

RESEARCH ARTICLE

Incongruent Reduction of Serotonin Transporter Associated with Suicide Attempts in Patients with Major Depressive Disorder: A Positron Emission Tomography Study with 4-^[18F]-ADAM

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Abstract

Background: Much evidence supports the role of the serotonin transporter (SERT) in the pathophysiology and pharmacotherapy of major depressive disorder (MDD) and suicidal behaviors.

Methods: In this study, we recruited 17 antidepressant-naïve patients with MDD and 17 age- and gender-matched healthy controls. SERT availability was measured *in vivo* with *N,N*-dimethyl-2-(2-amino-4-^[18F]fluorophenylthio)benzylamine (4-^[18F]-ADAM) positron emission tomography (PET) imaging. The 21-item Hamilton Depression Rating Scale (HDRS) and Beck Scale for Suicide Ideation were used to assess the severity of depression and the intent of suicide ideation prior to PET imaging. All subjects with MDD were in a current state of depression with HDRS scores ≥ 18 . Subjects who attempted suicide within two weeks of the study onset were recruited in the depressed suicidal group ($n = 8$). Subjects with MDD who denied any prior suicide attempt were recruited into the depressed non-suicidal group ($n = 9$).

Results: A significant reduction of SERT availability in the midbrain, thalamus, and striatum was noted in the MDD group relative to the control group (Bonferroni-adjusted p -value < 0.05). Moreover, this effect was more pronounced in the depressed suicidal group compared to the control group (Bonferroni-adjusted p -value < 0.01). Relative to both the depressed non-suicidal

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and control groups, the depressed suicidal group showed an increased prefrontal cortex (PFC)/midbrain SERT binding ratio (Bonferroni-adjusted p -value < 0.01).

Conclusions: This study suggests an incongruent reduction of PFC SERT binding relative to the midbrain might discriminate between depressed suicide attempters and non-attempters in patients with MDD and may be involved in the pathophysiology of suicide behaviors.

Keywords: 4- $^{[18F]}$ -ADAM, major depressive disorder, positron emission tomography, serotonin transporter, suicide

Introduction

Much evidence suggests that dysfunction of the serotonergic system is involved in the modulation of depression, impulsivity, and suicidal behaviors (Meltzer et al., 1998; Mann, 1999 2013; Arango et al., 2002). Serotonergic neurons originate in the dorsal raphe nuclei (DRN), and have rich innervation patterns throughout the brain (Purselle and Nemeroff, 2003). The serotonin transporter (SERT), located on serotonergic neurons, is believed to be primarily responsible for the regulation of 5-hydroxytryptamine (5-HT) levels through reuptake of 5-HT from the extracellular space into the presynaptic neuron. Moreover, many antidepressants, such as selective serotonin reuptake inhibitors and certain tricyclic antidepressants, exert their function by blocking SERT to increase synaptic levels of 5-HT. Therefore, it has been implied that SERT plays a crucial role in the pathophysiology of depression (Owens and Nemeroff, 1994) and suicide (Purselle and Nemeroff, 2003).

Post-mortem studies using autoradiography have shown that victims of suicide exhibit inconsistent findings in SERT binding in different brain regions, such as decreased SERT binding in the prefrontal cortex (PFC; Arango et al., 1995; Mann et al., 2000) and increased SERT binding in the hippocampus (Gross-Isseroff et al., 1989). However, no differences in SERT binding or messenger RNA expression have been found in other studies (Little et al., 1997; Bligh-Glover et al., 2000; Arango et al., 2001) between victims of suicide and healthy controls.

In vivo imaging provides a noninvasive method for exploring SERT availability in subjects with major depressive disorder (MDD) and in individuals who have attempted suicide. However, previous *in vivo* imaging studies on SERT binding in patients with MDD have yielded contradictory results, which may be due to methodological differences, recruiting samples with mixed diagnoses or psychiatric comorbidity, or utilizing various radioligands with lower selective binding for SERT (Brust et al., 2006; Meyer, 2007), such as [(123I)]beta-CIT (2beta-carbomethoxy-3beta-(4-iodophenyl)tropane) ([123 I]- β -CIT) in single-photon emission computed tomography (SPECT) or rel-(6R,10bS)-6-[4-(Methylsulfonyl)phenyl]-1,2,3,5,6,10b-hexahydropyrrolo[2,1- α]isoquinoline ([11 C](+)McN5652) in positron emission tomography (PET). Although using highly-selective SERT radioligands, such as 2-([2-(dimethylamino)methyl]phenyl)thio)-5-(123I)-iodophenylamine ([123 I]ADAM) SPECT (Newberg et al., 2005 2012; Ho et al., 2013) and [(11C)] 3-amino-4-(2-dimethylaminomethyl-phenylsulfonyl)benzotrile ([11 C]DASB) PET (Reimold et al., 2008; Selvaraj et al., 2011), have revealed diminished SERT binding in some brain regions in patients with MDD relative to healthy controls, other studies have reported no such difference (Meyer et al., 2004; Miller et al., 2013) or elevated SERT binding (Cannon et al., 2007) between subjects with MDD and controls using the same radioligand.

Another drawback to the current literature on SERT and suicide is that most of the previous studies focused on merely analyzing individuals who had attempted suicide in the past, but did not explore their current intensity of suicidal ideations. Additionally, previous reports neglected to acknowledge the

time interval between an individual's last suicide attempt and the completion of imaging acquisition. Thus, the association between the availability of SERT and major depression as well as suicide attempts remains inconclusive. A recent study found that the availability of SERT in projection areas could be modulated by tonic firing of serotonergic neurons through 5-hydroxytryptamine (serotonin) receptor 1A (5-HT $_{1A}$) autoreceptors to achieve 5-HT homeostasis (Best et al., 2010). Therefore, the pathophysiology of depression and suicidal behaviors may not solely rely on regional SERT density, but also on the interplay of SERT between serotonergic projecting terminal regions and the DRN (Lanzenberger et al., 2012).

In the present study, antidepressant-naïve participants were recruited and classified into three subgroups. Subjects with MDD who had attempted suicide within two weeks of the study onset were recruited into the depressed suicidal (DS) subgroup. Subjects with MDD who denied any prior suicide attempt were recruited into the depressed non-suicidal (DNS) subgroup. Age- and gender-matched healthy volunteers were recruited for comparison. In contrast to the previous reports that focused on past suicide history, we attempted to clarify the direct impact of suicide attempts on SERT binding. In order to do this, we used a highly selective radiotracer, N,N-dimethyl-2-(2-amino-4-[18 F]fluorophenylthio)benzylamine (4-[18 F]-ADAM; Shiue et al., 2003; Huang et al., 2010; Huang et al., 2013), to assess SERT availability in the following two experiments. First we investigated whether regional SERT binding potential (BP) or terminal region/midbrain (DRN) SERT BP ratios differed among DS, DNS, and control subgroups. We then explored the correlation between regional SERT (BP) or terminal region/midbrain SERT BP ratios and the real-time intensity of suicidal ideation, as well as severity of depression in depressed subjects.

Methods

Patient Selection

The experimental protocol was approved by the Institutional Review Board for the Protection of Human Subjects at the Tri-Service General Hospital, National Defense Medical Center in Taipei, Taiwan. All participants gave written informed consent. A Chinese version of the modified Schedule of Affective Disorder and the Schizophrenia-Lifetime (SADS-L) was used to screen psychiatric conditions in both patients and control subjects (Endicott and Spitzer, 1978; Huang et al., 2004). Inter-rater reliability for SADS-L ratings were good to excellent for major depression, bipolar disorder, anxiety disorder, schizophrenia, substance abuse/dependence, and personality disorders. The inclusion criteria for the patient group were as follows: (1) age between 20 to 65 years; (2) meeting MDD according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (American Psychiatric Association, 2000);

(3) a score of ≥ 18 on the 21-item Hamilton Depression Rating Scale (HDRS), indicative of moderate to severe MDD (Hamilton, 1960); and (4) subjects with a suicide attempt (defined as self-harm with at least some intent to end one's life) made within two weeks of the study onset were recruited into the DS group, while subjects who denied any prior suicide attempt were recruited into the DNS group.

The exclusion criteria were as follows: (1) patients diagnosed with other comorbid Axis I and/or Axis II disorders, with the exception of patients with nicotine dependence; (2) HDRS score of < 18 , indicative of a mild degree of depression; (3) significant physical illness; (4) women who were pregnant or lactating; (5) previous head trauma with loss of consciousness, epilepsy, and/or thyroid disease; (6) previous exposure to psychotropic medication, for example, antidepressants, mood stabilizers, or antipsychotics; (7) repetitive bodily harm without intent of suicide; or (8) a suicide attempt made more than two weeks before the study onset.

The patient group consisted of 17 antidepressant-naïve patients diagnosed with MDD that were recruited from the inpatient psychiatric ward on hospital day 1 in the Tri-Service General Hospital. There were eight patients in the DS group, and nine patients in the DNS group. PET imaging was arranged, on average, 3.5 days after informed consent was obtained. For safety issues, depressed subjects at suicidal risk underwent intervention for suicide prevention. A psychiatrist and an assistant would send the participant to the PET center and provide successive suicide prevention throughout the whole imaging procedure until sending the subject back to the inpatient ward. Antidepressants were first prescribed for these patients after completing PET imaging. The control group included 17 age- and gender-matched healthy volunteers recruited from the community. The control subjects were free of past or present major or minor mental illnesses, as determined by the SADS-L interview. Moreover, none of the first-degree relatives of the control subjects had histories of psychiatric disorders, substance abuse/dependence, or attempted suicide.

Clinical Assessments for Depression and Suicide

The 21-item HDRS (Hamilton, 1960) and Beck Scale for Suicide Ideation (BSS; Beck et al., 1979) were used to assess the severity of depression and intensity of suicide ideation prior to brain imaging. The HDRS includes 21 items rated on a 0–2 or 0–4 scale by a clinician, with a total score ranging from 0 to 66. A higher score indicates a greater degree of depression. The BSS is also a clinician-rated scale and is formatted as a semi-structured interview to evaluate a patient's current suicide risk. The interview consists of 21 items that evaluate three dimensions of suicidal ideation: active suicidal desire, specific plans for suicide, and passive suicidal desire. Each item is rated on a 3-point scale from 0 to 2, with a range for the total score from 0 to 42. A higher score indicates greater suicidal intent.

PET Procedure

The 4- ^{18}F -ADAM used in the present study was synthesized in an automated synthesizer as previously described (Peng et al., 2008). The preparations for 4- ^{18}F -ADAM synthesis were carried out in our PET cGMP laboratory and were inspected by the Council of Atomic Energy and the Department of Health of Taiwan. All 34 participants underwent a low-dose computed tomography (CT) scan (130 kVp, 50 mAs, 0.8 s tube rotation, 4 mm slice collimation, and pitch 3) and then a static PET

scan in three-dimensional mode using a BIOGRAPH PET/CT scanner (Biograph Duo, Siemens). Total PET imaging time was 20 min and began 120 to 140 min after intravenous bolus injection of 283.72 ± 35.74 MBq (7.67 ± 0.97 mCi) 4- ^{18}F -ADAM. The doses administered were weight-independent, as previously described (Huang et al., 2013). This scanner had a transverse field of view of 58.5 cm, an axial field of view of 15.5 cm, and a spatial resolution of 4.8 mm. PET images were reconstructed in a $512 \times 512 \times 64$ matrix with a pixel size of $0.519 \times 0.519 \times 2.4$ mm using the ordered subset expectation maximization method (six iterations and 16 subsets) with a Gaussian filter of 3 mm full-width half maximum.

Image Acquisition

Images were analyzed with PMOD software for Windows (version 3.0, PMOD group). Each subject's PET image was automatically co-registered with the corresponding CT scan and was then manually adjusted by an experienced physician. The CT was loaded to provide an anatomical reference, and regions of interest (ROIs) were defined on reconstructed and summated PET images. ROIs were drawn over the midbrain, striatum, thalamus, and PFC, where loci of serotonergic cell bodies and the main projection regions could be found (Supplementary Figure 1). We used the cerebellum as a background reference because of its low SERT concentration when compared to other brain regions (Kish et al., 2005). To reduce the confounding effects of SERT binding in the cerebellum, the white matter and vermis were excluded, and only the posterior half of the cerebellar cortex was delineated. The BP of 4- ^{18}F -ADAM was quantified using a previously-described ratio method by comparing specifically-bound radioligands to nondisplaceable radioligands in brain tissue (BP_{ND}) at equilibrium (Shiue et al., 2003; Chen et al., 2012; Huang et al., 2013). Because the uptake of 4- ^{18}F -ADAM has been reported to reach equilibrium about 120–140 min after its injection in human subjects (Huang et al., 2013), we selected the 120 min frames for the equilibrium ratio method in our modeling.

The BP_{ND} of 4- ^{18}F -ADAM in the target region was calculated by subtracting the mean counts per pixel in the nondisplaceable region (C_{CB}) from the mean counts per pixel in the target region (C_{ROI}), and then dividing the result by the mean counts per pixel in the nondisplaceable region: $\text{BP}_{\text{ND}} = (C_{\text{ROI}} - C_{\text{CB}}) / C_{\text{CB}} = (\text{tissue radioligand activity of target region} - \text{tissue radioligand activity of the cerebellum}) / \text{tissue radioligand activity volume of the cerebellum}$. The investigator using PMOD software to analyze images was blinded to all participant information.

Statistical Analysis

Normally-distributed continuous variables were analyzed by the student's *t*-test for independent samples, and non-normally distributed continuous variables were analyzed using the Mann-Whitney *U* test. Differences between nominal variables were tested using the Pearson χ^2 test; Fisher's exact test was used when the sample size was smaller than expected ($n < 5$). BP_{ND} data were analyzed by linear mixed-effects models, with brain region and diagnostic group as fixed effects and subjects as the random effect. Other fixed effects considered in the linear mixed-effects models included sex, age, smoking status, suicide attempt, depression severity, and suicide intensity factors. Bonferroni correction was used for multiple comparisons in the post hoc analysis. Pearson's correlation was utilized to examine the correlation between 4- ^{18}F -ADAM BP_{ND} and BSS scores, as well as HDRS score. All data were analyzed with SPSS software

for Windows (version 17). A p value of less than 0.05 was considered statistically significant (two-tailed).

Results

Demographic Data and Effect on SERT BP_{ND}

The characteristics of all participants are summarized in [Table 1](#). All participants were Han Chinese recruited from Taipei, Taiwan. There were no significant differences in age, gender, or body mass index (BMI) between the total MDD group (pooled DNS and DS subgroups) and control group. Daily smoking amount, number of major depressive episodes, and HDRS and BSS scores were higher in depressed subjects than in controls. Between DS and DNS subgroups, there were no differences in age, age of onset, gender, BMI, daily smoking amount, number of depressive episode, and HDRS scores, but there were differences in BSS scores and number of suicide attempts. Sixteen depressed subjects were early-onset MDD (onset age prior to 45), and only one subject with late-onset MDD was within the DS group. There was no difference in the dose of 4-[¹⁸F]-ADAM between MDD and control groups and between DS and DNS subgroups. The methods used by the DS group to attempt suicide included drug overdose in five subjects, arm cutting in two subjects, and carbon monoxide intoxication in one subject. Therefore, five subjects were non-violent suicide attempters (drug overdose), and three subjects were violent suicide attempters (arm cutting and carbon monoxide intoxication).

There was a significant effect of brain region on SERT binding ($F = 112.142$, $df = 3,99$, $p < 0.001$). The value of BP_{ND} from greatest to lowest, in the four ROIs was midbrain, thalamus, striatum, PFC. Across the four ROIs, there was no effect of sex ($F = 0.147$, $df = 1,31$, $p = 0.704$) or age ($F = 0.798$, $df = 1,31$, $p = 0.378$) on SERT binding in the combined sample. Moreover, no interactions were detected between age and diagnosis ($F = 0.115$, $df = 2,30$, $p = 0.737$) or age and region ($F = 1.131$, $df = 3,96$, $p = 0.340$) on SERT binding. Finally, the amount of cigarette smoking ($F = 0.145$, $df = 1,31$, $p = 0.706$) and BMI ($F = 1.423$, $df = 1,31$, $p = 0.242$) did not significantly influence SERT binding, and there was no effect of the presence of cigarette smoking on SERT binding ($F = 0.457$, $df = 1,31$, $p = 0.504$).

Effect of MDD Diagnosis and Depression Severity on SERT BP_{ND}

A scatter plot of raw BP_{ND} values in the four ROIs of subjects with MDD and healthy controls is presented in [Figure 1](#). Across the ROIs, BP_{ND} was significantly different according to MDD diagnosis ($F = 9.617$, $df = 1,32$, $p = 0.004$). A post hoc test revealed significantly lower BP_{ND} in the midbrain ($t = -3.066$, $df = 1,64$, uncorrected $p = 0.003$), thalamus ($t = -3.519$, $df = 1,64$, uncorrected $p = 0.001$), and striatum ($t = -2.691$, $df = 1,64$, uncorrected $p = 0.009$) of subjects with MDD relative to control subjects. Furthermore, a MDD diagnostic effect remained significant in the midbrain, thalamus, and striatum after Bonferroni correction for multiple comparisons (Bonferroni-adjusted p -value = 0.012, 0.003, and 0.035, respectively). Within the depressed group, the midbrain BP_{ND} negatively correlated with HDRS scores ($r = -0.513$, $p < 0.05$; [Figure 2](#), [Supplementary Table 1](#)).

Effect of Recent Suicide Attempt and Suicide Intensity on SERT BP_{ND}

A group-by-region interaction was found in the comparison of BP_{ND} in DS, DNS, and control groups ($F = 5.273$, $df = 2,31$, $p = 0.011$).

A post hoc analysis revealed that this difference was due to lower BP_{ND} in the midbrain ($t = -4.851$, $df = 1,57$, uncorrected $p < 0.001$), thalamus ($t = -3.635$, $df = 1,57$, uncorrected $p < 0.001$), and striatum ($t = -2.321$, $df = 1,57$, uncorrected $p = 0.023$) in the DS group compared to the control group. After Bonferroni correction for multiple testing, the BP_{ND} in the midbrain and thalamus remained significant (Bonferroni-adjusted p -value = 0.002 and 0.004, respectively). Within the depressed group, no significant correlation was found between the SERT BP_{ND} in any of the ROIs and the suicide intensity assessed by BSS. Within the DS group, there was no significant difference in SERT BP_{ND} between violent suicide attempters and non-violent suicide attempters ($F = 0.497$, $df = 1,6$, $p = 0.507$).

Association Between Projection Area/Midbrain SERT BP Ratios and Suicide Attempt and Intensity

We calculated the SERT binding ratio by dividing the SERT BP_{ND} in the projection area by the SERT BP_{ND} in the midbrain. The PFC/midbrain SERT BP ratio differed significantly among DS, DNS, and control groups. A post hoc examination showed a higher PFC/midbrain SERT binding ratio in the DS group compared to the DNS (uncorrected $p = 0.002$) and control groups (uncorrected $p = 0.001$). Moreover, this significance remained even after Bonferroni correction for multiple comparisons (Bonferroni-adjusted p -value = 0.007 and 0.004, respectively; [Figure 3](#)). No difference in the PFC/midbrain SERT binding ratio was found between DNS and control groups. Within the MDD group, BSS scores positively correlated to the PFC/midbrain SERT binding ratio ($r = 0.500$, $p < 0.05$; [Figure 4](#)). The thalamus/midbrain and striatum/midbrain SERT binding ratios did not differ among the three subgroups. Additionally, the thalamus/midbrain and striatum/midbrain SERT binding ratios did not correlate with BSS scores ([Supplementary Table 1](#)).

Discussion

In the present study, we observed that SERT BP_{ND} in the midbrain, thalamus, and striatum was significantly lower in patients with antidepressant-naïve MDD than in healthy controls. This finding is concordant with previous imaging studies that reported a reduction of SERT binding in the midbrains ([Newberg et al., 2005](#); [Parsey et al., 2006](#); [Selvaraj et al., 2011](#); [Miller et al., 2013](#)) and thalami ([Reimold et al., 2008](#); [Selvaraj et al., 2011](#); [Ho et al., 2013](#)) of subjects with major depression. In addition, we found that midbrain SERT BP_{ND} inversely correlated with HDRS scores, suggesting that more severe symptoms of depression are associated with greater reductions in SERT BP_{ND}. However, our result differs from those of other studies that reported no difference ([Lindstrom et al., 2004](#); [Meyer et al., 2004](#); [Ryding et al., 2006](#)) or elevated SERT binding ([Ichimiya et al., 2002](#); [Reivich et al., 2004](#); [Cannon et al., 2007](#)) in subjects with MDD relative to healthy controls.

Of the various subgroups we examined, we found that the DS group exhibited the lowest SERT availability in the midbrain and thalamus compared to DNS and control groups. This finding is partially consistent with a previous study that used [¹¹C]-ZIENT PET to show that SERT BP_{ND} was significantly decreased in the midbrain of patients with major depression that had previously attempted suicide ([Nye et al., 2013](#)). However, the decrease of SERT binding in the thalamus, but not the putamen (a part of the striatum), was inconsistent with the results reported by [Nye et al. \(2013\)](#). Our results were also similar to the results of a study where binding was the lowest in suicide attempters ([Miller](#)

Table 1 Clinical and Demographic Characteristics of the Sample (n = 34)

	Healthy controls (n = 17)		Total MDD (n = 17)		Control vs. total MDD		Total MDD					
							Depressed non-suicide (n = 9)		Depressed suicide (n = 8)		Depressed non-suicide vs. depressed suicide	
	n (%)	n (%)	n (%)	n (%)	χ^2 (df = 1)	p	n (%)	n (%)	n (%)	n (%)	χ^2 (df = 1)	p
Gender												
Male	8 (47.1)	8 (47.1)	8 (47.1)	8 (47.1)	0	1.00	5 (47.1)	3 (37.5)	5 (55.6)	3 (37.5)	0.55	0.64
Female	9 (52.9)	9 (52.9)	9 (52.9)	9 (52.9)			4 (52.9)	5 (62.5)	4 (44.4)	3 (37.5)		
Smoking status												
Smoker	2 (11.8)	10 (58.8)	10 (58.8)	7 (41.2)	8.24	0.01*	5 (55.6)	5 (62.5)	4 (44.4)	3 (37.5)	0.08	1.00
Non-smoker	15 (88.2)	7 (41.2)	7 (41.2)	10 (58.8)			4 (44.4)	3 (37.5)	4 (44.4)	5 (62.5)		
Major depressive episode												
First episode	-	7 (41.2)	7 (41.2)	10 (58.8)			5 (55.6)	2 (25.0)	4 (44.4)	6 (75.0)	1.63	0.34
Recurrent episode	-	10 (58.8)	10 (58.8)				4 (44.4)	6 (75.0)				
Age (years)	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	t (df = 32)	p	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	t (df = 15)	p
Onset age (years)	34.7 ± 7.3	35.2 ± 7.5	35.2 ± 7.5	30.7 ± 9.8	-0.19	0.85	32.9 ± 5.6	37.8 ± 8.8	32.9 ± 5.6	37.8 ± 8.8	-1.37	0.19
Weight (kg)	64.9 ± 19.1	57.8 ± 12.8	57.8 ± 12.8	22.6 ± 4.1	1.05	0.31	31.3 ± 6.8	30.0 ± 13.0	31.3 ± 6.8	30.0 ± 13.0	0.27	0.79
Body mass index (kg/m ²)	23.4 ± 4.2	12.5 ± 13.1	12.5 ± 13.1	1.8 ± 5.3	0.58	0.57	57.0 ± 13.4	58.3 ± 13.5	57.0 ± 13.4	58.3 ± 13.5	-0.15	0.88
Daily smoking amount (cigarette/day)	0	1.9 ± 0.9	1.9 ± 0.9	1.1 ± 1.5	-3.12	0.01*	23.5 ± 4.9	21.5 ± 3.0	23.5 ± 4.9	21.5 ± 3.0	0.98	0.34
Number of major depressive episode	0	1.1 ± 1.5	1.1 ± 1.5	0.3 ± 0.6	-8.37	<0.001***	10.6 ± 11.3	14.6 ± 15.3	10.6 ± 11.3	14.6 ± 15.3	-0.62	0.54
Number of suicide attempts	0	1.1 ± 1.5	1.1 ± 1.5	0	-2.95	0.01*	1.6 ± 0.7	2.3 ± 1.0	1.6 ± 0.7	2.3 ± 1.0	-1.62	0.13
HDRS score	0	25.4 ± 5.2	25.4 ± 5.2	13.8 ± 11.4	-19.67	<0.001***	23.9 ± 5.4	27.0 ± 4.8	23.9 ± 5.4	27.0 ± 4.8	-4.58	<0.001***
BSS score	0	13.8 ± 11.4	13.8 ± 11.4	277.1 ± 38.4	-5.02	<0.001***	5.1 ± 5.6	23.6 ± 7.2	5.1 ± 5.6	23.6 ± 7.2	-5.95	<0.001***
4- ^[18F] -ADAM dosage (MBq)	277.1 ± 38.4	290.4 ± 32.7	290.4 ± 32.7		-1.09	0.29	299.0 ± 31.4	280.6 ± 33.3	299.0 ± 31.4	280.6 ± 33.3	1.17	0.26

BSS: Beck Scale for Suicide Ideation; HDRS: 21-item Hamilton Depression Rating Scale; MDD: Major depressive disorder; SD: standard deviation. Total MDD group indicates pooled depressed suicidal subgroup and depressed non-suicidal subgroup *p < 0.05, ***p < 0.001.

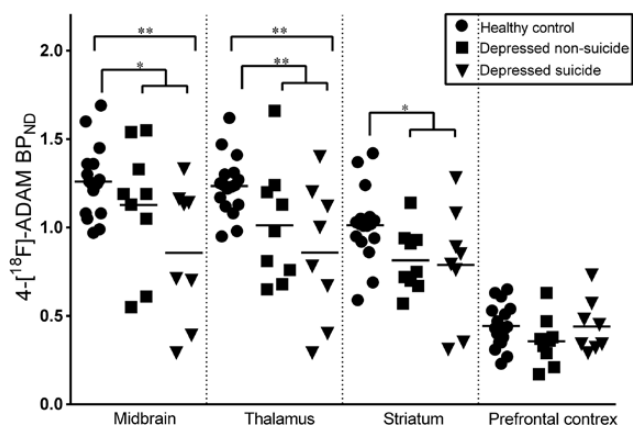


Figure 1. The scatter plot shows the *N,N*-dimethyl-2-(2-amino-4- ^{18}F -fluorophenylthio)benzylamine (4- ^{18}F -ADAM) binding potential of nondisplaceable region in the four regions of interest among depressed suicide patients, depressed non-suicide patients, and healthy controls. *Bonferroni-adjusted p -value < 0.05, **Bonferroni-adjusted p -value < 0.01 for multiple comparisons.

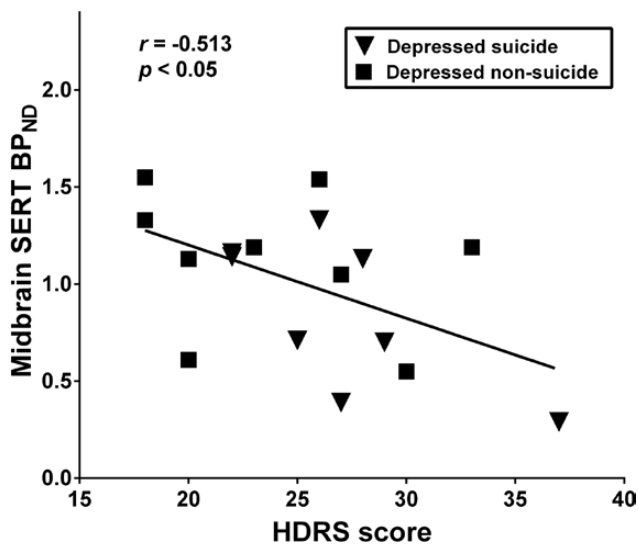


Figure 2. Correlation between Hamilton Depression Rating Scale (HDRS) scores and midbrain *N,N*-dimethyl-2-(2-amino-4- ^{18}F -fluorophenylthio)benzylamine binding potential of nondisplaceable region (BP_{ND}). SERT, serotonin transporter.

et al., 2013). Nevertheless, Miller et al. (2013) found no difference in SERT BP when comparing depressed subjects with healthy controls.

These conflicting results may be attributed to several factors. First, to clarify the effect of suicide attempts on SERT binding, we emphasized that only subjects who had recently attempted suicide could be recruited to the DS group. Therefore, compared to the findings of previous studies, which recruited subjects with past histories of attempted suicide (Parsey et al., 2006; Miller et al., 2013), our results may be more reflective of the effect of the subjects' last suicide attempt on SERT binding. Furthermore, in agreement with Miller et al. (2013), a higher percentage of individuals who had attempted suicide fell within the MDD group (47.1% in the present study), and this could have produced more significant differences on SERT binding between MDD and control groups. Second, antidepressants have been shown to either downregulate (Benmansour et al., 2002; Baudry et al., 2010) or upregulate (Qiu et al., 2013; Shrestha et al., 2014) SERT expression in animal studies. Therefore, SERT expression in the brain

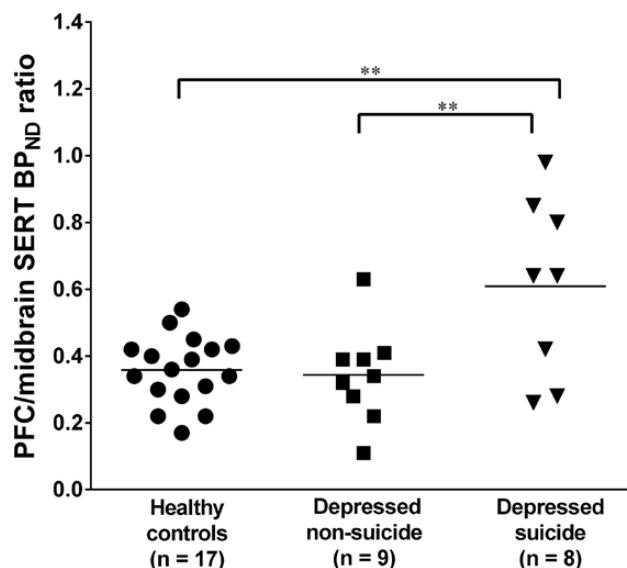


Figure 3. The *N,N*-dimethyl-2-(2-amino-4- ^{18}F -fluorophenylthio)benzylamine binding potential of nondisplaceable region (BP_{ND}) ratio between prefrontal cortex and midbrain among depressed suicide, depressed non-suicide patients, and healthy controls. Bars represent mean values. **Bonferroni-adjusted p -value < 0.01 for multiple comparisons. PFC, prefrontal cortex; SERT, serotonin transporter.

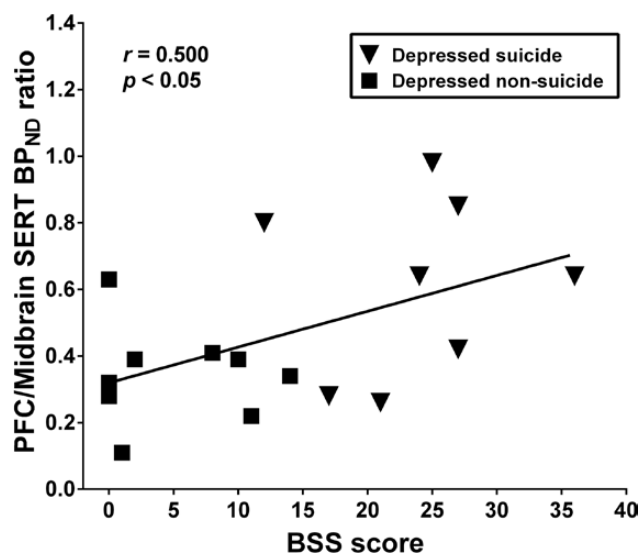


Figure 4. Correlation between Beck Scale for Suicide Ideation (BSS) scores and prefrontal cortex (PFC)/midbrain serotonin transporter (SERT) binding potential of nondisplaceable region (BP_{ND}) ratio.

may be altered in subjects with depression who have received antidepressant treatment. To overcome this limitation, our participants were antidepressant-naïve subjects with MDD; thus, we may have been able to obtain more accurate SERT binding data than what has been collected from antidepressant users.

Furthermore, non-specific binding due to the use of various radioligands should be taken into account when discussing previous studies (Brust et al., 2006; Meyer, 2007), such as ^{123}I - β -CIT in SPECT (Lindstrom et al., 2004; Ryding et al., 2006) or ^{11}C (+)-McN5652 in PET (Parsey et al., 2006). Previous studies using ^{123}I - β -CIT SPECT (Lindstrom et al., 2004; Ryding et al., 2006) to measure SERT binding have shown no significant difference between

suicide attempters and controls. Nevertheless, the radioligand of [¹²³I]-β-CIT presents lack of specific binding to the SERT and near equal affinity of the radioligand for the dopamine transporter (DAT; Meyer, 2007). Moreover, increased striatal DAT has been found in subjects with MDD (Brunswick et al., 2003; Yang et al., 2008). Therefore, the net effect of SERT and DAT measured by [¹²³I]-β-CIT SPECT may veil true SERT binding, leading to no difference of BP between depressed suicide attempters and controls. Another study using [¹¹C](+)McN5652 PET (Parsey et al., 2006) demonstrated significant differences in BP in the midbrain and amygdala between depressed subjects and healthy controls, but no difference between subjects with MDD who had attempted suicide and control subjects. Although the radioligand of [¹¹C](+)McN5652 shows greater selectivity for SERT compared with other monoamine transporters, it has a low ratio of specific binding relative to free and non-specific binding (Brust et al., 2006). In the above studies, participants had various psychiatric diagnoses in addition to major depression (Lindstrom et al., 2004; Ryding et al., 2006) or other psychiatric comorbidity (Parsey et al., 2006); thus, these factors may have confounded the results of previous investigations. In the present study, we only enrolled subjects with MDD in current major depressive episodes and excluded other subjects showing psychiatric comorbidity to avoid confounding effects. Thus, our study may reflect the diagnostic effect of MDD rather than the effect of different psychiatric disorders.

The second major finding of our study was a higher PFC/midbrain SERT BP ratio in the DS group than in both DNS and control groups. Neither midbrain SERT binding nor projection area SERT binding levels individually correlated with suicide intensity assessed by BSS in patients with MDD. However, the PFC/midbrain SERT BP ratio positively correlated with BSS scores. This incongruent reduction of SERT availability in innervating terminals (PFC) of serotonergic neurons relative to the midbrain (DRN) indicated that a great decrease of SERT in the midbrain raphe rather than an increase of SERT in the projecting PFC region might distinguish depressed suicidal subjects from depressed non-suicidal subjects. Although decreased SERT binding in the PFC has been suggested to reduce serotonin input to the PFC and may thereby predispose subjects to act on their suicidal ideations (Arango et al., 2002), we suggest that an unequal reduction of SERT BP_{ND} in the PFC and midbrain may be involved in the pathogenesis of suicide behaviors.

We propose two possible mechanisms for the incongruent reduction of SERT BP in different brain regions. First, post-mortem studies have shown lower brainstem 5-HT and its metabolite, 5-hydroxyindoleacetic acid, in suicide victims than in non-suicide controls (Kamali et al., 2001; Pandey, 2013). Cerebrospinal fluid 5-hydroxyindoleacetic acid levels are lower in high-lethality suicide attempters than low-lethality suicide attempters with major depression (Mann and Malone, 1997). Therefore, low SERT binding in the midbrain in depressed suicidal subjects may be a result of SERT internalization in response to low 5-HT levels (Ramamoorthy and Blakely, 1999). Moreover, fewer serotonergic axons and fewer serotonergic neurons in depressed suicidal subjects would reduce SERT levels in projecting terminals (Austin et al., 2002), which might be compensated partly by upregulation of SERT in the remaining serotonergic axon terminals of the PFC to maintain 5-HT homeostasis (Arango et al., 2002). Second, PFC pyramidal neurons could modulate DRN serotonergic neural activity and extracellular 5-HT levels through 5-HT_{1A} autoreceptors and glutaminergic and gamma-aminobutyric acid (GABA)ergic pathways in a complex neuronal circuit in animal models of stress (Celada et al., 2001; Artigas, 2013). Therefore, depressed suicidal attempters might be reacting to environmental stress that activates PFC pyramidal neurons to enhance SERT

levels in the PFC region, and then reduces midbrain DRN serotonergic neuron activity and SERT levels through a process of complex negative feedback (Warden et al., 2012; Artigas, 2013). The increased PFC/midbrain SERT BP ratio demonstrated lower levels of 5-HT neurotransmission at the PFC region because of increased reuptake, while there were higher levels of 5-HT at the midbrain raphe nuclei, resulting in decreased serotonergic firing through activation of 5-HT_{1A} autoreceptors (Best et al., 2010; Lanzenberger et al., 2012). Therefore, the net effect would be a great reduction of 5-HT neurotransmission in the PFC that would lead to suicidal behaviors. Our preliminary data may provide the first evidence that the PFC/midbrain SERT binding ratio may play an important role in suicidal behaviors.

Finally, it should be mentioned that the striatum and cortico-basal ganglia pathway have been suggested to play a crucial role in the neuropathology of affective disorders and subsequent suicide behaviors (Marchand et al., 2012). In addition, the basal ganglia are associated with reward prediction, which is involved in decision-making (Tanaka et al., 2004). As previously mentioned, we found that the DS group had the lowest SERT availability in the midbrain, thalamus, and striatum (marginal association). This may contribute to the development of suicidal action through decreasing subcortical function of impulse control and increasing cortical executive performance of suicidal plans.

A few limitations of the present study should be addressed. Our small sample size (n = 8 for the DS subgroup and n = 9 for the DNS subgroup) was probably insufficient to see an association between genotype on each group's SERT BP. Our findings are preliminary and will need further replication. However, our previous study had shown no effect of either 5-hydroxytryptamine transporter-linked polymorphic region or ST_{in} variable number tandem repeat polymorphisms on SERT binding using [¹²³I]ADAM SPECT (Ho et al., 2013), but we can still not rule out other genetic or epigenetic effects on SERT binding. Second, there was no collection of child abuse/trauma history in this study. Therefore, we were unable to explore the possible effects of childhood experiences and environment × gene interaction on SERT availability. Third, the equilibrium ratio method for 4-[¹⁸F]-ADAM PET imaging was applied to this study; however, there is a lack of arterial input compartment modeling in human studies.

Conclusion

Our result demonstrated reduced SERT availability in the midbrain and thalamus of antidepressant-naïve patients with MDD, especially in depressed suicide attempters relative to healthy controls. This study also provided initial evidence of a higher PFC/midbrain SERT binding ratio in suicide attempters than in non-attempters, which may be involved in the pathogenesis of suicide behaviors. Further prospective follow-up studies are required to establish consistency of the incongruent reduction of SERT availability in serotonergic projection region relative to the DRN.

Supplementary Material

For supplementary material accompanying this paper, visit <http://www.ijnp.oxfordjournals.org/>

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Statement of Interest

None.

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