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Higher Serotonin 1A Binding in a Second Major Depression Cohort: Modeling and Reference Region Considerations

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Abstract

Background—Serotonin 1A receptors (5-HT_{1A}) are implicated in major depressive disorder (MDD). We previously reported higher 5-HT_{1A} binding potential ($BP_F = B_{avail}/K_D$) in antidepressant naïve MDD subjects compared to controls while other studies report lower BP_{ND} ($BP_{ND} = f_{ND}B_{avail}/K_D$). Discrepancies can be related to differences in study population or methodology. We sought to replicate our findings in a novel cohort and determine whether choice of reference region and outcome measure could explain discrepancies.

Methods—Nine new controls and 22 new not recently medicated (4 years, NRM) MDD subjects underwent positron emission tomography with [¹¹C]WAY100635. BP_F and BP_{ND} were determined using a metabolite and free fraction corrected arterial input function. BP_{ND} was also determined using cerebellar gray matter (CGM) and cerebellar white matter (CWM) reference regions as input functions.

Results— BP_F is higher in the new NRM cohort ($p=0.037$) compared to new controls, which is comparable to the effect in the previous cohort ($p=0.04$). We combined the cohorts to examine the effects of the reference region and outcome measure. In the combined cohort, BP_F is higher in the

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NRM compared to controls ($p=0.0001$). Neither BP_{ND} using CWM ($p=0.86$) nor V_T ($p=0.374$) differs between groups. When CGM is used, the NRM group has lower $5HT_{1A}$ BP_{ND} compared with controls ($p=0.03$). CGM V_T is higher in NRM compared to controls ($p=0.007$).

Conclusions—Choice of reference region and outcome measure can produce different $5-HT_{1A}$ findings in MDD. Higher $5-HT_{1A}$ BP_F in MDD was found with the method with fewest assumptions about nonspecific binding and a reference region without receptors.

Keywords

Unipolar; genotype; polymorphism; medication; antidepressants; modeling

Introduction

[^{11}C]WAY-100635 is a serotonin $1A$ receptor ($5-HT_{1A}$) antagonist PET ligand.(1-9) We first reported higher $5-HT_{1A}$ binding potential ($BP_F=B_{avail}/K_D$) in antidepressant-naïve (AN) subjects with major depressive disorder (MDD) compared to antidepressant-exposed (AE) MDD subjects and controls.(10) Also, raphe nuclei (RN) BP_F increases with G allele load of the functional $5-HT_{1A}$ G(-1019)C promoter polymorphism(10), elevated BP_F predicts non-response to antidepressants(11), and remitted depressed have higher BP_F compared to controls.(12) Other PET studies have reported lower $5-HT_{1A}$ $BP_{ND}=f_{ND}B_{avail}/K_D$ in MDD.(9,13)

Two explanations are differences in study population or in methodology. We sought to replicate our findings and reconcile the seemingly disparate findings. For the former, we studied a new cohort of 9 controls and 22 MDD. For the latter we compared two different reference regions (RR) and two different outcome measures (BP_F and BP_{ND}) in the combined new and previous cohorts of 51 controls and 30 not recently medicated (NRM). Previously, we found a small number of $5-HT_{1A}$ receptors in the cerebellar vermis and gray matter (CGM) compared to cerebellar white matter (CWM).(14) We concluded that CWM is the best RR for [^{11}C]WAY-100635 studies because it appears devoid of receptors, is best fit with a one-tissue compartment model, has the lowest binding, better test-retest reliability, identifiability, and time stability. Perhaps unique to [^{11}C]WAY-100635, CWM has the same non-specific binding as CGM *ex vivo*(15), and therefore can be used to estimate free and non-specific binding in gray matter regions. To determine *in vivo* extensibility, we have reanalyzed previous $5-HT_{1A}$ blocking studies.(16) We hypothesized that [^{11}C]WAY-100635 binding in CWM would not change following pindolol, but decrease in CGM. Additionally, we calculated BP_{ND} using compartmental modeling and the simplified reference tissue method (SRTM)(17) using both CGM and CWM.

Methods and Materials

Subjects

Thirty subjects who met DSM-IV(18) criteria for a current major depressive episode (MDE) and 51 control subjects were included. Inclusion criteria were assessed through history, chart review, Structured Clinical Interview for DSM IV (SCID I)(19), review of systems, physical examination, routine blood tests, pregnancy test, urine toxicology and EKG. The Beck Depression Inventory(20), Hamilton Depression Rating Scale(21), and Global Assessment Scale(22) assessed subjective and objective depression severity and functional impairment, respectively (Table 1). Criteria for depressed subjects included: 1) age 18 to 65 years; 2) DSM-IV criteria for current MDE and MDD; 3) capacity to provide informed consent and *absence of*: 4) psychotropic medications for at least 2 weeks (6 weeks for fluoxetine, 4 weeks for neuroleptics); 5) lifetime history of alcohol or substance abuse or dependence; 6)

life-time exposure to 3,4-methylenedioxymethamphetamine; 7) significant medical conditions; 8) pregnancy; and 9) psychosis, bipolar disorder, or schizophrenia. Criteria for controls were similar except for the required absence of medical, neurological and psychiatric history or a history of a mood or psychotic disorder in a first-degree relative. The Institutional Review Board of the New York State Psychiatric Institute approved the protocol. Subjects gave written informed consent after an explanation of the study.

[¹¹C]WAY-100635 pindolol data come from a separate sample of nine controls, of which six had usable data.(16) Briefly, subjects underwent a baseline [¹¹C]WAY-100635 scan, followed by titrated doses of pindolol CR (7.5 mg/day for 7 days, 22.5 mg/day on day 8, and then 30 mg/day on day 9); a [¹¹C]WAY-100635 scan occurred four hours after the 30 mg dose on day 9.

Prior Medication History

We have shown that AE, medication free MDD have lower BP_F values than medication free AN MDD. We defined AE as those being on an adequate dose of antidepressant for at least 4 weeks. Subjects who could not recall the name, dose, or duration of medication were classified as indeterminate (n=13) and not used in imaging data analysis; they were used for genetic analyses. Because we used more stringent criteria for classification, 5 subjects from the previous cohort were reclassified as indeterminate. Since the subjects off of medications for greater than 4 years (n=7) did not differ from the AN subjects in their composite binding data (df=28, t=1.39, p=0.175), we combine these groups and call them not-recently-medicated (NRM). There was no significant difference in demographic or clinical variables between the new and old cohorts (data not shown).

Radiochemistry and Input Function Measurement

Preparation of [¹¹C]WAY-100635 and measurement of the arterial input function, metabolites, and plasma free fraction (f_p) were conducted as described previously.(7,14,23) Metabolite data are collected during the first 60 minutes; we are unable to quantify metabolites beyond this time point. Injected dose (ID), injected mass (IM), clearance and f_p are presented in Table S1 (see Supplement 1). The NRM had a significantly lower IM (df=79; t=3.82; p<0.001) and ID (df=79; t=2.43; p=0.02) than controls. Later studies were done after our human dosimetry study (24) determined the ID (and consequently IM) needed to be lowered. No correlation was found within groups between IM or ID and BP_F in any region (data not shown). Analyses were done covarying for IM and ID.

Image Acquisition and Analysis

PET imaging was performed on an ECAT EXACT HR+ (Siemens/CTI, Knoxville, TN). Emission data were collected for 110 minutes as 20 successive frames of increasing duration. Image analysis was performed using MATLAB 2006b (The Mathworks, Natick, MA) with extensions to the following: Functional Magnetic Resonance Imaging of the Brain's Linear Image Registration Tool (FLIRT) v5.2.(25), Brain Extraction Tool v1.2(26), Statistical Parametric Mapping (SPM5) normalization(27), and segmentation routines.(28) To correct for subject motion during the PET scan, de-noising filter techniques were applied to all PET images starting at frame five. All frames were aligned using rigid body FLIRT to the eighth frame. A mean of motion corrected frames eight through eighteen was registered to the MRI using FLIRT.

Acquisition of T1-weighted magnetic resonance image (MRI) 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, Milwaukee, WI).(7) ROIs were hand drawn on the MRI by experienced technicians trained

to reliably approximate these regions using brain atlases(29,30) and published reports. (31,32) ROIs included the amygdala (AMY), hippocampus (HIP), parahippocampal gyrus (PHG), temporal cortex (TEM), anterior cingulate (ACN), cingulate cortex (CIN), dorsolateral prefrontal cortex (DLPFC), medial PFC (MPFC), ventral PFC (VPFC), insular cortex (INS), occipital cortex (OCC) and parietal cortex (PAR). A fixed volume elliptical ROI (2cm^3) was placed on the raphe nuclei (RN) in the dorsal midbrain on a mean PET image, as described in(10) Cylindrical ROIs were drawn in the CWM(14) and CGM, as far as possible from the occipital cortex. The ROI contours were refined using the segmented MRI to reflect the gyral pattern and differences between the PET and MRI fields of view.

Derivation of Regional Outcome Measures

The outcome measure of choice in PET studies is B_{avail} , the total number of available receptors. Without multiple injections and occupancy of the receptors, current technology permits measurement of $BP_F = B_{\text{avail}}/K_D$. Fortunately, there is no evidence for alterations in 5-HT_{1A} K_D in depression.(33) $BP_{ND} = f_{ND}B_{\text{avail}}/K_D$ requires a further assumption: that depressed subjects and controls do not differ in f_{ND} .

Regional distribution volumes of [¹¹C]WAY-100635 are derived from kinetic analysis using the arterial input function and a two-tissue compartment (2T) model.(7) V_{ND} and V_S are defined as the distribution volumes of the nondisplaceable and specific compartments, respectively.(34-36) V_T is defined as the total regional equilibrium distribution volume, $=V_{ND}+V_S$. Time activity curves were fit with a 2T model, with the K_1/k_2 ratio fixed to that of the CWM (RR). For CWM, a 1T model was used.(14) BP_F was calculated as $(V_{T(\text{ROI})}-V_{T(\text{CER})})/f_p$. The contribution of plasma total activity to the regional activity was calculated assuming a 5% blood volume in the ROI and subtracted from the regional activity prior to analysis. Kinetic parameters were derived by nonlinear regression using MATLAB. For comparison purposes, BP_{ND} was calculated using V_T from the 2T model $(V_{T(\text{ROI})}-V_{T(\text{CER})}/V_{T(\text{CER})})$ and directly from SRTM(17), with both CGM and CWM as RR. For our additional examination of RR using human pindolol data, we estimated V_T/f_p . This measure of binding does not require a RR, and corrects for differences in free tracer in plasma.(37) We compared V_T/f_p before and after blockade with pindolol in CGM and CWM. CGM V_T was fit with a 2T model, as previously described.(14)

Voxel Based Analysis

V_T maps were created by applying Basis Pursuit on a voxel-by-voxel level using DEPICT. (38) BP_F maps were transformed onto the MRI by applying the PET to MRI transformation parameters obtained during co-registration. Each subjects' MRI image was processed with Freesurfer v4.05 (<http://surfer.nmr.mgh.harvard.edu/>). Freesurfer was used in conjunction with SPM5 derived gray, white and cerebrospinal fluid maps to remove the skull from MRI images after they were bias corrected and intensity normalized. Skull stripped MRI images were non-linearly registered to SPM5 single subject Montreal Neurological Institute (MNI) space using the Automated Registration Toolbox (ART).(39) The resulting non-linear transforms were applied to MRI co-registered BP_F maps to bring them into MNI space. Before analysis, all images were smoothed using an 8-mm full width half maximum gaussian kernel. Using SPM5, a student t-test assuming independence and unequal variance with no covariates was conducted when comparing groups. A mask was applied to remove the non-brain areas and the entire cerebellum from the analysis. Grand-mean scaling or global normalization was not applied, as BP_F is an absolute measurement. Height threshold was set a priori to $p<0.005$ with False Discovery Rate correction, the extent threshold was set to $p<0.05$ corresponding to a cluster size $k>5000$ voxels.

Genotyping the C(-1019)G Polymorphism

Genotyping of the C(-1019)G polymorphism of the 5-HT_{1A} receptor gene was performed using allele-specific polymerase chain reaction (PCR) amplification.(10)

Statistical analysis

To borrow strength across all ROIs and to properly account for correlation among ROIs in the same subject, we fit linear mixed-effects models to the ROI-level BP_F or BP_{ND} estimates with region and diagnostic group as fixed effects and subject as the random effect. Such a model provides a powerful test of our primary hypothesis without the necessity of dealing with multiple comparison issues. Letting Y_{ij} denote the response from the i th region and j th subject, the model is $Y_{ij} = \mu + \gamma_i + B_j + \varepsilon_{ij}$, where μ represents an overall mean, γ_i the effect of the i th region, B_j the random subject effect, assumed to be normally distributed, independently of the other subject effects, with mean zero and unknown variance σ_B^2 , and ε_{ij} is the error, also assumed to be independent of the other errors and of the subject effects and normally distributed with mean zero and variance σ^2 . In our analyses we used log-transformed binding potentials as responses. For our data we have available estimated standard errors computed using a bootstrap algorithm that takes into account errors in metabolite, plasma, and brain data(48) and thus we weighted the observations accordingly.

Additional statistical analyses include Student's and paired t-tests, and Fisher's exact test performed in Excel (Microsoft, 2003) or SPSS 17.0 (SPSS Statistics, 2008).

Results

Replication

The previous cohort NRM have higher [¹¹C]WAY-100635 BP_F compared to previous controls (df=1, 48; F=4.621; p=0.037). The new cohort NRM have higher [¹¹C]WAY-100635 BP_F compared to the new controls (df=1,29; F=4.606; p=0.04, Figure 1). Combining the previous and new cohort NRM and controls, the NRM have higher BP_F than controls (df=1,79; F=17.77; p=0.0001; Figure 2). As mentioned above, groups differed in ID and IM. When IM is added to the model the p value is unchanged. When ID is added, p=0.0006. Within the combined group of 51 controls, we also find higher BP_F in females compared to males (df=1,49; F=5.16; p=0.0276) and an inverse correlation between total lifetime aggression and BP_F in the raphe (R²=0.12; p=0.001) and VPFC (R²=0.05; p=0.025), consistent with previous findings(49). When sex and aggression are included in the analysis the group effect remains (df=1,75; F=16.59; p=0.0001).

Reconciliation

All subsequent analyses consider the combined previous and new cohorts of NRM and controls. We find no differences between the NRM and controls when BP_{ND} is estimated from a 2T model with CWM as a RR (df=1,79; F=0.03; p=0.86). There is no difference when using BP_{ND} from SRTM with CWM as RR (df=1,77; F=0.00; p=0.99). However, a group effect is found when BP_{ND} is estimated using SRTM with CGM, not CWM, as the RR (df=1,78; F=4.63; p=0.034; Figure 3). This could be explained by a difference in CGM V_T between NRM and controls, as there are a small but quantifiable number of 5-HT_{1A} in the CGM.(14) In fact, CWM V_T is different from CGM V_T (df=1,18; t=16.2; p < 0.0001). CGM V_T generates a group effect (df=1,79; F=7.59; p=0.0072; Figure S1 in Supplement 1). CWM V_T has no group effect (df=1,79; F=0.76; p=0.39). Although there is no difference between groups in V_T across all 13 ROIs (df=1,79; F=0.199; p=0.374), we do find higher V_T/f_p in NRM than in controls (df=1,79; F=17.9; p=0.00001; Figure 4).

Genotype

The MDD group differs significantly from controls in both the C(-1019)G polymorphism genotype (Fisher's Exact test; $p=0.0032$) and allele frequency (Fisher's Exact test; $p=0.0082$; Table S2 in Supplement 1). A description of genotype by cohort can be found in Table S2 of Supplement 1. Assuming the difference in binding is the same between the CC and the CG groups as it is between the CG and GG groups and not considering effects of diagnosis, raphe nuclei BP_F increases as expected with G allele load (GG>CG>CC; $df=1,78$; $F=7.13$; $p=0.009$; Figure S2 in Supplement 1). However, the effect does not remain when BP_{ND} from a 2T model is used in place of BP_F ($df=1,78$; $F=0.152$; $p=0.697$).

Free fraction

There is no difference in f_p between NRM and controls in the previous cohort ($df=29$; $t=1.82$; $p=0.074$). However, f_p does differ in the new cohort ($df=29$; $t=2.20$; $p=0.036$) and combined cohort ($df=79$; $t=3.86$, $p < 0.001$; Table S1 in Supplement 1).

Pindolol

In a separate group of subjects, there is no difference in CWM V_T/f_p between baseline and pindolol blockade ($df=5$, $t=1.99$, $p=0.10$), yet a 29.1% reduction in CGM V_T/f_p following pindolol blockade ($df=5$, $t=2.94$, $p=0.032$). Estimating BP_{ND} for CGM using a 2T model with CWM as the RR, we find a 34.9% reduction in CGM BP_{ND} following pindolol blockade ($df=5$, $t=2.63$, $p=0.047$). K_1 for CWM ($df=5$, $t=0.064$, $p=0.95$) and CGM ($df=5$, $t=0.032$, $p=0.98$) is unchanged between baseline and pindolol blockade (Table 2).

Discussion

Replication

We sought to replicate our findings and to reconcile our data with previous publications. In the previous cohort, new cohort and in the combined cohort of 51 controls and 30 NRM MDD subjects, NRM have higher 5HT_{1A} BP_F compared to controls (Figure 1). This was the case even though the second cohort included only 9 healthy controls. Strikingly, the magnitude of difference between control and NRM MDD subjects is nearly identical to that observed in the previous cohort (32.2% vs 32.6%).

Several lines of corroborating evidence support higher 5-HT_{1A} in depression. There is higher 5-HT_{1A} binding in the dorsal raphe of a mouse strain in-bred for learned helplessness.(50) Fluoxetine treatment simultaneously reduced 5-HT_{1A} and depressive behavior. 5-HT_{1A} is increased along with increased depressive phenotype in mice treated with retinoic acid.(51) 5-HT_{1A} is increased in isolation-housed mice, and there are greater changes in 5-HT_{1A} after citalopram treatment in isolation-housed mice than in group-housed mice.(52) 5-HT_{1A} mRNA is increased in response to isolation-housing(53) and other stressors like repeated exposure to forced swimming.(54) Prenatal stress in rats induced 5-HT_{1A} mRNA increases that were associated with behavioral changes, which were reversed by antidepressant treatment.(55)

Genetic studies have established a relationship between higher 5-HT_{1A} and depressive phenotype. The GG polymorphism of the C(-1019)G promoter, which is associated with depression and suicide, (56,57) is also associated with higher 5-HT_{1A} binding(10,11) and with a greater likelihood of developing interferon-induced depression.(58)

Treatment studies support the hypothesis of higher 5-HT_{1A} in depression. The proposed mechanism of action of SSRIs hinges on the presence of higher autoinhibitory 5-HT_{1A} receptors in depression.(59) Chronic, but not acute, exposure to SSRIs desensitizes

somatodendritic 5-HT_{1A} autoreceptors in the RN, but not hippocampal postsynaptic 5-HT_{1A} receptors, providing a mechanism of action for antidepressant medication that explains the lag in clinical treatment response.(60) Pindolol augmentation during SSRI treatment is thought to speed the efficacy of SSRIs by blocking 5-HT_{1A} receptors.(61,62) However, pindolol occupancy of 5-HT_{1A} receptors is relatively low; thus, higher doses (e.g., 15 mg/day) may be more effective.(63) Gur *et al.* found that chronic repetitive transcranial magnetic stimulation, associated with clinical remission, induced subsensitivity of presynaptic 5-HT_{1A} receptors in RN.(64)

There is considerable discrepancy among post-mortem studies of 5-HT_{1A} in depression, some finding no change, (65-68) increased, (33,69-73) and decreased binding.(74)

Overall, there is substantial evidence supporting an increase of 5-HT_{1A} receptors in MDD.

Model—We have shown 5-HT_{1A} BP_F (B_{avail}/K_D) is higher in NRM subjects compared to controls. Further, we have shown that BP_F in the RN increases with increasing G alleles of the functional G(-1019)C promoter polymorphism.(10,40) A greater “load” of G alleles has been associated in some studies with less response to SSRIs.(75) In a naturalistic study, we have shown that non-responders to antidepressants have both a higher frequency of the G allele and higher BP_F.(11) These data suggest a model of depression driven by this polymorphism. In these subjects, the presence of more G alleles increases the number of auto-inhibitory 5-HT_{1A} receptors in the RN, resulting in decreased release of 5-HT from serotonergic neurons in cortical regions. Lower serotonin release could potentially be associated with a homeostatic upregulation of the postsynaptic 5-HT_{1A}. Certainly, it is likely that not all depressive syndromes emerge via this pathway.

Reconciliation

The second goal of this study was to reconcile our findings with others. We examined alternate modeling methods and RRs. We report increased 5-HT_{1A} in NRM compared to controls using BP_F from a 2T model; yet BP_{ND} from a 2T model yields no difference between groups. This finding is not due to method, as there is also no difference between groups if we use BP_{ND} from SRTM. However, if CGM is used as the RR, despite the very low concentration of 5-HT_{1A}, there is a small but statistically significant difference in BP_{ND} between groups, *in the opposite direction*; depressed subjects have lower BP_{ND} compared to controls. BP_{ND} ($BP_P/V_{T(REF)}$) is sensitive to small changes in $V_{T(REF)}$ particularly when $V_{T(REF)}$ is $\ll 1$, as is the case for [¹¹C]WAY-100635. NRM have increased 5-HT_{1A} $V_{T(REF)}$ in CGM compared to controls. No differences in $V_{T(REF)}$ are found in CWM. This is not dependent on the placement of the RR ROIs, for either for CWM or CGM. If we compare V_T determined by our ROI in the CWM to V_T determined from the entire CWM, there is no difference ($R^2=0.975$, slope=0.97, intercept=0.16, $p < 0.001$, $n=39$). Similarly, if we measure V_T in the CGM ROI and compare it to the total gray matter V_T in the cerebellum, there is no difference ($R^2=0.979$, slope=1.03, intercept=-0.33, $p < 0.001$, $n=11$). Therefore ROI positioning should have no effect on our outcome measures or conclusions.

Additional support of CWM as opposed to CGM as a RR for [¹¹C]WAY-100635 comes from our pindolol data. Whereas there is no change in CWM V_T/f_P following blockade, there is a reduction in CGM V_T/f_P and BP_{ND}. This demonstrates significant, specific, competitive binding to the 5-HT_{1A} receptor in CGM, which leads to systematic bias when using CGM as a RR. Because BP_{ND} is based on k_3 and k_4 , changes following pindolol are entirely due to changes in specific binding. Conversely, the lack of change in CWM following pindolol blockade bolsters its use as a valid measure of nonspecific binding. While it is possible that pindolol administration may have led to reductions in blood flow,

thus impacting cerebellar V_T/f_P measures, we observed no change in K_1 between baseline and pindolol block scans, suggestive that this did not contribute significantly to our findings.

$[^{11}\text{C}]\text{WAY-100635}$ has an excellent signal-to-noise ratio. The very low non-specific binding (as evidenced by CWM and CGM $V_{T(\text{REF})}$ values of 0.2 to 0.6) makes the quantification of binding in the RR difficult. When the concentration of radioactivity in a region of the brain is this low, quantification can be affected by several factors including the presence of a radioactive metabolite, improper scatter correction, improper blood volume correction, etc. While SRTM is very appropriate for some tracers, this very low non-specific binding makes quantification of $[^{11}\text{C}]\text{WAY-100635}$ binding very difficult. For example, if we simulate data using the 2T model and then fit with SRTM, SRTM always underestimates BP_{ND} in the range of physiological cerebellar $V_{T(\text{REF})}$ (Figure S3 in Supplement 1). Large differences in estimated BP_{ND} (from ~ 7.5 to 5.5 , approximately a 30% change) can arise from very small changes (0.3 to 0.5) in cerebellar $V_{T(\text{REF})}$. The implications of this are that 5-HT_{1A} studies conducted *in vivo* with $[^{11}\text{C}]\text{WAY-100635}$ are best done with an arterial input function and the outcome measure of either BP_F or V_T/f_P .

These findings indicate that the choice of the outcome measure and RR profoundly influence the results when using $[^{11}\text{C}]\text{WAY-100635}$. One other publication showed lower BP_P in depressed subjects, but did not confirm decreased BP_{ND} using either CGM or CWM.(41) These authors did not measure f_P ; hence, BP_F is not available. When we replicate their modeling methods (60 minutes of scanning, a 2T model, restricted to first episode subjects, etc) we cannot replicate the BP_P findings (data not shown). Potential discrepancies are genetics (unavailable), image processing methods or power. We are working with the corresponding author to understand this dataset better.

Interestingly, one group has an *in vitro* study showing higher 5-HT_{1A} in a monkey model of depression(76), while the PET study, using BP_{ND} as outcome measure and cerebellar cortex as RR, showed lower 5-HT_{1A} in the same animals.(77) Choice of outcome measure and RR may perturb results from the PET studies, thus accounting for the seemingly discrepant findings within the same lab.

Finally, to help choose an outcome measure, we compared PET outcome measures to data obtained from other modalities. Post mortem studies have demonstrated higher 5-HT_{1A} in females compared to males.(33) Genetic studies predict higher 5-HT_{1A} in the RN with increasing G allele load(56) and predict lack of response to SSRIs.(75) Challenge studies have shown an inverse relationship between aggression and 5-HT_{1A}.(78) In each of these cases, BP_F and not BP_{ND} demonstrates the expected result giving indirect support for this outcome measure (Table 3).

Free Fraction—While f_P is different between groups, this effect does not drive the primary finding. In the previous cohort, f_P was not different and we still had higher 5-HT_{1A} in NRM. Also, if we use an outcome measure independent of f_P ($\text{BP}_P = f_P \text{BP}_{\text{avail}} / K_D$), we detect differences between groups in the expected direction ($p=0.0001$, data not shown). Furthermore, the differences in mean f_P are small and the values overlap to such an extent that they could not be used as surrogate markers for depression.

Statistical Analysis—One of the concerns of brain imaging studies is the issue of multiple comparisons and the high degree of correlation. We addressed these issues by testing for a group difference simultaneously for all regions in the model while allowing for region effects. If the group effect is proportional to mean binding in each region, additional power can be gained by analyzing the log-transformed data, which has the side benefit of stabilizing the variance. The log transform was used in previous PET studies by our group

and others to address these issues(10,11,40-45). Others have alternate strategies, including linearizing transformation(46) and non-parametric testing(47). As the natural log is a monotone transformation, demonstrating a difference in $\log(BP_F)$ is equivalent to demonstrating a difference (in the same direction) in BP_F . Our principal hypothesis that there is a difference between groups specifies that differences in each ROI are proportional to each ROI's binding level.

Conclusions

We find increased 5-HT_{1A} BP_F in all brain regions of NRM compared to controls. This may be due in part to greater 5-HT_{1A} gene expression. More autoreceptor binding may result in less serotonin neuron firing and hypofunction consistent with the indoleamine hypothesis of major depression. Reversal of that effect by antidepressants may exert an effect in some subjects but perhaps not in treatment resistant subjects, highlighting the need for antidepressants with alternative mechanisms of action. Additionally, the choice of outcome measure and RR profoundly affects the interpretation of the results.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Binding potential (BP_F) differs in not recently medicated (NRM) subjects compared to controls in the old cohort ($p=0.037$) and new cohort ($p=0.04$). The height of the bars indicates the weighted mean BP_F for each ROI (RN=raphe nuclei, AMY=amygdala, HIP=hippocampus, PHG=parahippocampal gyrus, TEM=temporal cortex, ACN=anterior cingulate, CIN=cingulate body, DLPFC=dorsolateral prefrontal cortex, MPFC=medial prefrontal cortex, VPFC=ventrolateral prefrontal cortex, INS=insula, OCC=occipital cortex, PAR=parietal cortex); the error bars represent the corresponding equivalent of the standard deviations of the weighted means for each ROI.

Figure 2.

Voxel based analysis. All PET data was registered using each individual's MRI to the MNI single subject MRI template (first row). Individual BP_F maps were averaged voxel-by-voxel in the control (CTR; second row) and NRM (third row) groups. The CTR average image is subtracted from the NRM average image (fourth row) to show regions that have greater average 5-HT_{1A} receptor binding in NRM subjects. Statistically significant regions (p < 0.005) with greater BP_F in the NRM group were calculated with an SPM5 parametric analysis, False Discovery Rate corrected for multiple comparisons, extent threshold set to p < 0.05 corresponding to a cluster size k>5000 voxels (fifth row). The top colorbar represents the BP_F value in the images of rows two to four. The bottom colorbar represents the t score

in row five image, where a t score of $t > 2.75$ corresponds to $p = 0.005$. MNI coordinates of displayed cross sections are given above SPM images.

Figure 3. BP_{ND} calculated using the simplified reference tissue model (SRTM) using cerebellar gray matter as the RR. The groups differ significantly ($df=1,78$; $F=4.63$; $p=0.034$).

Figure 4.

The volume of distribution corrected for the free fraction of [¹¹C]WAY 100635 in plasma (V_T/f_p) across 13 ROIs differs in control and NRM subjects ($df=1,79$; $F=17.9$; $p=0.00001$). The height of the bars represents the weighted mean V_T/f_p for each ROI; the error bars are the corresponding equivalent of the standard deviation of the weighted means.

Table 1
Clinical and Demographic Data

	Controls (n=51)	Not Recently Medicated (n=30)	<i>p</i>-value (Con vs. NRM)
Age	37.35 ± 14.44	40.59 ± 13.05	0.316
Hamilton Depression Rating Scale	0.73 ± 0.98	26.20 ± 6.98	<0.001
Years of Education	16.56 ± 2.89	14.36 ± 3.67	0.004
Beck Depression Inventory	1.59 ± 2.53	26.43 ± 9.68	<0.001
Lifetime Aggression	13.89 ± 3.72	17.38 ± 4.90	0.001
Global Assessment Scale	90.25 ± 4.76	54.53 ± 11.11	<0.001
Hopelessness	1.61 ± 2.33	9.60 ± 6.33	<0.001
Age of Onset		25.04 ± 13.73	
No. of MDE's		13.04 ± 27.41	
Length of Current MDE		56.32 ± 125.66	
% Female	56.86%	73.33%	0.16
Family History of MDD		56.67%	
Melancholic Depression		33.33%	
History of Post-traumatic Stress Disorder		26.7%	
History of Suicide Attempt		23.3%	
History of Panic Disorder		20.0%	
History of Dysthymia		13.3%	
History of Social Phobia		13.3%	

Table 2
Means and Standard Deviations of V_T/f_P and K_1 for Cerebellar White and Gray Matter

	Outcome Measure	Baseline	Pindolol Blockade
Cerebellar White Matter	V_T/f_P (mL/cm ³)	7.33 ± 1.19	6.02 ± 0.63
	K_1 (mL/cm ³ /min)	0.056 ± 0.019	0.055 ± 0.012
Cerebellar Gray Matter	V_T/f_P (mL/cm ³)	13.18 ± 2.47	8.95 ± 1.17
	K_1 (mL/cm ³ /min)	0.081 ± 0.023	0.080 ± 0.018

Table 3
Comparison of Outcome Measure: Contrasts Based on Known Group Differences from Non-imaging Modalities

Outcome Measure	Sex effect		Genotype	Treatment Responders vs. Non Responders		Lifetime Aggression Score
	BP _F	Females > Males; p = 0.028		GG>CG>CC; p = 0.009	NonResponders > Responders; p = 0.03	
	BP _{ND}	No difference; p = 0.497	No difference; p = 0.697	No Difference; p = 0.453		Inverse correlation; R ² = 0.12, p = 0.001
						No significant relationship