Official Title: PET and MRI Brain Imaging of Bipolar Disorder

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COMMITTEES ON RESEARCH INVOLVING HUMAN SUBJECTS
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Protocol Summary Form

Title: Pathophysiology and Treatment of Bipolar Disorder as Assessed by In Vivo Imaging
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Department: Psychiatry and Behavioral Science

A. Specific Aims

1. Quantify serotonin transporter (5-HTT) binding potential (BP) in vivo in bipolar disorder patients (BPD) during a major depressive episode (MDE): Using an older PET ligand for 5-HTT, [^{11}C]McN5652, we find lower 5-HTT BP_1 in medication-free bipolar patients in many brain regions compared to healthy volunteers. [^{11}C]DASB also offers better signal to noise than [^{11}C]McN5652 (Frankle et al., 2004) permitting assay of 5-HTT in the cortex. We hypothesize that untreated bipolar depression will be characterized by lower BP in both subcortical (amygdala, hippocampus, midbrain, thalamus, putamen) and cortical (anterior cingulate and prefrontal cortex) regions as assessed by region of interest and voxel based analyses, reflecting a more widespread abnormality of the serotonergic system than in unipolar depression.

2. Assess the effect of lithium treatment of bipolar disorder on 5-HTT. We propose to rescan BPD patients with [^{11}C]DASB 6-10 weeks from the time of therapeutic or highest tolerable dose of lithium monotherapy. We hypothesize that lithium will increase the low 5-HTT BP in BPD patients in the temporal, frontal, and entorhinal cortices towards 'normal' levels. We will determine the relationship between BP change and antidepressant effect.

3. Assess the effect of lithium treatment of bipolar disorder on 5-HT_{1A} BP. Chronic lithium in animal studies does not affect somatodendritic 5-HT_{1A} B_{max} in the dorsal raphe nucleus but decreases 5-HT_{1A} B_{max} in frontal cortex and hippocampus. We propose to perform baseline 5-HT_{1A} scans with [^{11}C]CUMI-101 or [^{18}F]MeFWAY and repeat scans 6-10 weeks from the time of therapeutic or highest tolerable dose of lithium monotherapy. Our preliminary 5-HT_{1A} data in bipolar subjects suggest that as a group they have slightly higher 5-HT_{1A} BP than controls with a sex by diagnosis interaction. We hypothesize that regardless of sex, 5-HT_{1A} BP will downregulate in response to chronic lithium.

4. Assess the effect of lamotrigine treatment of bipolar disorder on 5-HTT and 5-HT_{1A} BP. We estimate based on our experience with bipolar subjects in our clinic that 50% of the subjects will not be eligible for lithium monotherapy. We propose to treat this group with lamotrigine. Lamotrigine, like lithium, alters 5-HT_{1A} B_{max} and also activity. There are no data regarding lamotrigine and its effects on 5-HTT. We propose to explore the effect of lamotrigine on 5-HTT and 5-HT_{1A} in order to determine if this medication also acts via alteration of these proteins. We will determine the relationship between BP change and antidepressant effect.

5. ***Assess the effect of lithium treatment of unipolar depression on 5-HTT BP.*** We will perform baseline and

post treatment [^{11}C]DASB scans in 10 unipolar depressed subjects. We will determine if the effects of lithium treatment are specific to bipolar depression.

B. Background and Significance

Bipolar disorder is a brain disorder characterized by recurrent manic and major depressive episodes, the latter being clinically similar to those observed in unipolar or major depressive disorder. The one year prevalence rate for Bipolar I or II is estimated at 1-2% for all ages.[1] Bipolar disorder ranked 20th in terms of causes of loss of disability-adjusted life-years in 1999.[2] Yet, its neurobiological underpinnings are poorly understood and most research has focused on functional brain abnormalities rather than alterations in neurotransmission.[3] Pharmacotherapy development has focused on testing molecules that target neuroreceptors, transporters and heavily on serendipitous findings regarding lithium and anticonvulsants where the precise mechanism of therapeutic action is unknown. Further, treatments for bipolar depression show remission rates only modestly better than placebo.[4] As a potential aid to rational drug development and to elucidate its pathophysiology, neurotransmission deficits present in bipolar depression require attention.

Our group has been interested in determining the role of key neurotransmitter receptors and transporters in both unipolar and bipolar depression. In unipolar depression this initial work has lead to a working model that incorporates 5-HT$_{1A}$ receptors, the 5-HTT, and genetic polymorphisms.[9,21,22] In parallel with this work in unipolar depression we have started 5-HT$_{1A}$ and 5-HTT studies in bipolar disorder, preliminary data will be presented in Sections C.1 and C.2. Our goal is to develop a neurotransmitter based model of bipolar disorder to complement important findings from fMRI and MR that will enhance understanding of the pathophysiology and the action of drugs with proven beneficial effects to accelerate development of more specific or novel treatment strategies.

There is very little in vivo neuroreceptor data in bipolar disorder. The proposed studies would help in determining if there are abnormalities in the 5-HTT and 5-HT$_{1A}$ proteins in bipolar depressed subjects scanned during a major depressive episode. Further, we propose to examine the mechanism of action of two approved medications for the treatment of bipolar depression (lithium and lamotrigine). We will also examine the diagnostic specificity of lithium response by studying 10 unipolar depressed subjects in an identical manner. Based on our preliminary data of 5-HTT and 5-HT$_{1A}$ in bipolar depression and unipolar depression, the preclinical evidence, and years of working with this patient population, we believe we are in a position to address these fundamental and critical questions in this devastating mental illness.

[^{11}C]WAY-100635 has been very well characterized and validated. It has high sensitivity and specificity for the 5-HT$_{1A}$ receptor, and high correlation to the gold standard for receptor quantification, human post-mortem autoradiography. [^{18}F]MeFWAY was developed to be a close structural analog of [^{11}C]WAY-100635.[40] Due to its 109-minute half-life, this tracer can be shipped to hospitals and research centers and would not require a cyclotron on site for synthesis. This is the main advantage of using [^{18}F]MeFWAY vs [^{11}C]WAY-100635. Further, [^{18}F]MeFWAY was developed to be relatively more stable to metabolism, be easily synthesized, and retain high affinity and selectivity for the 5-HT$_{1A}$ receptors. Also, placing a fluorine on a primary carbon (rather than a secondary carbon as in ^{18}F-FCWAY) enhances the compound's stability toward defluorination *in vivo*. [40] Data suggest that it is a feasible alternative PET tracer to quantify 5-HT$_{1A}$ receptors. When necessary [^{18}F]MeFWAY will be used to complete the aims of this study.

Background for Optional <u>Psychophysiological Assessment</u>

Event-related potentials (ERPs) have been widely utilized to determine differences between healthy individuals and patients with depression. Interestingly, some ERP tasks may be sensitive to monoaminergic activity. For example, the loudness dependence auditory evoked potential (LDAEP) is thought to correlate with serotonergic

activity, such that a high LDAEP reflects low serotonergic neurotransmission (Hegerl et al 1998; Juckel et al 1999; Strobel et al 2003). It is thought that the neural generator of the LDAEP is the primary auditory cortex (Juckel et al 1997), which is a region that has a high concentration of serotonin (Azmitia & Ganno, 1986; Lewis et al 1986). In LDAEP studies, participants listen to an increasing sound intensity (usually between 60-100 dB) and the amplitude of specific ERP components (e.g. N1 and P2) should increase with each intensity increase. Although few studies have found significant effects of depression on the LDAEP (Brocke et al 2000), many have found increased LDAEP in responders to selective serotonin reuptake inhibitor (SSRI) treatment (Paige et al 1994; Linka et al 2004; Lee et al 2005; Mulert et al 2007). Interestingly, one study found that depressed patients with a high LDAEP did not respond to a norepinephrine reuptake inhibitor (NRI; Linka et al 2005). These studies support the notion that LDAEP may be sensitive to serotonergic changes that may result in psychopathological conditions that are thought to involve deficiencies in serotonin, such as depression. By including the LDAEP task in this proposal, we can 1) examine the relationship between the LDAEP (as a proxy of serotonergic neurotransmission) and 5-HT1A receptor binding (as measured by our PET scan using the CUMI tracer), and 2) examine if baseline LDAEP level is predictive of lamictal or lithium treatment response.

C. Preliminary Studies

C.1. 5-HTT Binding in Bipolar Disorder:

Prior to the use of [^{11}C]McN5652 in patient populations we carefully evaluated the ligand to determine the optimal data acquisition and analysis methods.[7] Demographics of the 18 subjects (10 female, 8 male) who met DSM-IV(1994) criteria for bipolar disorder with a major depressive episode (MDE) and 41 healthy volunteers (19 female, 22 male) who participated in this study are presented in Table 1. Inclusion and exclusion criteria were evaluated by clinical history, chart review, Structured Clinical Interview for DSM IV (SCID I)[133], Hamilton Depression Rating Scale for depression (HAM-D)[134], review of systems, physical examination, routine blood tests, pregnancy test, and urine toxicology. The healthy volunteer group was free of lifetime psychiatric history and had no first-degree relatives with mood disorders.

Table 1. Demographics of the [^{11}C]McN5652 Bipolar Study Groups

	Healthy volunteers (N=41)	Depressed Bipolars (N=18)	t-test	χ^2-test	p-value
Male	22	8			
Female	19	10		0.425	0.514
Age (years)	38.1 ± 9.7	39.3 ± 16.0	0.349		0.729
Bipolar I / Bipolar II	NA	10 / 8			
HDRS-24	0.61 ± 0.89	23.72 ± 6.27	15.579		<.001
BDI	1.5 ± 2.1	28.9 ± 10.1	16.711		<.001
GAS	89.5 ± 4.7	48.7 ± 10.1	21.262		<.001
1st Degree Relative with MDD	NA	16 (89%)			NA
1st Degree Relative with BD	NA	3 (17%)			NA
Age at first MDE (years)	NA	18.9 ± 7.89			NA
Age at first Manic/Hypomanic Episode (years)	NA	28.59 ± 11.36			NA
Previous Suicide Attempts	NA	9 (50%)			NA
5-HTTLPR Genotype (%)					
Ll	10 (25%)	8 (44%)			
Ls	19 (47.5%)	10 (56%)			
Ss	11 (27.5%)	0 (0%)		6.623	0.036
5-HTTLPR Allele Frequency					
L	39 (49%)	26 (72%)			
S	41 (51%)	10 (28%)		5.552	0.018

Criteria for study entry for bipolar subjects included: 1) age 18 to 65 years; 2) DSM-IV Major Depressive Episode, bipolar disorder) 3) absence of alcohol or substance abuse or dependence in the prior 6 months; 4) absence of family history of schizophrenia 5) absence of exposure to 3,4-methylenedioxymethamphetamine (MDMA, ecstasy); 6) absence of significant medical conditions; 7) absence

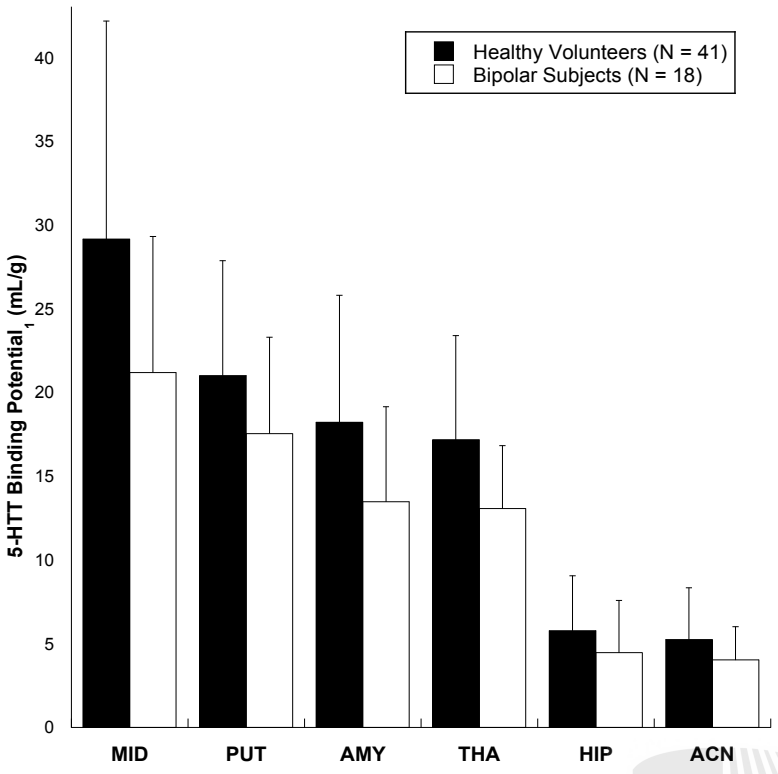

Figure 1: Lower [^{11}C]McN 5652 BP_1 to the serotonin transporter (5-HTT) in non-medicated bipolar subjects in six regions of interest (MID = midbrain; PUT = putamen; AMY = amygdala; HIP = hippocampus; ACN = anterior cingulate cortex; THA = thalamus) compared to healthy volunteers ($F = 5.41$, $df = 1, 58$, $p = .024$). Bars represent the mean LEGA modeled BP_1 (V_T(region) – V_T(cerebellum)) for each region +/- one standard deviation.

of pregnancy; and 8) capacity to provide informed consent; 9) able to tolerate a washout of current psychotropic medications and be medication free for at least 2 weeks prior to the scans (6 weeks for fluoxetine, 3 weeks for oral neuroleptics), except benzodiazepines, which were discontinued 24 hours prior to the scan. Two (2, 11%) subjects had a remission of MDE during medication washout, but all subjects were scanned within a month of full MDE. Only five of the bipolar subjects had no previous exposure to psychiatric medication.

Study criteria for healthy volunteers were similar except for the absence of a psychiatric history, including alcohol and substance abuse and absence of mood or psychotic disorder in their first-degree relatives. The Institutional Review Boards of Columbia University Presbyterian Hospital and the New York State Psychiatric Institute approved the protocol. Subjects gave written informed consent after detailed explanation of the study.

Healthy volunteer and bipolar disorder groups were matched for age (range 18 to 65 years; $t = 0.35$, $p = 0.73$) and sex ($\chi^2 = 0.43$, $p = 0.51$). Eight bipolar subjects met the criteria for Bipolar II disorder, 10 met criteria for Bipolar I disorder. Other Axis I disorders included Binge Eating (2), Obsessive-Compulsive Disorder (3), Simple Phobia (1), Generalized Anxiety Disorder (1), Post-Traumatic Stress Disorder (PTSD, 2). Nine bipolar subjects had made suicide attempts (2.33 ± 1.87 lifetime attempts, range 1 - 7). Bipolar subjects averaged 23.72 ± 6.27 on the Hamilton depression rating scale (24 item), while the healthy volunteer group averaged 0.61 ± 0.89. Data from this healthy volunteer sample was reported in a previous study involving Major Depressive Disorder.[9] The injected dose of [^{11}C]McN5652 injected was not different between healthy volunteers (11.89 ± 4.10 mCi) and bipolar subjects (12.79 ± 4.85 mCi, Students' $t = -0.733$, $p = 0.466$). Specific activity (healthy volunteers = 0.97 ± 0.69 mCi/nmol, bipolar = 0.81 ± 0.40 mCi/nmol, Students' $t = 0.921$, $p = 0.361$) and injected mass (healthy volunteers = 70.45 ± 69.10 μmole, bipolar = 50.40 ± 23.22 μmole, Students' $t = 1.20$, $p = 0.236$) also did not differ.

A linear mixed effects model of regional BP_1, demonstrated that bipolar subjects had lower 5-HTT BP_1 ($F = 5.41$, $df = 1, 58$, $p = 0.024$) across all six brain regions examined (Figure 1): midbrain (27%), amygdala (26%), hippocampus (23%), thalamus (23%), putamen (16%), and anterior cingulate cortex (23%). There was no evidence that the deficit differed across regions ($F = 1.34$, $df = 5, 285$, $p = 0.247$) despite the seemingly uniform reduction in 5-HTT BP_1 (Figure_2). Moreover, this effect was independent of depression severity rated by either self-report or clinical assessment. Results on the triallelic genotype (data not shown) are comparable to the biallelic genotype results (see Table 1). There was no relationship between5-HTTLPR genotype based on functional classification and BP_1 in the six regions considered in bipolar subjects.

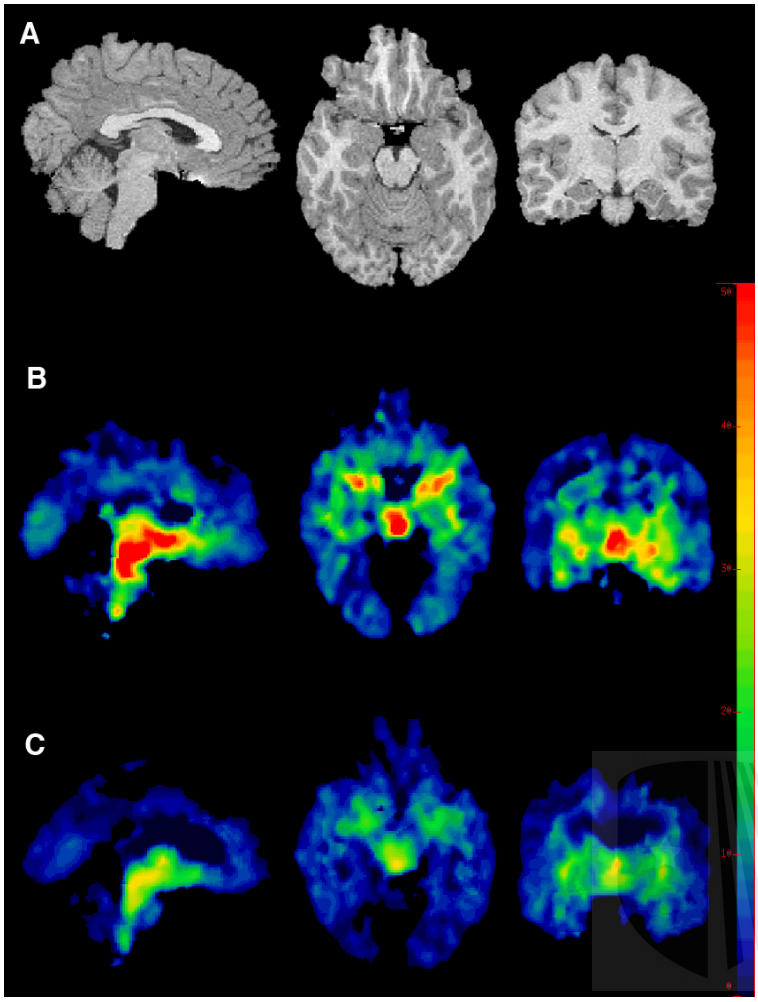

Figure 2: Maps of serotonin transporter (5-HTT) binding from a healthy volunteer (B) and a bipolar subject (C) whose midbrain BP_1 was closest to the respective group mean. Subject maps were co-registered to the MRI (A) from the healthy volunteer. Each voxel intensity is a single BP_1 measurement. The color-bar represents mL/g. 5-HTT BP_1 was significant lower in midbrain ($1^{st}/2^{nd}$ column), amygdala ($2^{nd}/3^{rd}$ column), thalamus ($1^{st}/3^{rd}$ column), anterior cingulate cortex (1^{st} column), hippocampus (2^{nd} column), and putamen (not shown).

In contrast to unipolar depressed subjects where BP_1 was 16% lower in amygdala and midbrain, using the same methodology, depressed bipolar subjects showed 16% to 26% lower BP_1 in all six brain regions examined: amygdala, midbrain, anterior cingulate cortex, hippocampus, putamen, and thalamus. The current findings suggest that lower 5-HTT binding is more pronounced and possibly more widespread in bipolar depression.

Cerebellar 5-HTT V_T (V_2) was higher in healthy volunteers than depressed bipolar subjects (21.49 ± 5.11 mL/g vs. 17.04 ± 2.58 mL/g, Students' $t = 4.43$, $df = 56$, $p < .001$), and therefore cannot explain our results. Such an effect leads to an underestimate of the 5-HTT BP_1 in the regions of interest, with the effect being more pronounced in the healthy volunteers. Thus, observed differences in BP_1 between healthy and depressed bipolar subjects are likely an underestimation of the actual physiological difference. Because of the difference in cerebellar V_T, outcome measures that are more sensitive to this measure ($BP_2 = BP_1/V_T$) show no difference between bipolar subjects and controls. A major limitation of studies that do not utilize arterial sampling is that BP_2 is the *only* outcome measure that can be obtained.

Whether lower 5-HTT BP_1 observed in depressed bipolar subjects is due to primary deficits in the serotonin system or secondary to other neurochemical abnormalities and whether this abnormality is a trait or only present during the depressed state requires further inquiry. Studies examining 5-HTT binding during periods of mania or euthymia or measuring 5-HTT binding concomitantly with GABA or glutamate levels in brain may elucidate these issues. Nonetheless, these findings, the first in vivo evidence of a brain abnormality of this magnitude in bipolar disorder, suggest that bipolar depression is associated with more extensive serotonergic dysfunction than unipolar depression and may be a more severe manifestation of illness along a spectrum of mood disorders.

C.2. 5-HT_{1A} in Bipolar Disorder.

Carson 2004	Laruelle 1994[23]	Carson 1997[24]	Relationship to receptor parameters	Equilibrium calculation	Kinetic calculation	Graphical calculation
BP	V_3	S'	B_{max}/K_D	C_3/C_1f_1	$K_1k_3/k_2k_4f_1$	$(V_T - V_{REF})/f_1$
BP_1	V'_3	S	f_1B_{max}/K_D	C_3/C_1	K_1k_3/k_2k_4	$V_T - V_{REF}$
BP_2	V''_3	R	f_2B_{max}/K_D	C_3/C_2	k_3/k_4	$(V_T/V_{REF}) - 1$

Table 2: Adapted from [25]. B_{max} = density of available binding sites; $1/K_D$ = affinity of the radiotracer for available binding sites; f_1 = radiotracer plasma free fraction; f_2 = radiotracer intracerebral free fraction $V_{REF} = V_2$ = distribution volume of nondisplaceable (free and nonspecifically bound) compartment; K_1 and k_2 = rate constants for transit of the radiotracer between plasma and nondisplaceable compartment; k_3 and k_4 = rate constants for transit of the radiotracer between nondisplaceable and specific binding compartment; V_T = total tissue distribution volume relative to total radiotracer concentration.

Prior to the use of [^{11}C]WAY-100635 in patient populations we carefully evaluated this ligand to determine the optimal data acquisition and analysis methods.[6,11,152,153] Twenty subjects who met DSM-IV[154] criteria for a bipolar disorder and a current major depressive episode and 41 control subjects have been scanned to date and are included in the data presented below. Inclusion criteria were assessed through history, chart review, Structured Clinical Interview for DSM IV (SCID I)[133], review of systems, physical examination, routine blood tests, pregnancy test, urine toxicology and EKG. The Beck Depression Inventory (BDI)[155], Hamilton Depression Rating Scale (HAMD)[134], and Global Assessment Scale[156] assessed subjective and objective depression severity and functional impairment, respectively.

Study criteria for bipolar subjects included: 1) age 18 to 65 years; 2) DSM-IV criteria for bipolar disorder and a current MDE; 3) absence of any psychotropic medications for at least 2 weeks (6 weeks for fluoxetine, 4 weeks for neuroleptics), except benzodiazepines, which were discontinued three days prior to the scan; 4) absence of lifetime history of alcohol or substance abuse or dependence; 5) absence of life-time exposure to 3,4-methylenedioxymethamphetamine ("ecstasy"); 6) absence of significant medical conditions; 7) absence of pregnancy; and 8) capacity to provide informed consent.

Study criteria for controls were similar except for the absence of medical, neurological and psychiatric history or a history of a mood or psychotic disorder in a first-degree relative. The Institutional Review Boards of Columbia University Medical Center and the New York State Psychiatric Institute approved the protocol. Subjects gave written informed consent after an explanation of the study.

Data acquisition and analysis was identical to those previously described.[9] Briefly, a ten-minute transmission scan was acquired prior to the bolus injection of [^{11}C]WAY-100635. Emission data were collected in frames of increasing duration for 110 minutes. Image analysis was performed using MEDX software (Sensor Systems, Inc., Sterling, VA). Each of the 20 frames was coregistered to the eighth frame using FMRIB's Linear Image Registration Tool (FLIRT) v5.0.[142] Mean PET images were coregistered to the corresponding MRI using FLIRT. No attempt was made to correct for transmission emission mismatch in reconstruction. ROIs were traced based on brain atlases[143] and published reports[144] and verified by a

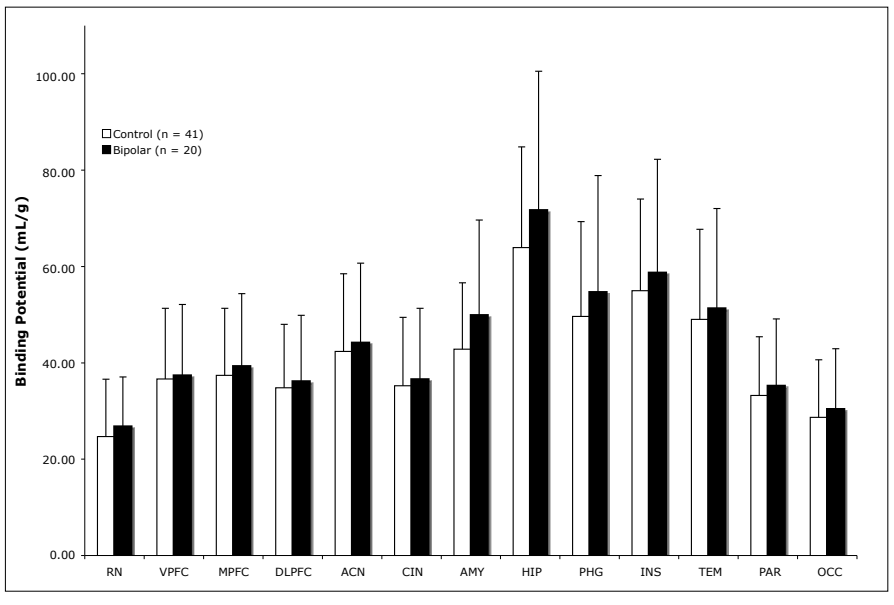

Figure 3: No difference in 5-HT_{1A} BP between controls and bipolar subjects scanned during a major depressive episode.

neuroanatomist (VA). ROIs included the ventral prefrontal cortex (VPFC), medial PFC (MPFC), dorsolateral PFC (DLPFC), anterior cingulate (ACN), cingulate cortex (CIN), amygdala (AMY), hippocampus (HIP), parahippocampal gyrus (PHG), insular cortex (INS), temporal cortex (TEM), parietal cortex (PAR), and occipital cortex (OCC). A fixed volume elliptical ROI (2 cm^3) was placed on the raphe nuclei (RN) in the dorsal midbrain: a composite of mostly the dorsal and median raphe nuclei, on a mean PET image for each subject since the boundaries of this structure cannot be identified on MRI. A cylindrical ROI was drawn in the cerebellar white matter.[11] MRIs were segmented utilizing the exbrain v.2 utility[145] and within the cortex only the voxels classified as gray matter were used to measure PET activity distribution. Data analysts were blind to subject identity or group. Binding potential (BP) is equal to the product of the receptor density (B_{max}, nM per g of tissue) and affinity ($1/K_D$, nM per mL of brain water, Table 2.[23,157] V_T was defined as the total regional equilibrium distribution volume, equal to the sum of V_2 and V_3, distribution volumes of the second (nondisplaceable) and third (specific) compartments, respectively. Cerebellar V_T from a one tissue (1T) compartment model was used as a constraint for the K1/k2 ratio in the two tissue (2T) kinetic modeling of the regions of interest. Binding potential (BP) was calculated as $(V_T - V_2)/f_1$ where f_1 is the free fraction in plasma.

Statistical analyses performed included Student's t-test, one way analysis of variance, and linear mixed models analysis with subject as the random effect. Model fitting was computed using both SPSS 11 for Mac OSX (www.spss.com) and R (www.R-project.org). When multiple regions were considered in a single analysis, region was included as a fixed effect, and the analysis was performed on the natural log of BP, in order to account for heterogeneity of variances across regions. Significance was defined as $p < 0.05$ and p-values are reported without multiple comparison adjustment. All tests were two-sided. In all models, there were no interactions, first or higher order, therefore, the interaction terms were not included in the models. Data are presented as mean ± standard deviation.

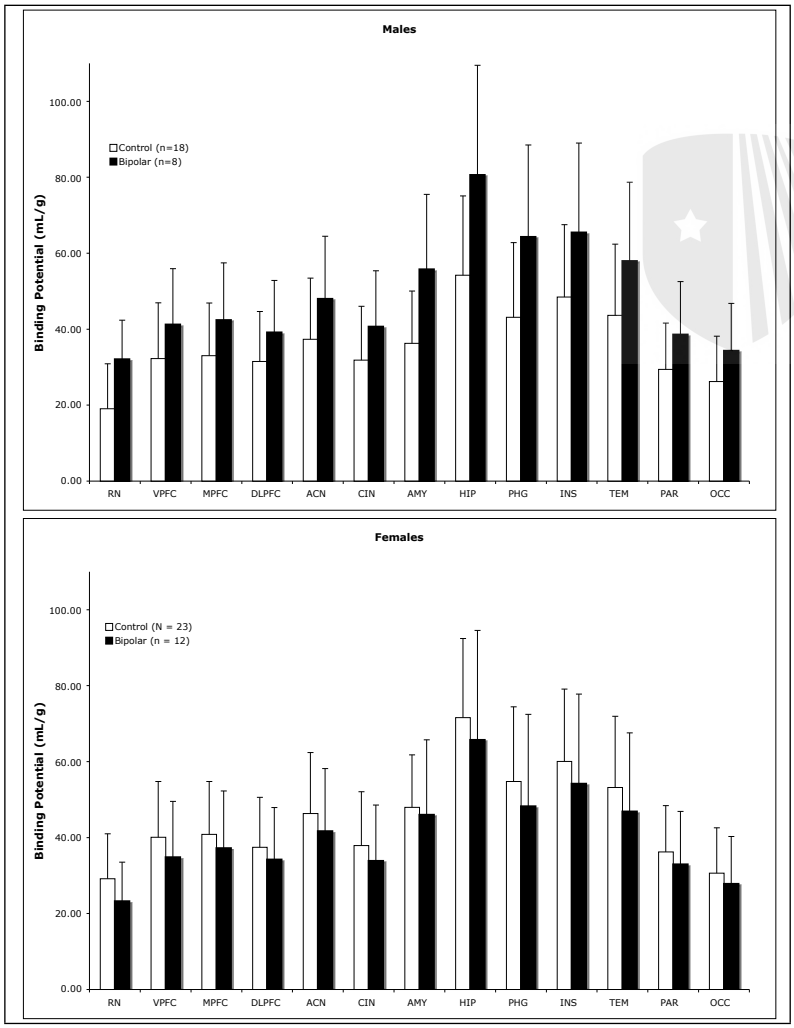

Figure 4: Trends for higher 5-HT_{1A} BP in male bipolar patients and lower 5-HT_{1A} BP in female bipolar patients.

There was no difference in percentage of females between controls (56%) and bipolar subjects (60). They also did not differ in age (controls = 37.3 ± 14.5, BPD = 39.04 ± 10.0, p = 0.640). The control population presented here is the same as in our previous publication.[22]

Across all regions there was slightly higher BP in BPD subjects scanned during a MDE compared to controls but this did not reach statistical significance ($F = 0.592$; $df = 1, 54$; $p = 0.444$; Figure 3). We have previously reported that females have significantly higher 5-HT_{1A} BP than men and that there is a correlation between lifetime aggression scores and BP.[153] As this is preliminary data, we do not have lifetime aggression scores for all the subjects but when we include gender in the linear mixed effects model we get a significant gender by diagnosis interaction ($F = 5.99$; $df = 1, 54$; $p = 0.018$). In contrast to the our findings in unipolar depression, it appears that male bipolar subjects

have higher 5-HT_{1A} BP compared to male controls while female bipolar patients have lower BP compared to female controls (Figure 4). We have also shown that prior medication status has a major effect on 5-HT_{1A} BP[22] however 90% of the bipolar subjects had been previously exposed to medication.

There is no compelling evidence of a differential response to lithium based on sex. In a review of 17 studies and over 1500 patients, males and females responded equally well to lithium[158], therefore we hypothesize that chronic lithium treatment will result in decreasing cortical but not somatodendritic 5-HT_{1A} receptors in both males and females. If our preliminary data are correct, this would result in 'normalizing' 5-HT_{1A} BP in males and possibly lowering 5-HT_{1A} BP in females below control levels. This significance of this can only be assessed in the larger context of the completed dataset.

D. Research Design and Methods

1. Rationale/Overview

Bipolar disorder is a brain disorder characterized by recurrent manic and major depressive episodes, the latter being clinically similar to those observed in unipolar or major depressive disorder. The one-year prevalence rate for Bipolar I or II is estimated at 1-2% for all ages. Bipolar disorder ranked 20th in terms of causes of loss of disability-adjusted life-years in 1999. Yet, its neurobiological underpinnings are poorly understood and most research has focused on functional brain abnormalities rather than alterations in neurotransmission. Pharmacotherapy development has focused on testing molecules that target neuroreceptors, transporters and heavily on serendipitous findings regarding lithium and anticonvulsants where the precise mechanism of therapeutic action is unknown. Further, treatments for bipolar depression show remission rates only modestly better than placebo. As a potential aid to rational drug development and to elucidate its pathophysiology, neurotransmission deficits present in bipolar depression require attention.

Our group has been interested in determining the role of key neurotransmitter receptors and transporters in both unipolar and bipolar depression. In unipolar depression this initial work has lead to a working model that incorporates 5-HT_{1A} receptors, the 5-HTT, and genetic polymorphisms. In parallel with this work in unipolar depression we have started 5-HT_{1A} and 5-HTT studies in bipolar disorder. Our goal with this study is to develop a neurotransmitter based model of bipolar disorder to complement important findings from fMRI and PET that will enhance understanding of the pathophysiology and the action of drugs with proven beneficial effects (lithium and lamotrigine) to accelerate development of more specific or novel treatment strategies.

We will use the radiotracer [^{11}C]DASB to measure serotonin (5-HT) transporter binding and [^{11}C]CUMI-101 to measure serotonin receptors in the brain of depressed or recently depressed bipolar subjects using Positron Emission Tomography (PET) before and after treatment with a mood stabilizer. We propose to assess how the clinical responses to two types of mood stabilizer treatment relate to the state of the serotonin system. The two treatments we intend to compare are lithium and lamotrigine. We propose to perform [^{11}C]CUMI-101 and [^{11}C]DASB in 38 medication free bipolar subjects during a major depressive episode and compare 5-HTT and 5-HT_{1A} binding potential in 38 healthy volunteers. We will also examine the diagnostic specificity of lithium response by studying 10 unipolar depressed subjects in an identical manner.

We will obtain blood samples from all subjects in the protocol in order to be able to evaluate their genetic information. We plan to analyze the DNA from the blood to assess for genome wide and single nucleotide polymorphisms and their associations with MRI and PET brain imaging measures. For example, the effects of the C(-1019)G 5-HT1A promoter polymorphism has been shown to affect the binding of our 5-HT1A tracers in humans. The genetic information may also be used to study the general pathophysiology of major depression, bipolar disorder or past suicidal ideation in the subjects. Blood RNA will also be evaluated because it tells us whether the gene variants are associated with altered gene expression. We expect gene variants with altered expression levels to be of greater pathophysiological consequence.

2. Research Site

MRI scans, research interviews, clinical assessments and treatment will take place at Stony Brook University Medical Center. PET scans will take place at the Yale University PET Center in New Haven, CT.

3. Study Sample

86 subjects in total are proposed. The power calculations are below.

Age range: 18-70

Total subjects: N = 86 (38 bipolar, 38 healthy volunteer, 10 unipolar)

Gender: Male and female representation are about equal.

No ethnic/racial/gender group is excluded.

4. Screening

All patients will be assessed clinically through history, chart review, Structured Clinical Interview for DSM IV (SCID I)[133], review of systems, physical examination, routine blood tests, pregnancy test, urine toxicology and electrocardiogram. The Beck Depression Inventory (BDI)[155], Hamilton Depression Rating Scale (HDRS)[134], and Global Assessment Scale[156] will be used to assess subjective and objective depression severity and functional impairment, respectively. Patients who currently meet criteria for bipolar disorder, as assessed by the Structured Clinical Interview for DSM-IV (SCID I), and who have a 17-Item HDRS score of at least 15 or a 17-Item HDRS score of 10 to 14 in conjunction with a BDI score of at least 29 will be recruited for this study. Patients who currently meet criteria unipolar depression and are in a current major depressive episode, as assessed by the Structured Clinical Interview for DSM-IV (SCID I), and who have a 17-Item HDRS score of at least 15 or a 17-Item HDRS score of 10 to 14 in conjunction with a BDI score of at least 29 will be recruited for this study.

Patients with prior antidepressant treatment will undergo a three-week medication washout prior to enrollment in the study. Although this is a relatively prolonged washout, we have shown that depressed patients who are washed out of antidepressants that they had failed to respond to do not worsen, rather report modest improvement in their symptoms.[159]

Normal controls are recruited to provide normative values on the clinical and brain imaging measures used. Subjects are screened for mental and physical health. Family history of psychiatric disorder is assessed using the Family Interview for Genetic Studies (FIGS). Urinary drug screen, physical examination and laboratory testing are performed prior to acceptance as a control subject. The normal control sample is selected to match the mean, variance and shape of the distributions across the clinical sample, at a group level. Matching variables are age, gender, socioeconomic status, education, marital and employment status, and race.

BIPOLAR PATIENTS:

INCLUSION:	
(1) Patients diagnosed with bipolar disorder. Patients on psychiatric medication will have failed their current regimen for the treatment of their depression: they will meet criteria for depression, be seeking treatment for it, and have been on an adequate dose of antidepressant or mood stabilizer (as defined by the Antidepressant Treatment Form—see Oquendo et al., 2003) for 4 weeks or more	As defined by the DSM-IV by means of the SCID

(2) Of sufficient severity to score at least 15 on the first 17 items of the Hamilton Depression Rating Scale or a score of 10 to 14 on the first 17 items of the Hamilton Depression Rating Scale in conjunction with a score of at least 29 on the Beck Depression Inventory	Hamilton Depression Rating Scale (HDRS), Beck Depression Inventory (BDI)
(3) Age range 18-70 years	Interview
(4) Able to stop all psychotropic and other types of drugs likely to interact with serotonin transporters and 5-HT_{1A} receptors for at least 21 days before PET scans. Allowed short-acting benzodiazepines for distressing anxiety or insomnia (up to 24 hours prior to each PET scan). Able to be off neuroleptics for 3 weeks and off fluoxetine for 6 weeks prior to study. Able to be off serotonin depleting drugs such as reserpine for 3 months. Able to be off anti-coagulant/anti-platelet treatment such as Coumadin, with the exception of aspirin, for 10 days before PET scans.	History, chart and urine drug screen
(5) Willing to travel to Yale University for PET scanning	Interview
EXCLUSION:	
(1) Other major psychiatric disorders such as schizophrenia, schizoaffective illness; Current substance abuse (within the past 2 months) or recent dependence (within the past 6 months) except for cannabis use disorders, which are not exclusionary; anorexia nervosa or bulimia nervosa, purging type in the past year, except for bulimia nervosa, non-purging type plus a normal BMI, which is not exclusionary; IV drug use in the past 5 years or ecstasy (MDMA) use more than 15 times in the past 10 years or any MDMA use in the past month.	SCID; Urine drug screen
(2) Significant active physical illness particularly those that may affect the brain or serotonergic system including blood dyscrasias lymphomas, hypersplenism, endocrinopathies, renal failure or chronic obstructive lung disease, autonomic neuropathies, peripheral vascular disease, diabetes, low hemoglobin and malignancy, significant anemic disease or blood loss and the lab parameters platelet count < 80,000	Medical history (by a physician), physical exam, screening lab tests (comprehensive metabolic panel, CBC with differential, thyroid function tests [TSH and free T4], PT/PTT, urine test for pregnancy, and urine drug screen) (approximately 15.2 ml of blood drawn) and an ECG. A physician will review lab findings to ensure that there are no anemia related contraindications to participation
(3) Lacks capacity to consent	Clinical interview
(4) Actively suicidal-begins expressing a plan for suicide during the washout phase or develop suicidal	Clinical interview

ideation that warrants admission or requires medication or treatment intervention	
(5) ECT within the past 6 months	History, chart review
(6) Pregnancy, currently lactating; planning to conceive during the course of study participation or abortion in the past two months	HCG pregnancy test to rule out pregnancy or unwilling/unable to use a medically acceptable means of birth control through the course of the study. Urine pregnancy test will be done at screening. A urine pregnancy test will be done on the day of the PET scan prior to scanning. In addition, each female subject must confirm that she is using an acceptable method of birth control for at least two weeks prior to the PET scan
(7) Metal implants, pacemaker or metal prostheses or orthodontic appliances, the presence of shrapnel	History
(8) Current, past or anticipated exposure to radiation, that may include: - being badged for radiation exposure in the workplace - participation in nuclear medicine procedures, including research protocols in the last year.*	1. Subjects will undergo a clinical interview, during which they will be asked whether or not they have worked with radioactive substances or have been badged in the past. In addition, they are asked about any prior chemotherapy or radiation treatment. 2. A research assistant will check our database to ensure that the subject has not participated in an imaging study within our department in the past year**.**
(9) A neurological disease or loss of consciousness for more than a few minutes	Clinical interview
(10) Medicinal patch that cannot be removed**	Medical history (by a physician), physical exam.
(11)Patients who are responding satisfactorily to psychiatric medications, because they will not be washed-out for purposes of this study	History
(12) A documented history of a lack of response to a trial of adequate dose and duration of both lithium and lamotrigine defined as minimal clinical response to lamotrigine 200 mgs for at least 4 weeks or lithium serum levels of at least 0.8 (or dose >= 900 mgs) for at least 4 weeks	History
(13) Patient is unlikely to be able to tolerate medication washout	Clinical interview
(14) Claustrophobia	Clinical interview

(15) Blood donation within 8 weeks of the start of the study.	Clinical interview
(16) History of a bleeding disorder or are currently taking anticoagulants (such as Coumadin, Heparin, Pradaxa, Xarelto).	History

* Subjects will be eligible if the injected dose and dosimetry of the radiotracer are known and the cumulative annual exposure of the previous study and this study is lower than the annual limit for research subjects defined by the FDA (see below)

** In case the subject does have a medicinal patch, they will be asked to remove it prior to the MRI scan session

HEALTHY VOLUNTEERS:

INCLUSION:	
(1) No lifetime history of Axis I disorders other than specific phobia, social phobia, and/or adjustment disorder.	Structured Clinical Interviews I for DSM IV; clinical history and interview
(2) Age range 18-70 years.	Interview
(3) Willing to travel to Yale University for PET scanning.	Interview
EXCLUSION:	
(1) Past or present alcohol/substance abuse or dependence except for cannabis use disorders, which are not exclusionary; IV drug use in the past 5 years; IV drug use in the past 5 years or ecstasy (MDMA) use more than 15 times in the past 10 years or any MDMA use in the past month.	SCID; Urine drug screen
(2) A first-degree relative with history of major depression if participant is less than 44 years old (median age of onset plus one quartile)[174], schizophrenia, schizoaffective disorder, or suicide attempt; two or more first degree relatives with a history of substance dependence if the participant is less than 27 years old (median age of onset plus one quartile).[174].	Clinical interview, Family Interview for Genetic Studies (FIGS)
(3) Significant active physical illness particularly those that may affect the brain or serotonergic system including blood dyscrasias lymphomas, hypersplenism, endocrinopathies, renal failure or chronic obstructive lung disease, autonomic neuropathies, peripheral vascular disease, diabetes, low hemoglobin and malignancy, significant anemic disease or blood loss, and the following lab parameters: platelet count < 80,000	Medical history (by a physician), physical exam, screening lab tests (comprehensive metabolic panel, CBC with differential, thyroid function tests [TSH and free T4], PT/PTT, urine test for pregnancy, and urine drug screen) (approximately 15.2 ml of blood drawn). A physician will review lab findings to ensure that there are no anemia related

	contraindications to participation.
(4) Lacks capacity to consent	Clinical interview
(5) Pregnancy, currently lactating; planning to conceive during the course of study participation or abortion in the past two months	HCG pregnancy test to rule out pregnancy or unwilling/unable to use a medically acceptable means of birth control through the course of the study. Urine pregnancy test will be done at screening. A urine pregnancy test will be done on the day of the PET scan prior to scanning. In addition, each female subject must confirm that she is using an acceptable method of birth control for at least two weeks prior to the PET scan.
(6) Metal implants, pacemaker or metal prostheses or orthodontic appliances, the presence of shrapnel	History
(7) Current, past or anticipated exposure to radiation, that may include: - being badged for radiation exposure in the workplace - participation in nuclear medicine procedures, including research protocols in the last year *	1. Subjects will undergo a clinical interview, during which they will be asked whether or not they have worked with radioactive substances or have been badged in the past. In addition, they are asked about any prior chemotherapy or radiation treatment. 2. A research assistant will check our database to ensure that the subject has not participated in an imaging study within our department in the past year**.**
(8) A neurological disease or loss of consciousness for more than a few minutes	Clinical interview
(9) Medicinal Patch that cannot be removed*	Medical history (by a physician), physical exam.
(10) Unable to stop drugs or medication that affect the serotonin system prior to the PET scans.	History
(11) Claustrophobia	Clinical Interview
(12) Blood donation within 8 weeks of the start of the study.	
(13) History of a bleeding disorder or are currently taking anticoagulants (such as Coumadin, Heparin, Pradaxa, Xarelto).	History

* Subjects will be eligible if the injected dose and dosimetry of the radiotracer are known and the cumulative annual exposure of the previous study and this study is lower than the annual limit for research subjects defined by the FDA (see below).

** In case the subject does have a medicinal patch, they will be asked to remove it prior to the MRI scan session.

UNIPOLAR PATIENTS:

INCLUSION:	
(1) Unipolar patients suffering from a major depressive episode currently or recently (in the month prior to scanning). Patients on psychiatric medication will have failed their current regimen for the treatment of their depression: they will meet criteria for depression, be seeking treatment for it, and have been on an adequate dose of antidepressant or mood stabilizer (as defined by the Antidepressant Treatment Form—see Oquendo et al., 2003) for 4 weeks or more	As defined by the DSM-IV by means of the SCID
(2) Of sufficient severity to score at least 15 on the first 17 items of the Hamilton Depression Rating Scale or a score of 10 to 14 on the first 17 items of the Hamilton Depression Rating Scale in conjunction with a score of at least 29 on the Beck Depression Inventory	Hamilton Depression Rating Scale (HDRS), Beck Depression Inventory (BDI)
(3) Age range 18-70 years	Interview
(4) Able to stop all psychotropic and other types of drugs likely to interact with serotonin transporters and 5-HT_{1A} receptors for at least 21 days before PET scans. Allowed short-acting benzodiazepines for distressing anxiety or insomnia (up to 24 hours prior to each PET scan). Able to be off neuroleptics for 3 weeks and off fluoxetine for 6 weeks prior to study. Able to be off serotonin depleting drugs such as reserpine for 3 months. Able to be off anti-coagulant/anti-platelet treatment such as Coumadin, with the exception of aspirin, for 10 days before PET scans.	History, chart and urine drug screen
(5) Willing to travel to Yale University for PET scanning***	Interview
EXCLUSION:	
(1) Other major psychiatric disorders such as schizophrenia, schizoaffective illness; Current substance abuse (within the past 2 months) or recent dependence (within the past 6 months) except for cannabis use disorders, which are not exclusionary; anorexia nervosa or bulimia nervosa, purging type in the past year, except for bulimia nervosa, non-	SCID; Urine drug screen

purging type plus a normal BMI, which is not exclusionary; IV drug use in the past 5 years or ecstasy (MDMA) use more than 15 times in the past 10 years or any MDMA use in the past month.	
(2) Significant active physical illness particularly those that may affect the brain or serotonergic system including blood dyscrasias lymphomas, hypersplenism, endocrinopathies, renal failure or chronic obstructive lung disease, autonomic neuropathies, peripheral vascular disease, diabetes, low hemoglobin and malignancy, significant anemic disease or blood loss and the lab parameters platelet count < 80,000	Medical history (by a physician), physical exam, screening lab tests (comprehensive metabolic panel, CBC with differential, thyroid function tests [TSH and free T4], PT/PTT, urine test for pregnancy, and urine drug screen) (approximately 15.2 ml of blood drawn) and an ECG. A physician will review lab findings to ensure that there are no anemia related contraindications to participation
(3) Lacks capacity to consent	Clinical interview
(4) Actively suicidal-begins expressing a plan for suicide during the washout phase or develop suicidal ideation that warrants admission or requires medication or treatment intervention	Clinical interview
(5) ECT within the past 6 months	History, chart review
(6) Pregnancy, currently lactating; planning to conceive during the course of study participation or abortion in the past two months	HCG pregnancy test to rule out pregnancy or unwilling/unable to use a medically acceptable means of birth control through the course of the study. Urine pregnancy test will be done at screening. A urine pregnancy test will be done on the day of the PET scan prior to scanning. In addition, each female subject must confirm that she is using an acceptable method of birth control for at least two weeks prior to the PET scan
(7) Metal implants, pacemaker or metal prostheses or orthodontic appliances, the presence of shrapnel	History
(8) Current, past or anticipated exposure to radiation, that may include: - being badged for radiation exposure in the workplace - participation in nuclear medicine procedures, including research protocols in the last year.*	1. Subjects will undergo a clinical interview, during which they will be asked whether or not they have worked with radioactive substances or have been badged in the past. In addition, they are asked about any prior chemotherapy or radiation treatment. 2. A research assistant will check our database to ensure that the subject has not participated in an imaging study within our department in the past year**.**

(9) A neurological disease or loss of consciousness for more than a few minutes	Clinical interview
(10) Medicinal patch that cannot be removed**	Medical history (by a physician), physical exam.
(11)Patients who are responding satisfactorily to psychiatric medications, because they will not be washed-out for purposes of this study	History
(12) A documented history of a lack of response to a trial of adequate dose and duration of lithium defined as minimal clinical response to lithium serum levels of at least 0.8 (or dose >= 900 mgs) for at least 4 weeks	History
(13) Patient is unlikely to be able to tolerate medication washout	Clinical interview
(14) Claustrophobia	Clinical interview
(15) Blood donation within 8 weeks of the start of the study.	Clinical interview
(16) History of a bleeding disorder or are currently taking anticoagulants (such as Coumadin, Heparin, Pradaxa, Xarelto).	History

* Subjects will be eligible if the injected dose and dosimetry of the radiotracer are known and the cumulative annual exposure of the previous study and this study is lower than the annual limit for research subjects defined by the FDA (see below)

** In case the subject does have a medicinal patch, they will be asked to remove it prior to the MRI scan session

*** In case the subject cannot travel to Yale, scans will be done at the Siemens Biograph mMR-System located at the Ambulatory Imaging Center at Stony Brook Medicine.

Screening Tests

All subjects will have a complete medical history and clinical examination to exclude significant physical illness as described above. Approximately 15.2 ml of blood will be drawn at the screening visit for laboratory testing. A urine sample will also be collected. The following tests will be performed before the study: 1) clinical blood count including differential; 2) metabolic panel (14) comprehensive (Alanine aminotransferase (ALT/SGPT); albumin:globulin (A:G) ratio; albumin, serum; alkaline phosphatase, serum; aspartate aminotransferase (AST/SGOT); bilirubin, total; BUN; BUN:creatinine ratio; calcium, serum; carbon dioxide, total; chloride, serum; creatinine, serum; globulin, total; glucose, serum; potassium, serum; protein, total, serum; sodium, serum); 3) thyroid function tests (TSH and free T4) 4) PT/PTT 5) drug screen; 6) and pregnancy test. Other tests will be performed if there are specific indications.

5. Procedures

Collection of Genetic Information

Whole blood (approximately 8 ml) will be drawn for assays of candidate gene expression and polymorphisms (i.e., genetic polymorphism of tryptophan hydroxylase, serotonin transporter, etc.).

MRI Scan
An MRI scan will be performed to localize anatomic regions of interest on the PET scan. MRI scans will be performed at the Stony Brook University SCAN Center. Participants will receive an array of structural and functional MRI scans (e.g. T1 and T2-weighted structural images, diffusion tensor imaging [DTI], pseudocontinuous arterial spin labeling [pCASL], etc.) over the course of approximately 1 to 1.5 hours. Patients will be monitored by the MRI technician and research assistant, who will be available at all times. A study physician will also be available if it is necessary. The procedure will be immediately stopped if subjects exhibit significant distress.

Repeat MRI: patient participants will have a second MRI scan following their treatment period (scheduled approximately one week before or after the second set of PET scans). Control participants will be asked to return for a second MRI approximately 12 weeks after their initial MRI. This is optional for control participants.

PET Studies
Approximately 8ml of blood will be drawn the day of the PET scans for female participants to assess their stage in the menstrual cycle.

PET experiments will be conducted at the Yale University PET Center, 800 Howard Avenue, in New Haven, CT. [^{11}C]CUMI-101 and [^{11}C]DASB scans will be performed. Participants may have only one of these scans, depending on radiotracer availability. The PET scans will preferably be performed after the MRI scan, but the order may be reversed if needed.

Participants will be provided with transportation from Stony Brook University to Yale University by a combination of taxi/car service, ferry, and/or train. Participants will be accompanied by a staff member while traveling to/from Yale University. Participants will arrive the night before the scheduled scan day(s) and be given accommodations at a nearby hotel at no cost to them.

On the day of the scans, a urine pregnancy test will be done on females to ensure that pregnancy has not occurred between the time of the screening and the PET scans. All participants will also have a urine drug screen. The preparation of the subject will include the placement of one venous lines (for radiotracer injection), a second venous line (for and blood sampling), and an arterial line (for blood sampling). An experienced physician will place the arterial line after infiltration of the skin with 1% lidocaine. A transmission scan is then obtained. Before injection of the radiotracer, approximately 8ml of venous blood will be collected. For the [^{11}C]CUMI-101 scan, up to 20 mCi of [^{11}C]CUMI-101 (mass dose of 5 μg or less) will be administered i.v. after the transmission scan. Emission data will be collected for up to 120 minutes. At the time of injection, arterial sampling will be initiated. In order to demonstrate that the arterial and venous metabolites are the same for late time points, a 4 mL venous sample will be initiated at 60 and 90 minutes. 90 ml or less will be collected during scan time.

At the end of the scan, subjects will be provided with a light meal and be allowed to relax. For the [^{11}C]DASB scan, the subject will go back on the scanner table and an injection of up to 20 mCi of [^{11}C]DASB, (mass dose of 10 μg or less) will be administered. Emission scan and arterial sampling will be resumed as described above. A 4 mL venous sample will be initiated at 50 and 80 minutes. Thus, maximal blood sampling during the entire PET procedure will be approximately 180 ml (about 12 tablespoons).

The order of [^{11}C]CUMI-101 and [^{11}C]DASB administration may be reversed, or subjects may participate in only one scan (either [^{11}C]CUMI-101 or [^{11}C]DASB),.

In cases where the subject has a hemoglobin level less than 12 for males and less than 11 for females, we would forgo blood drawing for the [^{11}C]DASB scan except for one sample to be taken at the 50 minute time point, as outlined in the simultaneous estimation (SIME) approach. Likewise, we would forego blood drawing for the [^{11}C]CUMI-101 scan except for one sample at the 60 minute time point. We have recently confirmed that this provides an excellent estimate of the plasma parameters necessary for quantification and modeling of this radioligand. It involves drawing one 12 cc sample off the arterial line at the 50 min post-radioligand injection time point. Therefore, instead of 120 cc being drawn during the DASB scan, we would reduce the blood drawn to 12 cc.

In the case that the two injections are planned but cannot be done on the same day once the PET procedures have been started, subjects may be asked to return on a separate day in order to complete the scans. If the PET procedure has to be rescheduled and placement of another aline is required, subject consent will be sought.

After the acute treatment period, the patients who are on either lithium or lamotrigine will have a second PET scanning session that is identical to the first: up to 20 mCi or less of [^{11}C]CUMI-101 and up to 20 mCi of [^{11}C]DASB. Healthy volunteers will not have follow up scans.

Alternate Location and Tracer for PET Scans

Due to limited availability of PET scans at the Yale PET center, alternative arrangements will be made for subjects at Stony Brook Medicine. In cases where the subject cannot accommodate a trip to the Yale PET center or the Yale PET center cannot accommodate for enough scans due to decreased scan staff, PET/MRI scans may take place at the the Siemens Biograph mMR-System located at the Ambulatory Imaging Center using [^{18}F]MeFWAY. Similar to the [^{11}C]CUMI-101 scans being performed at the Yale PET center, [^{18}F]MeFWAY also has the capability to examine 5-HT_{1A} binding. Only bipolar and unipolar patients will be scanned at Stony Brook, when Yale is unable to accommodate the number of scans necessary. Healthy controls will continue to be scanned at Yale PET Center.

Participants will complete a metal screening form prior to procedure to ensure there are no MRI contraindications (e.g. pacemaker, non-MR safe metal implants). A pregnancy test will be performed on the day of the scan for female participants to confirm that pregnancy has not occurred. And we will also measure female hormones by blood samples to ensure if contraceptives were used and if not, the results of the hormone levels can be used as covariates for the data analysis. Preparation of the subject will include the placement of venous lines (for radiotracer injection and blood sampling), and an arterial line (for blood sampling only). At the time of injection, blood sampling will be initiated. 120 mL or less (about 4 ounces or 1/2 cup) will be collected during each scan. The wrist scanner will also be placed around the subject's wrist.

Participants will be asked to urinate in a cup to determine the validity of the dosimetry for future studies.

Bolus Protocol: For each scan, participants will receive a venous injection (bolus delivery) of no more than 2.5 mCi of the radiotracer [^{18}F]MeFWAY, as per RDRC protocol.

Simultaneous MRI Scan: An MRI scan will be performed simultaneously with the PET scan. Participants will receive an array of structural and functional MRI scans (e.g. T1 and T2-weighted structural images,

diffusion tensor imaging [DTI], pseudocontinuous arterial spin labeling [pCASL], etc.) The anatomical image (T1) will be used to localize anatomic regions of interest on the PET scan. During this time, subjects will be monitored by the MRI technician. During the rest of the PET acquisition, participants will receive an array of structural and functional MRI scans.

Structural scans may include:

Diffusion Tensor or Spectrum Imaging to measure white matter tracts. These sequences are used to evaluate orientation and diffusion characteristics of white matter (WM) and, by inference, WM microstructure.[85] Fractional anisotropy (FA), or the disorganization of water molecules, is a common measure used in DTI to determine integrity of WM fibers. Characteristics of healthy WM include parallel organization of white matter fibers and myelination, which leads to reduced movement of water. FA values range from zero (isotropic diffusion) to one (anisotropic diffusion), with higher values denoting greater WM microstructure integrity.[86] In the past several years, FA measures have been used to study the correlation between WM microstructure and mood disorders.[87,88,89,90] A 2009 meta-analysis of DTI studies demonstrated that, in 21 of the 27 studies, subjects with mood disorders had significantly lower fractional anisotropy in the frontal and temporal lobes and tracts.[91]

Arterial Spin Labeling (ASL) to measure cerebral blood flow. In a previously published correlation of HAM-D scores and regional blood flow in depressed subjects, correlation coefficients between 0.69 and 0.84 were observed.[92]

Functional scans may include:

Resting-state functional MRI (fMRI) reveals the network of the brain active when a subject is not performing a task. During this functional task, the subject will be asked to keep their eyes open and try to clear their mind, fixate on an image of cross-hairs on a screen. Analysis of functional MR images indicates that some brain regions are more active during rest, and that these same regions have routinely decreased activity during the performance of tasks.[93,94] This led to the hypothesis that there is a "default mode" of the brain that remains active in a structured fashion when the brain is at rest.[94,95] Further, it has been shown that this default mode network is disrupted in MDD. [179-186]

Optional <u>Psychophysiological Assessment</u>

We will ask participants if they would be willing to participate in a brief optional electroencephalograph EEG procedure at baseline and follow-up. For patient participants, the baseline EEG would be before they begin medication treatment.

The EEG assesses brain electrical activity through surface recording disks (electrodes) which are placed near the participant's head. The electrodes transmit the signals, which are then amplified and stored on a computer. The procedure is entirely non-invasive.

A custom designed 32-electrode Lycra cap will be placed on the participant's head. In order to record the brain's activity, these disks need to be filled will a gel which allows the electrodes to better record brain activity at the scalp. Therefore, the participants in this study will need to clean their hair after participation. The gel is completely water soluble, and the procedure is painless. The laboratory has sinks and towels, and the investigators will help to make sure the participant has thoroughly rinsed all the gel from their hair. After an accurate signal is assured, the signal derived from the electrode cap is then amplified, transmitted to a computer, and stored for later analysis.

In addition to the EEG recording, skin conductance, heart rate, and muscle activity from around the eyes will be recorded from the participants. Skin conductance is recorded by placing two recording sensors on the participant's forefinger and middle finger; the procedure is entirely non-invasive and painless. Heart rate is recorded by placing a sensor on the participant's pinky finger. This procedure is completely non-invasive and painless. Muscle activity from the face and around the eyes is recorded by small sensors that will be placed

above and below the participant's right eye, as well as on the outer canthi (just below the eyebrow) of each eye, and above the eye.

Tasks

1. Loudness dependence auditory evoked potential (LDAEP)

The LDAEP will be performed in accordance with Linka et al (2007). Participants will be presented with binaural 1000-Hz tone bursts lasting 80 ms (including a 10 ms rise and 10 ms fall time). The tone bursts will be presented at one of five intensity levels: 60, 70, 80, 90 and 100 dB (presented in random order). There will be an interstimulus interval randomized between 500-900 ms. Participants will be instructed to attend to the sounds that are being presented. All participants will first perform a practice block of 10 trials; the actual experiment will consist of 2 blocks of 500 trials (1000 trials). Between blocks, participants will be able to take a short break. The actual experiment will last approximately 10 minutes.

Optional Adult Attachment Interview

We will ask controls if they would be willing to participate in an adult attachment interview. The interview is approximately 45-75 minutes long and can be completed over the phone or in person. The interviews are audio recorded and transcribed. The recorded interviews are kept confidential with a participant number. There are no risks or benefits associated with this optional interview.

Treatment Procedures

Site of Treatment

Patients will be treated on an outpatient basis at Stony Brook University Medical Center. Patients may visit the Clinical Research Center or a LabCorp collection site for sample collection, if needed on rare occasions.

Treatment Choice

At baseline, patients will have a [^{11}C]CUMI-101 and [^{11}C]DASB PET scan. All patients will then receive outpatient pharmacologic treatment with lithium administered as follows: Day 1, 2 and 3, 300 mg bid; Days 4-7 lithium 300 qam and 600 qhs. A blood sample will be drawn (approximately 8.5 ml) to check the lithium level as close to Day 7 as possible. The lithium level will be titrated to a therapeutic plasma level of 0.8-1.2 mEq/l; the lithium level will be checked weekly via a blood sample of approximately 8.5 ml until this level is reached. Patients may have additional blood samples drawn to check the lithium level after reaching this level, if needed. Patients will not undergo lithium monotherapy if they have a documented history of at least two failed trials of lithium of at least 4 weeks duration with therapeutic blood levels for a major depressive episode. If they have not responded to adequate prior lithium treatment while depressed or refuse lithium, they will be given lamotrigine. Lamotrigine will be started at 25 mg bid and increased to 50 mg bid after 2 weeks and again increased to 100 mg bid after an additional 2 weeks.

After 6-10 weeks from the time of therapeutic or highest tolerable dose of treatment with either agent they will have repeat [^{11}C]CUMI-101 and [^{11}C]DASB PET scans. Blood levels of the lithium will be measured at the time of the repeat scan. At 6 weeks, if patients have not shown at least a 50% improvement in their HDRS score from the baseline interview they will be switched to lamotrigine. Similarly, if patients cannot tolerate treatment, they will be switched to lamotrigine and we will repeat both [^{11}C]CUMI-101 and [^{11}C]DASB scans 4-10 weeks after subjects are on therapeutic levels of lamotrigine. If subjects cannot tolerate either treatment, or if the subject scores a CGI-I (severity of illness) score of 4 ("moderately ill") or more after the week 4 visit for two visits in a row, and/or a CGI-II (global improvement) score of 6 ("much worse") or 7 ("very much worse") for 2 visits in a row, the subject will be considered a non-responder and will receive the repeat [^{11}C]CUMI-101 and [^{11}C]DASB scans. They will then receive open treatment in our clinic.

Neither lithium nor lamotrigine is approved by the FDA for acute treatment of depressive episodes in patients with bipolar disorder, and neither drug is approved for treatment of unipolar depression. Thus, the use of both drugs as treatment for depression in this study is off-label. Nevertheless, both drugs represent reasonable treatment options for depression which has failed to respond to traditional antidepressants in either bipolar or unipolar depressive disorders. There have been several reports of the efficacy of lithium and lamotrigine in both bipolar and unipolar depression (175-178). In this study, unipolar depressed patients will only be treated with lithium.

Subjects will be offered up to 6 months of outpatient treatment and one month of medication at no cost. If the lithium or lamotrigine treatment is effective, it will be continued; if not, the subjects will be switched to open treatment with other drugs or other modalities. If subjects should still need additional treatment beyond the allotted time, they will be given referrals for continuing treatment elsewhere. In order to monitor renal and thyroid function, subjects taking lithium will have blood drawn (approximately 12.5 ml) for a comprehensive (14) metabolic panel, CBC with differential and Thyroid function tests (TSH and free T4) as well as an ECG after 3 months and 6 months from the start of treatment.

Clinical Monitoring

Personnel

Patients will be treated and monitored by psychiatrists in the Stony Brook Department of Psychiatry.

Frequency of clinical evaluations

For the initial 4 weeks of medication treatment, patients will see a psychiatrist in person at least once/week, and more frequently if clinical conditions warrant. If it is not possible to schedule an in-person visit every week, weekly phone contact may be substituted. Depending on each individual's clinical status, additional initial contact by the treating clinician may be either in person or by phone.

After 4 weeks, frequency of visits will be every two weeks up until the follow up PET scan, depending on combined evaluation of treatment response, and side effect issues. At minimum, all patients in active treatment will be seen monthly.

It should be noted that this outlines the minimum contact that each patient will have with their physician. In the course of the initial phases of treatment, patients may have multiple other contacts with clinically licensed personnel in the research program, such as psychologists, who will be involved in the conduct of this labor-intensive protocol. These staff members will be in frequent direct contact with treating physicians and will, therefore, constitute an extension of clinical monitoring.

Side effects

Side effects will be monitored in standard clinical fashion. MDs will interview patients about possible side effects at each contact and patients will be encouraged to call with any questions which may arise at any time. Response to side effects will depend on the circumstance. Doses may be titrated or patients may have medication discontinued, if clinically indicated.

Instruments

A battery of assessments will be administered to assess mood, suicidality, and character traits. Ratings will be collected at multiple time points, including at baseline (2 hours), biweekly during medication treatment (1/2 hour), within one week of an MRI/PET scan (or at the conclusion of a mood-stabilizer trial), (1/2 hour), and at designated follow-up time points (2 hours). Patients may be video or audio-recorded with their consent during the administration of these scales.

Examples of measures that will obtained include:

- Hamilton Depression Rating Scale (15 min)
- Hamilton Anxiety Scale (15 min) (at baseline and repeat PET scan)
- Beck Depression Inventory (10 min)
- GAS (2 min) (administered by treating physician during clinical check-ins)

Data Analysis

PET data will be reconstructed into images using the appropriate software, coregistered to the MRI and regional time activity curves will be measured. The arterial input function will be used to derive the regional density of 5-HT transporters and 5–HT_{1A} receptors with kinetic modeling.

E. Statistics

Power Analyses

A power analysis was conducted to determine required sample size for detecting a difference in populations with 80% probability using a linear mixed-effect model with region and diagnosis as fixed effects and subject as random effect. This was accomplished by means of a simulation study for a variety of sample sizes. The noise level, the subject effect variance, and mean levels for each region were extracted from the linear mixed-effect model fitted to preliminary DASB studies. The diagnosis effect was taken from the 5-HTT data in bipolar disorder. To stabilize variance among regions, analysis will be performed on the log-transformed BP data. Results of the analysis indicate that 80% power will be achieved with 38 bipolar subjects and 38 controls and 10 unipolar subjects. Note that these results are for the simplest analysis – comparing bipolar patients with normal controls over six regions. If genotype or other covariates are found to have an effect, then including these factors in the model can be reasonably expected to control more of the variance and thus increase power of testing for a diagnosis effect.

To assess the impact of lithium on the neuroreceptor measures we conservatively use the higher test/retest variability of the [^{11}C]DASB compound and assume a 20-25% change in both 5-HT_{1A} and 5-HTT after chronic lithium based on preclinical data. [14,16-18] Assuming 50% of the subjects complete the lithium treatment and repeat scans and this degree of change, we have a power of between 79% and 90% as this is a paired analysis. Estimated outcome measures will be analyzed for all ROIs and all patients in a linear mixed model with patient as random effect and ROI and remission status as fixed effects. Our previous studies have demonstrated that the standard deviation of BP across patients increases approximately linearly with the mean of the region, and so the BP values are first log-transformed in order to satisfy the assumptions of the modeling (constant variance across regions). For the pre/post imaging of the non-remitters, a linear mixed model will again be used with ROI and condition (before/after) as fixed effects and with two levels of random effects -- a patient effect and a study within patient effect. Many potential covariates will also be measured for each patient and will be included in the model when appropriate. Modeling will be done using R software (www.r-project.org).

F. Funding Status, Details

The study is currently funded through the National Institutes of Health (NIH) through May 31, 2017.
Grant Title: Lithium's Molecular Mechanism of Action and the Pathology of Bipolar Disorder

G. Human Subjects Research Protection from Risk

1. Risk to Subjects and 2. Adequacy of Protection Against Risks

Risks associated with participation in this study are related to 1) Drug-free interval; 2) placement of intra-arterial catheter; 3) blood sampling; 4) discomfort during scanning (PET and MRI); 5) MRI scan; 6) radiation exposure; 7) toxicology (idiosyncratic reaction to the tracer); 8) genetic testing/confidentiality; 9) pregnancy and 10) risks associated with lithium/lamotrigine use.

1. Drug-free interval

There is a risk that drug washout will result in a worsening of the patient's condition which could include worsening of depression of induction of a manic episode. During the medication washout, we employ either inpatient supervision or outpatient supervision by a psychiatrist. If the responsible physician (inpatient or outpatient) determines that treatment cannot be delayed safely or the patient withdraws from the study, all necessary treatment is offered. Patients on a mood stabilizer are excluded unless they are medication non-responsive. Patients are offered up to 3mg of Ativan daily to relieve symptoms during this phase. If the subject clinically deteriorates (worsening depression or mania) in the course of the drug washout to the extent that they feel they cannot tolerate the washout, or they are observed objectively to become significantly more depressed, manic, or develop suicidal ideation such that admission to hospital or medication is required clinically, we will drop them from the protocol and commence immediate appropriate treatment.

2. Intra-arterial catheter

Radial arterial catheterization is needed for repeated arterial blood samples to construct tracer input curves. Arterial sampling may be associated with mild-to-moderate pain, hematoma, inflammation, bleeding, or bruising at the puncture site. If this occurs, signs and symptoms will dissipate over time, usually 24 to 72 hours after the event. Certain individuals may feel light-headed during arterial catheter placement.

In rare instances blocking of the artery, tearing of the artery, arterial leakage, poor healing, or infection at the catheter insertion site may occur. In a review of the literature between 1978-2001, Scheer[21] found that among 19,617 radial artery catheterizations, temporary and benign occlusion occurred in 19.7% of patients. Thrombosis persisted as a major complication in just 0.09% of the cases. Septic complications occurred in 0.13% of cases. In studies conducted at Columbia University/NYSPI we have found that of 1,132 arterial lines in 924 subjects, there was 1 instance of symptomatic thrombotic occlusion (0.09%), documented by Doppler ultrasound in a depressed female patient. There was no associated ischemic damage, and the condition resolved over a period of weeks without intervention.[22] There has never been a serious adverse event related to an arterial line. In some cases, hematomas have formed after removal or the arterial line. But in all cases of hematoma formation this has occurred during the period immediately following removal of the line and after the routine several minutes of pressure applied by the physician removing the line. In all these cases, pressure was immediately reapplied and the hematoma stabilized after release of the pressure. No incidents or adverse events related to arterial line placement occurred in any of the subjects after leaving the Columbia University/NYSPI PET suite.

In the proposed study, arterial catheters will remain in place no more than 12 hours. The risks of radial artery cannulation are minimized by having the procedure performed by an experienced physician. Pain is minimized by local anesthesia. Bleeding is prevented by local pressure applied for a minimum of 15 minutes after catheter removal. Subjects will have their hand and finger blood supply examined after arterial cannulation and again following catheter removal. Also, subjects will be asked to abstain from aspirin and other NSAIDs for 7-10 days prior to arterial line insertion and 7-10 days following arterial line removal.Subjects will be provided a 24 hour emergency physician telephone number to call if they encounter pain, discoloration, numbness, tingling, coolness, hematoma, inflammation, or any other unusual symptoms in the wrist or hand, or fever, chills or drainage from the vascular puncture sites, following the procedure. In addition, if an emergency arises at the time of cannulation or scanning, 911 will be called, and the subject will be sent to the Emergency Department for evaluation and treatment. Nurses will provide the subjects an instruction sheet documenting problems to watch for and procedures to follow should such problems occur. Infection is avoided by adequate cleansing of the skin prior to intravascular line insertion.

Moreover, the physician will do no more than 3 arterial punctures in each arm, including the successful insertion of the catheter. Before each attempt, the subject will be assessed for pain/discomfort and asked if they are amenable to another attempt. At anytime they can decline to proceed. In the case that the arterial line has to be placed another time due to rescheduling of the scan, the subject will be reminded of the risks associated with the procedure.

In the event that an arterial line cannot be placed, we will use the simultaneous estimation (SIME) approach as discussed in the PET scan procedure section. This involves drawing one 12 cc sample via an arterial stick at 50 min post-radioligand injection for DASB, and at the 60 min post-radioligand injection timepoint for CUMI. This approach reduces the risk and burden to patients as it does not involve placing an indwelling catheter, but rather drawing a single blood sample. If the SIME approach fails, we will only draw from the venous line at the 50 and 80 time points for DASB and the 60 and 90 min time points or CUMI as described in the PET scan section.

3. Blood sampling & IV line insertion

Drawing blood and inserting an intravenous line (IV) into an arm vein are safe and standard medical procedures. Sometimes a bruise will occur at the puncture site and rarely, a blood clot or infection will occur in the vein. Certain individuals may feel light-headed during venipuncture. The volume of blood collected during the PET scans, will be approximately 16 tablespoons at most. At screening, 15.2ml will be drawn. An additional 8.5ml of blood will be drawn weekly until Lithium levels are therapeutic. This is not expected to have any serious negative effects on a study participant.

The risks of bruising, clotting, and infection will be minimized by having venipuncture performed by trained and experienced personnel using aseptic technique. To avoid injury due to fainting, the antecubital vein catheter will be inserted when the subjects are recumbent. The blood draws during PET scanning sessions will be obtained from the already inserted catheter, to minimize discomfort.

For patients who have low hemoglobin levels, possible adverse effects of blood sampling will be minimized by using the SIME approach. No additional risks are introduced by the simultaneous estimation (SIME) approach.

4. Discomfort during scanning (PET and MRI)
It may be uncomfortable to lie motionless in the cameras (both PET and MRI) and it may cause some subjects to feel anxious. Our staff will be available to provide support, reduce anxiety, optimize the comfort of the subject and remove the subject from the machine if requested.

5. MRI/fMRI Scan
MRI: While there have been no reports of any ill long-term effects caused by magnets of the same or even higher strength, the long-term effects of being placed in a magnet of this strength are unknown. The MRI scanner uses a large magnet to take pictures of the brain and is not associated with any known medical risks, except for persons who have a heart pacemaker, or have metal in their body (e.g. shrapnel or surgical prostheses) which may be affected by the magnet. Patients will be asked to notify us if this is the case. There is also the risk of burns from medicinal patches during the MRI; therefore, subjects will be asked to remove any patches prior to the scanning session. Also, although there are no known risks associated with pregnancy, we will not scan someone who is pregnant. Some people have reported sensations during the MRI scan, such as "tingling" or "twitching" (or, very rarely, a painful sensation), which are caused by changes in the magnetic field that can stimulate nerves in the body. If the subject experiences sensations and feels uncomfortable, the MR technologist will stop the scan immediately. Occasionally, some people experience nervousness or

claustrophobic feelings due to the scanner's small space. Despite these sensations, in our experience, no one has had sensations from the scanning that did not stop as soon as the scanning stopped. The MRI scan is not painful, but having to lie still in the enclosed space of the scanning table is uncomfortable for some people.

fMRI: The risks associated with the fMRI due to the magnet strength are exactly the same as the risks associated with the MRI.

6. Radiation Exposure (PET)
The Yale-New Haven Hospital Radiation Safety Committee (RSC) will review the use of radiation in this research study, and no subjects will be enrolled until RSC approval is obtained. This research study involves exposure to radiation from [^{11}C]CUMI-101 and [^{11}C]DASB PET scanning. This radiation exposure is not necessary for medical care and is for research purposes only.

The targeted amount of radiation an individual subject will receive in this study per year is from up to 2 injection(s) of ≤ 20 mCi from [^{11}C]CUMI-101 and from up to 2 injection(s) of ≤ 20 mCi from [^{11}C]DASB plus transmission scans.

Although each organ will receive a different dose, the maximum amount of radiation exposure subjects will receive per year from this study is equal to an effective dose equivalent of 2.4 rem for a total of 2 injection(s) of up to 20 mCi of [^{11}C]CUMI-101 and 2 injection(s) of up to 20 mCi of [^{11}C]DASB.

The amount of radiation subjects will receive in this study is below the dose guidelines established by the FDA and monitored by the Yale-New Haven Hospital Radiation Safety Committee for research subjects. This guideline sets an effective dose limit of 5 rem per year.

The dosimetry tables below provide the absorbed radiation dose calculation for [^{18}F]MeFWAY based on a dosimetry studies in humans.[187] [^{18}F]MeFWAY dosimetry estimates[101,102] are shown in Table 4. By voiding the urinary bladder at 90 minutes, as recommended by a recent human [^{18}F]MeFWAY dosimetry study, [187]the radiation dose is expected to be halved. By emptying the bladder at 90 minutes, the single study exposure to the critical organ (urinary bladder) with the minimum dose of 2.0 mCi and maximum dose of 2.5 mCi, as per Stony Brook RDRC protocol, results in radiation dosage of up to 1.9 Rad (Rem) for males and 2.46 Rad (Rem), which is under the 5 Rem single dose limit under the FDA 21 361.1.

For the maximum number of scans (3), the dose to the critical organ would be 5.7 Rad (Rem) for males and 7.38 Rad (Rem) for females, which is less than the 15 Rem annual dose limit under FDA 21 361.1. 22.8. It will be necessary to void the urinary bladder at 90 minutes. Each scan will be about 120 minutes or less, however, all participants will be required to void at 90 minutes. The scan will not continue if they cannot void their urinary bladder. We will encourage fluids including coffee, tea, and water intake. The half-life of 1[^{8}F]MeFWAY is 109 minutes and at least half of the radioactivity from the radiotracers, is expected to disappear by voiding the urinary bladder at 90 minutes. The rest of the tracer will be excreted through general dissipation and from urine from the body within 24 hours. Because the radiation dosage is close to the maximum annual radiation dose limit, we will ask about the participant's prior radiation exposure during the initial evaluation to ensure the radiation dose they are receiving from all research studies is less than the annual dose limit.

Table 3 shows the absorbed radiation dose calculation for [^{11}C]CUMI-101 based on our human dosimetry data (MIRDOSE calculation based on baboon data). Similarly, Table 3 provides the absorbed radiation dose calculation for [^{11}C]DASB based on the dosimetry study of [^{11}C]DASB in humans (Lu et al., 2004). The critical

organ is the testes in males and bladder wall in females for [^{11}C]CUMI-101, and the lungs for [^{11}C]DASB. The exposure after a single injection of [^{11}C]CUMI-101 and [^{11}C]DASB is below the single dose limit under the FDA 21 361 dose limits for research subjects (3 rem per injection, 5 rem annually for whole body, active blood forming organs, lens of the eye and gonads and 5 rem per injection, 15 rem annually for other organs).

Single Study Limit

[^{11}C]CUMI-101: For the [^{11}C]CUMI compound, the single study exposure to the critical organ (testes in males, bladder wall in females) with the maximum dose of 20 mCi, is 2.4 rem (males) and 1.5 rem (females) is below the 3(testes)/5(bladder wall) rem *single dose limit* under the FDA 21 361.1.

[^{11}C]DASB: Table 3 provides the absorbed radiation dose calculation for [^{11}C]DASB. The dose estimates indicate that the maximum permissible single study dosage of [^{11}C]DASB in human subjects remain below the 21 CFR 361.1 dose limit for research subjects which is 20 mCi (i.e., calculation based upon the lung as the critical organ; 3 rads per single study for the whole body, active blood forming organs, lens of the eyes and gonads; 5 rads for other organ per single study limit).

[^{18}F]MeFWAY

While no adverse events have been observed to date in human research with this drug, since fewer than 15 individuals have been injected with [^{18}F]MeFWAY worldwide, even common and serious effects may not be known yet.

Yearly Cumulative Exposure

The radiation exposure table indicated that the total exposure resulting from the study will remain well below the FDA 21 361.1 dose limits for yearly cumulative exposure to research subjects (dose limits of 5 rads per year for whole body, active blood forming organs, lens of the eye and gonads; 15 rads per year for other organs). Each subject will receive up to 4 injections, (up to 2 [^{11}C]DASB and up to 2[^{11}C]CUMI-101 scans). The cumulative exposure due to four injections remains below the FDA annual limits.

Table 3 (Human Data)

Organ	^{11}C-CUMI-101 Single Study maximal exposure (20 mCi)	DASB Single Study maximal exposure (20mCi)	Cumulative Exposure (1 DASB + 1 CUMI)	
Units	mGy/MBq	mGy/MBq	**mrem/Year**	**%of limit**
Adrenals	3.71E-03	3.16E-03	5.08E+02	3.4
Brain	1.04E-02	5.77E-03	1.20E+03	8.0
Breasts	1.79E-03	2.36E-03	3.07E+02	2.0
Gallbladder Wall	3.65E-03	9.27E-03	9.56E+02	6.4
LLI Wall	1.27E-03	2.11E-03	2.50E+02	1.7
Small Intestine	1.61E-03	2.18E-03	2.80E+02	1.9
Stomach Wall	2.58E-03	2.46E-03	3.73E+02	2.5
ULI Wall	1.74E-03	2.21E-03	2.92E+02	1.9
Heart Wall	2.51E-03	8.21E-03	7.93E+02	5.3
Kidneys	9.86E-03	9.28E-03	1.42E+03	9.4
Liver	1.84E-02	6.41E-03	1.84E+03	12.2

Lungs	8.60E-03	3.28E-02	3.06E+03	20.4
Muscle	1.95E-03	2.09E-03	2.99E+02	2.0
Pancreas	3.20E-02	3.03E-03	2.59E+03	17.3
Red marrow	1.77E-03	2.33E-03	3.03E+02	6.1
Osteogenic Cells	1.57E-03	2.25E-03	2.83E+02	1.9
Skin	1.50E-03	1.60E-03	2.29E+02	1.5
Spleen	1.45E-02	7.40E-03	1.62E+03	10.8
Testes	1.52E-03	1.74E-03	2.41E+02	4.8
Thymus	2.08E-03	2.98E-03	3.74E+02	2.5
Thyroid	4.55E-03	2.06E-03	4.89E+02	3.3
Urinary Bladder Wall	1.90E-03	1.20E-02	1.03E+03	6.9
Lens of the Eye	0.00E+00	N/A	N/A	
Total Body	0.00E+00	2.75E-03	2.04E+02	
	mSv/MBq	mSv/MBq	mrem/Year	
Effective Dose Equivalent	0.00E+00	8.01E-03	5.93E+02	
Effective Dose	5.33E-03	6.98E-03	9.11E+02	18.2

Note: The effective dose (ED) as a consequence of exposure to typical transmission scans on ECAT EXACT HR+ scanner is 2.7 x 10^{-4} mSv/MBq X h (Almeida et al, 1998). There are 3 Ge68 rod sources on HR+ (<5mCi each). The transmission scan for this study is 10 minutes, which would result in a total additional ED of 2.7 X 10^{-4}/MBq x (5x3x37) MBq x 10/60 hr = 0.025 mSv = 2.5 mrem per scan.

The dose of radiation will be submitted for approval to the Yale-New Haven Hospital Radiation Safety Committee (Y-NHH RSC). All scans will be done in the presence of medical supervision and trained staff in an institution specifically designed to support imaging studies. In the event of serious medical complications, the PET scan facilities have immediate access to or consultation with specialized medical units at the Yale-New Haven Hospital. Preparation of radiopharmaceuticals and performance of PET scans will be by radiochemists, physicians, and technologists of the Department of Diagnostic Radiology, Yale University School of Medicine. These professionals are qualified by training and experience in the safe use and handling of radiopharmaceuticals.

Subjects will be asked about their previous radiation and those who have had research exposure within the past year will be excluded if cumulative annual exposure (including the present study) exceeds FDA limits. The information on the previous radiation exposure of study subjects will be notified to the study doctor.

No PET studies will be performed on pregnant or potentially pregnant women, as confirmed by pregnancy testing during evaluation and on each scan day before initiation of any scan procedures. If subjects are breastfeeding they will not be able to participate in this research study.

Table 4: Human Dosimetry of [^{18}F]MeFWAY[101,102]

	mSv/MBq		mRad/2.5mCi	
Organ	Female	Male	Female	Male
Adrenals	1.59E-02	1.25E-02	147.25	116
Brain	8.58E-03	5.79E-03	79.25	53.5

Breasts	7.96E-03	5.99E-03	73.75	55.5
Gallbladder Wall	2.04E-02	1.68E-02	188.5	155
LLI Wall	2.32E-02	1.90E-02	215	176
Small Intestine	1.67E-02	1.44E-02	154.25	133.25
Stomach Wall	1.18E-02	9.23E-03	109.5	85.5
ULI Wall	1.58E-02	1.25E-02	146.25	115.75
Heart Wall	1.25E-02	7.43E-03	115.25	68.75
Kidneys	3.36E-02	4.95E-02	310	457.5
Liver	7.54E-02	5.23E-02	697.5	485
Lungs	2.28E-02	1.32E-02	210.5	122
Muscle	1.20E-02	9.74E-03	110.75	90
Ovaries	2.29E-02	N/A	211.5	N/A
Pancreas	1.51E-02	1.19E-02	139.75	110.25
Red marrow	1.45E-02	1.22E-02	133.75	113.25
Osteogenic Cells	1.75E-02	1.29E-02	162	119.5
Skin	7.68E-03	6.19E-03	171	57.25
Spleen	1.10E-02	8.85E-03	102	81.75
Testes	N/A	1.36E-02	N/A	125.75
Thymus	9.43E-03	7.01E-03	87.25	64.75
Thyroid	7.64E-03	6.36E-03	70.75	58.75
Urinary Bladder Wall*	**5.32E-01**	**4.10E-01**	**4925**	**3800**
Uterus	3.67E-02	N/A	340	N/A
Lens of the Eye	N/A	N/A	N/A	N/A
Total Body	N/A	N/A	N/A	N/A

*For the urinary bladder wall, by voiding the bladder at 90 minutes, the radiation dose is expected to decrease to 1.9 Rem for males and 2.46 Rem for females, which is under which is under the 5 Rem single dose limit under the FDA 21 361.1 as mentioned above.

7. Toxicology of Drugs and Radiotracers

[^{11}C]CUMI-101: We have performed the FDA required toxicity study (intravenous) of CUMI-101 (CUNBID-101) in Sprague-Dawley rats sponsored by National Institute of Mental Health (NIMH) in the Toxicology Laboratory of SRI International. We proposed a total human dose of 10 microgram per injection and based on this dose male and female Sprague-Dawley rats (10/sex/group) were given a single iv dose of CUMI-101 at 881 μg/kg (5286 μg/m^2, 1000 times the human dose, Group 2) or at 88.1 μg/kg (528.6 μg/m^2, 100 times the human dose, Group 4), or 44.05 μg/kg (264.3 μg/m^2, 50 times the human dose, Group 5) on Day 1 or twice a day at 440.5 μg/kg/injection (2643 μg/m^2/injection, 500 times the human dose; total dose 881 μg/kg and 1000x human dose, Group 3). A control group (10/sex), Group 1, was given a single iv dose of vehicle, 5% ethanol in sterile saline, at an equivalent volume on Day 1. Animals were sacrificed on Day 3 or Day 15 (interim and terminal necropsy, respectively). The following parameters were evaluated for the toxicity evaluation: mortality/morbidity, clinical observations, body weights, food consumption, clinical pathology (hematology and serum chemistry), organ weights, and, at necropsy, macroscopic observation and microscopic histopathology.

All animals survived until their scheduled necropsy. Male and female rats in the mid and high dose groups (Groups 2-4) displayed slight to moderate hypoactivity on Day 1 immediately after dose administration. The severity of the hypoactivity appeared to be dose dependent, with the highest severity observed in the high dose group (Group 2). The animals recovered quickly from the hypoactivity within a few hours and appeared normal by the last time point clinical observations were performed on Day 1. Rats in the low dose group at 44.05 μg/kg (264.3 μg/m^2, 50x human dose) and the control group did not exhibit hypoactivity after dose administration. No drug-related effects were found for body weights, food consumption, organ weights, and macroscopic and microscopic evaluations.

In conclusion, iv administration of CUMI-101 to male and female Sprague-Dawley rats for a single or twice a day administration did not produce overt biologically or toxicologically significant adverse effects except hypoactivity in the mid and high dose groups, which is not considered to be a dose limiting toxic effect. No adverse effects were observed in the low dose group. The no observed adverse effect level (NOAEL) is considered to be 44.05 μg/kg (264.3 μg/m^2) for a single iv dose administration. The maximum tolerated dose (MTD) is considered to be at least 881 μg/kg (5286 μg/m^2) for a single iv dose administration. Although the groups with 1000-100 times human dose based on a 10 microgram showed only slight hypoactivity, we reduced the maximum injected mass of CUMI-101 to human subjects as 5 microgram per dose considering the no hypoactivity for a 50 times dose. Based on this dose the group 5 is now 100 times dosage based on a 5 microgram human dose and tolerate well in the toxicology studies.

CUMI-101 has been successfully tested by us in 8 human volunteers with two doses of the tracer given to each subject. No adverse effects were observed and the tracer gives a very reliable finding outcome measure with less than 10% CV on test retest. It has also been tested by Robert Innis at the NIH in 8 human volunteers with no adverse effects.

The total dose of [^{11}C]CUMI-101 used for the scan in this study is below 5 μg which is negligible and has no pharmacological effect. The safety of [^{11}C]CUMI-101 in humans is supported by SRI International Toxicology Report. The risk of an idiosyncratic reaction is acknowledged in the consent form. A physician will be present at the time of each injection of the radiotracer. Any adverse reaction to the radioactive drug (radiation related or not) will be reported to the IRB and the RDRC & IRB at Stony Brook University.

[^{11}C]DASB: The dose of [^{11}C]DASB (equal or below 10 μg) will be administered at tracer levels. The risk of an idiosyncratic reaction is acknowledged in the consent form. A physician will be present at the time of each injection of the radiotracer. Any adverse reaction to the radioactive drug (radiation related or not) will be reported to the IRB and JRSC, as specified in 21 CFR 361.1 (d8).

The dose of radiation for [^{18}F]MeFWAY has been submitted for approval to the Stony Brook Hospital Radiation Safety Committee and the Stony Brook Radioactive Drug Research Committee (RDRC). All scans will be done in the presence of trained staff in an institution specifically designed to support imaging studies. In the event of serious medical complications, the PET scan facilities have immediate access to or consultation with specialized medical units at Stony Brook Hospital, as well as the responsible study physician, Dr. Kunkel. Preparation of radiopharmaceuticals and execution of PET scans will be performed by radiochemists and technologists of Stony Brook University, including the Director of Radiochemistry, Dr. Peter Smith-Jones and authorized users Drs. Dinko Franceschi and Robert Matthews. These professionals are qualified by training and experience in the safe use and handling of radionuclides. Subjects will be asked about their previous radiation exposure, and those who have had research exposure within the past year will be excluded if their cumulative annual exposure (including the present study) exceeds FDA limits. According to the CFR Title 21, part/section 361.1 “Radioactive drugs for certain research uses”, single dose for whole body, active blood-forming organs, lens of the eye, and gonads should not exceed 3 Rems and annual and total dose commitment should not exceed

5 Rems. For the current proposal, the estimated single study exposure to these organs is below 0.423 Rem (423 mrem). For the maximum number of scans (3), the dose to these organs is below 1.27 Rem (1,270 mrem), which is less than the 5 Rem annual dose limit. As for the other organs, Rems should not exceed 5 Rems for a single dose and 15 Rems for annual and total dose commitment. For the current proposal, the estimated single study exposure to all other organs is below 1.27 Rem (1,270 mrem). For the maximum number of scans (3), the dose to all other organs is below 3.81 Rem (3,810 mrem), which is less than the 15 Rem annual dose limit. Further, the radiation dose is expected to be halved when the participant voids at 90 minutes. The scan will not continue if the participant does not void at 90 minutes.

8. Genetic samples and confidentiality
Samples will be de-identified and coded only with a number; no personal identifying or clinical information is provided to the testing laboratories. Therefore, the risk of a breach in confidentiality is remote.

9. Pregnancy
Studies involving radiation are contraindicated during pregnancy because of possible risk to the fetus. Women of child-bearing age will be required to have a urine pregnancy test administered on the evaluation day as well as on the day of the PET session prior to scanning. Subjects will not be charged for the pregnancy test. In addition, nursing mothers will also be excluded from the study.

10. Other Risks Associated with Lithium and Lamotrigine Use
Lithium: possible side effects include diarrhea, vomiting, shakiness, drowsiness, slowed thinking, weakening of muscles, difficulty concentrating, acne, weight gain, change in thyroid function and increased urination. Because of the possibility of becoming sleepy, patients will be told to be cautious about driving especially when the medication is started or the dose is raised. Patients will be told to tell their doctor if they develop any of these symptoms. Patients will also be told not to take pain medications such as ibuprofen (Advil, Motrin, or Naproxen [Anaprox]) while on lithium. Diuretics (water pills) such as thiazide or furosemide (Lasix) should not be taken with lithium. Lithium may react with other medications. While taking lithium, before adding another medication patients will be asked to check with their doctor to be sure it is safe.

Lamotrigine: Possible side effects include serious rashes that may need to be treated in a hospital or cause permanent disability or death; fever; swelling of the face, throat, tongue, lips, eyes, hands, feet, ankles, or lower legs; hoarseness; difficulty breathing or swallowing; upset stomach; extreme tiredness; unusual bruising or bleeding; lack of energy; loss of appetite; pain in the upper right part of the stomach; yellowing of the skin or eyes; flu-like symptoms; pale skin; headache; dizziness; fast heartbeat; weakness; shortness of breath; sore throat, fever, chills, and other signs of infection; dark red or cola-colored urine; muscle weakness or aching; or painful sores in the mouth or around the eyes. Patients will also be told not to take valproic acid (Depakene) or divalproex (Depakote) while on lamotrigine.

11. Risks of optional EEG procedure
Potential risks include psychological distress and fatigue from performing the tasks. Participants may take short breaks between the various tasks. During the EEG recording, there is a small possibility of mild skin redness where the electrode contacts the skin. This, however, is rare and usually temporary.

3. Potential Benefits of Proposed Research to the Subjects and Others
There will be potential benefit to the patients in terms of free clinical and diagnostic assessments. Patients will get a thorough psychiatric and medical work-up and then a free inpatient or outpatient initial treatment course of a mood stabilizer. The benefits to society and to future patients may be considerable if enhanced understanding of biology of bipolar disorder and the action of mood stabilizers is gained.

4. Importance of the Knowledge to be Gained

Bipolar disorder is a major health problem in which the serotonin system may play a role in pathogenesis and/or treatment. An urgent need exists for a reliable biochemical test for bipolar disorder. Therefore, study of the serotonergic system should be conducted in the untreated depressed state where these measures may have potential as diagnostic tools as well as providing important information about the pathogenesis of bipolar disorder and biochemical heterogeneity of depressive disorders. Repeating these tests after treatment will assist in determining the mechanism of action of mood stabilizers, the potential of this type of PET scan as a biochemical monitor of clinical response as well as help to determine whether alterations in function are state or trait-dependent. Such studies can also improve knowledge regarding the mechanism of action of mood stabilizers, and may lead to the design of better medications.

H. Data Safety Monitoring Plan (for more than minimal risk studies)

Access to research data will be allowed only to members of the research team or institutional personnel as part of a routine audit. Records may be reviewed by state or federal regulatory agencies and their personnel. Research records, like other medical and clinical records, will be kept confidential to the extent permitted by law. There are legal advocacy organizations that have the authority under state law to access otherwise confidential subject records, though they cannot disclose this information without the subject's consent. All hard copies of records are kept in locked files. Coded computer files will be stored in a database which is password protected and behind an institute and department firewall.

Once a patient enrolls in the project they are given a code number which is used for all subsequent computer data and/or lab forms. The code list and patient names as well as all data are kept in locked files in locked offices with access limited to those directly responsible for maintenance of these files by the research team.
Subjects whose history is obtained through the collection of family history information (from the interviewee) are also considered research subjects. They are subjected to minimal risk because all information is confidential. There are procedures to safeguard confidentiality of the information gathered about them from other family members, including names or identifying information kept on the family history form or in the records. All hard copies of records are kept in locked files. Computer files will be stored in a database which is password protected. The database is stored on a secured server. Only essential staff will be allowed access to this information. The study could not be completed without this information.

Blood samples for genetic testing will be de-identified and coded only with a number; no personally identifying or clinical information will be stored with the samples or provided to testing laboratories.

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