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Eur Neuropsychopharmacol. Author manuscript; available in PMC 2018 October 01.

Published in final edited form as:

Eur Neuropsychopharmacol. 2017 October ; 27(10): 1011–1021. doi:10.1016/j.euroneuro.2017.07.009.

A positron emission tomography study of the serotonergic system in relation to anxiety in depression

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Abstract

Symptoms of anxiety are highly comorbid with major depressive disorder (MDD) and are known to alter the course of the disease. To help elucidate the biological underpinnings of these prevalent disorders, we previously examined the relationship between components of anxiety (somatic, psychic and motoric) and serotonin 1A receptor (5-HT_{1A}) binding in MDD and found that higher psychic and lower somatic anxiety was associated with greater 5-HT_{1A} binding. In this work, we sought to examine the correlation between these anxiety symptom dimensions and 5-HTT.

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Conflict of Interest

The authors declare no conflict of interest.

Contributors

ZI undertook the stepwise regression analysis, contributed to the literature search and wrote the manuscript. GR helped in literature search and wrote the introduction and discussion. SR and MM helped in literature search. JY and MZ performed the remaining part of the statistical analysis. JM, GMS, PS, MAO, JJM, and RVP wrote the manuscript. CD coordinated the whole process and wrote the manuscript.

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Positron emission tomography with [^{11}C]-3-amino-4-(3-dimethylamino-methylphenylsulfanyl)-benzonitrile ([^{11}C]DASB) and a metabolite-corrected arterial input function were used to estimate regional 5-HTT binding in 55 subjects with MDD and anxiety symptoms. Somatic anxiety was negatively correlated with 5-HTT binding in the thalamus (β=−.33, p=.025), amygdala (β=−.31, p=.007) and midbrain (β=−.72, p<.001). Psychic anxiety was positively correlated with 5-HTT binding in midbrain only (β=.46, p=.0025). To relate to our previous study, correlation between 5-HT_{1A} and 5-HTT binding was examined, and none was found. We also examined how much of the variance in anxiety symptom dimensions could be explained by *both* 5-HTT and 5-HT_{1A}. The developed model was able to explain 68% (p<.001), 38% (p=.012) and 32% (p=.038) of the total variance in somatic, psychic, and motoric anxiety, respectively. Results indicate the tight coupling between the serotonergic system and anxiety components, which may be confounded when using aggregate anxiety measures. Uncovering serotonin's role in anxiety and depression in this way may give way to a new generation of therapeutics and treatment strategies.

Keywords

anxiety; serotonin; positron emission tomography; depression

1. INTRODUCTION

Depressive and anxiety disorders have substantial comorbidity, however the shared pathophysiology between the two remains poorly understood. Studies of sequential comorbidity suggest that anxiety disorders are more often preceded by depressive disorders (Moffitt et al., 2007). Patients who are comorbid for both disorders have greater psychosocial disability and a poorer quality of life (Hirschfeld, 2001), and depression with anxious features is associated with worse treatment outcome (Fava et al., 2008).

Clinically, major depressive disorder (MDD) and generalized anxiety disorder share common core symptoms, which may reflect overlap in etiology. For example, the serotonergic (5-HT) system has been implicated in both the pathophysiology of MDD (Drevets et al., 1999), as well as modulation of anxiety symptoms (Olivier et al., 2013). Specifically, the serotonin transporter (5-HTT) is an important target for treatment of both depression and anxiety (Owens et al., 1997; Reimold et al., 2008; Tatsumi et al., 1997); selective serotonin reuptake inhibitors (SSRIs) are first-line treatments for both unipolar depression and anxiety disorders (Denys and de Geus, 2005; Feighner and Cohn, 1989; Olivier et al., 2013; Reimold et al., 2008).

The 5-HTT anxiety relationship has been explored in humans, and an association is reported between anxiety and polymorphisms of the 5-HTT (Cerasa et al., 2014; Liu et al., 2013; Pietrzak et al., 2013). Further, human PET imaging studies have consistently reported an inverse correlation between 5-HTT binding and the severity of depressive or anxiety symptoms; however, this relationship may be affected by the inclusion of heterogeneous MDD cohorts (Spies et al., 2015), particularly those with anxiety. In PTSD, lower 5-HTT binding in the midbrain and thalamus was associated with greater anxiety (Reimold et al., 2008). Similarly, an inverse correlation was found between 5-HTT expression and symptom

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severity in patients with PTSD (Frick et al., 2015b) But a study completed in patients with social anxiety disorder by the same group showed higher 5-HTT binding in this disorder compared to healthy control subjects (Frick et al., 2015a).

In some studies, patients with MDD exhibit lower 5-HTT binding in the amygdala (Murrough et al., 2011), midbrain (Malison et al., 1998; Parsey et al., 2006a) medial temporal lobe, and basal ganglia (Newberg et al., 2012) compared with healthy volunteers. However, using the ligand [^{11}C]-3-amino-4-(3-dimethylamino-methylphenylsulfanyl)-benzonitrile ([^{11}C]DASB), we previously found no differences in 5-HTT binding between MDD and healthy controls (Miller et al., 2013).

One of the challenges associated with relating neurobiology to anxiety is that the forms of anxiety co-occurring with MDD, whether subsyndromal or due to syndromal comorbid conditions (panic disorder, generalized anxiety disorder, social phobia), are heterogeneous in presentation. Therefore, breaking anxiety down into clinically distinguishable components may aid in defining its neurobiological underpinnings. This refined approach may also lead to treatments that can target specific anxiety symptoms. In a previous PET study, we used radioligand [^{11}C]WAY-100635 [N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridil) cyclohexanecarboxyamide] to relate 5-HT$_{1A}$ binding to three components of anxiety derived from a large sample of MDD patients: somatic (hypochondriasis, sweating, cardiovascular, respiratory, gastrointestinal and urination symptoms), psychic (anxiousness and irritability), and motoric (agitation) components. In that study, the severity of somatic and psychic anxiety correlated with 5-HT$_{1A}$ binding in anterior cingulate (negatively and positively, respectively), body of cingulate, orbital prefrontal and medial prefrontal cortices, along with temporal, parietal, and occipital cortices in patients with MDD (Sullivan et al., 2005). These regional relationships may explain why buspirone, a 5-HT$_{1A}$ partial agonist, can improve psychic anxiety more rapidly than somatic anxiety (Feighner and Cohn, 1989).

In this present study, we estimate regional brain 5-HTT binding in MDD subjects using [^{11}C]DASB and relate this binding to anxiety symptom dimensions. This overcomes the limitations of previous studies by specifically examining anxiety components within MDD, instead of confounding MDD group differences. To relate this analysis to our previous work examining 5-HT$_{1A}$ binding, we examined the correlation between 5-HTT and 5-HT$_{1A}$ in the regions implicated in the previous study with non-negligible levels of [^{11}C]DASB binding (anterior cingulate cortex, amygdala, midbrain). To further conceptualize the relationship between the serotonergic system and anxiety, we developed a model in which both 5-HT$_{1A}$ and 5-HTT were used to predict anxiety components. This will provide a more comprehensive view of serotonergic function in major depression with co-morbid anxiety symptoms, which is needed to develop the next generation of therapeutics.

2. EXPERIMENTAL PROCEDURES

Eighty subjects between 18 and 64 years of age (mean: 34 ± 12, SD) who met the criteria for major depressive disorder in a current major depressive episode using Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (American Psychiatric Association, 2000) participated in the study. None of the subjects were in remission.

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Inclusion criteria required subjects to be off all psychotropic medications and to discontinue use of any medications that interact with the serotonin transporter or 5-HT_{1A} for at least 2 weeks. Only co-medications allowed were short acting benzodiazepines or chloral hydrate for distressing anxiety of insomnia, but not within three days of the PET scans. All subjects were divided into two groups composed of 55 and 70 subjects. In Table 1, details of these groups are given.

In the cohort used to examine the relationship between 5-HTT and anxiety, 55 subjects (31 female, 24 male) were examined (right side of Table 2). 21 subjects were recently (in the last four years) treated with antidepressants. 6 subjects' medication history was unknown. The remaining 28 subjects were antidepressant naïve (AN) or not recently medicated (NRM). All were included in the 5-HTT analysis because a previous study from our group showed no effect from antidepressant exposure on 5-HTT binding (Miller et al., 2013). Twenty-four subjects (44%) also possessed at least one AXIS1 comorbidity, including attention deficit hyperactivity disorder (ADHD) (n=3), agoraphobia (n=1), binge eating (n=1), delusional disorder (n=1), dysthymia (n=5), generalized anxiety disorder (n=4), obsessive-compulsive disorder (OCD) (n=2), panic disorder (n=6), PTSD (n=11), specific phobia (n=3), and social phobia (n=9). All subjects received PET scans using the [^{11}C]DASB ligand (as described below). The mean and SD of the difference between the date of psychiatric ratings and the date of scans were 2 ± 2 days, for both HDRS and BPRS. Data from 54 of the 55 subjects have been reported before (Miller et al., 2013) in a study comparing [^{11}C]DASB binding between depressed suicide attempters, depressed non-attempters and healthy controls. However, in that study, anxiety components were not examined.

To examine the relationship between 5-HTT and 5-HT_{1A} binding, 70 (41 female, 29 male) MDD subjects receiving both [^{11}C]DASB (5-HTT tracer) and [^{11}C]WAY-100635 (5-HT_{1A} tracer) were examined. 68 of these 70 subjects have been reported before (Miller et al., 2009; Miller et al., 2013; Parsey et al., 2006a; Sullivan et al., 2005). In (Sullivan et al., 2005), the relationship between 5-HT_{1A} binding and anxiety components was examined. In (Miller et al., 2009), authors compared 5-HT_{1A} binding in patients with remitted MDD with currently depressed and healthy controls. In (Parsey et al., 2006a), authors evaluated the effects of antidepressant exposure and the role of C(−1019)G polymorphism on 5-HT_{1A} in MDD. 45 of the subjects in these studies were included in the group of 55 ([^{11}C]DASB only) subjects presented in Table 2. (The remaining 25 were not used in the anxiety component analysis because their [^{11}C]DASB scans were not acquired within one week of clinical ratings.) Of the 70 subjects, 9 subjects were excluded from the further analysis because previous medication history, which can affect [^{11}C]WAY-100635 binding (Gray et al., 2013), was unknown. Therefore, 61 subjects were included in analyses studying the relationship between [^{11}C]DASB and [^{11}C]WAY-100635 binding. Descriptive statistics for this group are given in Table 3. Two of these subjects were not included in the model where both 5-HT_{1A} and 5-HTT binding predicted anxiety measures due to missing scale items for these subjects.

In Table 3, AN and NRM groups were combined as suggested in (Gray et al., 2013; Parsey et al., 2010). Patients were off medications for at least 14 days prior to being scanned. After complete description of the study, written informed consent was obtained as approved by the

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Institutional Review Boards of Columbia University and New York State Psychiatric Institute for all subjects.

Clinical Assessments

Somatic, motoric and psychic anxiety factors for each subject were calculated according to a previous factor analysis (Sullivan et al., 2005) of the 24-item Hamilton Depression Rating Scale (HDRS-24) (Hamilton, 1960) and the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962). In that study, principal component analysis (PCA) was applied to HDRS-24 and BPRS items from 288 subjects (Table 2), yielding component loading factors (provided in Table 4). In Table 2, it can be seen that the sample from the previous study (Sullivan et al., 2005) and the sample in the current study (relating 5-HTT to anxiety components) are comparable in terms of sex ratio and mean HDRS and BPRS scores.

HDRS-24 and BPRS scores were used to estimate anxiety factors according to component loading factors.

Radiochemistry and Input Function Measurement

[^{11}C]WAY-100635 and [^{11}C]DASB were prepared as described in (Parsey et al., 2000) and (Belanger et al., 2004), respectively. Arterial input functions were collected for [^{11}C]WAY-100635 and [^{11}C]DASB as described in (Parsey et al., 2005; Parsey et al., 2000) and (Ogden et al., 2007; Parsey et al., 2006c), respectively. Briefly, [^{11}C]WAY-100635 parent fraction levels were fit with the Hill function (Gunn et al., 1998) while [^{11}C]DASB parent fraction levels were fit with a biexponential function, which was damped by a power function to take into account the low initial value. For both tracers, the input function was the product of total plasma counts and interpolated unmetabolized tracer fraction and fit with a straight line from time zero to the peak followed by the sum of three exponentials after the peak (Parsey et al., 2005). Free plasma fraction estimation was done in the same way for both tracers (Parsey et al., 2000). Scan duration was between 80 min and 120 min. The mean and the SD of the injected doses, specific activities, and the calculated masses were 16 ± 2 mCi, 1 ± 1 mCi/nmol, and 5 ± 2 ug.

Image Acquisition and Analysis

All PET scans were acquired with the same PET scanner: ECAT EXACT HR+ (Siemens/CTI, Knoxville, TN, USA) in Kreitchman PET Center at Columbia University Medical Center. FMRIB linear image registration tool (FLIRT, FMRIB Image Analysis Group, Oxford, UK) was used to align individual PET frames, in order to correct for subject motion. The mean, motion-corrected PET image was then coregistered to the subject's MRI to apply region of interests (ROIs) to the mean PET image, and individual PET frames. The ROI labeling process was described in (Parsey et al., 2000). Linear PET-to-MRI transformations were computed using FLIRT with a mutual information cost function, six degrees of freedom, and trilinear interpolation (DeLorenzo et al., 2009). After coregistration, time activity curves (mean activity within each ROI over time) were generated.

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Modeling

For [^{11}C]DASB, time activity curves were fit using a graphical approach, likelihood estimation in graphical analysis (LEGA), which was developed to remove the noise dependent bias inherent in the Logan graphical approach (Ogden, 2003; Parsey et al., 2003). LEGA was found to be the optimal modeling approach for [^{11}C]DASB studies. (Ogden et al., 2007).

For [^{11}C]WAY-100635, time activity curves were fit using a constrained two-tissue compartment model, in which the ratio of K_1/k_2 is constrained to that of the reference region (white matter of the cerebellum) (Parsey et al., 2000).

Outcome measures

Binding potential, $BP_F = (V_T - V_{ND})/f_P$ (mL/cm^3), is the closest measure of receptor density to that of *in vitro* studies, where V_T is the total regional equilibrium distribution volume, V_{ND} is the volume of distribution of the nondisplaceable compartment (reference region) and f_P, is the fraction of the ligand that is not bound to plasma proteins at equilibrium (Innis et al., 2007). For [^{11}C]WAY-100635 ligand, this measure was used. Since an optimal estimate of V_{ND} does not exist for [^{11}C]DASB (Parsey et al., 2006b), V_T/f_P, which does not depend on a reference region was used as the outcome measure for this ligand (Miller et al., 2013). Standard measurement error for calculated binding values, *SE*, was computed with a bootstrap algorithm that incorporates errors in metabolite, plasma, and PET image data (Ogden and Tarpey, 2006).

Statistical Analysis

Stepwise Linear Regression (SLR) for [^{11}C]DASB V_T/f_P vs. Anxiety—In a previous analysis (Sullivan et al., 2005) we found a relationship between anxiety factors and [^{11}C]WAY-100635 binding (BP_F) using regression analysis. In this study, we performed the same type of regression analysis to determine the relationship between anxiety factors and [^{11}C]DASB binding (V_T/f_P). As in the previous analysis, a weighted stepwise linear regression was performed between the z-values of natural logarithm of binding (in this case V_T/f_P) for each region and the z-values of three predictors: somatic, motoric and psychic anxiety component scores. As the scales for the predictors were different, we performed the commonly used (Huguelet et al., 2016) z-value standardization in order to ensure that the contributions of the variables to the analysis will be same. The natural logarithm of the outcome measure was applied to improve model fitting as the residuals become closer to normal distribution when this transformation is applied. Weighted regression uses information about the precision of the observations in the fitting. Weights were calculated as $1/(SE)^2$. We conducted this analysis in *a priori* ROIs of amygdala, midbrain, hippocampus, dorsal putamen, and thalamus, regions with highest 5-HTT binding. Exploratory ROIs examined were the anterior cingulate cortex and cerebellar gray matter. Sub-cortical regions were of primary interest as there is little [^{11}C]DASB binding in the cortex.

Although the previous regression analysis (of 5-HT$_{1A}$ and anxiety) used sex as a covariate, previous studies have found no sex differences in [^{11}C]DASB binding (Martinez et al., 2013). Therefore, it was not included in regression models for [^{11}C]DASB in the current

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study. Regression started with the intercept value and after this, the best predictor (i.e. somatic, motoric, psychic anxiety component scores) was added to the model if the F-test for adding the predictor had p-value<0.05. When no other predictor can be added, the current predictors in the model were assessed and, if the F-test for removing the predictor had p>0.10, the predictor was removed. This process continued until no predictors could be added or removed. p-value thresholds were the default values, which are commonly used in different statistical packages and were the same thresholds used in our previous analysis (Sullivan et al., 2005). MATLAB Release 2012b (The MathWorks, Inc., Natick, Massachusetts, United States) was used for the implementation of SLR method.

[^{11}C]WAY-100635 BP_F vs. [^{11}C]DASB V_T/f_P—A linear regression model incorporating measurement error in predictors was used to study the relationship between [^{11}C]WAY-100635 BP_F and [^{11}C]DASB V_T/f_P, after adjusting for sex and medication status as they have been shown to affect [^{11}C]WAY-100635 binding (Kaufman et al., 2015; Parsey et al., 2006a; Parsey et al., 2002). Both BP_F and V_T/f_P were log-transformed in order to ensure model assumptions were met. The SE of log(BP_F) was incorporated using weighted least square fit. The SE of V_T was incorporated into the model by the Simulation Extrapolation (SIMEX) (Carroll et al., 2006; Cook and Stefanski, 1994), an approach to deal with measurement error in predictors. Statistical inference of such estimation is conducted via nonparametric Jackknife resampling (Lederer and H., 2006). The SIMEX method was implemented using R package “simex” version 1.5.

The relationship between [^{11}C]WAY-100635 BP_F and [^{11}C]DASB V_T/f_P was evaluated in the three *a priori* regions: anterior cingulate, amygdala and midbrain (where the raphe is located). These regions were chosen because they were implicated in anxiety in our previous study (Sullivan et al., 2005) (anterior cingulate) and the current study (amygdala and midbrain) and they have measureable [^{11}C]WAY-100635 and [^{11}C]DASB uptake. We also examined this relationship between the anterior cingulate and amygdala 5-HTT and the raphe 5-HT$_{1A}$ because the neuronal cell bodies of serotonergic projections reside in the raphe and project broadly to the other regions of the brain (Hornung, 2003). We did not include midbrain 5-HTT because the raphe is located within the midbrain. Therefore, there are five comparisons in total.

[^{11}C]WAY-100635 BP_F and [^{11}C]DASB V_T/f_P prediction of anxiety—While the above SLR technique used anxiety dimensions as the independent variable predicting 5-HTT, we also sought the examine a model predicting anxiety from 5-HTT and 5-HT$_{1A}$ density. A model that predicts the somatic, motoric and psychic anxiety component scores using both [^{11}C]WAY-100635 BP_F and [^{11}C]DASB V_T/f_P was evaluated using the three *a priori* regions from above: anterior cingulate, amygdala and raphe. The methods in the calculation of the model parameters are same as the measurement error model for comparing [^{11}C]WAY-100635 BP_F with [^{11}C]DASB V_T/f_P.

In all analyses above, adjusted R^2 values were calculated as a measure of the explained variance.

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3. RESULTS

Relationship between [^{11}C]DASB V_T/f_P and Anxiety Factors

5-HTT binding in midbrain, amygdala and thalamus correlated significantly with anxiety measures (somatic and psychic) after Bonferroni correction, as shown in Table 5 and Figure 1. In thalamus, amygdala and midbrain, somatic anxiety was negatively related to 5-HTT binding, while psychic anxiety was positively related in midbrain. The highest adjusted R^2 was obtained in midbrain (R^2=0.54), where both psychic and somatic anxiety component scores correlated significantly with 5-HTT binding (p-value<0.001), but in opposite directions. Motoric anxiety was excluded from the model by the SLR method. There was no significant correlation in any exploratory region.

Figure 1 shows the 5-HTT binding (V_T/f_P) vs somatic and psychic anxieties in specific brain regions. Standard measurement errors show the reliability of the binding values.

[^{11}C]WAY-100635 BP_F vs. [^{11}C]DASB V_T/f_P

log(BP_F) was compared to log(V_T/f_P) with sex and medication status as covariates. Coefficient estimates and corresponding p-values (adjusted for multiple comparisons using Bonferroni adjustment) are shown in Table 6. No significant relationship was observed.

[^{11}C]WAY-100635 BP_F and [^{11}C]DASB V_T/f_P vs anxiety

In this model, anxiety symptom dimensions were predicted by log(BP_F) and log(V_T/f_P) with sex and medication status as covariates. Adjusted R^2 values, coefficient estimates and corresponding p-values (adjusted for multiple comparisons using Bonferroni adjustment) are shown in Table 7. 5-HTT binding and 5-HT_{1A} binding at three brain regions (anterior cingulate, amygdala and midbrain) explained 68% (p<.001), 38% (p=.012) and 32% (p=.038) of the variance respectively for somatic anxiety, psychic anxiety and motoric anxiety symptom dimensions. For somatic anxiety, the greatest and the only significant contribution to variance in the model in terms of R^2 value was the 5-HTT binding at anterior cingulate cortex (ACC) at 44%. For psychic anxiety none of the regional binding estimates alone reached significance as predictors. The greatest and the only significant contribution to variance in motoric anxiety was the 5-HTT binding at midbrain, explaining 34% of the variance in terms of R^2 value (p=0.049).

4. DISCUSSION

A previous publication from our group (Sullivan et al., 2005) found that patients with MDD and comorbid panic disorder had lower 5-HT_{1A} binding in several areas relative to those with MDD alone. Breaking anxiety down into psychic, motoric and somatic dimensions, that study predicted more than 50% of variance in 5-HT_{1A} binding in several regions. Since 5-HT_{1A} and 5-HTT are both extensively implicated in MDD and anxiety disorders, the natural next question was to examine correlation between 5-HTT binding and anxiety symptom dimensions in these same subjects, between 5-HTT and 5-HT_{1A}, and to determine how much of the variance in anxiety could be explained by both the 5-HTT and 5-HT_{1A} levels, as has been done in this study.

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Interrelationship between 5-HTT and Anxiety Components in the Thalamus, Amygdala and Midbrain

Following the same approach for determining the relationship between 5-HTT binding and anxiety, in this study, we identified negative correlations between 5-HTT binding and somatic anxiety in thalamus, midbrain and amygdala, and a positive correlation between 5-HTT binding and psychic anxiety in midbrain. These results align with previous literature. Following the initial animal studies showing the role these regions play in fear, multiple pharmacological and MRI imaging studies have consistently implicated the thalamus and amygdala in anxiety (Davis, 1992) and panic disorder (Kim et al., 2012) in humans. Though the midbrain has been implicated in some studies of panic or other anxiety disorders (Damsa et al., 2009; Mochcovitch et al., 2014; Nikolaus et al., 2010; Pannekoek et al., 2013), this finding has not been consistent across imaging studies.

In preliminary PET investigations into the relationship between 5-HTT binding in MDD and anxiety symptom severity, the thalamus appears to be consistently implicated. Specifically, two studies reported negative correlations between anxiety symptom severity and the 5-HTT binding in the thalamus (Reimold et al., 2008) (Reimold et al., 2011), and one reported such correlations in the midbrain and amygdala regions as well (Reimold et al., 2008). In studies examining panic disorder, one found a positive correlation between panic disorder symptoms and binding in all areas except the hippocampus (Maron et al., 2011) and, in the other, the relationship was only seen in the anterior cingulate cortex and midbrain (Martinez et al., 2013). In both studies, this relationship was observed only in male patients and not controls or females. The inconsistencies between studies may be due to differences in cohorts (panic vs anxiety) and heterogeneity of subjects (e.g. mixing cohorts with OCD symptoms). Our study partially validates these findings, which almost all implicate the thalamus, by reporting a negative correlation in an MDD cohort between anxiety and 5-HTT binding in the thalamus ($p=0.007$). However, we further extend these findings by showing that this relationship is specific to somatic anxiety. Breaking anxiety into components in this way could further reduce ambiguity between studies. For example, the observed positive correlation between panic symptoms and midbrain binding is consistent with the positive correlation we observe in this brain region. However, our results suggest that this relationship is specific to psychic anxiety.

Examining the role of the midbrain in MDD, unlike previous [^{11}C]DASB studies (Cannon et al., 2007; Meyer et al., 2004; Selvaraj et al., 2011), our previous study (Miller et al., 2013) did not report a difference in 5-HTT binding between MDD and control subjects. However, other studies showed low regional midbrain 5-HTT binding (using [^{11}C]DASB and BP_{ND} as an outcome measure) in patients with comorbid anxiety with MDD (Martinez et al., 2013; Reimold et al., 2008). Taken together, it appears that the pathophysiology in the midbrain is inconsistently implicated in studies that examine MDD in isolation. Part of this inconsistency may stem from the inclusion of patients with anxiety symptoms in depressed cohorts and vice versa, as well as the inclusion of people with different types of anxiety. Our study identified opposite directions of the correlations in midbrain for psychic and somatic anxiety. The opposite direction of the correlation in our study could be due to the role of midbrain in both somatic (e.g. cardiovascular control) and psychic (e.g. anxiousness)

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symptoms. More studies are needed to replicate this finding and explain its neurobiological basis.

Choice of PET Outcome Measure

Besides evaluating specific components of anxiety to provide a more nuanced understanding of the relationship between anxiety and 5-HTT binding in MDD, another significant difference between our study and all previous imaging studies cited is that those studies used BP_{ND} as the outcome measure. BP_{ND} is defined as the ratio at equilibrium of specific binding to non-displaceable uptake (as measured in a reference region, which is assumed to be devoid of any target binding) and was estimated using the simplified reference tissue model with cerebellum as the reference region. However, as there is no ideal reference region that is completely devoid of 5-HTT and some 5-HTT binding in the cerebellum could confound study results. By using BP_F or V_T/f_P, therefore, our study should have greater sensitivity to detect relationships with anxiety.

Relationship between 5-HT_{1A} and 5-HTT

To gain a better understanding of the relationship between 5-HT_{1A} and 5-HTT binding, the correlation between these entities was examined. In humans, the correlation between [^{11}C]WAY-100635 BP_F and [^{11}C]DASB V_T/f_P has been examined in five previous studies, which were all done in healthy controls, with inconsistent results. Two studies found positive correlations between 5-HT_{1A} and 5-HTT binding – one found correlations in both the raphe and the hippocampus (Lundberg et al., 2007) and the other found the correlation in the hippocampus only in women (Jovanovic et al., 2008). One study found a non-linear relationship between the 5-HT_{1A} binding in the raphe and the 5-HTT binding in the insula/superior temporal gyrus (Bose et al., 2011). The remaining two studies did not find any correlation (Strupp-Levitsky et al., 2016; Takano et al., 2011). Of these five studies there are two studies that overlap with our methodology – in terms of outcome measures and ligands used (Bose et al., 2011; Strupp-Levitsky et al., 2016). The other studies used BP_{ND} to measure binding outcomes.

In this study, we did not find a correlation between 5-HT_{1A} and 5-HTT binding within a region or between raphe 5-HT_{1A} and regional 5-HTT binding. The correlation with raphe nucleus binding was examined because serotonergic projections from the raphe nucleus extend to multiple areas in the brain including the hypothalamus, brain stem, parietal and frontal cortex, hippocampus and amygdala (Hornung, 2003). However, one reason a correlation may not have been observed between these regions is because these projections are complex and the relationship between 5-HT_{1A} binding at the raphe and 5-HTT binding at cortical and subcortical sites may be a non-linear one (Strupp-Levitsky et al., 2016). Further, all previous human studies done were in healthy controls, our study is the first one done in depressed subjects.

Incorporating 5-HT_{1A} and 5-HTT binding into a Model of Anxiety

In a model incorporating sex, medication status and serotonin receptor binding, we found that serotonin binding (5-HT_{1A} and 5-HTT) at three regions (anterior cingulate, thalamus and midbrain) could explain 68% of the variance (adjusted R^2) in somatic anxiety symptom

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scores. Binding at the anterior cingulate alone could explain 44% (p=0.01) of the variance (R^2) in somatic symptom severity. The anterior cingulate, which is a critical primary area, along with amygdala and the periaqueductal gray (located near the raphe in the midbrain) are implicated in the neurobiological basis of somatic symptoms (Duval et al., 2015; Graeff and Del-Ben, 2008). The ACC modulates emotional processing and as such, may trigger somatic anxiety symptom expression (Martin et al., 2009).

Interestingly, no correlation between 5-HTT binding at exploratory regions (including the ACC) and anxiety symptom severities was found. The anterior cingulate cortex's contribution to somatic anxiety expression in this combined 5-HT_{1A} and 5-HTT model with no correlation to 5-HTT directly seemingly indicates that the serotonergic relationship with somatic anxiety in this region is dependent on contributions from transporter binding as well as the serotonin 1A receptor and other covariates in the model.

Similarly, contributions to psychic and motoric anxiety variance are interesting to examine in this model in comparison to using 5-HTT alone as a predictor. We reported a positive correlation between psychic anxiety and midbrain transporter binding, whereas this model showed no significant contributions to the variance. The inverse is true for motoric anxiety, where our first model showed no results, and this model showed significant contributions from midbrain transporter binding. This could be due to the loss of power when using multiple predictors and covariates in the complex model. This underscores the robustness of the significant model findings.

Limitations of the study

Although the results were quite interesting, there were several limitations that should be mentioned. First, in this study anxiety was quantified using the 24-item HDRS and the BPRS. Other specific scales for anxiety like the Hamilton Anxiety Rating Scale are designed to provide greater detail on anxiety symptoms and may provide increased resolution. Second, unlike some of the previous studies, we did not analyze males and females separately because of the limited sample size. Further, a previous study from our group showed no influence of sex on 5-HTT [^{11}C]DASB binding in an MDD cohort with no anxiety comorbidity (Miller et al., 2013). Third, our results could have been affected by the inclusion of a mixture of suicidal and non-suicidal depressed subjects. In a previous study, lower midbrain 5-HTT binding was observed in suicidal versus non-suicidal MDD and healthy controls (Miller et al., 2013). However, it is unclear whether suicidality would affect these study results. In a larger cohort, other comorbities such as this can be examined.

Conclusions & Future studies

We sought to understand the biological underpinnings of two highly comorbid disorders – anxiety and MDD. This will aid in treatment, prognosis and design of novel therapeutics (Ball et al., 2014; Evans et al., 2006). Using these sensitive divisions of anxiety, our study showed negative correlations between somatic anxiety and 5-HTT binding in thalamus, midbrain and amygdala and positive correlation between psychic anxiety and 5-HTT binding in midbrain. We hypothesize that is this due to the multiple biological roles of the midbrain, but future studies will be required to study this further. The lack of a correlation

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between motoric symptoms and 5-HTT suggests that inclusion of these symptoms in studying serotonin in anxiety may result in a loss of sensitivity. To determine whether 5-HTT studies would explain more of the variance in anxiety symptoms than 5-HT_{1A} alone, we examined the 5-HT_{1A}/5-HTT binding correlation. The lack of correlation implied that including 5-HTT in a predictive model of anxiety might explain more variance than 5-HT_{1A} alone. Indeed, including both 5-HTT and 5-HT_{1A} in a predictive model explains most (68%) of the variance in somatic anxiety. This finding is of paramount importance considering we have few robust correlations between biology and behavior. The results of this study indicate the tight coupling between the serotonergic system and aspects of anxiety in MDD, shedding light on the biological underpinnings of anxiety in MDD. In future studies, specific scales developed for anxiety can be used for better assessment of anxiety components.

Acknowledgments

We would like to thank Tony Jin, Hao Chen, Ruofeng Wen, Mala Ananth, Judith Crowell, Laura Kunkel, Mohammad Zia, Greg Perlman, Joshua Kaufman from Stony Brook University and Francesca Zanderigo from Columbia University for their comments and help in data acquisition and processing. We acknowledge the biostatistical consultation and support from the Biostatistical Consulting Core at the School of Medicine, Stony Brook University.

Role of the Funding Source

This study was supported with the following grants: J.J.M.: R01 MH40695, P50 MH62185, R.V.P.: R01 MH074813-01 (NIMH), NARSAD – PTSD—Serotonin and Stress System Interactions, AFSP – Suicide in Depression Comorbid with PTSD: Serotonin and Stress System Interactions, RFMH – Localization of Selective Attention Deficits in High Lethality Suicide Attempters. CD: K01 MH91354 (NIMH). Moreover, the study has been partly funded by the Russian Academic Excellence Project ‘5–100’. Funding sources had no further role.

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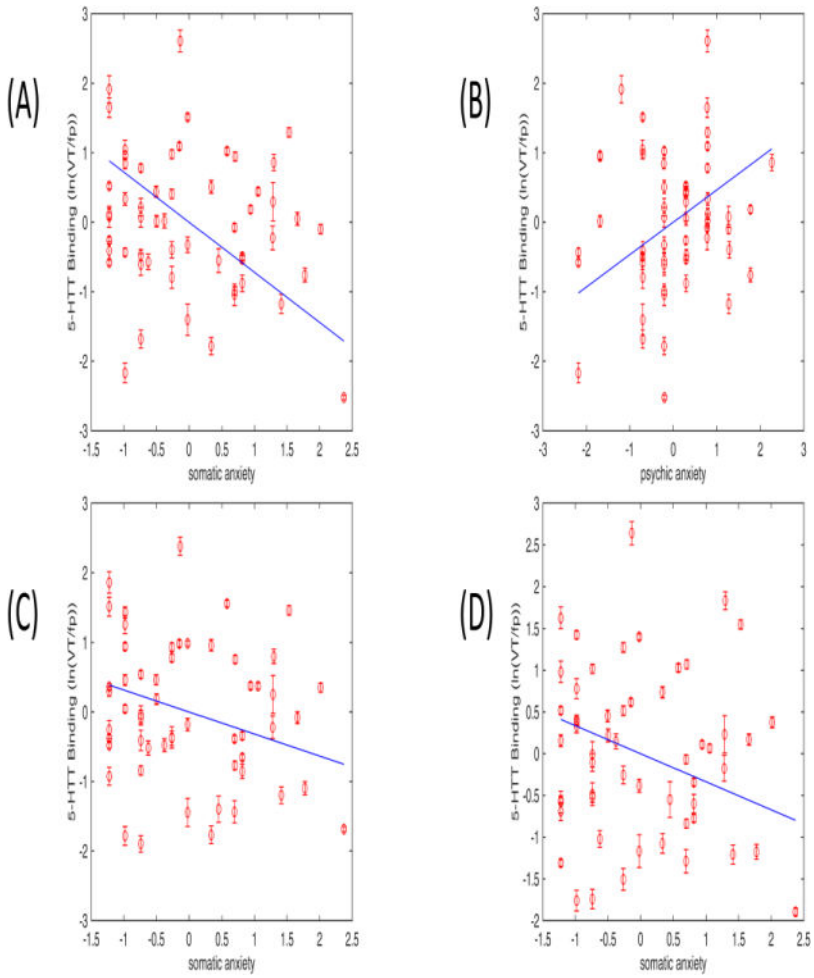

Figure 1.

Relationship between 5-HTT binding (ln(V_T/f_P)) and anxiety components in midbrain (A) somatic: β=−.72; B) psychic: β=.46), amygdala (C) somatic: β=−.31) and thalamus (D) somatic: β=−.33) (z scores). The linear regression fit and standard measurement errors are indicated.

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Table 1

Clinical Details for the two group of subjects participated in the study.

	Group 1 (N=55)	**Group 2 (N=70)**
Age (Mean ± SD)	40 ± 12	38 ± 12
In/Out Patients	13/42	13/57
Smoking Status:		
Non-smoker	42	56
Light smoker	6	8
Moderate Smoker	5	4
Heavy smoker	1	1
Unknown	1	1
Age of onset of first MDD episode (Mean ± SD)	20 ± 11	21 ± 11

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Table 2

Demographic information for the subjects from an MDD sample (n=288) used for the principal component analysis (PCA) and the current study (n=55).

Variable	**PCA sample (*n*=288)**		**PET sample (*n*=55)**	
Sex (% female)	60%		56%	
	Mean	SD	Mean	SD
HDRS-24 Score	28.1	7.9	24.6	6.4
BPRS Score	35.6	7.5	32.3	5.5

BPRS – Brief Psychiatric Rating Scale

HDRS- Hamilton Depression Rating Scale

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Table 3

Descriptive statistics of the subject group who received both [^{11}C]DASB (5-HTT tracer) and [^{11}C]WAY-100635 (5-HT$_{1A}$ tracer) (AE: Exposure to prior antidepressant medication (time off medication: 2–4 weeks), AN: Antidepressant naïve, NRM: Not recently (>4 years) medicated).

Variables	Levels	Count	Percent (%)
Sex	Female	36	59.02
	Male	25	40.98
MedicationStatus	AE	23	37.70
	AN & NRM	38	62.30

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Table 4

Rating Scale Items and related loadings generated by Principal Component Analysis (PCA). The scale items Hypochondriasis, Somatic Anxiety, Agitation, and Psychic Anxiety are HDRS-24 items 15, 2, 9, and 10, respectively. Somatic Concern, Tension, and Anxiety are BPRS items 1, 9, and 2, respectively.

Somatic Anxiety = (0.902 × Hypochondriasis) + (0.889 × Somatic Concern) + (0.603 × Anxiety Somatic)
Motoric Anxiety = (0.932 × Agitation) + (0.905 × Tension)
Psychic Anxiety = (0.855 × Anxiety Psychic) + (0.845 × Anxiety)

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Table 5

Weighted stepwise linear regression results for primary regions of interest (1–5) and exploratory regions (6–7). β denotes the standardized regression coefficients. The reported p-values are corrected for multiple comparisons.

	Adjusted R^2	__ANOVA__		Remaining	___Coefficients___		
ROI		***F*** **p-value**		**Predictors**	***β*** ***t*** **p-value**		
1. Amygdala	.16	11.4	.007*	Somatic anxiety	−.31	−3.36	.007*
2. Midbrain	.54	32.6	<.001*	Somatic anxiety	−.72	−7.96	<.001*
				Psychic anxiety	.46	3.71	.0025*
3. Hippocampus	–	–	–	–	–	–	–
4. Dorsal putamen	.08	5.59	.108	Somatic anxiety	−.24	−2.36	.108
5. Thalamus	.14	5.43	.036*	Somatic anxiety	−.33	−2.93	.025*
				Psychic anxiety	.32	2.51	.076
6. Anterior cingulate cortex	–	–	–	–	–	–	–
7. Cerebellar gray matter	–	–	–	–	–	–	–

*p<.05. Uncorrected p-values = corrected p-values/5.

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Table 6

Relationship between log(BP_F) and log(V_T/f_P) after adjusting for sex and medication status (N=61). Coefficient estimates and corresponding p values (corrected for multiple comparisons using Bonferroni adjustment) for testing whether the coefficient is different from 0. Uncorrected p-values = corrected p-values/5.

Models 5-HT_{1A} vs 5-HTT binding in designated brain region	Adjusted Coefficient estimate for Log(V_T/f_P)	p-value (Bonferroni correction)
anterior cingulate vs anterior cingulate	0.49	0.0965
amygdala vs amygdala	0.37	0.2984
raphe nuclei vs raphe nuclei	0.36	0.3131
raphe nuclei vs anterior cingulate	0.55	0.0944
raphe nuclei vs amygdala	0.28	0.8845

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Table 7

Coefficients of log(V_T/f_P) and log(BP_F) binding based on models: anxiety ~ log(BP_F) + log(V_T/f_P) + Gender + Med_Status (N=59). Coefficient estimates (β) and corresponding p values (corrected for multiple comparisons using Bonferroni adjustment) for testing whether the coefficient is different from 0.

	somatic anxiety Adjusted R^2 = .68 p=0.0006		**psychic anxiety Adjusted R^2 = .38 p=0.0123**		**motoric anxiety Adjusted R^2 = .32 p= 0.0382**	
	β	**p-value**	**β**	**p-value**	**β**	**p-value**
Anterior cingulate (V_T/f_P)	19.0808	0.0115 *	6.9681	0.4350	−2.0866	1
Amygdala (V_T/f_P)	−15.5454	0.1405	−9.7170	0.2808	−4.7272	0.3121
Midbrain (V_T/f_P)	−3.0819	1	2.1035	1	6.0133	0.0490 *
Anterior cingulate (BP_F)	−0.9478	1	−5.7782	0.2445	0.4928	1
Amygdala (BP_F)	−3.3293	1	3.1139	0.7378	−0.6416	1
Midbrain (BP_F)	3.1724	0.2164	1.5889	0.6484	0.1166	1

* p<.05. Uncorrected p-values = corrected p-values/3.