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PET QUANTIFICATION OF SEROTONIN_{1A} RECEPTOR BINDING IN MEDICATION-FREE BIPOLAR DEPRESSION

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Abstract

Background—Little is known about the serotonin_{1A} receptor (5-HT_{1A}) in bipolar depression despite altered 5-HT_{1A} binding in major depressive disorder (MDD). Utilizing PET and the radioligand [C-11]WAY100635, regional 5-HT_{1A} binding was compared between depressed bipolar disorder (BD) and control subjects.

Methods—Brain 5-HT_{1A} binding potential ($BP_F = B_{max}/K_D$, where B_{max} is the total number of available receptors and $1/K_D$ is the receptor affinity of the ligand) was measured in 32 currently depressed, medication-free BD subjects and 47 healthy controls. Participants were also genotyped for the 5-HT_{1A} promoter polymorphism C(-1019)G.

Results—The bipolar depressed group demonstrated higher 5-HT_{1A} BP_F across all regions of interest (ROIs; $p=0.022$). Post-hoc analyses indicated that male BD patients had higher 5-HT_{1A} BP_F than male controls ($p=0.025$), with higher 5-HT_{1A} BP_F found in every region (by 102% in raphe nuclei and 31–54% in the 12 forebrain ROIs). Whereas, female subgroups did not differ in 5-HT_{1A} BP_F ($p=0.32$). 5-HT_{1A} BP_F did not correlate with depression severity. The GG genotype was overrepresented at a trend level in the BD group ($p=0.057$). Number of copies of the G-allele was

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associated with higher 5-HT_{1A} BP_F in raphe ($p=0.0050$), amygdala ($p=0.022$), and hippocampus ($p=0.041$).

Conclusion—Higher 5-HT_{1A} BP_F in depressed males with BD suggests higher raphe autoreceptor binding, potentially causing less serotonin release and compensatory upregulation of postsynaptic 5-HT_{1A} receptors in forebrain regions. The raphe effect may be partly genetic. Lack of difference in 5-HT_{1A} BP_F between BD and control females may reflect greater effect of prior antidepressant exposure in BD females.

Keywords

Bipolar; depression; serotonin; 5-HT_{1A} receptor; positron emission tomography (PET)

INTRODUCTION

Bipolar disorder has high prevalence (1) and substantial morbidity (2) and mortality (3,4). Bipolar disorder is characterized by mania and major depressive episodes (MDEs) that are clinically indistinguishable from those of major depressive disorder (MDD). Most of the disease burden and almost all suicides in bipolar disorder are attributed to the occurrence of depressive episodes, the longest duration illness phase in this condition (5-7).

Impaired serotonergic (5-HT) function is implicated in the pathophysiology of depression and manic phases. Brainstem serotonin-1A autoreceptors (5-HT_{1A}) regulate 5-HT neuronal firing, and other 5-HT auto- and heteroreceptors mediate serotonin effects in many forebrain regions. The downregulation or desensitization of the brainstem 5-HT_{1A} autoreceptors in raphe nuclei that occurs over weeks with serotonin selective reuptake inhibitor (SSRI) treatment mediates greater 5-HT neurotransmission to forebrain regions and is thought to underlie the lag time to antidepressant action (8).

To date, *in vivo* positron emission tomography (PET) imaging studies report altered 5-HT_{1A} binding in MDD although both the direction of the effect and the brain regions involved differ among studies (9-11). We now report on *in vivo* 5-HT_{1A} binding using PET and [C-11] WAY100635, comparing 32 medication-free bipolar disorder patients in an MDE with 47 controls. Participants were also genotyped for the functional promoter C(-1019)G polymorphism in the 5-HT_{1A} gene to explore effects on 5-HT_{1A} binding in bipolar disorder. We hypothesized higher 5-HT_{1A} binding in bipolar depression, similar to the pattern we found in MDD, which would be partly dependent on the G allele promoter variant.

METHODS AND MATERIALS

PARTICIPANTS

Thirty-two patients who presented in a MDE and met DSM-IV criteria for bipolar I or bipolar II disorder on *Structured Clinical Interview for DSM-IV Axis I Disorders* (SCID-I/P) (12), psychiatric interview, and chart review were enrolled. Forty-seven healthy volunteer control participants were also enrolled if free of Axis I diagnosis by SCID-I-NP (12) and without history of mood or psychotic disorder or suicide in any first-degree relative. MA- or PhD-level psychologists performed all SCID interviews with independent diagnostic assessment by a research psychiatrist. Consensus diagnoses were determined by at least 3 senior clinicians. Controls were recruited concurrently with bipolar disorder patients. Data from 43 of the 47 controls have previously been reported (10). Control participants had their respective PET scans between 8/17/1999 and 6/6/2007. Bipolar participants had their respective PET scans between 6/14/1999 and 6/27/2007. All participants were 18-65 years old. Depressed bipolar patients scored ≥ 16 on the 17-item Hamilton Depression Rating Scale (HDRS-17) upon

screening. They were free of antidepressant and mood stabilizer medication for at least 14 days (42 days for fluoxetine) and oral antipsychotics for at least 21 days before PET scan. No patients were on depot antipsychotics. Small doses of shorter-acting benzodiazepines were the only psychotropic permitted, used only as needed until 72 hours before PET. For ethical reasons, no patients were tapered off of medications *effective* for their mood disorder for the purpose of this research. Exclusion criteria for all participants were: significant medical condition as determined by history, physical examination, routine blood and urine tests; positive urine toxicology; positive pregnancy test; history of alcohol or substance use disorder in lifetime for controls and within the previous 6 months for bipolar disorder patients; lifetime exposure to 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”); current or planned pregnancy; or lack of capacity to provide informed consent.

Only three of the 32 bipolar disorder participants were naïve to treatment with an antidepressant medication (12/32 had the last antidepressant discontinued > 120 days before PET; 17/32 had the last antidepressant discontinued 15-120 days before PET, mean $\pm$ SD, 41.0 $\pm$ 30.2 days). Comorbid Axis I disorders in the bipolar disorder group included panic disorder (n=7), posttraumatic stress disorder (n=6), obsessive compulsive disorder (n=5), simple phobia (n=2), social anxiety disorder (n=1), and generalized anxiety disorder (n=1). Twelve of the 32 met DSM-IV criteria for a past history (> 6 months before screening) of alcohol or substance use disorder (9 alcohol; 5 cannabis; 3 cocaine; 1 stimulant; 1 opiate).

All participants provided written informed consent after description of the study protocol as approved by the Institutional Review Boards of the New York State Psychiatric Institute and Columbia University Medical Center.

CLINICAL ASSESSMENTS

The HDRS-17 (13), the Beck Depression Inventory (BDI) (14), and the Global Assessment Scale (15) were utilized to assess depression severity and functional impairment. Lifetime aggression was rated using the Brown-Goodwin Aggression Inventory (16) and manic/hypomanic symptoms using the Young Mania Rating Scale (17).

RADIOCHEMISTRY AND INPUT FUNCTION MEASUREMENT

[Carbonyl-C-11]WAY100635 [N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridinyl) cyclohexane carboxamide] was prepared as previously described (18). Measurements of arterial input function, metabolites, and free plasma fraction (f_p) were made as described previously (18,19). No group differences between controls and bipolar disorder were present in: specific activity of [C-11]WAY100635 (1.56 ± 0.73 mCi/nmol versus 1.88 ± 1.04 mCi/nmol; $t=-1.50$, $df=51.3$, $p=.14$); injected mass (2.86 ± 1.88 μ g versus 2.31 ± 1.97 μ g; $t=1.27$, $df=77$, $p=.21$); or injected dose (8.17 ± 3.52 mCi versus 6.70 ± 3.58 mCi; $t=1.81$, $df=77$, $p=.08$).

IMAGE ACQUISITION AND ANALYSIS

Acquisitions of MRI and PET data were performed as described previously (19). Image analysis was performed using MATLAB 2006b (The Mathworks, Natick, MA) with extensions to the following open source packages: Functional Magnetic Resonance Imaging of the Brain's Linear Image Registration Tool (FLIRT) v5. (20), Brain Extraction Tool (BET) v1.2 (21), and University College of London's Statistical Parametric Mapping (SPM5) normalization (22) and segmentation routines (23). To correct for subject motion during the PET scan, de-noising filter techniques were applied to all PET images starting at frame five. The eighth frame was used as a reference to which all other frames were aligned using rigid body FLIRT. For registration, a mean of the motion corrected frames eight through 18 was registered using FLIRT.

Region of interests (ROI) were drawn on each subject's MRI as previously described (18) and included: the ventral prefrontal cortex (VPFC), medial prefrontal cortex (MPFC), dorsolateral prefrontal cortex (DLPFC), anterior cingulate cortex (ACC), cingulate (posterior) cortex (CIN), amygdala (AMY), hippocampus (HIP), parahippocampal gyrus (PHG), insular cortex (INS), temporal cortex (TEM), parietal cortex (PAR), and occipital cortex (OCC). Because the boundaries of the median and dorsal raphe nuclei are not identifiable on MRI, a 2 cm³ ellipsoid was manually placed on the raphe nuclei of each individual's mean PET image, completely encompassing the high [C-11]WAY100635 binding region of the posterior midbrain. A cylindrical reference region was drawn in cerebellar white matter, as this region of cerebellum is virtually devoid of 5-HT_{1A} (19). For cortical regions, the ROIs were modified to include only gray matter voxels using SPM5-generated gray matter probability (GMp) maps. SPM5 segments the structural MRI image of each subject using the default tissue probability maps as priors. It produces GMp maps for each subject using a single generative model that combines the registration and tissue classification components of segmentation, and includes parameters that account for image intensity nonuniformity (23). PET image voxels within a cortical ROI at each frame were multiplied by the corresponding gray matter probability map value. The average gray matter corrected intensities were obtained by taking the sum of the PET activity within each cortical ROI and dividing by the sum of the GMp map voxel values within that ROI. Data analysts were blind to subject identity and diagnostic grouping.

DERIVATION OF OUTCOME MEASURES

Regional distribution volumes (*V*) of [C-11]WAY100635 were derived as previously described (18). Consensus nomenclature for distribution and binding parameters, which we now employ, was recently established by an international group of experts in pharmacokinetic modeling (24). For comparison purposes with outcome data from other investigators, *BP_{ND}* was also derived by the simplified reference tissue model (SRTM) (25) as previously described (18), using the first 90 minutes of PET emission data and the cerebellar gray matter as the reference region.

GENOTYPING

The functional 5-HT_{1A} gene promoter region single nucleotide polymorphism (SNP) known as 5-HT_{1A} C(-1019)G (a.k.a. rs6295) was genotyped for a biallelic classification (CC, CG, or GG) for each participant as previously described (26,27).

STATISTICS

The log transform of *BP_F* was used to stabilize the variance across regions. Also, the necessity to address the non-normal distribution of binding of [C-11]WAY100635 for statistical comparisons has been recognized by our group and others (28-30). Linear mixed-effects models were fit to the ROI-level *BP_F* estimates with region, diagnostic group, sex, and lifetime aggression score as fixed effects and subject as the random effect. Standard errors (SE) were computed for each estimated *BP_F* value using a bootstrap algorithm that takes into account errors in metabolite, plasma, and brain data (31) and observations were weighted accordingly in the linear mixed-effects models.

RESULTS

The healthy volunteer and bipolar disorder groups were of comparable age ($t=-0.14$; $p=0.89$) and sex distribution (χ^2 , $p=0.87$). Table 1 provides socio-demographic and clinical data for the two groups. The ROI analysis of the [C-11]WAY100635 PET data revealed expected differences in the binding to 5-HT_{1A}, expressed as *BP_F*, across brain regions ($F_{12,900}=976.1$, $p<.0001$).

Controls vs. Bipolar Depressed

The depressed bipolar group had higher 5-HT_{1A} BP_F than the control group ($F_{1,74}=5.45$, $p=0.022$). There was also an effect of sex on 5-HT_{1A} BP_F that depended on brain region ($F_{12,900}=2.20$, $p=.010$); and a significant 3-way interaction indicated this pattern of influence also differed between bipolar disorder patients and controls ($F_{12,900}=2.60$, $p=.0021$). Based on a model that constrains equal effect size for all regions and both sexes, we estimate 25.1% higher 5-HT_{1A} BP_F for bipolar depressed subjects relative to controls.

Post hoc testing by brain region demonstrated higher 5-HT_{1A} BP_F in the bipolar group in all ROIs (see Table 2). Figure 1 shows the weighted mean $\pm$ standard error of 5-HT_{1A} BP_F for all ROIs in controls and bipolar disorder patients. For HIP, there was also a sex by diagnosis interaction ($F_{1,75}=4.32$, $p=0.041$). And for RN there was also an effect of sex ($F_{1,75}=5.49$, $p=0.022$) and a sex by diagnosis interaction ($F_{1,75}=12.51$, $p=0.00070$), indicating the binding pattern in RN at least contributed to the 3-way interaction in the main analysis.

Effects of Sex

As we reported previously (19,32), females controls have higher BP_F than male controls in all ROIs. To explore the sex differences in 5-HT_{1A} BP_F between diagnostic groups, *post hoc* testing for each sex was carried out across all ROIs. Among the males, both the main effect of diagnosis ($F_{1,31}=5.52$, $p=0.025$) and the region by diagnosis interaction ($F_{12,372}=2.78$, $p=0.0012$) terms were statistically significant. In contrast, 5-HT_{1A} BP_F in female bipolar patients did not differ from female controls ($F_{1,44}=1.03$, $p=0.32$), nor was the interaction term significant ($F_{12,528}=1.08$, $p=0.38$). In male bipolar patients, weighted means of 5-HT_{1A} BP_F were 102% higher in RN and 29-50% higher throughout the 12 forebrain ROIs compared to male controls.

Figure 2A and 2B show scatter plots for raphe nuclei and hippocampus of raw 5-HT_{1A} BP_F values for individual male subjects, also displaying the associated standard error (31) for each BP_F estimate. To demonstrate visually regions of higher binding in male bipolar patients, Figure 3 provides voxel maps of mean 5-HT_{1A} BP_F for the male bipolar patient subgroup and the male control subgroup. This illustrates how binding in bipolar males is elevated across the brain, including elevated binding in the brainstem raphe autoreceptors.

Clinical Correlates

No correlations were identified between depression or mania severity scores on the HDRS-17, YMRS or BDI total scores and the natural log of BP_F in any of the 13 ROIs. This included no significant correlations for the controls, bipolar disorder patients, bipolar disorder males, and bipolar disorder females.

Age did not correlate with the natural log of 5-HT_{1A} BP_F in any ROI for the total sample: ACC ($r=-.04$, $p=.74$), AMY ($r<.01$, $p=.98$), CIN ($r=-.12$, $p=.28$), DLPFC ($r=-.09$, $p=.45$), HIP ($r=-.02$, $p=.88$), INS ($r=-.10$, $p=.36$), MPFC ($r=-.05$, $p=.66$), OCC ($r=-.11$, $p=.33$), OPFC ($r=-.06$, $p=.62$), PAR ($r=-.12$, $p=.28$), PHG ($r=.01$, $p=0.92$), TEM ($r=-.05$, $p=.64$), and RN ($r=-.08$, $p=.51$). Nor were there any significant partial correlations between the natural log of 5-HT_{1A} BP_F and age when controlling for sex and diagnosis: ACC ($r=-.02$, $p=.89$), AMY ($r=.03$, $p=.80$), CIN ($r=-.11$, $p=.33$), DLPFC ($r=-.07$, $p=.54$), HIP ($r=.01$, $p=0.95$), INS ($r=-.09$, $p=.45$), MPFC ($r=-.03$, $p=.79$), OCC ($r=-.10$, $p=.40$), OPFC ($r=-.04$, $p=.74$), PAR ($r=-.11$, $p=.36$), PHG ($r=.01$, $p=.95$), TEM ($r=-.04$, $p=.77$), and RN ($r=-.06$, $p=.63$).

Other clinical variables did not affect results. There were no differences in 5-HT_{1A} BP_F between bipolar subtypes I versus II or related to suicide attempter status within the bipolar depressed group. Controlling for lifetime aggression history score, previously shown to

correlate inversely with 5-HT_{1A} BP_F in healthy volunteers (32), in the model did not alter findings related to bipolar disorder.

Plasma and Nonspecific Binding

Free fraction in plasma or f_p of [C-11]WAY100635 was higher in controls than in patients with bipolar disorder ($8.1 \pm 2.5\%$ v. $5.9 \pm 2.0\%$, respectively; $t_{77}=4.22$; $p<0.000067$). Cerebellar distribution volume or V_{ND} of [C-11]WAY100635 did not differ between controls and patients with bipolar depression (0.29 ± 0.11 v. 0.26 ± 0.10 mL/g respectively; $t_{77}=1.40$; $p=0.17$).

C(-1019)G Promoter Polymorphism

Genotyping for the 5-HT_{1A} C(-1019)G promoter polymorphism demonstrated that 8/31 (25.8%) of the bipolar patients and 4/46 (8.7%) of controls were homozygous for the G allele variant (Fisher's exact test, 2-tailed, $p=0.057$). Frequency of the G allele presence did not differ between males (66%) and females (73%; Fisher's exact test, $p=.47$). There was a relationship between 5-HT_{1A} BP_F and number of G alleles that was dependent on brain region ($F_{12,900}=2.39$, $p=0.0049$). *Post hoc* testing revealed that number of G alleles was associated with higher 5-HT_{1A} BP_F in AMY ($t=-2.34$, $p=0.022$), HIP ($t=-2.08$, $p=0.041$), and RN ($t=-2.90$, $p=0.0050$); and in RN there was also a sex by G-allele interaction ($t=2.07$, $p=0.042$), indicating number of G-alleles in male depressed bipolars was associated with higher 5-HT_{1A} BP_F in RN.

Alternative Reference Region and Outcome Measure

Several PET studies using [C-11]WAY100635 have not acquired an arterial input function, used shorter duration data acquisition, and employed the SRTM method of image analysis and used cerebellar gray (rather than white) matter as reference region (9,11,33). For SRTM, BP_{ND} is the only possible binding outcome measure, as total and free arterial plasma concentrations are necessary for calculation of BP_P and BP_F, respectively (see Innis et al (24) for nomenclature). To allow comparison of our findings in bipolar disorder with data from other groups using SRTM rather than the 2-tissue compartment model, we also performed an SRTM analysis (using only the first 90 min of PET emission data and employing cerebellar gray matter as reference region). By this method, we found no significant differences in BP_{ND} across all ROIs in the bipolar disorder group compared to controls ($F_{1,74}=3.32$; $p=0.073$), no effect of sex ($F_{1,74}=0.19$; $p=0.66$), no sex by diagnosis interaction ($F_{1,74}=1.04$; $p=0.31$), and no 3-way interaction of region, sex, and diagnosis ($F_{12,900}=0.92$; $p=0.53$). BP_{ND} was nonsignificantly lower by 5.1-13.9% across the ROIs in the bipolar group.

DISCUSSION

To our knowledge, this is the first published report employing PET to assess 5-HT_{1A} binding *in vivo* in a homogeneous, non-medicated, patient sample with bipolar depression. Using the 5-HT_{1A} antagonist radioligand [C-11]WAY100635, we identified in bipolar depression higher 5-HT_{1A} BP_F, in particular in the raphe autoreceptors and to a lesser degree across all terminal field ROIs. Post-hoc analyses indicated the higher 5-HT_{1A} BP_F was specific to male bipolar depressed patients compared with male controls. In contrast, female bipolar depressed patients did not differ in 5-HT_{1A} BP_F from female controls throughout the brain. Healthy females had higher binding than healthy males, as we previously reported (18,19).

It has been demonstrated *in vitro* that the C(-1019) allele (rs6295) of the polymorphic region of the 5-HT_{1A} gene promoter region is part of a 26 base pair imperfect palindrome that binds a transcriptional repressor, nuclear deformed epidermal autoregulatory factor (NUDR), that suppresses expression in raphe but not hippocampal neuron cultures (34). It would be predicted that the G allele would be associated with higher raphe autoreceptor binding and not with higher terminal field binding. Previously, we reported among MDD patients and controls that carriers

of at least one G allele have higher 5-HT_{1A} BP_F in raphe nuclei (10). In the present sample, there was a trend for an overrepresentation of the GG genotype the bipolar group (8.7%, controls; 25.8%, bipolar disorder). In raphe nuclei there was a relationship between number of copies of the G allele and higher raphe 5-HT_{1A} BP_F. Similar relationships identified in hippocampus and amygdala do not withstand correction for multiple comparisons. Yet the sample sizes in this study are not sufficient to differentiate effect of bipolar disorder diagnosis from effect of genotype on raphe nuclei 5-HT_{1A} expression; however, it is possible that the G allele is part of the reason for higher raphe autoreceptor binding in males with bipolar disorder. We did not find a sex difference in G allele frequency so that effect is explained by some other mechanism. Thus, the present results are at least consistent with a genetic mechanism by which G allele carriers have higher expression of 5-HT_{1A} autoreceptors in the raphe nuclei, potentially producing lower serotonin neuron firing and less serotonin release at nerve terminals. That in turn may result in some post-synaptic receptor upregulation, although preclinical data supporting upregulation of terminal field 5-HT_{1A} in serotonin depleted states is lacking.

We previously reported higher 5-HT_{1A} BP_F in antidepressant naïve patients with MDD (10). The present finding of higher 5-HT_{1A} BP_F in bipolar depression is similar to our prior finding in MDD. Although, in bipolar depression the estimated 25% difference relative to controls is slightly larger than that found in the prior antidepressant naïve MDD cohort, which was 19%. Also, the effect in bipolar depression is apparently not substantially diminished by prior history of antidepressant medication exposure. Unfortunately, only 3 of 32 in the present bipolar disorder sample were antidepressant naïve, precluding meaningful analysis by antidepressant exposure history. If indeed prior antidepressant exposure has a similar lasting reduction in 5-HT_{1A} density and function as revealed in preclinical models (35,36), an antidepressant-naïve sample of bipolar depressed males may demonstrate even higher 5-HT_{1A} BP_F.

Alternatively, prior antidepressant exposure in bipolar disorder may result in a sex-specific reduction in 5-HT_{1A} in the females and not males so that, among antidepressant-exposed patients, higher binding is only demonstrable in the males. In support of this hypothesis, female bipolars with prior antidepressant exposure greater than 120 days from PET (n=5) had higher mean 5-HT_{1A} BP_F than those with exposure between 15 and 120 days from PET (n=13; data not shown). Also, there was a high rate of 5-HT_{1A} C(-1019)G polymorphism GG genotype in our bipolar male subsample, 33%, compared with 21% in the bipolar female subsample. It may thus be speculated that prior antidepressant exposure in male bipolar did not result in a lasting reduction in 5-HT_{1A} binding as a result of deficient NUDR repressor function in 5-HT_{1A}-expressing raphe nuclei neurons. Such an effect might contribute to a poorer clinical response to antidepressants. In our prior MDD sample, higher pretreatment 5-HT_{1A} BP_F was associated with nonremission after one year of naturalistic treatment (37) and the GG genotype predicts poorer antidepressant treatment response (38).

Over half of our sample of bipolar patients had a history of suicide attempt, but we did not identify an effect of attempter status on 5-HT_{1A} BP_F. Our finding of higher 5-HT_{1A} BP_F in raphe nuclei in depressed bipolar disorder males is consistent with postmortem studies reporting higher dorsal raphe nucleus 5-HT_{1A} binding in male suicides with MDD using a 5-HT_{1A} agonist probe, [H-3]8-OH-DPAT (39,40) and higher 5-HT_{1A} mRNA in male depressed suicides in frontopolar cortex and hypothalamus (41).

Our post-hoc finding of no difference in 5-HT_{1A} BP_F between female bipolar disorder patients and female controls may be related to sex-specific characteristics of the 5-HT_{1A} receptor often observed in basic (42-46) and clinical (39,47-49) studies. It was recently reported using PET *in vivo* in healthy females that 5-HT_{1A} BP_{ND} in raphe nuclei increases between the follicular phase and luteal phase of the menstrual cycle (50). Yet, only when using BP_F, not BP_{ND}, as the outcome measure do we find sex differences in 5-HT_{1A} (19), as reported in postmortem

studies of 5-HT_{1A} (39,47); therefore this is unlikely to explain the lack of difference in 5-HT_{1A} BP_F between female bipolar patients and controls.

There has been apparent disagreement in the [C-11]WAY100635 PET literature regarding the direction of difference between depressed patients and controls in 5-HT_{1A} availability for binding, with other groups reporting *lower* binding in raphe nuclei (9) and specific forebrain regions (9,11,33). Yet, we have discussed crucial differences in PET image processing and modeling methodologies and that our outcome measure is BP_F , not BP_{ND} (10,19). Use of BP_{ND} for estimation of available 5-HT_{1A} using [C-11]WAY100635 assumes no between group differences in the free fraction of ligand in the nondisplaceable compartment, f_{ND} . Yet, f_{ND} is related to f_P , and we now report a significant between-group difference in f_P in bipolar depression. Moreover, we have shown that despite the very low concentration of 5-HT_{1A} receptors in cerebellar gray matter and vermis, BP_{ND} ($=BP_P/V_{ND}$) is exquisitely sensitive to small changes in V_{ND} when, as in the case of [C-11]WAY100635, V_{ND} is $\ll 1$ (19). When we employ SRTM and cerebellar gray instead of white matter, we find BP_{ND} is 5-14% *lower* in bipolar depression throughout the 13 ROIs. We believe that because of the very low specific and nonspecific binding of the [C-11]WAY100635 compound in the cerebellum, this approach is prone to error. Finally, BP_F is the outcome measure that estimates B_{max}/K_D , as it is the only expression of specific distribution volume that depends exclusively on receptor parameters. The other outcome measures require assumptions that either f_{ND} or f_P is not different between groups.

There are several limitations to the present study. In particular, the washout period of antidepressants before scanning was limited to 14 days for ethical reasons, and 10 (7 female, 3 male) of the 32 bipolar patients had stopped the last antidepressant(s) between 15 and 32 days before PET. This washout time may be insufficient for the effects of antidepressant treatment on the 5-HT system to fully abate. Prior substance and alcohol use disorder and present anxiety disorder comorbidity in some of the sample are also limitations, although we have demonstrated (data not shown) that neither can explain the finding of higher 5-HT_{1A} BP_F in the bipolar males. Also, although this is a relatively large sample for PET imaging in bipolar disorder, the numbers are not large enough to test if the higher raphe nuclei 5-HT_{1A} availability in bipolar depression is explained by presence of the C(-1019)G polymorphism.

In summary, we found higher 5-HT_{1A} BP_F in midbrain raphe nuclei and to a lesser extent across other brain regions in bipolar disorder within a major depressive episode compared with healthy volunteers. Lack of correlation of 5-HT_{1A} BP_F and mood severity measures suggests that future studies should assess bipolar subjects in a remitted state to determine whether higher 5-HT_{1A} BP_F is a state or trait effect. Such a potential trait effect on raphe autoreceptors appears related partly to the presence of the higher expressing G allele and future studies need to verify this possibility in a larger sample.

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Figure 1.

Radiolabeled [C-11]WAY-100635 binding potential (BP_F) estimates for the 5-HT_{1A} receptor in nonmedicated depressed bipolar patients and controls in 13 regions of interest. Comparison of 5-HT_{1A} BP_F between the bipolar group and control group demonstrates higher 5-HT_{1A} BP_F in bipolar patients ($p=.022$). Also, there was a region x sex interaction ($p=0.010$) and a 3-way interaction, region x sex x diagnosis ($p=0.0021$). Post-hoc comparisons demonstrate higher 5-HT_{1A} BP_F in the bipolar group in every ROI (see Table 2). ACN, anterior cingulate cortex; AMY, amygdala; CIN, cingulate body; DLPFC, dorsolateral prefrontal cortex; HIP, hippocampus; INS, insula; MPFC, medial prefrontal cortex; OCC, occipital cortex; PAR, parietal cortex; PHG, parahippocampal gyrus; TEM, temporal cortex; VPFC, ventral prefrontal cortex; RN, raphe nuclei. The height of each vertical bar represents the weighed means of 5-HT_{1A} BP_F for the region, and the error bar represent the standard error of the weighted estimate.

Figure 2.

Radiolabeled [C-11]WAY-100635 binding potential (BP_F) estimates for the 5-HT_{1A} receptor in male bipolar patients and male controls in **A**) raphe nuclei and **B**) hippocampus. Diamonds and squares represent single measurements of BP_F in controls and bipolar patients, respectively. Thin capped vertical error bars represent standard errors computed using a bootstrap algorithm that takes into account errors in metabolite, plasma, and brain data (31). Weighted group mean and standard error of the weighted mean of BP_F are represented by thick horizontal lines and thick capped vertical lines, respectively.

Figure 3.

Mean binding potential (BP_F) voxel maps for **A**) the male healthy volunteer group and **B**) the male bipolar depressed group. Each voxel intensity is the mean of the 5-HT_{1A} BP_F measurements for that voxel among each group. The color bar represents 5-HT_{1A} BP_F level in mL/cm³.

Table 1
Sociodemographic and Clinical Data of the Study Groups

	Controls (n = 47)	Bipolar Depressed (n = 32)	Statistic (df)	P value
Sex, M/F, No.	20/27	13/19	$\chi^2_{(1)} = 0.029$	NS (0.87)
Age, mean $\pm$ SD, y	38.1 $\pm$ 14.7	38.4 $\pm$ 9.7	$t_{(77,0)} = -0.143$	NS (0.89)
Bipolar I/bipolar II, No.	NA	18/14		
Inpatient/Outpatient, No.	NA	16/16		
Age at 1st major depressive episode, mean $\pm$ SD, y	NA	20.3 $\pm$ 9.8		
Age at 1st manic or hypomanic episode, mean $\pm$ SD, y	NA	28.0 $\pm$ 10.6		
Length of current major depressive episode, wk	NA	35.3 $\pm$ 64.0		
Antidepressant history, exposed/naïve, No.	NA	29/3		
Previous suicide attempts, No. (%)	NA	19 (59%)		
Number of suicide attempts in attempters (n = 19), No.	NA	1.6 $\pm$ 2.1		
HDRS (17-item) score, mean $\pm$ SD	0.6 $\pm$ 0.9	18.0 $\pm$ 4.9	$t_{(31,7)} = -17.9$	<0.001
Beck Depression Inventory score, mean $\pm$ SD	1.7 $\pm$ 2.6	25.7 $\pm$ 11.0	$t_{(33,4)} = -12.1$	<0.001
Young Mania Scale score	0.2 $\pm$ 0.6	6.4 $\pm$ 7.1	$t_{(30,3)} = -4.8$	<0.001
Global Assessment Scale score, mean $\pm$ SD [†]	90.1 $\pm$ 4.5	47.6 $\pm$ 10.5	$t_{(35,8)} = 21.0$	<0.001
Aggression Inventory Score (Brown-Goodwin) [†]	14.0 $\pm$ 3.7	20.0 $\pm$ 6.5	$t_{(43,4)} = -4.7$	<0.001
5-HT1APR C(-1019)G biallelic genotype, No. (%) [†]				
CC	15 (32.6)	8 (25.8)		
CG	27 (58.7)	15 (48.4)		
GG	4 (8.7)	8 (25.8)	FET*	NS (0.057)**

Abbreviations: HDRS, Hamilton Depression Rating Scale; NA, not applicable; NS, not significant

[†] Based on 46 healthy volunteers and 31 depressed bipolar patients

* Fisher's exact test (two-sided) comparing count data for homozygotes with GG v. non-GG homozygotes (i.e. CC and CG) between groups

** Fisher's exact test (one-sided) p=0.045

Table 2
Linear Mixed Effects Model Results for Each Region of Interest (ROI)

ROI	Effect of Diagnosis	
	<i>F</i> _(1,75)	<i>P</i>
Anterior cingulate cortex (ACC)	6.64	0.012
Amygdala (AMY)	6.52	0.013
Cingulate body (CIN)	8.55	0.005
Dorsolateral prefrontal cortex (DLPFC)	5.86	0.018
Hippocampus (HIP)	5.57	0.021
Insular cortex (INS)	5.68	0.020
Medial prefrontal cortex (MPFC)	8.91	0.004
Occipital cortex (OCC)	9.11	0.004
Orbital prefrontal cortex (OPFC)	6.56	0.012
Parietal cortex (PAR)	6.60	0.012
Parahippocampal gyrus (PHG)	9.24	0.003
Temporal cortex (TEM)	6.08	0.016
Raphe nuclei (RN)	18.32	0.00005