ORIGINAL ARTICLE

Brain Serotonin Transporter Binding Potential Measured With Carbon 11–Labeled DASB Positron Emission Tomography

Effects of Major Depressive Episodes and Severity of Dysfunctional Attitudes

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Background: Although brain serotonin transporter (5-HTT) density has been investigated in subjects with a history of major depressive episodes (MDE), there has never been an investigation of brain 5-HTT during a current MDE. Brain 5-HTT binding potential (BP) may have an important role during MDE due to major depressive disorder, because the 5-HTT regulates extracellular 5-HT. The BP is an index of receptor density. Carbon 11–labeled 3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile (DASB) positron emission tomography (PET) is the first brain imaging technique that can measure the 5-HTT BP in cortical and subcortical brain regions in vivo. The purposes of this study were to investigate 5-HTT BP during MDE and to determine the relationship between 5-HTT BP and negativistic dysfunctional attitudes during MDE. Dysfunctional attitudes are negatively biased assumptions and beliefs regarding oneself, the world, and the future. Our recent publication of increased serotonin$_2$ BP in MDE with severely negativistic dysfunctional attitudes suggests that this subgroup of MDE subjects has very low levels of extracellular serotonin.

Methods: Regional 5-HTT BP was measured in 20 nonsmoking medication-free (≥3 months) depressed subjects and 20 age-matched nonsmoking, medication-free, healthy subjects using [^{11}C]DASB PET. Dysfunctional attitudes were measured using the Dysfunctional Attitudes Scale.

Results: No difference in regional 5-HTT BP was found between MDE and healthy subjects; however, the subgroup of MDE subjects with highly negativistic dysfunctional attitudes had significantly higher 5-HTT BP compared with healthy subjects in brain regions mainly sampling serotonergic nerve terminals (prefrontal cortex, anterior cingulate, thalamus, bilateral caudate, and bilateral putamen; average, 21% greater; $F_{1,26}$, 5.6-12.2 [*P* values, .03-.002]). In the MDE subjects, increased 5-HTT BP was strongly associated with more negativistic dysfunctional attitudes in brain regions primarily sampling serotonergic nerve terminals (prefrontal cortex, anterior cingulate, thalamus, caudate, and putamen; r=0.64-0.74 [*P* values, .003 to <.001]).

Conclusions: Serotonin transporters play an important role during depression. The magnitude of regional 5-HTT BP can provide a vulnerability to low levels of extracellular serotonin and symptoms of extremely negativistic dysfunctional attitudes.

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SEROTONIN (5-HT) MAY BE ABnormally regulated during major depressive episodes (MDE) due to major depressive disorder. This assertion is based largely on abnormalities of serotonin turnover during MDE. For example, the level of the cerebrospinal fluid metabolite of serotonin, 5-hydroxyindoleacetic acid, is often low during MDE, especially when suicidal ideation is present.[1,2] Prolactin release is increased after administration of fenfluramine, a 5-HT–releasing drug, and it has been observed that the prolactin release after fenfluramine administration is attenuated during MDE.[3,4] A role for serotonin to modulate mood has been proposed, because mood lowering after tryptophan depletion is often observed in subjects with either a family history of depressive episodes or a history of depressive episodes.[5,6]

The primary mechanism by which extracellular levels of serotonin may be low during an MDE due to major depressive disorder is unknown. It has been proposed that increased serotonin transporter (5-HTT) density could lead to increased serotonin clearance from extracellular regions in the brain. The answer to this fundamentally important ques-

tion is not known because there are no investigations of 5-HTT density in subjects who are in the midst of a depressive episode due to major depressive disorder. Instead, a number of postmortem studies have investigated subjects with a history of an MDE. These studies usually do not distinguish between unipolar disorder and bipolar disorder, and the samples of unipolar subjects are often small. Most of these postmortem investigations report decreased 5-HTT density[7-9] or no difference in 5-HTT density[10-12] between depressed and healthy subjects. Although the regions investigated varied across these studies, all sampled the prefrontal cortex.

It is difficult for postmortem investigations to determine the relationship between 5-HTT density abnormalities and the presence of a current depressive episode, because the clinical data are gathered retrospectively. In theory, with receptor-ligand imaging in vivo, it is quite feasible to collect clinical data and determine the binding potential (BP), an index of receptor density, during a current depressive episode.

In the past, the barrier to investigating the 5-HTT BP with receptor-ligand imaging was the absence of a method that was both valid for multiple brain regions and reliable. Quantification of the 5-HTT BP in most brain regions, including the prefrontal cortex, was not possible with either of the 2 previous imaging methods, including carbon 11 [^{11}C]–labeled(+)McN5652 positron emission tomography (PET) and iodine I 123–labeled 2β-carbomethoxy-3β-(4-^{123}I-iodophenyl)-tropane (CIT) single-photon emission computed tomography (SPECT). To our knowledge, the test-retest reliability for regional 5-HTT BP found with [^{123}I]β-CIT SPECT or [^{11}C](+)-McN5652 PET has not been published. Relative to specific binding, [^{11}C](+)McN5652 had very high nonspecific uptake, and 5-HTT BP values were only detectable in the thalamus[13] and possibly the basal ganglia and midbrain (although measuring 5-HTT BP in the latter 2 regions may require concurrent arterial sampling of the radiotracer).[14] The SPECT radiotracer, [^{123}I]β-CIT, has been used to measure 5-HTT sites in the midbrain region in depressed subjects.[15,16] However, [^{123}I]β-CIT has similar affinity for the dopamine transporter (DAT) and the 5-HTT[17,18]; hence the midbrain [^{123}I]β-CIT is a combined measure of DAT BP in the substantia nigra and 5-HTT BP in the raphe nuclei.

Ichimiya et al[19] used [^{11}C](+)McN5652 PET to investigate the thalamus 5-HTT BP in 7 subjects with major depressive disorder. The data from these 7 subjects were pooled with those of 6 subjects who had bipolar disorder. That study did not address whether 5-HTT BP was abnormal during a current MDE. Only 5 subjects with a current MDE and major depressive disorder were enrolled in the study.[19]

Carbon 11–labeled 3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile (DASB) positron emission tomography (PET) is a new advance in brain imaging that measures 5-HTT BP in multiple brain regions, including the prefrontal cortex.[20-24] [^{11}C]DASB is highly selective, showing nanomolar affinity for the 5-HTT and negligible affinity for other receptors.[20,21] The main advantage of [^{11}C]DASB is that it has a much higher ratio of specific binding to nonspecific binding in vivo. As a result, with [^{11}C]DASB PET, 5-HTT BP values are quantifiable in prefrontal cortex and reasonably high in the basal ganglia and thalamus, even without arterial sampling.[22,23] Furthermore, the 5-HTT BP found with [^{11}C]DASB PET is reliable for most brain regions under test-retest conditions.[24]

This study had 2 main purposes. The first was to investigate the prefrontal and subcortical (thalamus, basal ganglia, and midbrain) 5-HTT BP in subjects who are currently experiencing an MDE secondary to major depressive disorder. The first purpose focused on whether brain 5-HTT abnormalities occur during a current MDE, an issue not resolved in earlier postmortem studies[8-12] or the PET investigation of thalamic 5-HTT BP by Ichimiya et al.[19]

The second main purpose was to determine the relationship between 5-HTT BP and dysfunctional attitudes in MDE. Dysfunctional attitudes are negatively biased assumptions and beliefs regarding oneself, the world, and the future. A modest degree of negativism is found in healthy subjects, whereas a high proportion of subjects with MDE have severely negativistic thinking. Our recent investigations suggest that a subgroup of subjects with MDE, rather than all subjects with MDE, have very low levels of extracellular serotonin. A previous study from our group found that increasing 5-HT agonism with a single dose of d-fenfluramine in healthy subjects lowered dysfunctional attitudes tremendously toward optimism (highly significant effect, $F_{1,25}=17$ [$P<.001$]).[25] This finding suggested a role for serotonin in humans as a modulator of dysfunctional attitudes. Given that serotonin can function as a modulator of dysfunctional attitudes, it seemed quite possible that low levels of serotonin could contribute to the increased severity of dysfunctional attitudes that often occur during MDE. In a second investigation in MDE subjects, we found that negativistic dysfunctional attitudes were associated with increased cortex serotonin$_2$ BP.[25] Furthermore, MDE subjects with severely negativistic dysfunctional attitudes had increased serotonin$_2$ BP compared with healthy subjects. Both findings were highly significant, especially in the prefrontal cortex ($r=0.56$ [$P=.009$] and $F_{1,19}=11$ [$P=.003$], respectively).[25] In animal models, serotonin$_2$ receptor density may increase after certain 5-HT–depleting paradigms (that reduce serotonin synthesis or storage for ≥2 weeks[26,27]) and decrease after certain 5-HT–increasing paradigms (monoamine oxidase inhibition for ≥2 weeks[28,29]). The BP is proportional to receptor density. A plausible explanation for the relationship between serotonin$_2$ BP and dysfunctional attitudes is that subjects who have a form of MDE with severely negativistic dysfunctional attitudes also have had an extended period of low levels of extracellular serotonin in the cerebral cortex.

We hypothesized that MDE subjects with greater 5-HTT BP would have more severely negativistic dysfunctional attitudes. The underlying model for this hypothesis is that MDE subjects with greater 5-HTT BP will remove more extracellular serotonin, have lower levels of extracellular serotonin, and experience more severe symptoms of pessimism. The prefrontal cortex region is the primary location for this hypothesis, because it is typically sampled in

investigations reporting increased serotonin$_2$ density in drug-free depressed suicide victims.[12,30] Furthermore, this region had the strongest correlation between the Dysfunctional Attitudes Scale (DAS) and serotonin$_2$ BP in a previous study by our group.[25] We also examined the relationship between dysfunctional attitudes and other sampled brain regions, including the midbrain. However, the 5-HTT in the midbrain region is proximal to serotonergic cell bodies[31] and would not be expected to directly modulate serotonin levels in distant brain regions.

METHODS

PARTICIPANTS

Twenty subjects with an MDE and major depressive disorder (mean age, 35 years [SD, 11 years]; 9 men and 11 women) and another 20 age-matched healthy subjects (mean age, 35 years [SD, 11 years]; 10 men and 10 women) were recruited. Ages ranged from 19 to 52 years. Healthy subjects were age matched within 3 years to depressed patients. All MDE and healthy subjects were physically healthy, had no history of alcohol or substance abuse or neurotoxin use, were free of psychotropic drug use for longer than 3 months plus 5 half-lives of any medication, and were nonsmoking.

Healthy subjects underwent screening to rule out Axis I disorders (current or in remission),[32] current suicidal ideation, and history of self-harm behavior, anger dyscontrol, or impulsive behavior. For each subject, written consent was obtained after the procedures had been fully explained.

The 20 subjects with MDE were obtained from a larger sample of 37 medication-free subjects with MDE who had enrolled in a study of antidepressant occupancy.[24,33] All were followed up for a minimum of 2 months by a psychiatrist (J.H.M.). The other 17 subjects were excluded on the basis of the criteria of nonsmoking and comorbid Axis I anxiety disorders. Diagnosis of MDE secondary to major depressive disorder was based on the Structured Clinical Interview for *DSM-IV* for Axis I disorders (SCID), (S.S.)[34] and a consultation by a psychiatrist (J.H.M). For subjects with MDE, the minimum severity of depression for enrollment was based on a cutoff score of 16 on the 17-item Hamilton Depression Rating Scale. The mean Hamilton Depression Rating Scale score was 20 (SD, 4). These recruiting methods are similar to what has been described previously.[25,35] Exclusion criteria included MDE with psychotic symptoms, bipolar disorder (type I or II), comorbid Axis I diagnoses, and history of self-harm or suicidality outside of episodes of depression, anger dyscontrol, impulsive behavior, and neuroleptic use. The exclusion of self-harm, anger dyscontrol, and impulsivity (taken from the Structured Clinical Interview for *DSM-IV* Axis II Disorders)[36] ruled out severe borderline personality disorder behaviors. People who have severe borderline personality disorder behaviors have abnormal severity of dysfunctional attitudes.[37] Fourteen of the 20 depressed patients had never received a trial of antidepressant treatment. No subject with MDE had received antidepressant treatment within the past 3 months. The recruitment of medication-free subjects has become a recognized standard in receptor-ligand imaging studies of MDE.[25,35,38-40]

All patients received common blood tests to rule out medical causes of disturbed mood (measurement of thyroid function, electrolyte levels, and complete blood cell count). Three of the MDE subjects who had received an antidepressant trial in the past also had a history of substance use (marijuana) that did not meet criteria for the SCID diagnosis of drug abuse. These 3 patients had not used marijuana within the previous year. Each underwent urine drug screening, the results of which were negative. All healthy subjects underwent urine drug screening.

All patients gave written consent after the procedure had been fully explained. The study and recruitment procedures were approved by the research ethics board for human subjects at the Centre for Addiction and Mental Health, Toronto, Ontario.

Additional information recorded included version A of the DAS; visual analog scales for mood, anxiety, and energy; and the Scale for Suicide Ideation.[41] Version A of the DAS is a 40-item self-report that requires subjects to indicate to what degree they agree with an individual statement. Each endorsement is converted into a score between 1 and 7. The total score for endorsement of pessimistic statements and disagreement with optimistic statements becomes the final measurement from the DAS. The DAS detects negativistic thinking during episodes of MDE,[42,43] has very good test-retest reliability across 6 to 8 weeks (r=0.83-0.84),[44,45] and has a high degree of internal consistency (Cronbach α=0.85-0.87).[45,46]

MEASUREMENT OF 5-HTT BP

The synthesis and imaging of [^{11}C]DASB has been described previously.[20-23] Before the PET scan, an intravenous bolus of 10 mCi (370 MBq) of [^{11}C]DASB was injected. The [^{11}C]DASB was of high radiochemical purity (>95%) and high specific activity (950±270 mCi/μmol [35±10 GBq/μmol] at the time of injection). The PET images were obtained using a GEMS 2048-15B camera (Scanditronix Medical, General Electric, Uppsala, Sweden; intrinsic resolution, full width at half maximum, 5.5 mm). The images were corrected for attenuation using a germanium Ge 68 transmission scan and reconstructed by filtered back projection (Hanning filter).

To obtain a measure of the 5-HTT BP with region-of-interest data, we chose the noninvasive method of Logan et al[47] (hereafter referred to as the Logan method) implemented within PMOD software (PMOD Technologies Ltd, Zurich, Switzerland).[48]

We used the noninvasive Logan method because it provides valid measurements of 5-HTT BP, and the between-subject variance in 5-HTT BP is low for most brain regions. The coefficient of variation (standard deviation/mean) using the Logan method[47] varied from 25% in the prefrontal cortex and midbrain to 15% in the putamen. Furthermore, the 5-HTT BP found with this method is very reproducible. In our data sets,[24] the absolute difference in the test-retest measurement of the 5-HTT BP with application of the Logan method[47] averages below 15% of the 5-HTT BP itself in all regions except the dorsolateral prefrontal cortex and midbrain, for which it is higher at about 15% to 20% of the mean 5-HTT BP. The less reliable test-retest measurement of 5-HTT BP in the dorsolateral prefrontal cortex region and midbrain reflects a lesser sensitivity of [^{11}C]DASB to detect the 5-HTT BP in these regions with this method. The measurement of 5-HTT BP with the noninvasive Logan method[47] is an underestimate of the same 5-HTT BP found with invasive arterial sampling and the single-tissue compartment model. This is quite acceptable because the between-subject variance of 5-HTT BP is also lower with the noninvasive Logan method,[47] and the regional 5-HTT BP found with the 2 methods is very strongly correlated. In general, we find that the 5-HTT BP measured using reference tissue–based methods is highly correlated with the ratio of the kinetically determined distribution volumes between regions with specific binding and the reference region.[23] As part of our assessment of the noninvasive Logan method,[47] we determined distribution volumes in the thalamus, striatum, frontal cortex, and cerebellum in a set of healthy subjects using an arterial input function and a single-tissue compartment model. Ratios of distribution volumes in regions with

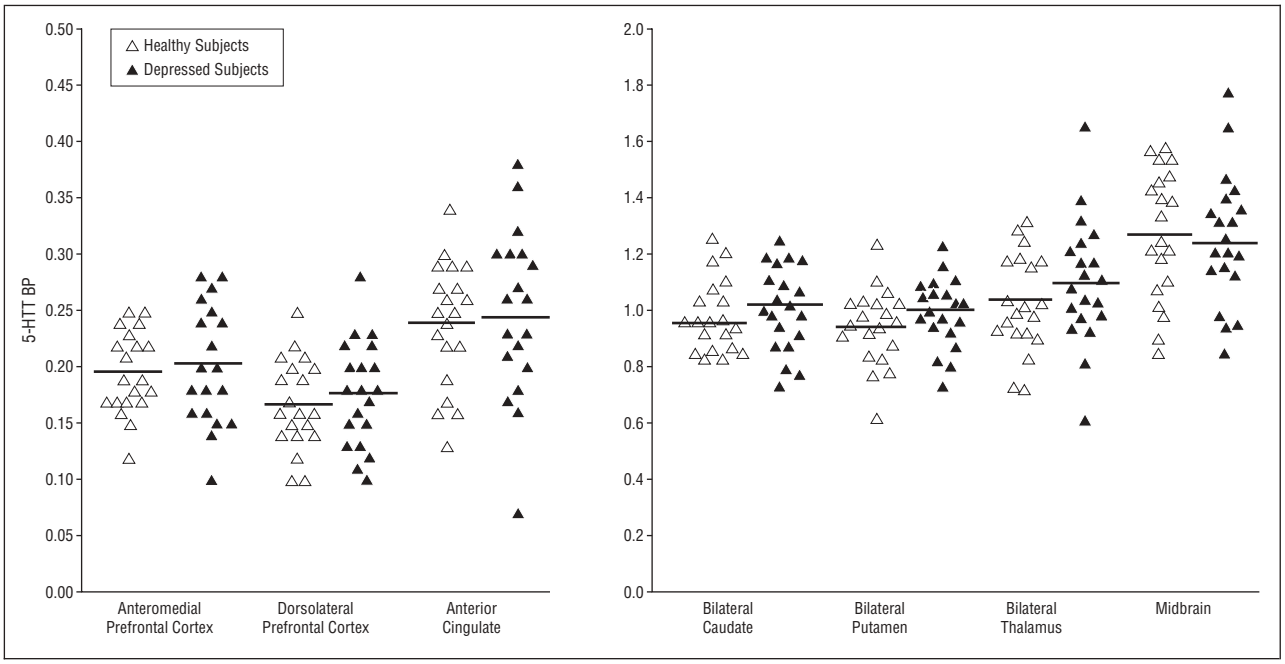

Figure 1. We found no difference in regional serotonin transporter binding potential (5-HTT BP) between 20 depressed and 20 healthy subjects (analysis of variance, effect of diagnosis on regional 5-HTT BP, $F_{1,38}$=0.09-1.4 [P=.77-.24]). Horizontal lines indicate group means.

specific binding to the distribution volume of the cerebellum were determined for each brain region, and this ratio was highly correlated with the binding potential found with the noninvasive Logan method (r=0.97).

For each region, except the midbrain, analyses were also carried out using the modified simplified reference tissue method,[49] which is also suitable for [^{11}C]DASB PET.[50] The lesser reversibility of radiotracer uptake in the midbrain precluded routine use of the modified simplified reference tissue model in this region.

A key assumption in the noninvasive models is that there is a reference region that does not contain specifically bound radioligand. For [^{11}C]DASB, the cerebellum is a suitable reference region because studies report either undetectable[51] or extremely low 5-HTT density.[52,53] More recently, it was found that the 5-HTT density in the cerebellum is less than 3% of the striatal value using the Western blot method (Stephen J. Kish, PhD, Yoshiaki Furukawa, MD, Li-Jan Chang, MSc, Junchao Tong, PhD, N.G., A.A.W., S.H., and J.H.M., unpublished data, May 2004).

The BP found with these noninvasive methods represents the ratio of specifically bound radiotracer to radiotracer in free and nonspecific compartments at equilibrium. This BP is equal to $(1/V_2) \times B_{max}/K_d$[54,55] where V_2 represents free and nonspecific binding at equilibrium; B_{max}, receptor density; and K_d, the dissociation constant. For [^{11}C]DASB PET, V_2 is sufficiently similar among subjects that it may be considered a constant.[23]

Regions chosen were bilateral anteromedial prefrontal cortex (Brodmann areas included were part of 8, 9, and 10), left and right dorsolateral prefrontal cortex (part of Brodmann areas 9 and 46), anterior cingulate, left and right caudate, left and right putamen, left and right thalamus, and midbrain. Prefrontal regions were included because indexes of increased serotonin$_2$ receptor density have been reported in prefrontal cortex in suicide victims[12,30,56-59] and medication-free depressed subjects who are suicidal[12,30] or have severely negativistic thinking.[25] The anterior cingulate was chosen because metabolic abnormalities have been reported in this general region in subgroups of depressed subjects.[60,61] Other regions (thalamus, basal ganglia, and midbrain) were chosen because the 5-HTT density is high in these regions.[51-53]

To assist with the region-of-interest measurement, each subject underwent a magnetic resonance imaging scan (GE Signa 1.5-T scanner; GE Medical Systems, Milwaukee, Wis) (spin-echo sequence proton density–weighted image; x, y, and z voxel dimensions, 0.78, 0.78, and 3 mm, respectively). Regions of interest were found using a semiautomated method[62,63] verified by visual assessment with reference to a coregistered magnetic resonance imaging scan. The only exception was the midbrain region, which was drawn within the superior 2 planes to the pons with reference to the coregistered magnetic resonance imaging scan. These methods have been described in more detail previously.[62]

RESULTS

EFFECT OF AGE AND SEX ON REGIONAL 5-HTT BP

There was no effect of age on regional 5-HTT BP in the healthy sample for any region (prefrontal cortex, anterior cingulate, bilateral caudate, bilateral putamen, bilateral thalamus, and midbrain) (1-way analysis of variance [ANOVA], $F_{1,18}$=0.01-3.0 [P=.92-.10]). There was no effect of sex on 5-HTT BP in these regions within the healthy sample (1-way ANOVA, $F_{1,18}$=0.001-1.0 [P=.97-.32]). The results for this analysis in peripheral regions using the modified simplified reference tissue model[49,50] were similar (effect of age, 1-way ANOVA, $F_{1,18}$=0.03-1.8 [P=.73-.20]; effect of sex, 1-way ANOVA, $F_{1,18}$=0.008-0.6 [P=.93-.45]).

EFFECT OF MDE ON REGIONAL 5-HTT BP

In the entire sample, MDE had no effect on regional 5-HTT BP for any individual region (1-way ANOVA, $F_{1,38}$=0.09-1.4 [P=.77-.24]); in fact, the mean regional 5-HTT BP within each group was very similar (**Figure 1**). The re-

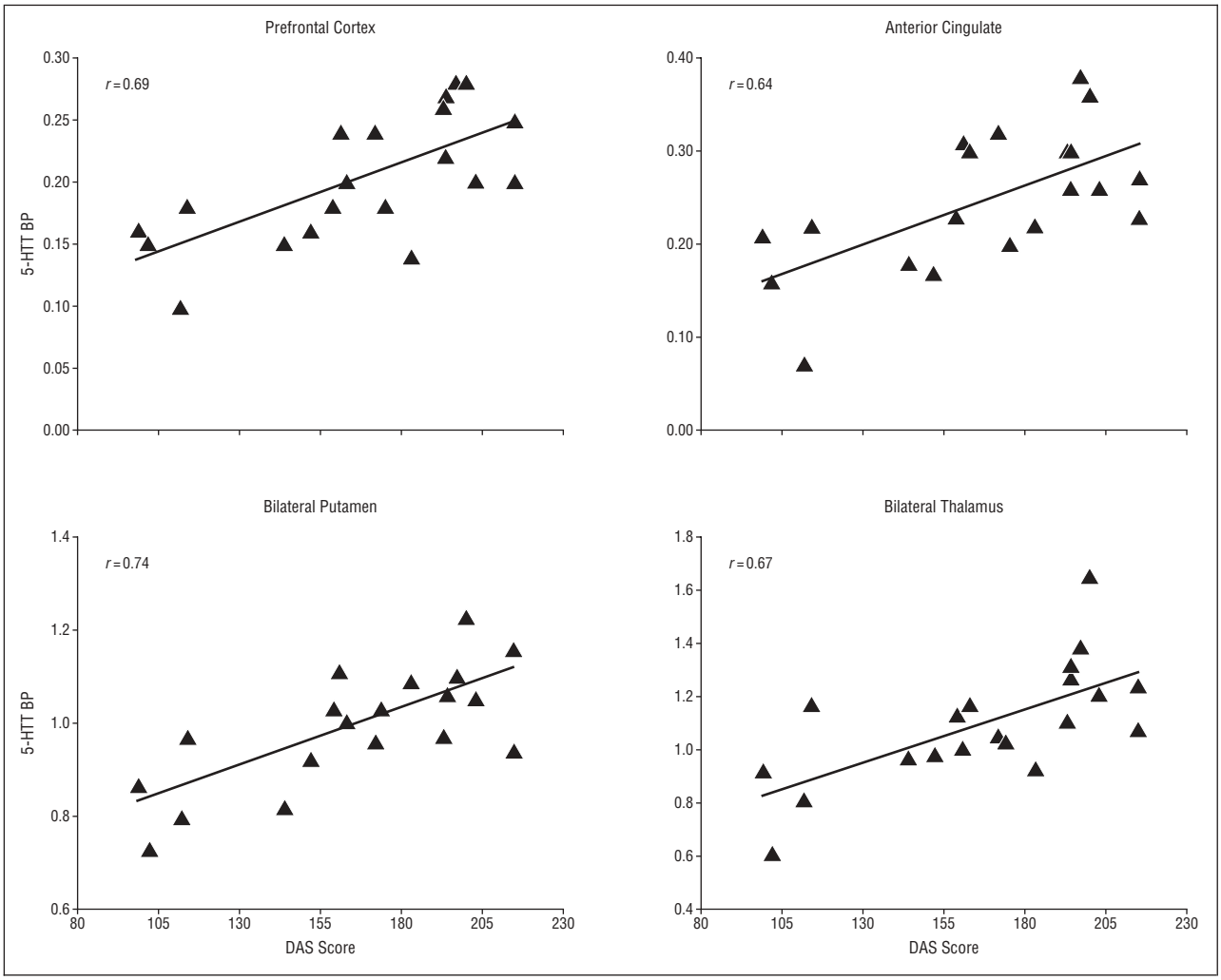

Figure 2. Correlations between the Dysfunctional Attitudes Scale (DAS) scores and serotonin transporter binding potential (5-HTT BP) in some of the larger regions in depressed subjects. The following highly significant correlations were found: prefrontal cortex ($P<.001$), anterior cingulate ($P=.003$), bilateral putamen ($P<.001$), and bilateral thalamus ($P=.001$).

sults for this analysis in peripheral regions using the modified simplified reference tissue model[49,50] were similar (effect of depression, 1-way ANOVA, $F_{1,38}=0.1\text{-}1.3$ [$P=.75\text{-}.26$]).

CORRELATIONS BETWEEN REGIONAL 5-HTT BP AND DYSFUNCTIONAL ATTITUDES

In MDE subjects, in all regions primarily sampling 5-HT nerve terminals (ie, everywhere except the midbrain), there were strong correlations between increasing DAS scores and increasing 5-HTT BP (**Figure 2**). In healthy subjects, the correlations between DAS and regional 5-HTT BP were nonsignificant. The correlation coefficients between the DAS and regional 5-HTT BP in healthy subjects were compared with the correlation coefficients between the DAS and regional 5-HTT BP found in MDE subjects. We performed the comparison by transforming the correlation coefficients into *z* scores with the Fisher transformation so that they then could be compared using the function of the gaussian distribution.[64] For all regions mainly sampling 5-HT nerve terminals, the correlation between regional 5-HTT BP and DAS was significantly different between MDE and healthy subjects (**Table**). For all of these analyses, extremely similar results were obtained using regional 5-HTT BP found with the modified simplified reference tissue method.[49,50]

COMPARISON BETWEEN DEPRESSED SUBGROUP WITH SEVERELY NEGATIVISTIC DYSFUNCTIONAL ATTITUDES AND HEALTHY SUBJECTS

Severely negativistic dysfunctional attitudes in the MDE subjects were defined as a DAS score of greater than 190. The cutoff score of 190 was chosen because this score is 3 SDs above the mean DAS score of our healthy subjects. Eight subjects with MDE had a DAS score greater than 190, and their regional 5-HTT BP was compared with that of the 20 healthy subjects. For all peripheral regions, the 5-HTT BP was higher in the MDE subjects with highly abnormal DAS scores (**Figure 3**). For these analyses, similar results were obtained using regional 5-HTT BP found with the modified simplified reference tissue method.[49,50]

Table. Correlations Between 5-HTT BP and DAS Scores

Region	Correlation Between 5-HTT BP and DAS, Subject Groups: Depressed r	Depressed P Value	Healthy r	Healthy P Value	Comparison Between Correlations λ*	Comparison P Value
Prefrontal cortex†	0.69	.001	−0.23	.33	3.15	.002
Left DLPFC‡	0.46	.04	−0.26	.26	2.23	.03
Right DLPFC‡	0.75	<.001	−0.28	.22	3.68	<.001
Anterior cingulate	0.64	.003	−0.34	.14	3.24	.001
Right thalamus	0.61	.004	−0.13	.58	2.45	.01
Left thalamus	0.71	<.001	−0.40	.08	3.82	<.001
Right caudate	0.52	.02	−0.25	.28	2.42	.02
Left caudate	0.67	.001	−0.13	.60	2.74	.006
Right putamen	0.72	<.001	−0.03	.89	2.73	.007
Left putamen	0.72	<.001	−0.02	.94	2.70	.007
Midbrain	0.33	.16	−0.28	.23	1.84	.07

Abbreviations: DAS, Dysfunctional Attitudes Scale; DLPFC, dorsolateral prefrontal cortex; 5-HTT BP, serotonin transporter binding potential.

*Using Fisher *z* transformation, the correlation coefficients (*r*) are transformed into *z* scores, and the λ statistic is derived from a comparison of the *z* scores.

†Indicates anteromedial prefrontal cortex (part of Brodmann areas 8, 9, and 10).

‡Indicates dorsolateral prefrontal cortex (part of Brodmann areas 9 and 46).

COMMENT

To our knowledge, this was the first investigation of regional 5-HTT BP with a selective radioligand in drug-free depressed subjects, and there were 3 main findings. First, there was no difference in regional 5-HTT BP in the entire sample of depressed subjects compared with the healthy subjects. Second, depressed subjects with greater regional 5-HTT BP had higher levels of dysfunctional attitudes. Third, a subgroup of depressed subjects with extremely high levels of dysfunctional attitudes had increased regional 5-HTT BP compared with healthy subjects.

An important function of the 5-HTT is to remove extracellular serotonin. This concept is primarily based on the findings that selective serotonin reuptake inhibitors inhibit the reuptake of 5-HT[65] and that knockout mice without the 5-HTT have increased levels of extracellular serotonin.[66] The findings of greater 5-HTT BP in MDE subjects with high levels of dysfunctional attitudes is consistent with this role. Greater 5-HTT BP could result in greater clearance of serotonin. On the basis of our previous findings,[25] the MDE subjects with more negativistic thinking should have lower levels of extracellular serotonin. (Previous findings were that increased serotonin release after d-fenfluramine administration decreased dysfunctional attitudes toward optimism.[25] Higher serotonin$_2$ BP was found in MDE with severe dysfunctional attitudes.[25] Other investigators have reported greater serotonin$_2$ density after 5-HT–depleting paradigms of ≥2 weeks' duration[26,27] and reduced serotonin$_2$ density after certain serotonin-increasing paradigms of ≥2 weeks' duration[28,29]). The association between negativistic thinking and higher 5-HTT BP is interpreted to reflect a vulnerability to greater extracellular clearance of serotonin when the 5-HTT BP is high. Greater extracellular clearance of serotonin then results in more severe symptoms. The earlier investigations[25] suggest that depressed patients with greater dysfunctional attitudes have a greater loss of extracellular serotonin, and the present study proposes that greater 5-HTT BP is a mechanism that could contribute to greater extracellular serotonin loss.

This interpretation suggests that the 5-HTT and DAT have similar roles of increasing vulnerability to symptoms during MDE. In a previous report of a separate sample of subjects, we found that MDE subjects with the lowest DAT BP were not motor retarded, whereas MDE subjects with higher DAT BP were impaired on neuropsychological measurement of motor speed.[35] The correlation between DAT BP and motor retardation was very strong (r=0.86 [P=.006]), and the subjects were also carefully recruited (drug free, nonsmoking, and no comorbidity).[35] The interpretation of this previous study was that greater DAT BP provides vulnerability to greater clearance of dopamine, resulting in lower levels of extracellular dopamine and more severe motor impairment, whereas lower DAT BP reduces clearance of extracellular dopamine, resulting in near-normal extracellular dopamine levels and negligible motor impairment.

We are characterizing the sample of MDE subjects with more severe dysfunctional attitudes as having more severe serotonergic disturbances with greater serotonin$_2$ BP in the previous sample,[25] and with greater 5-HTT BP in the current sample. This may have implications for suicide risk due to MDE. The DAS is highly correlated with the Beck Hopelessness Scale during MDE,[67,68] and greater scores on the Beck Hopelessness Scale are associated with higher likelihood of suicide.[69,70] Therefore, it is possible that during MDE, in some people, elevated 5-HTT BP may lead to lower serotonin levels, less stimulation via major serotonergic signal transduction pathways, and elevated serotonin$_2$ BP. It may be that these processes mediate greater negativistic thinking and eventually result in an elevated risk of suicide.

The correlations between DAS and 5-HTT BP were very strong in the brain regions that primarily sampled serotonergic nerve terminals. This is not surprising, because the 5-HTT BP tends to associate across these regions within subjects. For example, subjects with higher 5-HTT BP in the frontal cortex tend to have higher 5-HTT BP in the striatum.

In a large earlier postmortem study (53 patients vs 107 subjects with no history of a depressive episode), it was found that the 5-HTT density was lower in the patient sample.[7-9] There are several major differences in sampling between the earlier study and the present one. In our study, all subjects were in the midst of a current MDE due to major depressive disorder. The previous study sampled patients who had a history of unipolar or bipolar depressive episodes and were not necessarily in the midst of a depressive episode at the time of measurement. Another key difference was that the subjects in the present study were about a decade younger than the subjects in the postmortem study. If there are any long-term effects of mood disorder illnesses on 5-HTT den-

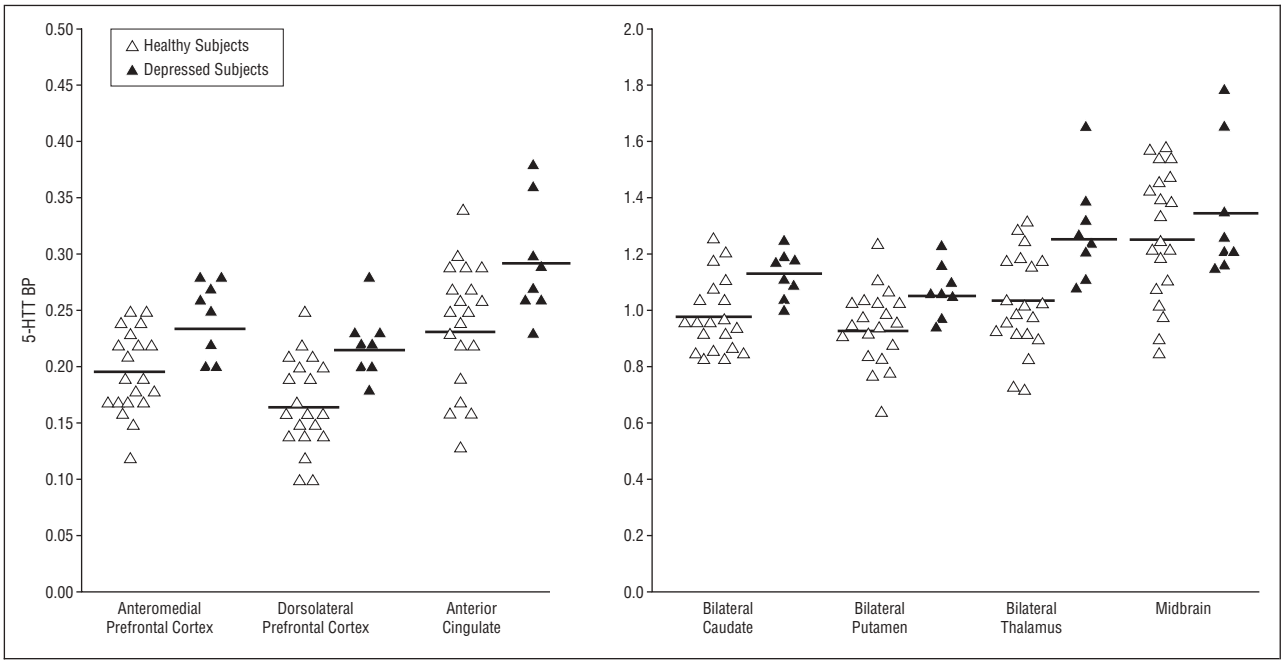

Figure 3. Comparison of regional serotonin transporter binding potential (5-HTT BP) between 8 depressed subjects with severely negativistic dysfunctional attitudes (Dysfunctional Attitudes Scale score >190) and 20 healthy subjects. For regions primarily sampling serotonergic nerve terminals (anteromedial prefrontal cortex, anterior cingulate, and bilateral caudate, putamen, and thalamus), the 5-HTT BP was significantly greater in the depressed group ($F_{1,26}$=5.6-12.2 [P=.03-.002]). The midbrain 5-HTT BP was not significantly different ($F_{1,26}$=0.5 [P=.49]). Horizontal lines indicate group means.

sity, the results of the studies would be expected to differ. Future longitudinal studies of 5-HTT BP in untreated remitted depressed subjects may help resolve these questions.

Whenever multiple regions are investigated in a brain imaging study, one should consider how multiple comparisons influence the interpretation of results. In this study, the 2 major findings (correlation between 5-HTT BP and dysfunctional attitudes during depression and higher 5-HTT BP in depressed subjects with severe dysfunctional attitudes) were present in all 6 regions of serotonin nerve terminal areas. The probability of having significant findings in all 6 brain regions sampling serotonergic nerve terminals by independent chance alone is exceedingly small. The probability of 6 significant, independent findings is 0.05^6 or 1.6×10^{-8}. Therefore, it is unlikely that the findings of this study represent independent chance events.

The results of this study show strong significant correlations between the 5-HTT BP and dysfunctional attitudes. However, there are limitations. Not all brain regions were analyzed; rather, we chose brain regions in which the 5-HTT BP could be reliably and validly measured. The 5-HTT BP is proportional to both 5-HTT density and affinity. Even so, the combined measurement of density and affinity obtained with the BP does not alter our interpretations, because both 5-HTT density and affinity should contribute to serotonin clearance. We also acknowledge that 5-HTT density and affinity are not the only indices of 5-HTT function. For example, it is possible for desensitization processes to occur for some receptors without changing either the density or the affinity of receptors. We cannot measure serotonin directly in the human brain, and in our interpretations we made inferences about extracellular serotonin based on the strong relationships between manipulations of serotonin and dysfunctional attitudes, as well as measures of serotonin$_2$ receptors and dysfunctional attitudes in our previous investigations.[25] We also recognize that there are alternative interpretations for the correlations between dysfunctional attitudes and regional 5-HTT BP. For example, it could be that in MDE with high dysfunctional attitudes, there is a greater density of neurons expressing 5-HTT, a greater density of dendrites expressing 5-HTT, a wider range of neurons expressing 5-HTT, an unusual regulation of 5-HTT in response to some other process, etc. The importance of the explanation we presented is that it most straightforwardly accounts for both existing knowledge regarding 5-HTT function[65,66] and the relationships between dysfunctional attitudes and serotonin measures in earlier investigations.[25]

This study was the first investigation of the 5-HTT BP in drug-free, nonsmoking, currently depressed subjects with a reasonably large sample size using a specific radioligand. We found that MDE subjects with more negativistic thinking had greater 5-HTT BP in MDE and that a subgroup of MDE subjects with severely negativistic thinking had a 21% higher 5-HTT BP compared with healthy subjects. There was no difference in 5-HTT BP between the entire sample of MDE and the healthy subjects. The findings are important because they suggest a role for 5-HTT in the pathophysiology of serotonin-related symptoms. During an MDE, elevated 5-HTT BP may result in excessive extracellular clearance of serotonin, providing vulnerability to serotonin-related symptoms. This interpretation is consistent with previous investigations that associate serotonin agonist effects (ie, d-fenfluramine effect and serotonin$_2$ BP) with dysfunctional attitudes.[25] Our data

argue that the 5-HTT BP is strongly associated with a symptom of excessive dysfunctional attitudes within an MDE rather than the MDE itself.

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