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# Genetic Variation in Brain-Derived Neurotrophic Factor val66met Allele is Associated with Altered Serotonin-1A Receptor Binding in Human Brain

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## Abstract

Brain Derived Neurotrophic Factor (BDNF) regulates brain synaptic plasticity. BDNF affects serotonin signaling, increases serotonin levels in brain tissue and prevents degeneration of serotonin neurons. These effects have hardly been studied in human brain. We examined the relationship of the functional val66met polymorphism of the BDNF gene to serotonin 1A (5-$HT_{1A}$) receptor binding *in vivo*. 50 healthy volunteers (HV) and 50 acutely depressed, unmedicated patients with major depressive disorder (MDD) underwent PET scanning with the 5-$HT_{1A}$ receptor ligand, [$^{11}$C]WAY-100635 and a metabolite corrected arterial input function. A linear mixed effects model compared 5-$HT_{1A}$ receptor binding potential ($BP_F$, proportional to the number of available receptors) in 13 brain regions of interest between met allele carriers (met/met and val/met) and noncarriers (val/val) using sex and C-1019G genotype of the 5-$HT_{1A}$ receptor promoter functional polymorphism as covariates. There was an interaction between diagnosis and allele ($F=4.23$, $df=1, 94$, $p=0.042$), such that met allele carriers had 17.4% lower $BP_F$ than non-met carriers in the HV group ($t=2.6$, $df=96$, $p=0.010$), but not in the MDD group ($t=-0.4$, $df=96$, $p=0.58$). These data are consistent with a model where the met allele of the val66met polymorphism causes less proliferation of serotonin synapses, and consequently fewer 5-$HT_{1A}$



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receptors. In MDD, however, the effect of the val66met polymorphism is not detectable, possibly due to a ceiling effect of over-expression of 5-$HT_{1A}$ receptors in mood disorders.

**Keywords**

BDNF; Brain; Serotonin; PET; [$^{11}$C]WAY-100635; Depression

## Introduction

Brain Derived Neurotrophic Factor (BDNF), an extracellular signaling molecule, plays a crucial role in mature brain function, primarily by affecting synaptic plasticity and axonal growth (Zagrebelsky and Korte 2013). Altering BDNF levels affects the serotonin signaling system *in vitro* (Martinowich and Lu 2008). BDNF infusions stimulate serotonin neuronal proliferation after spinal cord injury (Xu, Guenard et al. 1995, Bregman, McAtee et al. 1997) and prevent degeneration of serotonin neurons exposed to neurotoxins (Mamounas, Blue et al. 1995). BDNF infusions into rat brain also cause higher brain tissue levels of serotonin (Pelleymounter, Cullen et al. 1995, Siuciak, Boylan et al. 1996) and tryptophan hydroxylase, the rate limiting enzyme for serotonin synthesis (Siuciak, Clark et al. 1998). It is unknown if BDNF-related increases in serotonin occur through greater proliferation of serotonin synapses or through increased serotonin release. In animal models, BDNF infusions can have an antidepressant effect consistent with increased serotonin signaling (Siuciak, Lewis et al. 1997, Shirayama, Chen et al. 2002). Conversely, BDNF heterozygote knockout mice manifest increased inter-male aggressiveness (Lyons, Mamounas et al. 1999), and mice with the met allele of the val66met BDNF gene demonstrate symptoms of anxiety (Chen, Jing et al. 2006, Soliman, Glatt et al. 2010), consistent with a serotonin deficit. Less is known about the effect of BDNF on the human serotonin system.

Serotonin 1A (5-$HT_{1A}$) receptors are widely distributed in the brain. The somatodendritic autoreceptors located in midbrain regulate the firing rate of serotonin neurons. This rate of firing, in turn, determines release and intra-synaptic levels of serotonin. In rodents, the 5-$HT_{1A}$ receptor is affected by alterations in BDNF. A BDNF conditional knockout mouse strain had lower 5-$HT_{1A}$ receptor levels and mRNA levels in hippocampus, although not frontal cortex (Klein, Santini et al. 2010). The heterozygote knockout mouse has altered 5-$HT_{1A}$ receptor function, but not receptor levels (Hensler, Ladenheim et al. 2003, Hensler, Advani et al. 2007). Conversely, BDNF intracerebroventricular infusion into mice with a depressive phenotype increases 5-$HT_{1A}$ receptors in hippocampus (Naumenko, Kondaurova et al. 2012). In carriers of the met allele of the val66met human polymorphism, a precursor of BDNF, proBDNF, does not form as efficiently. BDNF intracellular trafficking becomes disrupted and BDNF function is subsequently disturbed (Egan, Kojima et al. 2003). Together, these data suggest that genetic differences affecting BDNF function impact 5-$HT_{1A}$ receptor quantity or function in humans.

In this study we sought to determine whether the val66met polymorphism was associated with 5-$HT_{1A}$ binding potential ($BP_F$) in human brain (a measure of receptor level and affinity) using positron emission tomography (PET) and the radiotracer [$^{11}$C] WAY–

100635. We conducted a secondary analysis of data acquired to evaluate the association between depression and 5-$HT_{1A}$ receptor [$^{11}$C] WAY–100635 $BP_F$. Based on the above-mentioned *in vitro* studies, we hypothesized that human met allele carriers would have less serotonin signaling in the brain than non-met carriers, and that this difference would affect the levels of the 5-$HT_{1A}$ receptor as measured by [$^{11}$C] WAY–100635 $BP_F$

## Methods

### Subjects

Fifty healthy volunteer (HV) subjects and fifty patients who met DSM-IV criteria for major depressive disorder (MDD) in a major depressive episode were included in the study. All subjects were evaluated by Structured Clinical Interview for DSM-IV (SCID I) (First 1994), clinical history, chart review, EKG and laboratory analysis. All clinical and PET imaging data, but not the genotyping data, in this study were previously published, and inclusion and exclusion criteria are detailed there (Parsey, Hastings et al. 2006, Sullivan, Ogden et al. 2009, Miller, Hesselgrave et al. 2013). In brief, MDD and HV subjects were adults aged 18–65 years, without active medical conditions, and had a negative urine pregnancy test and urine toxicology screen. HV subjects had no history of alcohol or substance use disorders, and depressed subjects had no substance use disorder history in the previous six months. No subject had a lifetime history of methylenedioxymethamphetamine (MDMA) use more than two times. HV subjects had no psychiatric history or first-degree relatives with a history of mood disorders, psychotic disorders or suicidal behavior. MDD subjects had a 17-item Hamilton Depression Rating Scale score of >15, were off all medications known to affect serotonin binding for at least 4 years, were off all psychotropic medications for at least two weeks prior to scanning (short-acting benzodiazepines could be used on an as-needed basis for anxiety or insomnia up to 72 hours before PET scanning), and did not take injectable antipsychotics. All subjects provided written informed consent as approved by the Institutional Review Board.

### Genotyping

The BDNF val66met polymorphism (GenBank dbSNP: rs6265; chr: position 27637492) was genotyped for each subject by PCR using the restriction enzyme BsaA I. A PCR fragment of 673 bp length was amplified using the oligonucleotide primers, sense MannBF-1F (5′-ATCCCGGTGAAAGAAAGCCCTAAC-3′) and antisense MannBF-1R (5′-CCCCTGCAGCCTTCTTTTGTGTAA-3′). PCR was conducted in a 20 μl volume, containing 100 ng DNA, 40 ng of each primer, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2 mM $MgCl_2$, 0.01% gelatin, 200 μM of each dNTP, and 0.8 U of REDTAQ Genomic DNA Polymerase (Sigma, MO, USA). Robocycler (Stratagene, CA, USA) was used to process the samples. Thirty temperature cycles of 30s at 95°C, 30s at 60°C and 40s at 72°C were followed by an extension step of 72°C for 4 min. The BsaA I restriction enzyme (NE Biolab, MA USA) was then used to digest the samples into 3 fragments consisting of 275, 321 and 77 bp when guanine is present, and 2 fragments of 321 and 352 bp if cytosine is present at the position. A 1.2% agarose gel was used to separate the PCR products. The allele C(-1019)G of the 5-$HT_{1A}$ promotor was genotyped for each subject as previously described (Wu and Comings 1999, Huang, Battistuzzi et al. 2004).

### Radiochemistry and Input Function Measurement

The preparation of [Carbonyl-C-11]WAY100635 [N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridinyl) cyclohexane carboxamide] was performed as previously reported (Hwang, Simpson et al. 1999). The calculation of the arterial input function was described previously (Parsey, Slifstein et al. 2000, Parsey, Arango et al. 2005) Briefly, at 30 timepoints during the PET scan, plasma was assessed for radioactivity to calculate the arterial input function. Metabolites were calculated after plasma was precipitated with acetonitrile followed by high pressure liquid chromatography (HPLC). Free fraction (fp) was calculated from triplicate aliquots of plasma collected before injection that were mixed with radiotracer; the ratio of ultrafiltrate activity to total plasma activity was calculated when normalized to the filter retention of free [$^{11}$C]WAY-100635.

### Image Acquisition and Analysis

PET data were acquired as described previously (Parsey, Arango et al. 2005, Sullivan, Ogden et al. 2009). Briefly, a head holder (Soule Medical, Tampa Florida) was used to reduce head motion. Images were acquired using ECAT EXACT HR+ camera (Siemens/CTI, Knoxville, Tennessee) in three-dimensional mode. A 10 minute transmission scan was acquired before an intravenous bolus injection of [$^{11}$C]WAY-100635. Emission data were acquired for 110 minutes. T1 weighted magnetic resonance images (MRI) were obtained for co-registration using either a 1.5T Signa Advantage or a 3T HDx scanner (General Electric Medical Systems, Milwaukee, Wisconsin).

Subject motion correction was achieved using de-noising filter applied to the frame five PET image using FSL version 3.3 (Functional Magnetic Resonance Imaging of the Brain's Linear Image Registration Tool [FLIRT] (Jenkinson and Smith 2001). PET/MRI coregistration was performed between a mean image of motion-corrected PET frames and the corresponding MRI using FLIRT as previously described (Parsey, Arango et al. 2005, Sullivan, Ogden et al. 2009). The Brain Extraction Tool of FSL version 3.3 was used for image cropping (Smith 2002) and the Statistical Parametric Mapping 5 was used for normalization (Ashburner and Friston 1999) and segmentation (Ashburner and Friston 2005).

Regions of interest (ROI's) were drawn on each subject's MRI for ventral prefrontal cortex (VPFC), anterior cingulate cortex (ACN), posterior cingulate cortex (CIN), amygdala (AMY), hippocampus (HIP), parahippocampal gyrus (PHG), insular cortex (INS), temporal cortex (TEM), parietal cortex (PAR) and occipital cortex (OCC) (Parsey, Slifstein et al. 2000). Cylindrical regions were drawn within the cerebellar white matter as a reference region and in the midbrain in the approximate location of the raphe nucleus (RN) (Parsey, Arango et al. 2005). A constrained two-tissue compartment (2T) model and arterial input function was used to calculate the outcome measure, $BP_F = B_{avail}/K_D$ where $B_{avail}$ is the total number of available receptors and $1/K_D$ is the affinity of the tracer for the receptor (Parsey, Slifstein et al. 2000).

We have outlined evidence supporting the use of $BP_F$ as the outcome measure for [$^{11}$C]WAY-100635 binding potential elsewhere (Parsey, Ogden et al. 2010, Lan, Hesselgrave et al. 2013, Miller, Hesselgrave et al. 2013). To allow a comparison of our data

with that of other groups, we included calculations here of $BP_{ND}$, calculated as $[V_{T(ROI)} - V_{T(REF)}]/V_{T(REF)}$ and $BP_P$, calculated as $V_{T(ROI)} - V_{T(REF)}$.

### Statistical Analysis

Differences in $BP_F$ between groups were evaluated using a linear mixed models analysis with subject as the random effect and region, diagnosis, sex, BDNF genotype group (met carrier or val/val) and 5-$HT_{1A}$ receptor C-1019G genotype as fixed effects (Parsey, Oquendo et al. 2002, Parsey, Oquendo et al. 2006, Parsey, Ogden et al. 2010). The analysis was performed on the natural log of $BP_F$ to correct some slight skewness in the outcome measure and to normalize variance across ROIs. A bootstrap algorithm was used to calculate standard errors (SE) for each $BP_F$ value taking into account errors in metabolite, plasma and brain data; the errors were then used to weight the data (Ogden and Tarpey 2006, Lan, Hesselgrave et al. 2013, Miller, Hesselgrave et al. 2013). Continuous demographic and clinical measures were evaluated using student's t-test to determine differences between groups, and categorical data were assessed using Fisher's exact test. Significance was defined as a p-value of less than 0.05 and all tests were two sided. SPSS 11 for Mac OSX (www.spss.com) and R (www.R-project.org) were used for calculations.

## Results

### Associations between PET binding and genotype

When all subjects were considered together, controlling for potential effects of diagnosis, sex and 5-$HT_{1A}$ receptor C-1019G genotype as covariates in the model, no binding difference was found between met allele carriers and non-carriers in 5-$HT_{1A}$ receptor [$^{11}$C]WAY100635 $BP_F$ ($F=2.21$, df=1, 95, $p=0.141$) and no allele by region interaction ($F=1.33$ df=12, 1176, $p=0.193$). We previously reported higher 5-$HT_{1A}$ receptor $BP_F$ of [$^{11}$C] WAY–100635 in MDD compared to HV groups (Parsey, Oquendo et al. 2006, Parsey, Ogden et al. 2010). Therefore, an interaction between diagnosis and allele was evaluated within the statistical model, and such an interaction was present ($F=4.23$, df=1, 94, $p=0.042$), indicating that the relationship between BDNF genotype and 5-$HT_{1A}$ binding differed as a function of diagnosis. In post-hoc testing, Met allele carriers had lower $BP_F$ than non-met carriers in the HV group ($t=2.51$, df=94, $p=0.014$), but not in the MDD group ($t=-0.47$, df=94, $p=0.64$, Figure 1). These data did not significantly change when the model did not include the 5-$HT_{1A}$ receptor C-1019G genotype as a covariate. Data from a representative region of interest with high 5-$HT_{1A}$ binding, the hippocampus, are displayed in Figure 2, showing 17.4% lower binding in HV met allele carriers compared to noncarriers, and an absence of this effect in MDD subjects. Free fraction (fp) was 20% higher in met carriers than non-met carriers in HV's ($p=0.03$), and was 14% trend higher in met carriers in MDD subjects ($p=0.12$).

### Clinical Factors

Met allele carriers and noncarriers (val/val genotype) were well-matched for age, sex, and 5-$HT_{1A}$ receptor C-1019G genotype (Table 1). MDD met allele carriers and non-carriers did not differ in HAM-D scores, rates of comorbid anxiety and substance use disorders, or rates of past exposure to psychiatric medications (Table 1). The MDD met allele carriers included

two subjects with ADHD, three subjects with dysthymia and one subject with a delusional disorder, and the MDD noncarriers included one subject with comorbid dysthymia.

In both the HV and MDD groups, the allele frequency of the BDNF gene was found to be in Hardy Weinberg equilibrium ($X^2$=0.065, p=0.80 for HV and $X^2$=1.81, p=0.18 for MDD). The MDD group met carriers had 0 subjects with AA and 16 subjects with AG and 34 subjects with GG allele. The HV group had 4 subjects with AA, 19 subjects with AG and 27 with GG allele. The allelic distribution (number of G alleles) showed a trend of a difference between MDD and HV groups in the vall66met allele (p=0.087), but no difference in the 5-$HT_{1A}$ receptor C-1019G allele (p=0.125). There were no differences in injected dose (ID), injected mass (IM) or specific activity (SA) between met and non-met carriers in HVs (ID, p=0.95; IM, p=0.80; SA, p=0.72) or MDD (ID, p=0.77; IM, p=0.27, SA, p=0.36).

### Alternate Outcome Measures

Allowing for an effect of diagnosis, sex and 5-$HT_{1A}$ receptor C-1019G genotype as covariates in the model, no differences between met carriers and non-met carriers were found when HV and MDD subjects were assessed together for $BP_{ND}$ (F=0.020, df=1,95, p=0.89) or $BP_P$ (F=1.08, df=1,95 p=0.30). In contrast to our findings with $BP_F$, the interaction term of allele and diagnosis was not significant within the model for $BP_{ND}$ (F=1.88, df=1,94, p=0.17) or $BP_P$ F=2.57, df=1,94, p=0.11).

## Discussion

In healthy volunteers (HV), BDNF met allele carriers have lower 5-$HT_{1A}$ receptor binding potential compared to non-carriers, consistent with our *a priori* hypothesis. Within MDD subjects, however, no differences in 5-$HT_{1A}$ receptor $BP_F$ of [$^{11}$C] WAY– 100635 were observed between met allele carriers and non-carriers. BDNF affects the serotonin system in the brain, but the molecular pathway has not been fully elucidated. Our data are consistent with a model in which BDNF induces synaptic connections and the axonal outgrowth of serotonin neurons (Pelleymounter, Cullen et al. 1995, Siuciak, Boylan et al. 1996). That model would suggest that met allele carriers would have lower BDNF levels, resulting in less extensive serotonin neuronal outgrowth, fewer terminals on target neurons, and fewer 5-$HT_{1A}$ receptors in the brain.

A previous PET study did not find differences in binding potential of [$^{11}$C]WAY100635 between met allele carriers and subjects with the val/val allele (Henningsson, Borg et al. 2009). The discrepancy with our finding may be due to PET outcome measures used. We utilized a fully quantitative method involving a metabolite-corrected input function with cerebellar white matter as a reference region, also accounting for plasma free fraction. Henningson et al used $BP_{ND}$ as their outcome measure that relied on whole cerebellum as a reference region for normalization without collecting metabolite or free fraction data. Our approach limits complications from nonspecific binding of the radiotracer in the cerebellum and susceptibility of the cerebellum to signal artifacts (Parsey, Ogden et al. 2010). We repeated our primary analyses using $BP_{ND}$ and $BP_P$ as the outcome measure to evaluate the impact of outcome measure on the observed BDNF effect. Our results confirm the importance of calculating the outcome measure for [$^{11}$C]WAY-100635 using a metabolite

corrected arterial input function that incorporates free fraction data of the radiotracer. The discrepancy of findings with alternate outcome measures is consistent with those that we previously observed with the depression effect using [$^{11}$C]WAY-100635 (Parsey, Ogden et al. 2010). Based on our data, we can not rule out the possibility that the difference in $BP_F$ in the brain between met carriers and non-met carriers is due to an effect that the val66met allele has on the binding of radiotracer in the blood. Repeating this analysis using a different radiotracer that binds to the serotonin-1A receptor would further verify these results.

Genetically engineered mice with disruptions in BDNF have been found to have behavioral abnormalities. A mouse strain with the met/met allele of the val66met BDNF gene demonstrates a significantly anxious phenotype (Chen, Jing et al. 2006). Heterozygote knockout mice display aggression and hyperphagia (Hen 1996), although most studies demonstrate no depressive phenotype with alterations of BDNF gene (Martinowich and Lu 2008). Our studies suggest further inquiry as to how the serotonin system may mediate these behavioral effects of BDNF gene alterations.

This alteration in the serotonin system could explain why the met allele of BDNF has been previously reported to be associated with susceptibility to psychiatric conditions. Several studies have identified genetic alterations associated with bipolar disorder that are consistent with alterations in BDNF function (Neves-Pereira, Mundo et al. 2002, Sklar, Gabriel et al. 2002). BDNF polymorphisms are also associated with certain features of MDD such as psychosis and suicidal thoughts (Iga, Ueno et al. 2007, Sarchiapone, Carli et al. 2008), although a meta-analysis found no association of the val66met allele with MDD (Verhagen, van der Meij et al. 2010). There is some evidence that genetic differences in BDNF are associated with geriatric (Hwang, Tsai et al. 2006) and childhood onset depression (Strauss, Barr et al. 2005). Our results suggest further inquiry is warranted to determine whether genetic alterations in BDNF function cause susceptibility to these psychiatric conditions via changes in the serotonin system.

Our data do not suggest that the effect of the val66met allele can explain the pathogenesis of MDD or responses to antidepressants, however, as BDNF genotype was not found to affect 5-$HT_{1A}$ receptor binding in MDD. These data are consistent with over-expression of the 5-$HT_{1A}$ receptor in MDD (Parsey, Oquendo et al. 2006, Parsey, Ogden et al. 2010), which may counteract or overwhelm the effect of the BDNF met allele on 5-$HT_{1A}$ receptor levels. A recent meta-analysis found decreased serum BDNF levels in bipolar subjects in depressive and manic episodes, with some evidence of reversal of this deficit with treatment (Fernandes, Gama et al. 2011). Similarly, two recent meta-analyses found decreased BDNF in the serum of patients with MDD, with some evidence of correction with antidepressant treatment (Sen, Duman et al. 2008, Bocchio-Chiavetto, Bagnardi et al. 2010). BDNF protein has been found to be lower in postmortem brain of depressed suicides (Dwivedi 2010) and stress has been reported to lower BDNF levels (Bath, Schilit et al. 2013). Such state dependent and environmental effects may be more pronounced in MDD than HV.

In animal models, treatment with antidepressants can increase BDNF in brain and block chronic stress effects that can lead to decreased brain BDNF (Duman and Monteggia 2006). This effect may be clinically important, in part because it occurs in the same timeframe as

the antidepressant behavioral response. Some evidence exists that BDNF function is needed for antidepressant effects of the medication. Mice with the met/met allele at the val66met site of the BDNF gene, for example, demonstrated a blunted anxiolytic response to fluoxetine (Chen, Jing et al. 2006) and mice without TrkB, the receptor for BDNF, showed a lack of antidepressant effect (Li, Luikart et al. 2008). These reports and our data are consistent with a feed-forward mechanism between BDNF and serotonin where higher serotonin levels at synapses from antidepressant treatment would cause increased BDNF levels in the brain, which in turn would cause more serotonin neuronal outgrowth and connections.

Our sample size is robust for a PET study. Previous studies, including ours, have found other genetic variations to affect PET radiotracer binding to the 5-$HT_{1A}$ receptor, including a functional polymorphism in the 5-$HT_{1A}$ gene itself, the triallelic polymorphism of the serotonin transporter, and the catechol-o-methyl transferase genotype (Parsey, Oquendo et al. 2006, Henningsson, Borg et al. 2009, Lothe, Boni et al. 2009, Parsey, Ogden et al. 2010, Baldinger, Hahn et al. 2013). The current results require replication in future molecular imaging studies or further animal model work. The genetic regulation of serotonin function is likely complex, but may play a key role in our understanding of human behavior and psychopathology.

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## Abbreviations

| | |
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| **BDNF** | Brain Derived Neurotrophic Factor |
| **5-HT1A** | Serotonin 1A |
| **HV** | Healthy Volunteer |
| **MDD** | Major Depressive Disorder |
| **met carriers** | Subjects with met/met or met/val genotype of val66met polymorphism |
| **met non-carriers** | Subjects with val/val genotype of val66met polymorphism |

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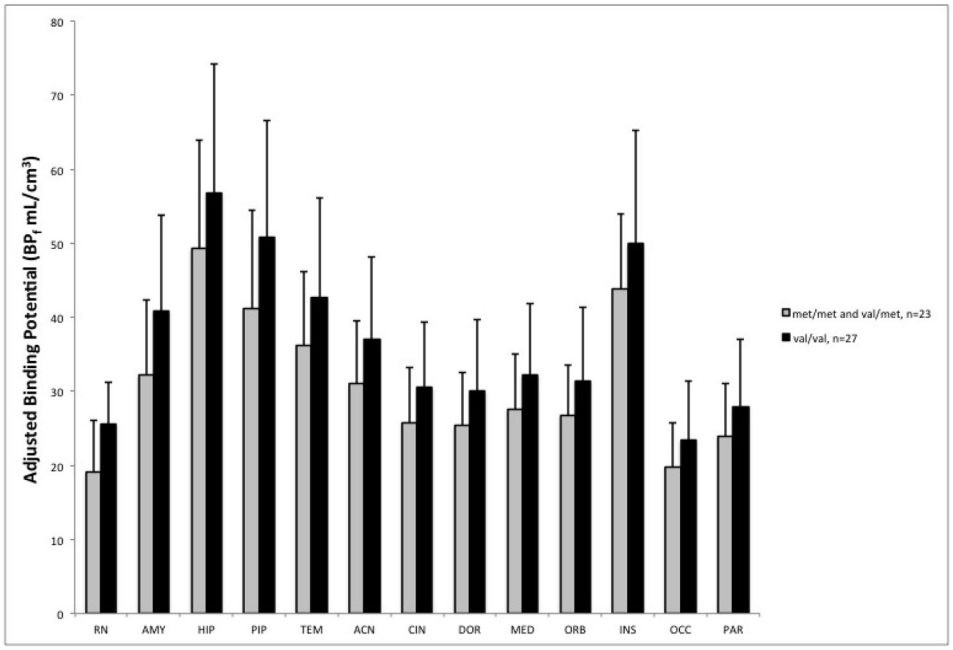


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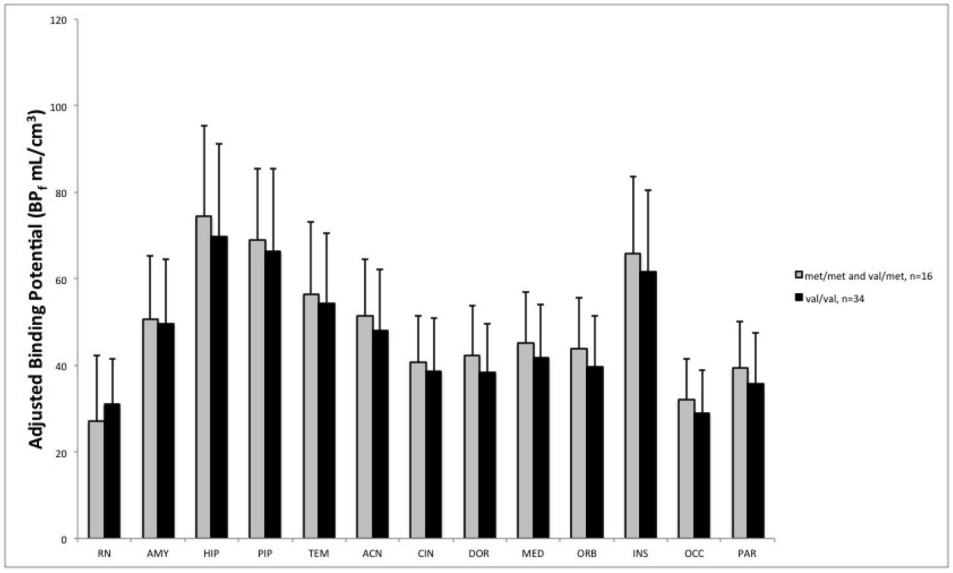


B

**Figure 1.**

5-$HT_{1A}$ receptor $BP_F$ values for [$^{11}$C]WAY-100635 in the brain for met allele carriers (subjects with met/met and val/met alleles) and subjects that were not met allele carriers (val/val allele) within the data for healthy volunteer subjects (Figure 1A) and MDD subjects in a major depressive episode (Figure 1B) adjusted for the effect of diagnosis, sex and 5-$HT_{1A}$ receptor C-1019G genotype. Data for the diagnostic groups are displayed separately because a statistically significant interaction between diagnosis and allele was found within

the dataset. HV subjects (p=0.014), but not MDD subjects (0.64), have an association between BDNF allele variant and 5-$HT_{1A}$ receptor $BP_F$ values.

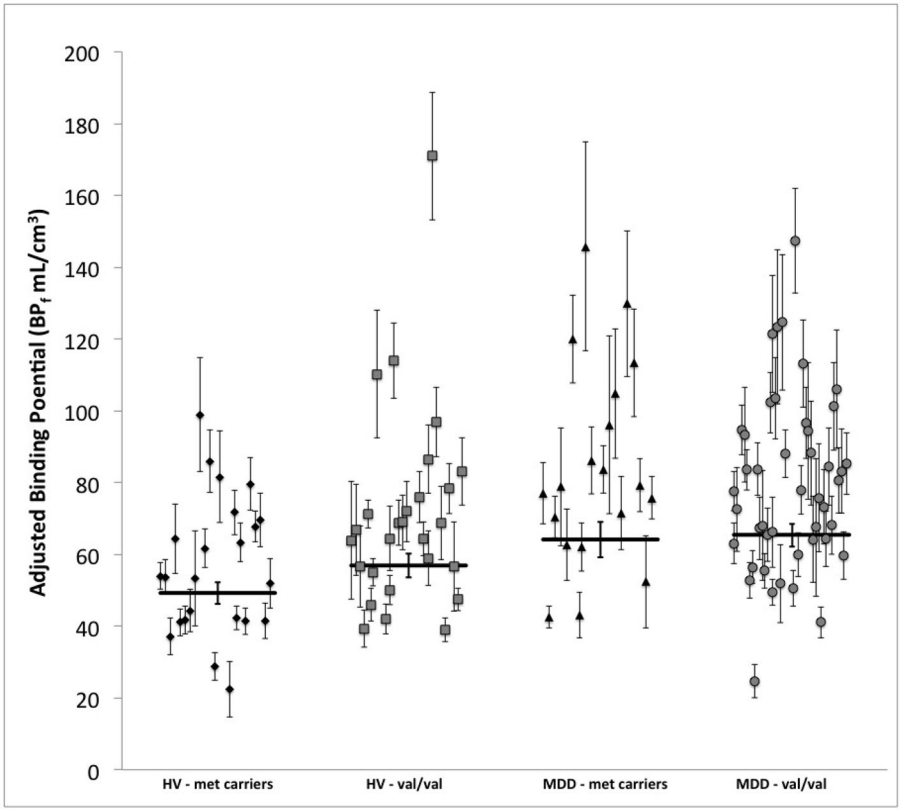


**Figure 2.**

A scatter plot of 5-$HT_{1A}$ receptor $BP_F$ values within the hippocampus, a representative region of interest adjusted for the effect of diagnosis, sex and 5-$HT_{1A}$ receptor C-1019G genotype. Data are divided into major depressive disorder (MDD) and healthy volunteer (HV) groups. Data are also divided into met carriers (subjects with met/met and val/met alleles) and subjects with val/val allele. Error bars indicate weights that quantify the level of uncertainty for each value. The weighted mean values are marked by horizontal lines. The distribution of data demonstrates the effect size of differences in $BP_F$ between allele genotype in the HV group that is not present in the MDD group.

**Table 1**

Demographic variables between met carriers and non-met carriers for both the HV and MDD groups. Values for age and HAMD are listed as group means with standard deviations in parentheses. The percentage of subjects with each polymorphism of the C-1019G allele are listed for each group. The number of subjects with co-morbid psychiatric disorders and exposure to past psychiatric medications are listed.

| | HV | | | MDD | | |
|---|---|---|---|---|---|---|
| | **Met carriers N=23** | **Non-met carriers N=27** | **P value** | **Met carriers N=16** | **Non-met carriers N=34** | **P value** |
| Age in years | 34.5(13.4) | 40.4(15.5) | 0.15[a] | 37.2(14.4) | 37.7(12.5) | 0.90[a] |
| Sex (# female) | 13(57%) | 16(59%) | 1.0[b] | 10(63%) | 24(71%) | 0.75[b] |
| HAMD | 0.5(1.1) | 0.9(0.9) | N/A | 25.0 (6.8) | 25.6(6.5) | 0.75[a] |
| 5HT1AR CC polymorphism | 43.5 | 26.9 | | 25.0 | 23.5 | |
| 5HT1AR CG polymorphism | 47.8 | 65.4 | | 50.0 | 55.9 | |
| 5HT1AR GG polymorphism | 8.7 | 7.7 | 0.41[c] | 25.0 | 20.6 | 0.92.[c] |
| Past anxiety disorders | N/A | N/A | N/A | 8(50%) | 14(41%) | 0.76[c] |
| Past substance use disorders | N/A | N/A | N/A | 3(19%) | 5(15%) | 0.70[c] |
| Antidepressant history | N/A | N/A | N/A | 5(31%) | 8(24%) | 0.73[c] |
| Mood stabilizer history | N/A | N/A | N/A | 0(0%) | 2(6%) | 1.00[c] |
| Benzodiazepine history | N/A | N/A | N/A | 1(6%) | 3(9%) | 1.00[c] |
| Antipsychotic history | N/A | N/A | N/A | 0(0%) | 2(6%) | 1.00[c] |

HAMD: Hamilton Depression Rating Scale value. 5-HT1A receptor polymorphism: Serotonin 1A receptor promoter C-1019G biallelic genotype.

[a]: Calculated with two tailed t test,

[b]: Calculated with Fisher's exact test.

[c]: Calculated using Fisher's exact test comparing count data for the three polymorphisms between the two groups