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Positron Emission Tomography Quantification of Serotonin Transporter Binding in Medication-Free Bipolar Disorder

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Abstract

Objectives—Bipolar disorder (BD) is associated with abnormalities in the serotonin transporter (5-HTT), but specific *in vivo* findings have been discrepant. Using positron emission tomography (PET) and [¹¹C]DASB, we compared 5-HTT binding between unmedicated depressed BD subjects and healthy volunteers (HVs).

Experimental Design—5-HTT binding in six brain regions was compared between 17 depressed, unmedicated BD subjects and 31 HVs, using the outcome measure of V_T/f_p (proportional to the total number of available transporters). Alternative outcome measures were examined as well. 47% of BD were BP I; 65% reported a prior suicide attempt.

Principal Observations—5-HTT binding (V_T/f_p) did not differ between BD and HV groups considering six brain regions of interest simultaneously ($p=0.24$). In contrast, alternative outcome measures (BP_F^* , BP_P^* , and BP_{ND}^*) indicated lower binding in BD compared with HV across these six regions of interest (BP_F^* : $p=0.047$; BP_P^* : $p=0.032$; BP_{ND}^* : $p=0.031$). 5-HTT binding was unrelated to suicide attempt history, depression severity, bipolar subtype, or history of past substance use disorder.

Conclusions—Choice of outcome measure strongly affects comparisons of serotonin transporter binding using PET with [¹¹C]DASB. We do not find evidence of abnormal 5-HTT binding in bipolar depression using our primary outcome measure, V_T/f_p . However, we did observe lower 5-HTT binding in BD with alternative outcome measures that are frequently used with [¹¹C]DASB. Relative merits and assumptions of different outcome measures are discussed. Evaluation in larger samples and during different mood states, including remission, is warranted.

Graphical abstract

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Keywords

DASB; PET imaging; mood disorders; 5-HTT; SERT

INTRODUCTION

Bipolar disorder (BD) is a prevalent psychiatric disorder (Kessler et al., 2005; Merikangas et al., 2007) that ranks as the sixth leading cause of disease burden worldwide (Murray, 1996; Murray and Lopez, 1997), and is characterized by episodes of mania and depression. It carries a substantially increased risk for nonfatal suicide attempts and completed suicide (Goodwin and Jamison, 2007; Jamison, 2000; Marangell et al., 2006). Most suicides in BD occur during major depressive episodes (MDEs) (Judd and Akiskal, 2003), which are clinically comparable to MDEs occurring in the context of major depressive disorder (MDD).

Abnormalities in the serotonin (5-HT) neurotransmitter system are implicated in the etiology of bipolar disorder (Oquendo and Mann, 2000). Positron emission tomography (PET) studies of bipolar disorder report altered regional serotonin transporter (5-HTT) binding, but disagree about which brain regions differ and the direction of binding difference relative to healthy volunteers. We have reported 16–26% lower 5-HTT binding across six brain regions in a group of depressed, medication-free bipolar subjects compared to controls using the radioligand [^{11}C]McN5652 and outcome measure BP_{P} (Oquendo et al., 2007). In contrast, another study of depressed, medication-free bipolar subjects reported regional heterogeneity, with higher 5-HTT binding in cortical and subcortical regions, and lower 5-HTT binding in the brainstem, compared with controls using the ligand [^{11}C]DASB and outcome measure BP_{ND} (Cannon et al., 2006a). A third study of six BD subjects found no difference in [^{11}C]McN5652 BP_{ND} between BD subjects and controls (Ichimiya et al., 2002). These inconsistencies may result from methodological factors, such as the use of different radiotracers and outcome measures to estimate the density of 5-HTT receptors. Alternatively, bipolar subtypes or mood state may be associated with different 5-HTT binding in BD. This possibility is suggested by a SPECT study of euthymic bipolar patients finding low midbrain 5-HTT binding in bipolar I subjects compared with bipolar II subjects and healthy controls (Chou et al., 2010), although this finding was not replicated by other *in vivo* imaging studies in BD (Cannon et al., 2006a; Oquendo et al., 2007).

Altered *in vivo* 5-HTT binding in BD may also be related to suicidal behavior. We and others have reported lower 5-HTT binding postmortem in suicides, although some studies disagree ((Arango et al., 1995; Mann et al., 2000; Purselle and Nemeroff, 2003), reviewed in (Stockmeier, 2003)). We recently found low *in vivo* 5-HTT binding in midbrain in currently depressed MDD subjects with a history of prior suicide attempt compared with depressed MDD non-attempters or controls (Miller et al., 2013a). In partial agreement, lower midbrain, but higher pregenual, 5-HTT binding *in vivo* has been reported in bipolar suicide attempters compared to bipolar non-attempters (Cannon et al., 2007).

To evaluate potential reasons for the above cited discrepant results, and to follow up on our previous finding using the first-generation radiotracer, [^{11}C]McN5652, we now report on 5-HTT binding in a new sample of 18 medication-free, bipolar subjects in a current MDE at the time of scanning and 32 controls using a second-generation 5-HTT radiotracer [^{11}C]DASB that permits fully quantitative methods, including arterial sampling for metabolite-corrected arterial input function and determination of free plasma radioligand fraction (f_p). In comparison to [^{11}C]McN5652, [^{11}C]DASB has better specific/nonspecific binding ratio and measurable plasma free fraction (f_p) (Frankle et al., 2004). We hypothesized lower 5-HTT binding in midbrain, putamen, amygdala, thalamus, hippocampus and anterior cingulate (ACN) in BD during depression compared with healthy controls. Based on postmortem findings (Arango et al., 2001; Arango et al., 1995), we further hypothesized that bipolar depressed individuals with suicide attempt history would have lower 5-HTT binding in ventral prefrontal cortex (VPFC), ACN, and midbrain compared with bipolar depressed individuals without prior suicide attempt. To facilitate comparisons to previous published findings results are reported with several alternative outcome measures for [^{11}C]DASB.

MATERIALS AND METHODS

Sample

18 subjects with bipolar disorder in a current MDE and 32 healthy volunteer controls initially enrolled in this study. One bipolar subject was an extreme low outlier, with binding (V_T/f_p) values 2.8–3.5 standard deviations below the bipolar group mean for all 6 ROIs; this subject was removed from all analyses. One healthy volunteer was subsequently determined not to meet eligibility criteria due to a diagnosis of past alcohol abuse, and was excluded from all analyses, leaving a final analyzed sample of 17 bipolar subjects and 31 healthy volunteers. Participants were recruited 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.

Psychiatric diagnoses were established by masters or PhD-level psychologists using the Structured Clinical Interview for DSM-IV (SCID) (First et al., 1995); diagnoses were based on a consensus conference with senior research clinicians to generate best-estimate diagnoses. The Beck Depression Inventory (BDI) (Beck et al., 1961), Hamilton Depression Rating Scale (HAM-D) (Hamilton, 1960), and Global Assessment Scale (GAS) (Endicott et al., 1976) assessed depression severity and functional impairment. Inclusion criteria for bipolar subjects included: 1) bipolar disorder with a current MDE; 2) 17-item Hamilton

Depression Rating Scale score 15 at time of screening; 3) 18–65 years of age; 4) off all psychotropic and other types of medications likely to interact with 5-HTT for at least 14 days (off neuroleptics for 3 weeks). Among the 16 of 18 bipolar subjects with prior psychotropic medication exposure, median duration off of such medications was 73 days (range: 16 to 3640 days). For those undergoing medication washout, short-acting benzodiazepines were allowed for distressing anxiety or insomnia up to 24 hours prior to PET scanning; 2 subjects used benzodiazepines for this purpose. Exclusion criteria included 1) other major psychiatric disorders such as schizophrenia or schizoaffective disorder; anorexia nervosa or bulimia nervosa in the past year; drug or alcohol abuse within the past 2 months or dependence within the past six 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 males and 26.8 in females (Loranger, 1984)); 3) significant active physical illness; 4) lack of capacity to consent to study participation; 5) pregnancy or lactation among women; or 6) previous head injury with loss of consciousness requiring some medical evaluation.

For healthy controls, 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; or 3) age 18–65 yr. Exclusion criteria included: 1) significant active physical illness; 2) past or present alcohol/substance abuse or dependence; 3) previous head injury with loss of consciousness as above; or 4) first-degree relative with history of major depression, schizophrenia, schizoaffective disorder, or suicide attempt; two or more first-degree relatives with a history of substance dependence. Thirteen BD subjects had comorbid current or lifetime Axis I diagnoses, which included PTSD (n=7 current, 4 remitted), remitted substance abuse or dependence (n=8), panic disorder (n=6 current, 1 remitted), obsessive-compulsive disorder (n=3 current, 2 remitted), social phobia (n=2 current, 1 remitted), and simple phobia (n=1 current).

Radiochemistry and input function measurement

Preparation of [^{11}C]DASB, measurement of arterial input function, metabolites, and plasma free fraction (f_p) were performed as previously described (Belanger et al., 2004; Ogden et al., 2007). The chemical purity of [^{11}C]DASB was 95%. Injected mass, injected dose, and f_p did not differ between bipolar subjects and controls (Table 1).

PET Protocol

Details of the PET protocol are described elsewhere (Ogden et al., 2007). Briefly, a venous catheter was used for injection of the radiotracer and an arterial catheter to obtain arterial samples for the input function. PET imaging was performed with the ECAT HR+ (Siemens/CTI, Knoxville, TN, USA). A 10-minute transmission scan was obtained prior to radiotracer injection. At the end of the transmission scan, 16.21 ± 2.34 mCi of [^{11}C]DASB was administered intravenously as a bolus over 30 seconds. Emission data were collected in 3D mode for 100 minutes with 19 frames of increasing duration.

Magnetic Resonance Imaging

Acquisition of T1-weighted MRI images for co-registration of PET images and identification of regions of interest (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, WI) (Parsey et al., 2000).

Image Analysis

The FMRIB linear image registration tool (FLIRT), version 5.0 (FMRIB Image Analysis Group, Oxford, UK) was used to correct for subject motion during the scan. An automated algorithm was used to obtain ROIs in this study (midbrain, amygdala, putamen, thalamus, hippocampus, anterior cingulate, prefrontal cortex, and cerebellar gray matter) using nonlinear registration techniques to warp 18 manually outlined MRIs to the target image (Milak et al., 2010). At each voxel, the probability of being within an ROI was calculated as the number of transformed template ROIs assigned to that voxel divided by 18. Non-midline ROIs were averaged across hemispheres. In previous work, this approach yielded very high correlations of binding as compared to those obtained with manually-drawn ROIs ($r^2 > 0.98$; (Milak et al., 2010)). The mean PET image was coregistered to each subject's MRI so that the regions of interest could be applied to the mean PET image, as well as individual PET frames. PET-to-MRI transformations were computed using FLIRT with a mutual information cost function, six degrees of freedom, and trilinear interpolation, optimized as previously described (DeLorenzo et al., 2009). Time activity curves were generated by plotting the measured activity within a region over the time course of the PET acquisition.

Outcome Measure Estimation

Because no brain region is devoid of specific binding with [^{11}C]DASB (Parsey et al., 2006c), we used an outcome measure that does not rely on a reference region: V_T/f_p (where V_T is the volume of distribution in the region of interest). This outcome measure has been used in several studies by different groups where a reference region is not available (Chin et al., 2011; Esterlis et al., 2010; Fujita et al., 2012; Ichise, 2009; Mukhin et al., 2008). [^{11}C]DASB regional volume of distribution (V_T) values were derived using likelihood estimation in the graphical approach (LEGA), which reduces the noise-dependent bias inherent in the graphical approach (Ogden, 2003; Parsey et al., 2003). Brain activity was corrected for the contribution of plasma activity assuming a 5% blood volume in the regions of interest (Mintun et al., 1984). The primary outcome measure V_T/f_p was calculated as total distribution volume corrected for plasma free fraction. For comparison purposes to other published studies, we also calculated the following outcome measures, using cerebellar gray matter (CGM) as reference region: $BP_F^* = (V_{T(\text{ROI})} - V_{T(\text{REF})})/f_p$, $BP_P^* = (V_{T(\text{ROI})} - V_{T(\text{REF})})$, and $BP_{ND}^* = (V_{T(\text{ROI})} - V_{T(\text{REF})})/V_{T(\text{REF})}$. Asterisks are added to consensus terminology designation (Innis et al., 2007), to emphasize that the “reference” region does in fact have some appreciable specific binding (Parsey et al., 2006d), and thus these outcome measures are biased, the extent of the bias depending on the level of specific binding in the purported reference region. All binding potential measures involve a difference between the V_T of the target region and the V_T of the reference region, which is taken to be an estimate of V_{ND} , the level of nondisplaceable binding (3). Therefore, any specific binding in the

purported reference region will cause the estimate of V_{ND} to have a positive bias, and thus the estimate of any of the binding potential outcomes will have a negative bias.

Statistics

To borrow strength across all ROIs and to 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 or binding potential estimates with region and diagnostic group as fixed effects and subject as the random effect. Data entered in linear mixed-effects models were first log transformed; this was performed to remedy slight skewness of binding estimates (Hirvonen et al., 2008; Meltzer et al., 2004; Rabiner et al., 2002) to stabilize the variance, and because our principal hypothesis of a difference between groups specifies differences in each ROI proportional to each ROI's binding level. Log transformation has been used in multiple previous PET studies by our group and others to address these issues (Hirvonen et al., 2008; Miller et al., 2009a; Miller et al., 2013a; Miller et al., 2013b; Miller et al., 2009b; Oquendo et al., 2007; Parsey et al., 2006a; Parsey et al., 2006b; Parsey et al., 2010; Parsey et al., 2006e; Parsey et al., 2006f; Sullivan et al., 2005). Other groups have used related statistical approaches, including linearizing transformation (Rabiner et al., 2002) and non-parametric testing (Meltzer et al., 2004) to address these issues in analyzing PET data. 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 binding values were weighted in the model according to standard errors computed using a bootstrap algorithm that takes into account errors in metabolite, plasma, and brain data (Ogden and Tarpey, 2006). To prevent any individual data points from dominating the analysis due to chance underestimation of standard errors, the weights were Winsorized at the 30th percentile within each region (Potter, 1988; Potter, 1990). Linear mixed effects models of binding and Fisher's exact tests were performed in R 2.10.0 (<http://cran.r-project.org>). T-tests were performed in SPSS Statistics 18 (<http://www.spss.com/software/statistics/>).

RESULTS

Demographic and clinical data for bipolar and control subjects are in Table 2. The two groups had comparable sex distribution ($p=0.13$, by Fisher's exact test) and age (range 21–61 years; $t = 1.4$; $p=0.17$). Thirteen BD subjects had comorbid current or lifetime Axis I diagnoses, as described above.

Bipolar depressed versus healthy controls

A linear mixed-effects model of 5-HTT V_T/f_P did not show a difference between BD and control groups across midbrain, amygdala, putamen, thalamus, hippocampus, and ACN ($F_{1,46}=1.42$, $p=0.24$; Figure 1 and Table 3). Although a region $\times$ diagnosis interaction ($F_{5,230}=3.26$, $p=0.0072$) was present, indicating that the relationship between diagnosis and 5-HTT V_T/f_P was heterogeneous across examined brain regions, in post-hoc testing V_T/f_P did not differ significantly between BD and control groups in any individual region.

There was no effect of depression severity on 5-HTT V_T/f_P values, as assessed using the Beck Depression Inventory ($F_{1,46} = 2.63$, $p = 0.11$) or the Hamilton Depression Rating Scale

($F_{1,46} = 1.55$, $p = 0.22$), nor was there an effect of age on 5-HTT V_T/f_P values ($F_{1,45} = 2.03$, $p = 0.16$).

Possible covariates

Eleven of 17 bipolar subjects (65%) had a suicide attempt history. V_T/f_P did not differ between BD attempters and BD non-attempters in the *a priori* regions of midbrain, VPFC, and ACN ($F_{1,15} = 0.28$, $p = 0.61$). Eight of 17 bipolar subjects (47%) were BD I. Bipolar subtype was unrelated to V_T/f_P ($F_{1,46} = 0.19$, $p = 0.67$) in the initial 6 ROIs. We did not observe an effect of past substance use disorder history on V_T/f_P ($F_{1,45} = 1.78$, $p = 0.19$). Since only two of the BD participants were antidepressant treatment naïve, it was not possible to determine the effect of exposure to antidepressant medication on 5-HTT binding.

Alternative outcome measures

Previous PET studies estimated 5-HTT binding in BD subjects using other outcome measures. For comparison purposes, we therefore modeled our data with the outcome measures BP_F^* , BP_P^* , and BP_{ND}^* . Across the six ROIs examined, BD had lower BP_F^* , BP_P^* , and BP_{ND}^* than controls (Table 3: BP_F^* : $F_{1,46} = 4.17$, $p = 0.047$; BP_P^* : $F_{1,46} = 4.92$, $p = 0.032$; BP_{ND}^* : $F_{1,46} = 4.95$, $p = 0.031$). Reference region binding ($V_{T(REF)}$, in cerebellar gray matter (CGM)) did not differ between BD subjects and controls ($F_1 = 1.47$, $p = 0.25$).

DISCUSSION

Using [^{11}C]DASB and the outcome measure V_T/f_P , we did not find a difference in 5-HTT binding between BD subjects and healthy controls across six *a priori* ROIs, unlike our previous findings in BD (Oquendo et al., 2007). We did observe a modest group by brain region interaction that suggests heterogeneity of the binding comparison between groups across regions; however, no individual region showed a significant difference in binding on post-hoc testing. Unlike MDD, we did not observe an effect of prior suicide attempt history on 5-HTT binding. Consistent with our *a priori* hypotheses, we found no relationship between 5-HTT binding and bipolar subtype. In contrast, using three other outcome measures that are frequently used in [^{11}C]DASB studies (BP_F^* , BP_P^* , and BP_{ND}^*), we observed lower 5-HTT binding across all ROIs in bipolar subjects compared to controls. The possible reasons for different findings across outcome measures are considered in detail below.

Comparison to previous *in vivo* imaging studies

Using the radiotracer [^{11}C]McN5652 with the outcome measure BP_P , we previously observed low BP_P across all regions examined in a cohort of 18 BD as compared to 41 HV subjects (Oquendo et al., 2007). We do not replicate this finding in the current study with the primary outcome measure V_T/f_P , although we do replicate it using all alternative outcome measures, including the outcome measure used in the previous publication (BP_P^*). In partial agreement with current findings with binding potential measures, a previous study using [^{11}C]DASB found lower brainstem 5-HTT BP_{ND} but higher cortical and subcortical BP_{ND} in BD (Cannon et al., 2006b). Thus, our findings using V_T/f_P do not replicate those of

previous studies of bipolar depression, but our findings with alternative binding potential outcome measures partially or fully replicate previous work by our group and others.

We chose the outcome measure V_T/f_p to quantify [^{11}C]DASB binding primarily because there is measurable specific binding throughout the traditional reference region of the cerebellum, including cerebellar gray matter (Parsey et al., 2006d), that violates the basic assumption that the reference region is free of specific binding. V_T/f_p does not require a reference region, but as a result does not account for non-specific binding in the brain, which is high with most 5-HTT tracers. This could potentially explain a negative finding with V_T/f_p and positive findings with binding potential measures, as we observe in the current data. Of note, we found no differences between bipolar subjects and healthy volunteers in reference region binding ($V_{T(\text{REF})}$). This may mitigate problems arising from the use of binding potential (BP) outcomes in this instance.

An additional advantage of V_T/f_p is that it corrects for the plasma free fraction of radioligand (f_p), which is available to bind 5-HTT target sites. BP_p^* , by contrast, is calculated using total plasma concentration of radioligand, and assumes comparable f_p in bipolar and control groups. A potential disadvantage of outcome measures that correct for f_p , including BP_p^* and V_T/f_p , is the potentially for measurement noise in the assessment of f_p that may obscure a group difference that might be detected by alternative binding potential measures. In the current study, variance in f_p measurements was low, and f_p did not differ between groups.

The use of different outcome measures across studies in the bipolar PET literature therefore appears to be consequential. Using all BP^* measures, we did identify lower binding in bipolar subjects than controls, comparable to our previous findings in BD (Oquendo et al., 2007). We and others are actively working to develop methodology that will overcome the problem of specific binding in a putative reference region, using reference-free mathematical models to estimate V_{ND} (Ogden et al., 2015).

Interpretation of findings

The finding with our primary outcome measure argues against a large abnormality in brain 5-HTT in the pathophysiology of BD. However, if findings with alternative BP^* measures are reflective of underlying biology, low 5-HTT binding in bipolar subjects could represent fewer serotonin transporter sites available to bind [^{11}C]DASB, based on postmortem studies that assayed transporter binding density and affinity (Perry et al., 1983). The cause could be low intrasynaptic serotonin levels, that may in turn, enhance internalization of serotonin transporters into the presynaptic terminal (Ramamoorthy and Blakely, 1999). Indeed, we have previously shown the rapid responsiveness of 5-HTT to intra-synaptic levels through tryptophan depletion studies (Milak et al., 2005). Fewer 5-HTT binding sites could also reflect reduced innervation by serotonergic neurons or neuronal processes (Mann et al., 2000; Soucy et al., 1994).

The absolute magnitude of group differences in binding potential measures is small, with a range of 5 to 27% across regions and binding potential outcomes, with significant group overlap. These findings are smaller in magnitude than group differences observed with other

serotonergic markers in bipolar disorder, such as 5-HT_{1A} binding assessed by [¹¹C]WAY-100635 (Sullivan et al., 2009). This suggests that 5-HTT quantification is unlikely to be used as a diagnostic biomarker for bipolar disorder. Some of the variance in 5-HTT binding within the bipolar group may reflect the heterogeneity of this illness, present at both phenomenological and biological levels. Parsing such heterogeneity in the future through approaches such as NIMH's Research Domain Criteria (RDoC, (Cuthbert and Insel, 2013)) may identify an aspect of neurocognitive functioning more closely associated with 5-HTT binding than the categorical diagnosis of bipolar disorder.

Strengths, limitations and conclusions

The limitations of this study include modest sample and the limitations of the binding outcome measure described above. Moreover, we previously reported that depressed, AD-naïve MDD patients have lower 5-HTT binding than AD-exposed counterparts (Parsey et al., 2006b). If this pattern is also present in bipolar depression, our current results would underestimate possible 5-HTT binding deficits in bipolar disorder, as our sample included only two AD-naïve bipolar patients, and 38% of the BD subjects had been exposed to AD medications within nine weeks of scanning. Another potential limitation is the psychiatric comorbidity of the bipolar sample, including current and past anxiety disorders, as well as remitted substance use disorders. Our sample is highly representative of bipolar illness in this regard: 75% of individuals with bipolar disorder experience comorbid anxiety disorders and 42% experience comorbid substance use disorders (Merikangas et al., 2007). We repeated the primary contrast (bipolar vs. healthy volunteers across 6 primary ROIs) using only the subset of bipolar individuals with no history of current or past anxiety disorders; effect sizes with all outcome measures were comparable to those obtained when considering the entire bipolar sample (data not shown). However, we cannot exclude the possibility that anxiety comorbidity may represent a confound in the current analyses.

The strengths of this study include rigorous diagnostic evaluation of all subjects; a radioligand with high sensitivity and specificity for the serotonin transporter; and consideration of a range of possible PET outcome measures derived through fully quantitative estimation, including a metabolite-corrected arterial input function and measurement of f_p to allow comparison to the existing literature.

In summary, we do not find evidence of a widespread difference in 5-HTT binding in BD using our primary outcome measure. We do observe 5-HTT binding deficits in BD using alternative outcome measures. Future studies are needed to resolve these discrepancies and further clarify serotonergic abnormalities in bipolar disorder.

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Figure 1.

Comparison of [^{11}C]DASB binding potential (V_T/f_P) between bipolar and control groups. Bar heights represent the weighted means for each region of interest. Error bars indicate the corresponding standard deviations of the weighted means. No group difference was observed across the six regions considered simultaneously ($p=0.24$).

Table 1^[11C]DASB radiochemistry

	Healthy Volunteers (n=31)	Depressed Patients with Bipolar Disorder (n=17)	<i>t</i> test	<i>P</i> value
Injected Dose (mCi)	16.14 ± 2.36	16.30 ± 2.44	0.21	.83
Injected Mass (μg)	4.36 ± 2.31	5.38 ± 2.27	1.5	.15
Free Fraction (f _p)	0.12 ± 0.03	0.11 ± 0.02	.92	.36

Table 2

Demographic and clinical data for bipolar and control subjects

	Healthy Volunteers (n=31)	Depressed Patients With Bipolar Disorder (n=17)	<i>t</i> statistic	<i>P</i> value
Sex, M/F, No.	15/16	4/13	NA	0.13*
Age, Mean±SD, y	32.6±11.3	37.1±8.9	1.40	0.17
Hamilton Depression Rating Scale (24 items) score, mean ± SD	1.7 ± 2.4	26.8±7.6	17.1	< 0.00001
Beck Depression Inventory score, mean ± SD	1.3 ± 1.7	23.5 ± 9.2	13.1	< 0.00001
Global Assessment Scale score, mean ± SD	90.0 ± 6.4	52.6 ± 9.6	15.9	< 0.00001
Bipolar I/bipolar II, n	NA	8/9	NA	NA
Inpatients/outpatients, n	NA	10/7	NA	NA
Antidepressant history, exposed/naïve, n	NA	15/2	NA	NA
Suicide attempters/nonattempters, n	NA	11/6	NA	NA
Number of suicide attempts in attempters (n=11), n	NA	1.82±2.51	NA	NA
First-degree relative with MDD, n (%)	NA	10(59)	NA	NA
First-degree relative with bipolar depression, n (%)	NA	4(22)	NA	NA

* Fisher's Exact Test

Table 3

Comparison of [^{11}C]DASB binding using different outcome measures. Estimated weighted mean binding levels are shown with associated standard errors.

V_T/f_p	Bipolar	Control	% Difference (Control-Bipolar)
Anterior Cingulate	117.65 (5.79)	127.54 (4.64)	7.75
Amygdala	183.83 (9.01)	205.01 (7.43)	10.33
Dorsal Putamen	193.21 (9.51)	199.89 (7.26)	3.34
Hippocampus	123.84 (6.09)	131.42 (4.78)	5.77
Midbrain	248.06 (12.25)	275.33 (10.05)	9.9
Thalamus	182.80 (9.01)	188.89 (6.89)	3.22
Cerebellar Grey Matter	86.05 (4.87)	87.00 (3.30)	1.09
BP_F	Bipolar	Control	% Difference (Control-Bipolar)
Anterior Cingulate	28.06 (1.84)	36.55 (1.75)	23.23
Amygdala	94.86 (6.01)	114.66 (5.32)	17.27
Dorsal Putamen	104.18 (6.67)	109.50 (5.14)	4.86
Hippocampus	34.07 (2.24)	40.15 (1.92)	15.14
Midbrain	159.20 (10.31)	184.51 (8.75)	13.72
Thalamus	93.38 (6.01)	98.05 (4.64)	4.76
BP_P	Bipolar	Control	% Difference (Control-Bipolar)
Anterior Cingulate	3.07 (0.24)	4.19 (0.24)	26.73
Amygdala	10.34 (0.78)	13.12 (0.73)	21.19
Dorsal Putamen	11.37 (0.86)	12.53 (0.70)	9.26
Hippocampus	3.72 (0.29)	4.60 (0.26)	19.13
Midbrain	17.36 (1.32)	21.10 (1.18)	17.73
Thalamus	10.18 (0.77)	11.22 (0.63)	9.27
BP_{ND}	Bipolar	Control	% Difference (Control-Bipolar)
Anterior Cingulate	0.32 (0.02)	0.41 (0.02)	21.95
Amygdala	1.07 (0.06)	1.28 (0.05)	16.41
Dorsal Putamen	1.17 (0.06)	1.22 (0.05)	4.1
Hippocampus	0.39 (0.02)	0.45 (0.02)	13.33
Midbrain	1.78 (0.10)	2.05 (0.09)	13.17
Thalamus	1.04 (0.06)	1.09 (0.05)	4.59