 2 attempters out of 12 MDD subjects, which did not allow direct examination of an effect of suicide attempt status on binding (20).

There are some clinical and demographic differences between the MDD attempters and nonattempters in our sample: while depression severity did not differ between attempters and nonattempters, attempters had an earlier onset of major depressive illness, consistent with previous studies (79). This raises the possibility that low midbrain 5-HTT binding among attempters is driven by specific genetic loading associated with early-onset depression (80). Moreover, attempters had a trend toward greater depression chronicity as measured by number of prior major depressive episodes. While this may be a potential confound in the interpretation of our results, we did not find a relationship between age of onset of major depressive illness and number of 5-HTT alleles.

We found no effect of suicide attempt status in vPFC or ACN. The low signal-to-noise ratio in vPFC (binding is only 14% higher
in vPFC than in cerebellar gray matter) may have limited our ability to detect group differences. We did not examine the relationship between regional 5-HTT binding and suicide attempt lethality or objective medical damage, given the limited range of lethality in the current sample.

**Interpretation of Findings**

Low regional 5-HTT binding among MDD attempters may be due to less gene expression. Consistent with our previous findings, 5-HTT binding was not associated with 5-HTTLPR genotype in this study, but other functional promoter 5-HTT loci need to be examined. Additionally, epigenetic differences may drive differential 5-HTT binding: studies in nonhuman primates find that DNA methylation, but not 5-HTT genotype, is significantly associated with peripheral blood mononuclear cell 5-HTT messenger RNA expression (81). Discrepant findings reported regarding the potential association between 5-HTT binding and 5-HTTLPR genotype may also be due to biallelic versus triallelic genotyping and different brain imaging outcome measures, as well as racial stratification differences in study populations.

An alternative explanation for low regional 5-HTT binding among suicide attempters is that it is a result of accelerated 5-HTT internalization in response to low 5-HT release. Evidence supporting a 5-HT deficiency related to suicidal behavior includes low cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5-HIAA) associated with suicidal behavior and risk of suicide (82), post-mortem studies reporting lower brainstem 5-HT or 5-HIAA in suicides (83), and lower CSF 5-HIAA in more lethal suicide attempters with MDD (84).

Serotonergic abnormalities may contribute to suicidal behavior through effects on aggressive traits, decision making, or problem solving. Measures of aggression have been correlated with several serotonergic measures, including low in vivo serotonin 1A receptor binding (85), blunted prolactin responses to serotonergic challenge with fenfluramine (86,87), and low CSF 5-HIAA (88). However, we do not find an effect of lifetime aggression assessed via the Brown Goodwin Lifetime History of Aggression scale on 5-HTT V\(_{FP}\) in vPFC (\(F = .084, df = 1,77, p = .77\)).

**Reported Childhood Abuse**

In exploratory analyses, we did not replicate our previous finding of low 5-HTT in MDD with reported childhood abuse history compared with MDD without childhood abuse history (28). Given these discrepant findings and the report of lower 5-HTT binding in adult monkeys with a history of maternal deprivation (89), replication is required with a larger sample, using a validated measure of childhood abuse such as the Childhood Trauma Questionnaire (90). We did not observe a gene-environment interaction between reported childhood abuse and 5-HTTLPR genotype on 5-HTT binding within the MDD sample. A definitive examination of a gene-environment interaction affecting 5-HTT binding as a mediator of depression risk would necessitate a large sample stratified across a continuous range of depression severity.

**Strengths and Limitations**

Strengths of this imaging study include the large sample size, favorable properties of \(^{11}\text{C}\)DASB, quantitative estimation of V\(_{FP}\), using a metabolite-corrected arterial input function, and careful diagnostic assessment. A limitation of this study is the lack of age matching between MDD subjects and control subjects. This is unlikely to have impacted the reported findings for the following reasons: 1) we did not observe an effect of age on 5-HTT binding in our sample; 2) we covaried for age in all analyses; 3) age did not differ between MDD attempters and MDD nonattempters, who nonetheless differed in midbrain 5-HTT binding; and 4) if age-related decline in 5-HTT binding were present, it would bias our results toward lower binding in MDD subjects than control subjects, which we did not observe.

A longer minimum antidepressant-free interval than the 2-week minimum used in the present study may be preferable, but ethical requirements prevent this approach. An alternative strategy would be to recruit drug-naïve participants. Nonetheless, in this sample, the median antidepressant-free interval in those MDD subjects with prior antidepressant exposure was 11.5 weeks. Moreover, we did not observe a difference in 5-HTT V\(_{FP}\) as a function of prior antidepressant exposure status, nor did mean antidepressant-free interval differ between MDD attempters and MDD nonattempters, which makes the minimum antidepressant-free interval employed in this study an unlikely explanation for reported findings.

Other clinical and demographic factors have previously been associated with 5-HTT binding, including cigarette smoking (91,92) and anxiety (19). We did not include these as covariates in the current analysis, given the large number of covariates examined and as smoking history did not differ between groups and anxiety comorbidity did not differ between MDD attempters and nonattempters. It should be noted that while differences in midbrain V\(_{FP}\) between MDD suicide attempters and nonattempters are statistically significant, there is overlap between groups in binding, and as such, this measure cannot be used alone to differentiate these groups. Future studies with improved 5-HTT quantification and the combination of imaging and clinical measures may improve group differentiation.

A limitation common to most studies quantifying 5-HTT in vivo is the lack of a reference region in the brain that is devoid of 5-HTT. We chose one approach to address this issue, using the outcome measure V\(_{FP}\), thereby avoiding the error introduced when using other available outcome measures that subtract out, or subtract and then divide by, the volume of distribution measured in a reference region that actually contains specific binding. Use of V\(_{FP}\) does not account for nonspecific binding in the brain, and it is thus possible that low midbrain binding in MDD attempters is due to differences in nonspecific binding. Other approaches to address this methodological challenge have been proposed (52). However, our results were similar when using all other outcome measures that do attempt to correct for nonspecific binding in the brain using a reference region (BPND, BPF, and BPN), so this methodological issue is unlikely to be driving our reported findings.

**Conclusions**

Serotonin transporter binding is low in vivo in the midbrain of depressed suicide attempters. This abnormality is consistent with postmortem findings in suicides and with a serotonergic deficit model of suicidal behavior. We are currently studying the prognostic significance of low 5-HTT binding as a predictor of repeated suicide attempt.

*Research presented in this manuscript was supported by National Institute of Mental Health Grants 5P50 MH62185 (Dr. Mann, principal investigator) and 2 R01 MH040695 (Dr. Mann, principal investigator). Dr. Ogden and Ms. Hesselgrave report no biomedical financial interests or potential conflicts of interest. Dr. Miller has received.*
financial compensation for psychiatric evaluations of subjects enrolled in medication studies sponsored by Pfizer and Orexigen. Therapeutics unrelated to the current manuscript. His family owns stock in Johnson & Johnson. Dr. Sullivan serves as a member of the Scientific Advisory Board of TONIX Pharmaceuticals, Inc. and has received compensation in the form of stock shares; he has served as a consultant for Ono Pharma USA, Inc. and he has a US patent application for a use of tianeptine. None are related to the current manuscript. Dr. Mann received past unrelated grants from GlaxoSmithKline and Novartis and royalties for a rating scale, Columbia Suicide Severity Rating Scale. Dr. Oquendo receives royalties for the use of the Columbia Suicide Severity Rating Scale and received financial compensation from Pfizer for the safety evaluation of a clinical facility unrelated to the current manuscript. She was the recipient of a grant from Eli Lilly to support a year of the salary for the Lilly Suicide Scholar, Enrique Baca-Garcia, M.D., Ph.D. She has received unrestricted educational grants and/or lecture fees from Astra-Zeneca, Bristol Myers Squibb, Eli Lilly, Janssen, Otsuka, Pfizer, Sanofi-Aventis, and Shire. Her family owns stock in Bristol Myers Squibb. Dr. Parsey was the recipient of grants from Pfizer, Lundbeck, Sepracor, Novartis, and General Electric, all unrelated to this manuscript. He has a US patent on voxel-based methods for assessing subjects using positron emission tomography.

Supplementary material cited in this article is available online at http://dx.doi.org/10.1016/j.biopsych.2013.01.024.


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