Differentiating Unipolar and Bipolar Depression in Young Adults Using fMRI

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1. SPECIFIC AIMS

Overall Aim: It is critical to be able to differentiate between unipolar depression (UD) and bipolar disorder depression (BDD) both for understanding the etiology of these similar but at the same time very different illnesses as well as for determining the type of treatment (mood stabilizers alone, antidepressants alone or a combination) that is indicated. Despite phenomenological and neurobiological research for past several decades, no single clinical or biological finding is available at present that can reliably differentiate between the two illnesses. In young patients, bipolar disorder (BD) first presents itself as depression and a history of mania or hypomania is either not available or very difficult to elicit. Only with time, and usually after years of misdiagnosis and inappropriate treatment, a proportion of the young depressed subjects start suffering from episodes of overt (hypo)mania and reveal themselves to be actually suffering from BD. Therefore, it is particularly important to be able to differentiate between young UD patients at a high risk for developing BD (HRUD) and young UD patients with low risk of developing BD (LRUD). The primary investigator has been conducting functional magnetic resonance imaging (fMRI) studies for the past decade on resting state functional connectivity and task-induced activation abnormalities in BD and UD. Preliminary findings from more recent studies suggest that resting state corticoamygdalar connectivity and task-induced activation differences may be able to differentiate between BDD, UD, HRUD and LRUD subjects. This proposal will study 40 BD, 120 UD (60 HRUD and 60 LRUD) and 40 closely matched healthy controls (HC). Aims and specific hypotheses for the present proposal, based on preliminary data collected are as follows:

SPECIFIC AIM 1: To investigate resting state corticoamygdalar connectivity and activation abnormalities which are able to differentiate between young (15– 30 yrs of age) BDD, UD and HC subjects.

HYPOTHESIS 1: BDD subjects will be differentiated from UD and HC subjects on measures of resting state corticoamygdalar connectivity and task-induced activation: BDD subjects will have lower lateral orbitofrontal (LOFC)-amygdalar connectivity but higher ventral anterior cingulate cortex (vACC)-amygdalar connectivity compared to UD and HC subjects. On measures of activation, while performing a happy emotion NoGo-Go task, UD subjects will have less dorsal ACC (dACC) activation compared to BDD and HC subjects.

SPECIFIC AIM 2: To investigate similarities and differences between BDD, HRUD and LRUD on measures of corticoamygdalar connectivity and activation.

HYPOTHESIS 2: HRUD subjects will display a pattern of corticoamygdalar connectivity and task-induced activation which will have similarities and differences with both BDD and LRUD subjects: HRUD subjects will have lower LOFC-amygdalar connectivity similar to BDD subjects but also lower vACC-amygdalar connectivity similar to LURD subjects. On measures of activation, on the happy emotion NoGo-Go task, HRUD and LRUD subjects would have a similarly decreased dACC activation but on a negative facial emotion recognition task both HRUD and BDD will have lower insula activation compared to LRUD subjects.

EXPLORATORY AIM 3: Ultimately, only prospective data regarding which patients actually develop BD can validate the ability of the baseline imaging measures to predict a BDD or UD diagnosis. The main indicator of bipolarity is the presence of hypo(manic) symptoms. Beside conversion to (hypo)mania we will also measure occurrence of episodes of sub-threshold hypomania symptoms using the Young Mania rating Scale (YMRS) and the mania syndrome checklist of the Schedule for Depression and Schizophrenia (MSC-SADS) which has been recently used to study hypomania development in depressed patients. Besides looking at differences between converters and non-converters, an analysis will also be conducted of the correlation between peak change of YMRS and MSC-SADS scores as well as the slope of the change for mania symptoms ratings obtained over the course of the two years of follow-up. The data could be used to plan future large scale definitive studies.

EXPLORATORY HYPOTHESIS 3: Baseline imaging measures will differ in converters to BD diagnosis vs. non-converters as between BDD and UD in Hypothesis 1. Peak change and slope of change in scores for YMRS and, MSC-SADS will correlate negatively with baseline LOFC-amygdala resting state connectivity and posterior insula activation on the face emotion recognition task and positively with vACC-amygdala connectivity and dACC activation on the happy emotion NoGo-Go task.

2. RESEARCH STRATEGY

2a. SIGNIFICANCE:

2a(i). Bipolar Disorder (BD) characterized by acute episodes of (hypo)mania and depression affects 0.5-4% of the U.S. adult population depending on the severity criterion employed. **Unipolar Depression or Major Depressive Disorder (UD)** presents with depressive episodes only and is one of the most prevalent psychiatric illnesses (10 -15% prevalence). Though some debate continues (as reviewed below), the two illnesses are classified as separate mood disorders. In the absence of a clear-cut history of (hypo)mania it is difficult to decide whether a depressive episode is a manifestation of UD or of BD depression (BDD). However, currently the difference in the pathophysiologies of the two disorders is not known and there is no biological marker available to differentiate between the two illnesses. The age of onset of BD is typically in the late teens[6]. However, in young patients, frequently, the first episode(s) are of depression making it highly difficult to diagnose subjects with BDD. An early age of onset and a family history of BD in a first degree relative are thought to be the most reliable indicators for UD subjects with a high risk of developing BD (HRUD)[7] compared to UD patients with low risk of developing BD (LRUD). However, most first degree relatives of BD patients suffer only from UD[8] and would never develop BD and some UD patients can also have the onset of illness at an early age. Therefore, it is critical to develop additional biomarkers to differentiate between BDD and UD in young adults. This proposal will, for the first time, investigate whether specific functional magnetic resonance imaging (fMRI) measures of corticoamygdalar connectivity and activity can be used to differentiate between BD, UD, HRUD and LRUD in young adults.

2a(ii) Differentiation between Bipolar and Unipolar Depression:

2a(ii) A. Relationship between BDD and UD: Several different hypotheses have been proposed regarding the relationship between BDD and UD: 1) The two are the same illness at opposite ends of a spectrum; 2) The two are different manifestations of the same illness; 3) Mania and depression are two different illnesses which are comorbid; and 4) The two are entirely different illnesses [9, 10]. The arguments for the two being similar illnesses stem from the difficulty in differentiating between UD and BD based on history and presentation. Both frequently have a recurrent course and though some studies have reported that atypical symptoms, anxiety, and post-partum occurrence are associated with BDD other studies have been negative[9]. Relatives of BDD subjects frequently suffer only from UD[8] and do not necessarily convert to BDD. However, in clinical practice, it is clear that some patients have recurrent depression with hypomania and mania while others do not. The hypothesis that mania and depression are different illnesses which coincidentally co-occur in BDD but not in UD is an interesting one but does not take into account the frequent close temporal relationship between manic and depressive episodes and the tendency to switch from one to the other. The above considerations suggest that the mania and depression cycles in BD are probably caused by the same pathological process which may be distinct from that in UD. Therefore, it should be feasible to differentiate between BDD and UD using an objective neurobiological measure but none is available until present. The development of brain imaging technologies promises to deliver such a measure as the function of the brain can be measured directly.

2a(ii)B. Neuroimaging studies of corticolimbic connectivity and regional activation abnormalities BDD and UD: Neuroanatomical and neurophysiological studies have identified abnormalities of the putative mood regulating circuit (MRC) which includes cortical mood–regulating areas such as the medial prefrontal cortex (MFC)(including the ventral ACC (vACC)) and the medial and lateral orbitofrontal cortex (MOFC, LOFC) which regulate mood-generating limbic structures such as the amygdala (AMYG), pallidostriatum (PST), ventral striatum (VST), and dorsomedial thalamus (DMTHAL) [11-15]. In particular, functional connectivity and activation abnormalities of the amygdala have been repeatedly reported in mood disorders literature (please see review by P.I.: Brain Imaging Studies in Mood and Anxiety Disorders)[16](Appendix) and could provide clues to the differential pathophysiology of BDD and UD.

However, there have been very few neuroimaging studies which have concurrently studied BDD and UD to look at differences between the two illnesses. Most studies have either studied UD or BDD. In UD, a number

of studies have reported decreased frontal cortex metabolism/activation and increased limbic activation[15]. In BDD studies in which phase of illness has been characterized, increased limbic activation and decreased activation of cortical regions such as the DLPFC and ventral ACC has also been reported [14]. Structural studies which are less prone to state effects have also reported similar findings in BDD and UD such as increased[17, 18] or decreased amygdala volume [19] and changes in anterior [20] and posterior cingulate [21] , frontal cortex and other areas of the mood regulating circuit (MRC) [20, 22]. Functional studies using positron emission tomography (PET) or functional MRI (fMRI) have reported corticolimbic abnormalities pointing to abnormal functioning of the dorsolateral prefrontal cortex (DLPFC) [23-25], ventrolateral prefrontal cortex (VPFC) [26 27], and orbitofrontal cortex (OFC) [28] as well as the vACC. Studies of anterior cingulate cortex (ACC) activation have reported contradictory findings with some studies indicating increased activation and metabolism in mania, depression and euthymia [29-31] while others report decreased activation [32, 33]. Increased limbic activity has been reported in response to attentional tasks and attentional tasks associated with emotional stimuli [34, 35] in unmedicated euthymic bipolar subjects. Increased limbic activity has been reported in response to emotional stimuli in some [25] but not all studies [33].

As noted above, remarkably, very few imaging studies have concurrently investigated UD and BDD. Drevets and colleagues reported decreased vACC metabolism in familial UD and a small number of familial BDD patients[14]. The same investigators reported increased left amygdala metabolism in familial UD and BDD. [36]. One study has reported decreased reactivity to faces with positive emotional expression distinguishes UD from BDD subjects [37]. Using regional prefrontal cortical and amygdalar activation in response to happy faces, Almeida and colleagues [38] have reported differences in medial orbitomedial prefrontal cortex (OMPFC) and amygdala effective connectivity in UD and BDD subjects. They reported that abnormal left sided top down and right sided bottom-up effective connectivity between OMPFC and amygdala differentiated between the two illnesses. Most of the studies conducted until present have been with medicated subjects and the effect of medication was noted to be a possible confounding factor on some of the abnormalities reported. Though a number of studies of high risk adolescent offspring [39, 40] have been conducted to investigate the genetic and neurobiological basis of the disorder, very few or no studies have been conducted for young adults presenting with depression who may be at a high risk for developing BD. Studies conducted by the investigators suggest that connectivity abnormalities are intrinsic to the pathophysiology of mood disorders along with regional activation abnormalities. The P.I. has employed novel connectivity specific imaging methods namely resting state corticoamygdalar connectivity to investigate mood circuit abnormalities in UD[41] and BDD[42]. Findings from our previous studies as well as preliminary results (presented below) from ongoing studies suggest that these differences in connectivity patterns can be used to investigate differences between BDD and UD.

2a(iii). Resting state low frequency BOLD weighted temporal fluctuations (LFBF) measurement for elucidating connectivity between brain regions: Recently, there has been considerable interest generated from the discovery of spontaneous low-frequency (<0.08Hz) fluctuations in resting state blood oxygen level-dependent (BOLD) signal in echoplanar imaging (EPI) data [43]. It has been recognized that these LFBFs are not caused by instrumentations or physiological effects (such as cardiac and respiratory cycles) originating outside the brain and that these resting state signal changes reflect alterations in blood flow and oxygenation that may be coupled to neuronal activity [43-50]. It has been demonstrated that LFBF correlations in steady-state follow the neurophysiological architecture of the normal human brain [48]. Importantly, Biswal and colleagues demonstrated that LFBFs are phase locked between areas of plausible functional connectivity [44]. The correlation of LFBF between two areas is a measure of functional connectivity i.e. that two are in functional synchrony [51]. It has also been shown that in the resting state a default-mode pattern of contributions from the ACC, PCC, and the hippocampus. This pattern has been linked to organization of networks that support basic brain functions in the awake state such as consciousness, self reference and identity, spontaneous thought processes, vigilance for environmental dangers and the basal emotional state of the organism [41, 43, 52-55]. Therefore, abnormalities of LFBF correlations within the MRC are likely to be present in abnormal mood states.

The investigators were one of the first to report decreased corticolimbic connectivity in UD and BD using the LFBF method[41, 42]. This new perspective regarding brain function has also led to the use of correlation of resting state LFBFs as a measure of functional connectivity between distant functionally related brain regions. Published studies of connectivity abnormalities using LFBF correlation method have been reported in attention deficit hyperactivity disorder (ADHD) [56], schizophrenia [57-59], Alzheimer's Disease [60], substance abuse [61], multiple sclerosis [62], and autism [63]. The hypothesis for the proposed investigation is that if the pathophysiology of UD and BDD is different it is likely to be revealed in the investigation of resting state

connectivity besides the use of conventional fMRI regional activation paradigms of response to emotional stimuli. Preliminary findings (described below) from our studies support this hypothesis and provide rationale to conduct a definitive clinically relevant study.

2cii. Methods and Design:

We plan to recruit unmedicated 40 BDD, 60 HRUD, 60 LRUD as well as 40 HC subjects closely matched for age, gender and ethnicity for the study. We expect that at baseline 10% of subjects could be lost. During follow-up 15 - 20 % of HRUD and LRUD of patients may be lost before adequate data for development of (hypo)mania is collected. The number of subjects remaining will be adequate for statistical analysis proposed. For the exploratory aim, both the UD groups will be offered 24 months of antidepressant treatment with frequent mood assessments in the study. The other BDD and HC subjects will be followed up by phone for 24 months. During the 24 months all subjects will be rated on YMRS and the MSC-SADS for any periods suggestive of occurrence of hypomanic symptoms.

2c(ii)A. Human Subjects: The detailed inclusion and exclusion criteria and protection procedures are described in Human Subjects section E1a.

2c(ii)B. Recruitment and Timeline:

1. **Year 1:** Week 1 – 12: Training of study personnel and organization for initiation of study.
2. **Year 1 – Year 5:** Week 12 – Week 234: Recruitment of subjects and conduct of the study.
3. **Year 5:** Week 234 – Week 260: Continued follow-up of subjects and final data analysis and publication.

2c(ii)D. Training and Quality Assurance for rating scales: All raters will be trained on these scales by senior training research coordinators with several years of experience in training raters for federally sponsored studies and several are available within our department. Before start of the study the senior research coordinators will organize a training session for all personnel taking part in the ratings. Each interviewer will observe several interviews and also perform several interviews during the course of the training. A kappa of 0.90 or above will be required for major mood disorder diagnoses and rating scale rating and if this is not met further reliability testing will be carried out. When this level of agreement is not attained, additional practice ratings will be made in conjunction with review of scoring rules and techniques until it is achieved. Once the raters have been trained, an ongoing quality assurance program will continue with periodic checks on interrater reliability every 6 months. Training will be provided for both in-person (for baseline visit) as well as telephone ratings (for which our center has a number of years of experience and has many senior raters who can train research specialists).

2c(ii)E. Screening and follow-up procedures: All subjects will have a comprehensive baseline psychiatric diagnostic assessment for screening and if found eligible will be included in the study. BDD subjects will be referred to the treater of their choice after completing the baseline study. We have an extensive list of local treaters which can be provided to subjects and subjects guided how to get treatment. Using this process we are able to help subjects get access to treatment which many times they are not able to get on their own. Following the baseline scan, healthy subjects and BDD subjects will be given the option to be followed up by phone every three months for 24 months. Subjects will be monitored by phone for the emergence of sub-threshold or hypomanic/manic symptoms on diagnostic and symptom severity scales for depression and hypomania. UD subjects will be given the option to receive antidepressant treatment for 24 months and assessed after two weeks for first month, and every month up to 6 months for symptom improvement as well as for emergence of sub-threshold or hypomanic/manic symptoms on diagnostic and symptom severity scales for depression and hypomania. After 6 months subjects will be followed up with every 3 months visits. The baseline and follow-up procedures are detailed in Table 3.

Visit 1, the screening visit. Lasting approximately 3-6 hours, the screening will include a clinical psychiatric interview (DIGS: Diagnostic Interview for Genetic Studies or the KSADS: Kiddie Schedule for Affective Disorders and Schizophrenia)(Nurnberger et al 1994) specially designed to confirm Diagnostic and Statistical Manual (DSM-IV) diagnosis as well as provide a detailed assessment of the course of the illness and chronology of mood syndromes, and past medication and substance abuse history which has been extensively used in genetic studies of BD at our center. Subjects will also be

administered the following scales: The Mini International Neuropsychiatric Interview (M.I.N.I.) and the Family Interview for Genetic Studies (FIGS), the Structured Clinical Interview for DSM-IV-TR Axis II Personality Disorders (SCID II), Columbia Suicide Severity Rating Scale (CSSRS), Beck Scale for Suicide Ideation (BSSI), the Hamilton Depression Rating Scale 17-Item (HAMD) or the Children's Depression Rating Scale (CDRS-R), the Montgomery-Asberg Depression Scale (MADRS), the Hamilton Anxiety Rating Scale (HAMA) or the Screen for Child Anxiety Related Disorders (SCARED), the Young Mania Rating Scale (YMRS), the Childhood Events and Life Events Scales, the Hypomania Syndrome checklist of schedule for Affective Disorders and Schizophrenia (HSC-SADS), and the Clinical Global Impression Scale-for Bipolar Disorder (CGI-BP). Patients will then undergo a full physical examination (including heart rate, blood pressure, height and weight) and laboratory tests that will include complete blood count, comprehensive metabolic panel, thyroid function tests, electrocardiogram (EKG) urine examination and a urine toxicology screen. S100B and genetic blood draw will be completed at this time as well. There will also be a serum pregnancy test conducted at this visit. Full medication, medical, and psychiatric histories will be taken.

Visit 2 will consist of the mood questionnaires, Columbia Suicide Severity Rating Scale (CSSRS), Beck Scale for Suicide Ideation (BSSI), Visual Analog Mood Rating Scale (VAS), recording of vital signs and weight, and the baseline fMRI scan. The visit will last approximately 4 hours with approximately 2 hours being spent in the scanner itself. There are occasional circumstances that are beyond our control in which the subject will not be able to scan on the indicated week. If this occurs we will make every effort possible to scan the subject as soon as possible. Upon completion of the scan, UD patients, if they agree, will be started on antidepressant treatment. Patients will be educated in detail about the side effects of antidepressant treatment, ways to avoid side effects, and how to contact the research team in case of uncomfortable side effects. The study doctor will adjust the antidepressant dose as needed throughout the study. Genetic blood draw will also be done during this visit.
Only UD subjects who opt for treatment (and sign the appropriate consent) will complete the following visits:

Visit 3 will be one week following visit two and consist of mood questionnaires, Columbia Suicide Severity Rating Scale (CSSRS), Beck Scale for Suicide Ideation (BSSI), TESES (Treatment Emergent Side Effects Scale), and recording of vital signs and weight.

Visits 4 and 5 will be two weeks apart and consist of mood questionnaires, Columbia Suicide Severity Rating Scale (CSSRS), Beck Scale for Suicide Ideation (BSSI), TESES (Treatment Emergent Side Effects Scale), and recording of vital signs and weight.

Visit 6 through 10 will be one month apart and consist of mood questionnaires, Columbia Suicide Severity Rating Scale (CSSRS), Beck Scale for Suicide Ideation (BSSI), TESES (Treatment Emergent Side Effects Scale), and recording of vital signs and weight.

Visit 11 through 16 will be three months apart and consist of mood questionnaires, Columbia Suicide Severity Rating Scale (CSSRS), Beck Scale for Suicide Ideation (BSSI), TESES (Treatment Emergent Side Effects Scale), and recording of vital signs and weight.

BDD,Healthy control subjects, and UD subjects that discontinue medication treatment who opt for follow up (and sign the appropriate consent) will complete the following study procedures:

Phone assessments 1 through 8 will be three months apart and consist of mood questionnaires, and CSSRS/BSSI. These phone calls will take approximately 30 minutes. If subjects report significant mood changes they may be asked to come to the clinic for assessment. Assessment will be done with mood ratings done at V1 and V2.

Genetic Analysis:

A trained phlebotomist will collect the blood sample. Deoxyribonucleic Acid (DNA) is isolated from 10ml of blood using an organic procedure (Madisen et al.1987). The concentration and quality of DNA is determined using absorbance measurements at 260 and 280nm. The serotonin transporter (SLC6A4) proximal 5' regulatory polymorphism will be genotyped by DNA amplification following the protocol of Heils et al. (Heils et al. 1996). The primers stpr5; 5'-GGCGTTGCCGCTCTGAATTGC- 3' and stpr3; 5'-GAGGGACTGAGCTGGACAACCCAC-3' generate 484 and/or 528 base pair products. The amplification reactions are electrophoresed through 3% agarose and visualized by ethidium bromide staining using a UV transilluminator.

All gel photographs will be examined independently by at least two technical personnel before a genotype is assigned. If there are any ambiguities, the genotyping will be repeated on a new amplification product. The technical staff will be working with DNA numbers only and will not have knowledge of the clinical status of the individuals being genotyped. Individuals with known genotypes will be used as controls, and a negative control (no DNA added) will be included with each run. All necessary precautions will be taken to avoid contamination.

Micro arrays. We will collect 10 cc of blood at screening for micro array analysis of gene expression. Micro arrays, standard technique will be used to obtain RNA and to purify the RNA (RNeasy mini kit (Qiagen, Valencia, CA) from dissected BLA tissues. The quality and quantity of total RNA will be evaluated by 260/280 ratios and by 260nm UV absorption (Beckman, DU 640B spectrophotometer (Beckman Coulter, Fullerton, CA)). We will use Murine Genome U74A and Bv2 oligonucleotide arrays (Affymetrix, Santa Clara, CA) in a procedure recommended by manufacturers. Thus, T7-linked oligo (dT) primer will be used to reverse transcribe the mRNA, and biotin-labeled cDNA will be generated using the Enzo Bio Array High Yield RNA Transcript Labeling Kit (Enzo Diagnostics, Farmingdale NY). Samples will be hybridized at 45°C for 16 hours under constant rotation. Arrays will be washed and stained using the Affymetrix Fluidics Station 400 and will be scanned using Affymetrix GeneArray Scanner 2500 following manufacturer's recommendation.

Gene identification. The Affymetrix Interactive Query feature will be used to identify each gene name from the probe-set information. In the case where the Affymetrix website did not identify a known gene by name, a National Center for Biotechnology Information Blast analysis will be carried out to identify the closest known gene existing in the database.

S100B, Antibody, and Cytokines Analysis

A trained phlebotomist will also collect an additional 3.5 mL to 5.0 mL of blood at screening for S100B and cytokines analysis. The BD Vacutainer Tube will contain a clot activation chemical and inert silica gel to facilitate with the separation of the plasma from the whole blood. Blood will be allowed to clot for 20-30 minutes at room temperature and centrifuged at 2000 rpm for 10 minutes. The blood will then be processed and serum/plasma will be collected within 2-4 hours. The serum samples will be stored at 2-8°C for 24 hours. For longer storage samples will be frozen to below -80°C. Samples are stable for 6 months at -20°C. Frozen serum must be gently, but thoroughly mixed after thawing. Repeated freeze-thaw cycles will be avoided.

Samples which are grossly lipemic or contaminated will not be used. Samples containing precipitates will be centrifuged before testing. Heat-inactivated samples will not be used as well.

Samples will be stored in Dr. Damir Janigro's laboratory in the Lerner Research Institute until they are analyzed. All samples will be de-identified and assigned an internal number. Serum samples will then be stored at -80°C to be analyzed in batches. Serum's ID will be matched to consent forms and research record. S100B measurements will be performed using Elisa kits (98 wells, anti-human S100B, Diasorin) and reading will be done using a multi-plate fluorescent reader. Fluorescent signals were converted into ng/ml as per standard curve concentrations.

Antibodies to S100B have recently been measured and described as an indicator of remote and chronic elevation of S100B. The presence of the antibodies could also signify development of brain inflammation against astrocytes. Therefore, we will also measure S100B antibodies using previously described methods. Blood will also be collected for future analysis of other cytokines such as interleukins and TNF-alpha as elevated levels of these cytokines have also been implicated in the pathogenesis of mood disorders.

2c(ii)F. Antidepressant treatment: SSRIs are the most frequently prescribed antidepressants and are generally well tolerated. We will use SSRI antidepressants or Wellbutrin which are available in generic form i.e. either sertraline, fluoxetine, paroxetine, vilazodone, citalopram, or bupropion. We have elected to use these medications because there is some evidence that they are less likely to induce (hypo)mania. Subjects treatment will tailored according to clinical needs and the dose adjusted or the medication switched depending on response and side effects. If a patient satisfies criteria for conversion to BD (please see section 2c(ii)G below) at any time during follow-up, the study will be terminated, and either the antidepressant would be withdrawn and the patient treated with a mood stabilizer or continued and a mood stabilizer added upon. Once the patient is stabilized they will be referred to the treater of their choice or to an inpatient facility for appropriate treatment. If at any time point the patient requires other treatment e.g. due to treatment resistance or wants to be treated with psychotherapy only then they will be referred to a treater of their choice but would continue to come for follow-up visits for assessment of mood.

Patients with psychotic symptoms, who can be safely treated as on outpatient, will also be treated with an atypical neuroleptic of their choice per treatment guidelines[5]. The dosage and duration of use of these medication will be recorded, converted to a single standard equivalent and used in the data analysis as detailed in section 2c(ii)Ia.(adjusting for covariates)**.**

2c(ii)G. Rating Scales:

I. Diagnostic Instruments:

a. Diagnostic Interview for Genetic Studies (DIGS)[75](Appendix 2): The DIGS is a poly-diagnostic interview to fully assess bipolar affective disorder and comorbid psychiatric illnesses and is being used for all our current bipolar studies. The DIGS encompasses the diagnostic criteria for DSM-IIIR, DSM-IV, RDC and Modified RDC criteria for bipolar and unipolar affective disorder. It allows a detailed assessment of the onset of illness, course of illness, comorbid psychotic features, and treatment for affective disorders. Both qualitative and quantitative phenotypes can be assigned using this instrument. Additional phenomenology regarding affective illness will also be queried such as age of onset, number of episodes, irritability, mixed states, and suicidality. Assessment of Other Psychiatric Disorders with the DIGS. A complete lifetime psychiatric history will be obtained from each subject. This will cover psychotic disorders, personality disorders, anxiety disorders, PTSD, and substance dependence (alcohol, nicotine, and other illicit drugs) as well as an assessment of childhood and adult attention deficit symptoms. For prior psychotropic medication use**,** medication free period will be recorded. Prior medication use will be recorded and converted into equivalent dosages of the standard medication in the class (e.g., haldol for neuroleptics, fluoxetine for antidepressants, diazepam for benzodiazepines, and valproate for anticonvulsants). Both the medication free period and medication use will be used in an analysis of covariance model to control for prior use of medication as described in Section D6a. Prior history of substance use will be recorded in terms of alcohol and class of substance use (e.g. stimulants, opiates, psychedelics or polysubstance use). Patient groups with and without alcohol and/or substance use will be compared to patients with no such history using ANCOVA model as described in Section D6a.

b. Kiddie Schedule for Affective Disorders and Schizophrenia- Present and Life Time (KSADS-PL)[76]: the standard diagnostic instrument for childhood diagnosis would be used additionally for subjects <18 yrs.

II. Symptom Rating Scales:

i. Young Mania Rating Scale [77] . An 11-item, clinician-rated measure that queries symptoms of (hypo)mania.

ii. Screening Items list for Manic Syndrome From the Schedule for Affective Disorders and Schizophrenia (MSC-SADS)[3]: consists of five items that can be rated on a scale of 1- 6 with a possible score of 5 – 30. SADS a precursor of DSM diagnostic criteria has been shown to have high reliability and validity [3]. Pertinent to current proposal, the MSC-SADS uses a score rather than a dichotomous measure of presence of absence of (hypo)mania. We decided to include the MSC-SADS in addition to the YMRS because this screening questionnaire has been very recently reported to be able to screen for bipolarity in patients with unipolar depression and predict the development of (hypo)mania [1]. Dr. Fiedorowicz the primary author of the study has agreed to be a consultant for this proposal.

iii. Clinical Global Impressions of Severity Scale-Bipolar Version (CGI-BP)[78]. The CGI-BP is a modified version of the original CGI designed specifically for use in assessing global illness severity and/or change in patients with bipolar disorder and assesses overall bipolar illness, depression, and mania.
iv. 17-item Hamilton Depression Scale[79] and Hamilton Anxiety Scales[80]: are gold standards for assessing symptom severity of depression and anxiety respectively.
v. Life events scale [81] and childhood events scales would be used to document events which could contribute to development of depression. Scores would be used as covariates in the analysis.
vi. Treatment Emergent Side Effect Scale (TESES)
vii.Columbia Suicide Severity Rating Scale (CSSRS) Scale used for identifying the presence and severity of suicide ideation.
viii. Beck Scale for Suicide Ideation (BSSI) Scale used for identifying the presence and severity of suicide ideation.
ix. Structured Clinical Interview for DSM-IV Personality Disorders (SCID-II) Scale used for identifying the presences of DSM-IV personality disorders.
x. Montgomery-Asberg Depression Rating (MADRS) Scale used by clinicians to assess the severity of depression among patients who have a diagnosis of depression.
xi. Screen for Child Anxiety Related Disorders (SCARED) Scale used by clinicians to assess the severity of anxiety in pediatric patients.
xii. Children's Depression Rating Scale, Revised (CDRS-R) Scale used for rating the severity of depression in pediatric patients

Table 3. Baseline procedures for all subjects and Follow-up procedures for UD subjects

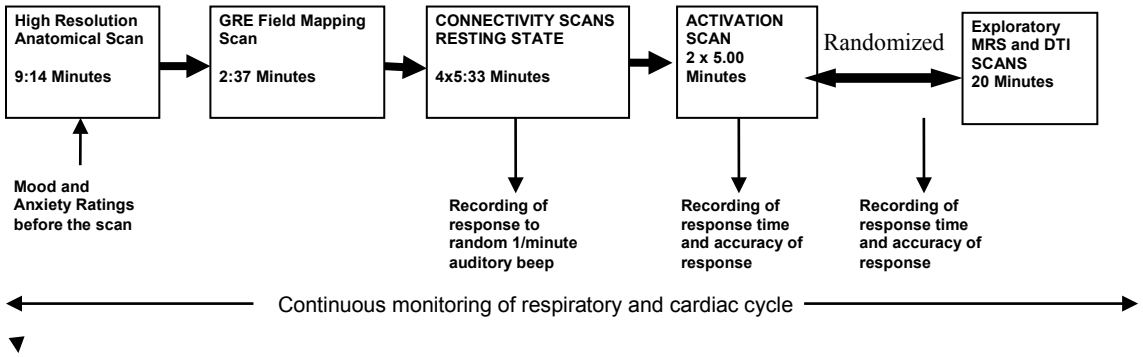
Questionnaires and Tools	Screening	Baseline	Wk 2	Wk 4	2 mon	3 mon	Every mon from 4 - 6 months	Every 3 Months from 6 months – 24 Months
Diagnostic Instrument for Genetic Studies Kiddie Schedule for Affective Disorders and Schizophrenia	X							
DIGS/KSADS Mood Disorders Section	X							
SCID II	X							
Visual Analog Scale	X							
MINI International Neuropsychiatric Interview (MINI)	X	X	X	X	X	X	X	X
Childhood Events and Life Events Scales	X							
Physical Examination	X							
Clinical Psychiatric Interview	X							
Family Interview for Genetic Studies (FIGS)[82]	X							
Laboratory Tests:	X							
fMRI SCAN		X						
Genetic blood draw	X							
S100B , antibody, and cytokine testing	X							
Antidepressant Treatment		X	X	X	X	X	X	X
Hypomania Syndrome Checklist of schedule for Affective Disorders and Schizophrenia (HSC-SADS)	X	X	X	X	X	X	X	X
17-Item Hamilton Depression Scale (HDRS)[79]- Children's Depression Rating Scale (CDRS-R) Hamilton Anxiety Scale (HAM-A) Screen for Child Anxiety Related Disorders (Scared)	X	X	X	X	X	X	X	X
Montgomery-Asberg Depression Scale (MADRS)	X	X	X	X	X	X	X	X
Young Mania Rating Scale (YMRS)[77]	X	X	X	X	X	X	X	X

Columbia Suicide Severity Rating Scale (CSSRS)	X	X	X	X	X	X	X	X
Beck Scale for Suicide Ideation (BSSI)	X	X	X	X	X	X	X	X
Treatment Emergent Side Effects Scale (TESES)			X	X	X	X	X	X
Clinical Global Impression Scale – for Bipolar Disorder (CGI-BP)[83]	X	X	X	X	X	X	X	X

III. Rating for any episodes of subthreshold or over threshold (hypo)manic symptoms: At each follow-up time point the subject will be asked for any sustained periods since the last visit in which they felt more high, irritable or energetic than their normal self[3]. If the patient answers in the affirmative then they would be rated on the YMRS and MSC-SADS for that period as well as for DSM-IV criteria for conversion to BD.

2c(ii)H. fMRI Scan methodology: Subjects will be imaged using state-of-the-art whole-body 3T Siemens Trio-Tim MRI scanner. Before the imaging session, subjects will be trained regarding experimental procedures. The imaging session duration will be approximately 90 minutes.

Figure 7. Imaging session:



2c(ii)H1. Image Acquisition: After a short scout imaging scan to survey head position and center the field of view (FOV), a high resolution 3D magnetization prepared rapid gradient echo (MPRAGE) scan will be performed and used for co-registration and normalization of the functional image volumes to a Montreal Neurological Institute (MNI) space, as well as for the structural analyses. This high-resolution anatomical volume will be comprised of 160 sagittal slices and have 1.0×1.0×1.2 mm voxel dimension, as optimized by the Alzheimer's Disease Neuroimaging Initiative protocol. Immediately following will be a 2:37 minute long gradient recalled echo (GRE) field mapping scan used for post-processing field inhomogeneity correction algorithms available in SPM's FieldMap extension. This dual echo (TE 4.97 ms; 7.43 ms) whole-brain (39 axial slices; FOV 220×220 mm; voxel dimension 2.5×2.5×3.5 mm) scan will be manually shimmed to ensure optimization of the signal, especially in the ventral areas of the brain.

Resting state connectivity scan: Functional connectivity and activation scans will be acquired using a T2*-weighted gradient echo echo-planar imaging (EPI) sequence TR/TE 2250/29 ms; with same slice locations and voxel dimension as in the GRE field mapping scan. The prospective motion correction algorithm (3D-PACE, Siemens) providing a dynamic, real-time adjustment for detected head motion will be enabled. An integrated parallel acquisition technique reduction factor of 2 will be implemented with a generalized autocalibrating partially parallel acquisition (GRAPPA) to improve spatial resolution, reduce geometric distortion and scan time. For resting state functional connectivity 145 image volumes will be acquired in 5:33 min and two connectivity scans completed to address within-subject variability. Functional connectivity image volumes will be acquired while subject is in the resting state with eyes open and fixated on a cross and asked to think nothing in particular.

Activation task: (3:00 min) developed by Hariri and colleagues to focus subject's attention on the negative facial emotion depicted reliably activates the amygdale and limbic regions[68]. A number of studies have reported altered activation of the limbic regions using this task. Increased limbic activation in BD has been reported with this task[84] and with other tasks using sad faces [85]. We are currently using this task as well as the Go-NoGo task in ongoing studies and have considerable experience administering and analyzing the results. Each trial consists of a presentation of three faces depicting a negative emotion in which the subject

has to match the top picture with one of the two bottom pictures. In the control task oval shapes are used instead of faces. Five trials are presented in 22.5 sec long blocks, with three face presentation blocks interspersed with four shape blocks. Subject's task performance (accuracy, response times) will be compared between groups and considered for use in the correlation analyses or as covariates.

Emotional Go-NoGo task combines presentations of nonemotional (letter) and emotional (sad or happy) faces. This task tests emotion regulation and previous studies in BD have reported abnormal anterior cingulate activation [86]. In the nonemotional task, subjects view a series of 12 letters presented in an 18 sec time period and respond to each letter (Go blocks) or to every letter except "X" (No-Go blocks). In the emotional task, subjects view happy and sad faces and press a response button for all faces (Go blocks) or specifically for happy or sad faces (No-Go blocks). Go and No-Go blocks of each category are repeated twice with the order of emotional and nonemotional blocks randomized within a 4:57 min scan. Subject and accuracy and response times compared across Go and No-Go conditions and disease states.

2c(ii)H2. Image Analyses: All analysis will be done blind to the subject's group status by trained analysts and inter-rater reliability will be obtained with a senior co-investigator(Dr. Dzemidzic)

a) Resting state functional connectivity (LFBF correlation): The time series data pre-processing (physiologic noise reduction, slice-timing correction, realignment, de-trending and bandpass filtering to extract low frequency BOLD fluctuations) will be accomplished using the AFNI software[87]. SPM's (Wellcome Department of Cognitive Neurology, London, United Kingdom) mutual information algorithm will be applied to co-register each subject's high-resolution MPRAGE anatomical image to the mean of all functional connectivity image volumes and segment it into its tissue components. Three mean time-series, calculated by averaging across all brain, white matter and cerebrospinal fluid voxels will be utilized to account for any remaining global effects from each voxel's time series [88]. The measures of functional connectivity will be evaluated on the AFNI pre-processed native-space connectivity volumes. We will either extract correlations between the mean time series of seed regions such as the amygdala and mean time series of another region such as the ventral anterior cingulate or calculate these correlations for all other voxels in the brain and thus generate connectivity maps. The more detailed description of the Pearson correlation coefficient calculation between the averaged LFBF time series of a-priori defined ROIs and its transformation to T-statistic was provided earlier[41] Based on ACC region of the Talairach Daemon [89], ventral ACC (vACC) is defined as the rostral ventral part of the ACC resembling rostral Brodmann Areas (BA) 24 and 32, the area that has recently been reported to be involved in corticoamygdalar connectivity and mood regulation [90, 91]. The amygdalar ROIs will be defined using MarsBar's Montreal Neurological Institute (MNI) anatomical library definition but 10 mm diameter spherical regions centered at location (-23, -5, -15) and (23, -5, -15) will also be evaluated as adopted from the Talairach Daemon dataset [89]. The medial and lateral OFC ROIs will also be defined from the MarsBar MNI anatomical library. The connectivity map approach used in our preliminary data analyses provides not only a measure of the regional connectivity but also its spatial distribution and can be utilized to fine-tune the seed region location, shape and size. These connectivity maps will be normalized to a common space by applying segmentation-generated transformation matrix, interpolated to 2 mm per side isotropic voxels and smoothed by a 6 mm full-width half-maximum (FWHM) Gaussian kernel. The resulting connectivity maps will be entered into SPM's second-level models (full factorial or paired-T design) to visualize as well as quantitate between-group differences. It should be noted that seed regions will be defined in the MNI space but that native-space ROIs will be checked using SPM's check-registration feature by overlaying them onto each subject's high-resolution anatomical and functional connectivity volumes. This procedure will detect low signal regions and normalization problems. Beside the above ROI seed based hypothesis driven analysis we will also explore other potential methods to conduct pattern analysis using independent and principal components analysis [74, 92].

b) Regional Activation Analysis: Functional image volumes in NoGo-Go task will be processed using SPM standard individual first level modeling. Group effects will be obtained using a second level analysis within SPM. Furthermore, a priori defined ROIs will be used to extract mean activation effects for between-group statistical analyses and to collect ROI percent signal change in response to various emotional and non-emotional NoGo-Go conditions. The primary contrast for analysis would be Happy NoGo-Go and Sad NoGo–Go compared to letter NoGo-Go to measure regional brain activation specifically for response inhibition to emotional stimuli. Corrections for multiple comparison/multiple hypothesis will be performed using the false discovery rates [93].

c) Volumetric Analysis: While structural analysis is not part of the aims of this proposal we will conduct structural analysis on the high-resolution anatomical scan to explore correlations with functional measures of activation and connectivity. A semiautomated cortical reconstruction and volumetric segmentation will be

performed as previously reported by the investigators [94] with the Freesurfer image analysis suite [95]. This process generates individual regional measures for cortical and subcortical anatomic structures. We will register individual maps to a standardized atlas and will apply a GLM approach to compare morphometric features across individual subjects and between study groups.

2c(ii)Ia. Statistical Analysis:

Specific Aim 1: Differentiation between BDD and UD: We will conduct a one way ANOVA with the connectivity and activation measures as dependent variables to look at differences between groups (BDD and UD). The analyses will be similar to those in our previous studies[41, 42]. The normality assumptions will be examined using the Kolmogorov-Smirnov test. Box-Cox type transformation methods will be employed if the normality assumption is violated for the outcome variable. To adjust for multiple comparisons accounting for multiple brain ROIs and ad-hoc comparisons conducted within an ANOVA model, Bonferroni adjustment will be used to control overall type I error rates (over-all significance level will be set to 0.05). A post ANOVA logistic regression based classification model will be constructed to build a classifier between BDD and UD. Specificity and sensitivity will be examined using receiver operating characteristics (ROC) curves. We will use leave-one out cross validation method to assess predictive performance of our classification model[96].

Specific Aim 2: Differentiation between BDD, HRUD, LRUD and healthy control: We will use corticoamygdalar connectivity, amygdalar-LOFC connectivity and ACC Activation in Emotion to perform one-way ANOVA among all four groups. Prior to the analysis, each of these variables will be transformed (using the Box-Cox transformation) so that they will made as close to Gaussian as possible. The normality assumptions will be examined using the Kolmogorov-Smirnov test. Specificity and sensitivity will be examined as in Aim 1.

Specific Aim 3: Identification of imaging measures significantly affecting the rate of conversion from UD to BD: The conversion of UD subjects into BDD is defined as the time to event phenomenon and will be modeled using Cox-proportional hazard model given by,

$$\log(h(t)) = h_0(t) + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_p X_p$$

where $h(t)$ is the hazard or rate of conversion at time (*t*), $h_0(t)$ is the baseline conversion rate and X_i for $i = 1,2,\cdots, p$ are covariates. The subjects will be followed in the fashion described in Table 3 with periodic assessments. The assumption of constant relative hazard rate over time will be tested and remedied if required following data transformation or by incorporating a piecewise constant hazard model. Based on this framework, we will test the significance of imaging and activation measures on the hazard rate. Our primary objective here is to identify those covariates that significantly affecting the hazard rate. The grouping of the UD (HRUD and LRUD) will be also treated as a separate covariate and put in the model as an indicator variable. To detect the progression of disease severity (episodes of (sub) threshold hypo(mania)) we will additionally use Young Mania Rating Scale (YMRS) and the mania syndrome checklist from the Schedule for Depression and Schizophrenia (MSC-SADS) between study visits over during follow-up. We will test the group difference between LRUD and HRUD using mixed-effects linear regression analyses [97] with repeated measurements taken over two years. Each model will include a random intercept and slope and fixed effects group, and time, as well as the stratification covariates. A likelihood ratio test will examine the incremental contribution of the group effect over time.

Adjusting for covariates: These include behavioral rating such as, attentional variables such as response time, and accuracy of response, and changes in physiological ratings; demographic variables such as age, gender, and ethnicity; scores on behavioral measures such as anxiety; treatment with concomitant medications such as atypical neuroleptics during the study; treatment history, substance abuse, and trauma history; and structural variables such as whole brain and ROI volumes. We will first identify the covariates with highly correlated with the outcome measures. Next, from this pool of highly correlated covariates to guard against multicollinearity and to conserve for degrees of freedom to ensure that we have sufficient power. To control for potential confounding effects from these covariates and not over-fit in any of our models, we will use the forward stepwise regression model building approach - so that only one covariate will be included at a time in the model - which allows us to screen out covariates that are not related to our outcome measures by using an elevated p-value (p=0.1) and reserve statistical power to a limited number of variables that are shown to be associated with outcome measures in our data[98].

2c(ii)Ib. Sample size and power consideration: Preliminary data obtained from BDD and UD subjects were compared with healthy subjects to obtain estimates of sample size at significance level set at 0.05. Proc Power of SAS software is used to estimate the sample size.

Specific Aim 1: We used our pilot data for Left LOFC-amygdala connectivity measure. This data yielded an effect size of 0.27 for difference between BDD and UD group. 40 samples in the BDD and 120 samples in UD group will give us more than 80% power to detect an effect size as low as 0.15 for the difference in Left LOFC-amygdala connectivity between two groups. Probability of type 1 error (alpha) was set always at 0.05. For ACC-left amygdalar connectivity measure, the effect size is 0.482. With previous mentioned sample size for each group we will have 90% power to detect an effect size as low as 0.3. Similar conclusion can be made for ACC activation in NoGo-Go task for which the data yielded an effect size of 0.726 for difference between BDD and UD group. Our proposed sample size is enough to detect the difference of effects with a power close to 90% at a significance level of 0.05. Even with 20% attrition rate (i.e. 32 BDD and 96 MDD) this sample size is large enough to ensure 80% power at alpha 0.05.

Specific Aim 2: For comparisons among BDD, HRUD, LRUD and healthy subjects all three connectivity and activation measures produce power more than 80% for 40 subject per group to detect a significant difference. In the present case with 40 BDD, 60HRUD and 60 LRUD samples the power calculation is little involved, nevertheless increased sample size ensures more than 80% power at alpha= 0.05. To compare HRUD vs. LRUD left LOFC-amygdala connectivity measure yields an effect size of -0.254. For 60 subjects/group this produces 88% power at alpha 0.05.

Specific Aim 3: Using a conservative assessment of a 6% conversion rate for the LRUD group and 14% conversion rate for HRUD group, we calculate that with a sample size of 60 per group (HRUD and LRUD) we will have 80% power with type I error fixed at 0.05, under the assumption of 20% attrition rate in both groups, to detect statistically significant imaging measures as well as differential rate of conversion. We have used Bacsafra's[96] web based program to arrive at this power calculation. For sample size requirement to differentiate between HRUD and LRUD subjects via MSC-SAD and YMRS score peak-change and slope of change we will compare the mean baseline score and the mean peak scores using two sample t-test. Peak value for each group are extrapolated from usual values seen for YMRS iduring hypomania. Under the assumption of homogeneity among groups, for both the scales a sample of size 60 will be adequate even after adjusting for 20% attrition is adequate to ensure 90% power at alpha 0.05.

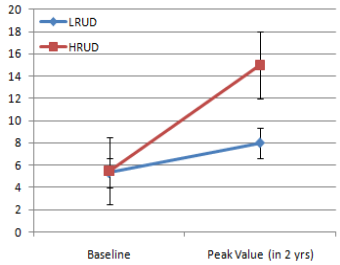


Fig. 8. Hypothetical YMRS peak score changes for HRUD and LRUD group over time

3. HUMAN SUBJECTS

E1a. Human Subjects Characteristics:

The human subjects age range 16 - 30 yrs (inclusive) involved in this study will be - 40 BDD, 60 HRUD, 60 LRUD and 40 healthy control subjects matched as a group for age, gender and ethnicity who will be studied at baseline. BDD and UD subjects will be matched for depression severity at the time of the scan. However, it is likely that, by definition the BDD and UD subjects will be different in terms of other illness characteristics such as duration of illness, number of episodes, and history of alcohol and substance abuse. We will treat those illness variables as covariates in our statistical analysis (as described in section 2c(ii)Ia above) to take into account the potential confounding effects of some of these variables.

Inclusion criteria for BDD patients:

1. Ages 15 - 30 years (inclusive) and able to give voluntary informed consent (consent will be taken from both parents and child for children under the age of 18)
2. Satisfy criteria for Diagnostic and Statistical Manual 4th edition (DSM-IV-TR) for Bipolar I or II disorder
3. Satisfy criteria for DSM-IV depressive episode-current
4. 17-item Hamilton Depression Rating Scale > 15 but < 25;
5. Young Mania Rating Scale score < 10
6. Satisfy criteria to undergo an MRI scan based on MRI screening questionnaire.
7. Able to be managed as outpatients during the study as ascertained by the following –
   i. Clinical Global Severity Scale < 5 i.e. moderately ill;
   ii. No significant suicidal or homicidal ideation or grossly disabled.

Inclusion criteria for All UD patients:

1. Ages 15 - 30 years and able to give voluntary informed consent (consent will be taken from both parents and child for children under the age of 18).
2. Satisfy criteria for DSM-IV-TR Major Depressive Episode using a Structured Interview
3. Never met criteria for mania or hypomania
4. 17-item Hamilton Depression Rating Scale score (HDRS) > 15 and < 25.
5. Young Mania Rating Scale (YMRS) score < 10
6. Satisfy safety criteria to undergo an MRI scan
7. Able to be managed as outpatients during the study as ascertained by the following –
    i. Clinical Global Severity Scale < 5 i.e. moderately ill;
    ii. No significant suicidal or homicidal ideation or grossly disabled

Additional Inclusion criteria for High Risk UD (HRUD) patients beside the inclusion criteria for UD:

These variables were chosen keeping in mind the most frequently cited risk factors for development of bipolar disorder in previous longitudinal studies of major depression

At least one of the following:

1. Family history of bipolar disorder in at least one first degree relative
2. History of any sub-threshold hypomania symptoms
3. History of mood episode related psychotic symptoms

Additional Inclusion criteria for Low Risk UD (HRUD) patients beside the inclusion criteria for UD:

These variables were chosen to minimize any overlap between the HRUD and LRUD groups.

1. No family history of BD in a first or a second degree relative
2. No past history of any sub-threshold hypomania symptoms
3. No history of psychotic symptoms

Exclusion criteria for UD and BDD patients:

1. Meeting DSM-IV criteria for schizophrenia, schizophreniform disorder, schizoaffective disorder, mental retardation, pervasive developmental disorder.
2. History of receiving electroconvulsive therapy in the past 1 year
3. Use of neuroleptics, mood stabilizers or benzodiazepines in the past 2 weeks.
4. Use of antidepressants in the past 2 weeks.
5. If on fluoxetine in the past, then should not have been on this medication for 5 weeks.
6. Acutely suicidal or homicidal or requiring inpatient treatment.
7. Meeting DSM-IV criteria for other substance/alcohol dependence within the past 6 months or abuse in the past 3 months, excluding caffeine or nicotine. The criteria will be evaluated by interview and urinary toxicology screening. The use of caffeine or nicotine will be recorded.
8. Use of alcohol in the past 1 week before the MRI scan.
9. No serious acute or chronic medical or neurological illness, including previously known HIV positive status (due to possible CNS involvement) as assessed by history, physical examination and laboratory examination including EKG, CBC and blood chemistry.
10. Current pregnancy or breast feeding.
11. Metallic implants or other contraindication to MRI.

Inclusion criteria for healthy subjects: Healthy subjects matched for age, gender and ethnicity will be included

1. Ages 15 - 30 years (inclusive) and able to give voluntary informed consent (consent will be taken from both parents and child for children under the age of 18)

2. No current or past history of psychiatric illness or substance abuse or dependence.
3. No current or past history of psychiatric illness or substance abuse or dependence in a first degree relative.

Exclusion criteria for healthy subjects:

1. Pregnant or breast feeding.
2. Metallic implants or other contraindication to MRI.
3. Significant family history of psychiatric or neurological illness.
4. Currently taking any prescription or centrally acting medications.
5. Serious acute or chronic medical or neurological illness as assessed by history, physical examination and laboratory examination including CBC and blood chemistry.
6. Use of alcohol in the past 1 week and not being able to avoid alcohol use during the course of the study.

E 1b. Potential Risks:

Risks associated with this study are those associated with the interview for psychiatric symptoms, venous blood drawing, MRI scan, potential delay in treatment, antidepressant treatment for UD patients, emergence of mania or hypomania, and loss of confidentiality.

E1b1. Interview for psychiatric symptoms: Some patients may become anxious when they focus on their symptoms.

E1b2 . Venous blood drawing for blood tests during screening: This can lead to pain and bruising at site. In rare cases inflammation or infection of the site can occur.

E1b3. MRI scan: There are almost no known significant physical risks associated with MRI. The only exceptions are individuals with certain surgical implants such as cardiac pacemakers. Subjects with such implants will be excluded from any study. A screening procedure will be used identical to that used for clinical MRI scanning. Subjects not passing the screening will be excluded from the study as being contra-indicated for MRI. Subjects will be fully informed about the fMRI procedures with a written consent form. We will also show them pictures of the MRI machine and the visual apparatus so that they are fully aware of the procedures involved. Patients reporting previous experience of anxiety in closed spaces will be excluded.

E1b4. Delay in treatment: patients will only be included in the study if they are unmedicated at the time of initial screening. We will not do a medication wash-out for the purpose of this study. However, patients need to be kept off medication between screening and time of the scan. This may delay their treatment for a few weeks and worsen their depression. Patients may become suicidal or significantly impaired in terms of their functioning.

E1b5. Worsening of symptoms: Patients may have worsening of their symptoms during the waiting period before the first scan and during the study. Patients may become severely depressed and grossly disabled or may develop suicidal or homicidal ideation. Patients who become more manic may act impulsively and have poor judgment leading to self harm or harm others.

E1b6. Loss of confidentiality: There may be a loss of confidentiality of information obtained from psychiatric interviews and of the brain imaging data.

E1b7. Tolerability of fMRI procedures by psychiatric patients: Patients and healthy subjects may become uncomfortable or agitated during the scan. The procedures for fMRI and stimulus induced activation measurements have been used extensively in our radiology facilities. In the depression and bipolar study for which preliminary data has been provided patients tolerated the procedures very well. No patients' scan had to be interrupted midway due to patient's mental or physical discomfort. Other investigators at IUSM have used similar procedures with patients with schizophrenia and substance abuse disorders and the procedures have been well tolerated. The radiology imaging area is well staffed with personnel who have extensive experience in doing fMRI studies. A crash cart and other equipment and medical personnel for medical emergencies are also readily available. Throughout the procedures the patients comfort will be closely monitored and the study will be terminated if needed.

E1b8. Antidepressant treatment: Antidepressants are generally well tolerated. Common early side effects are nausea or stomach irritation, jitteriness and increased anxiety, changes in appetite,

constipation, dizziness, dry mouth, headache, increased sweating, taste changes, weight changes, sleep disturbances, sexual dysfunction, and possible increase in suicidal ideation. Antidepressants can also precipitate mania. In line with the purpose of the study, all subjects will be monitored closely every month for the first 6 months and every 3 months thereafter for two years or more frequently as needed and the medication stopped or changed if there are unwanted side effects. In high doses Wellbutrin may cause seizures, subjects with a history of seizures will not be treated with Wellbutrin.

E1b9 Antipsychotic Treatment: Although antipsychotic medication, such as risperidol, has been given to subjects with good results before, the study medication may cause some side effects. You may experience none, some, or all of those listed below. A common side effect for this type of drug when beginning treatment is sleepiness, dizziness, rapid heartbeat, dry mouth, constipation, indigestion, feeling weak, swelling of arms and legs, weight gain, fainting, blurred vision, increased appetite, pain in the arms and legs,hyperglycemia (high blood sugar), or stuffy nose. In some cases there may be a change in the amount of white blood cells (cells in your blood to help fight infections). If you experience symptoms such as fever and/or sore throat and sores on the tongue or inside of the mouth, you should seek medical care, as such symptoms may be due to a decrease in white blood cells. Some patients have shown an increase in the amount of liver enzymes (indicating possible liver injury or damage), other enzymes, or fatty substances in the blood. More uncommon side effects are allergic reactions and seizures. An allergic reaction to this drug might start with the following symptoms: itching or hives, swelling in your face or hands, swelling or tingling in your mouth or throat, chest tightness, or trouble breathing. A rare side effect is priapism (long-lasting and painful erection). Two very rare side effects that may occur with the use of quetiapine include neuroleptic malignant syndrome (NMS) and tardive dyskinesia.Neuroleptic malignant syndrome is a serious, potentially life-threatening disorder that includes symptoms such as high fever, tight muscles, changes in blood pressure and heart rate, or changes in thinking and understanding. If you appear to be developing neuroleptic malignant syndrome, your participation in the study will be stopped. Tardive dyskinesia is a syndrome of potentially irreversible, abnormal, involuntary movements of the tongue, jaw, body, arms, and legs. There is no known treatment for tardive dyskinesia, although the symptoms may lessen or disappear completely if the antipsychotic medication is stopped.

E2. Protection Procedures:

E2a. Recruitment and Informed Consent: BDD and UD patients will be recruited from patients presenting to the outpatient clinic at University Hospital, University counseling and Psychological Services, the Child and Adolescent Clinic at Riley Hospital, and by advertisement. Unmedicated BDD and UD patients frequently present to the mood disorders clinic at IUSM for first time diagnosis, or for consultation for whether they should be on medication or not. Unmedicated BDD or UD patients in a mood episode will be either in a moderate depression (BDD; HAM-D >15 , < 25) There are a number of patients with mild to moderate forms of BD who present to our clinics or respond to advertisement who are not on medication.

To avoid any conflict of interest between the research and clinical missions of the Mood Disorders Clinic, patients who are in clinical treatment with Dr. Anand or Dr. Hulvershorn or in clinical treatment with another clinician supervised by them will only be recruited and consented to take part in the study by an independent clinician (Michael Webber, MD) not involved in the main study.

Patients in an unmedicated state who voluntarily wish to participate in the study will be recruited. Informed consent form approved by the Indiana University School of Medicine (IUSM) Investigational Review Board (IRB). For subjects under age 18 both parent and minor will sign the consent form. Subjects will have adequate time to read the consent form and make the decision to take part in the study by signing it. Subjects will be informed that their relationship with the treating clinic or the hospital will not be affected by whether they take part in the study or not. Subjects will take part in the study only after they have read and signed, in the presence of a witness, after being explained all the procedures of the study and had the opportunity to ask questions, the informed consent form approved by the IUSM IRB. Subjects will be given a copy of the signed

consent form and the original and copy will be kept for research records. Subjects will also be informed that they could terminate from the study at any time of their choice without affecting their relationship with the clinical staff or treating facility.

E2b. Protection against risk:

E2b1. Interview for psychiatric symptoms: experienced research personnel who will make every effort to minimize the subjects' apprehension will do the interview. Subjects will be clearly informed that they may stop the interview or not complete the tests at any time they choose. Subjects will also be informed that they can withdraw from the study at any time. In our experience, most subjects find going through their symptoms in detail helpful in understanding of their illness.

E2b2. Venous Blood draw for screening: a trained person who will make every effort to minimize pain or bruising will do Veni-puncture. If a subject has any of these complications then appropriate therapy will be instituted. The total amount of blood drawn in this study is around 10 cc or 1 tablespoon at the time of screening and 2 tablespoons on the day of the scan.

E2b3. MRI scan: A screening procedure will be used identical to that used for clinical MRI scanning. Subjects not passing the screening will be excluded from the study. Subjects will be fully informed about the fMRI procedures with a written consent form. We will also show them the MRI machine and the visual apparatus so that they are fully aware of the procedures involved. Patients reporting previous experience of anxiety in closed spaces will be excluded.

E2b4. Delay in treatment: Patients will only be included in the study if they are not on any psychotropic medications for at least 2 weeks at the time of the study. Therefore, there is no medications washout for the purposes of this study. Patients will undergo baseline ratings and MRI scans as soon as possible once they have been deemed to be eligible and have signed the consent form. From our experience of conducting the preliminary study we are usually able to arrange the baseline scan within a week once the patient is found to be eligible for the study and signs the consent form. Even in clinical settings, an evaluation period of 7 – 10 days is not uncommon before mild to moderately ill outpatients are started on medication. Subjects will be clinically assessed according to the criteria noted above, including lack of any significant suicidal or homicidal ideation and 17-item HAM-D score < 25, YMRS score < 10 and CGI severity score < 5 i.e., subjects will judged to be only moderately ill. Patients will also be assessed regarding whether they can tolerate to continue as outpatients while not being on medications for 7 – 10 days. If not, then subjects will not be included in the study and either started on treatment at Indiana University Hospital or referred to the clinician of their choice. It is expected that most of the patients identified by the inclusion and exclusion criteria will be moderately ill depressed outpatients and they will not need inpatient treatment. However, once the patient is included in the study and their condition worsens, an option is available for them to be admitted into an inpatient research unit and treated clinically as described below. The P.I. has been conducting imaging studies with this category of patients for past 15 years and has considerable experience in safely getting patients through these studies.

E2b5. Worsening of symptoms before the baseline scan: Several steps will be taken for close monitoring of the patients during this study to prevent significant worsening of symptoms. First, we will not include patients with very severe symptoms as described above in E2b4, who are grossly disabled because of their mood symptoms or who have any significant suicidal ideation, intent or plan. Second, once the unmedicated patients are included in the study, they will be monitored closely with clinical visits every 3 days until the time that they have the first scan. Patients will be monitored at each visit with the **(CSSRS)**. If patient's symptoms worsen, they will be monitored more frequently with the **(CSSRS)**. They will be given emergency phone numbers with which they can contact either the principal investigator or a psychiatrist on the research team who will be available 24 hours, 7 days a week. If despite these interventions, at any time a patient's symptoms worsen by more than 10 points on the HAM-D or the YMRS, or significant worsening is judged by clinical evaluation e.g. suicidal ideation, the patient will be taken out of the study and treated clinically as necessary with pharmacotherapy and/or psychotherapy as an outpatient or hospitalized as an inpatient. An inpatient clinical as well as research unit is available at the participating hospitals to hospitalize patients if necessary. Patients will be followed up clinically as outpatients or inpatients until their condition is

improved and stabilized. At that point they will be referred to the clinician of their choice and referral will be arranged.

E2b6. Occurrence of hypomania or mania in UD patients during supervised antidepressant treatment phase and during follow-up: the development of subthreshold hypomania or full blown hypomania or mania are the endpoints for this study after which the subject will be terminated from the study and appropriate clinical treatment instituted and after stabilization the subjects will be referred to the treater of their choice. If a patient is deemed to require hospitalization it would be arranged at one of inpatient psychiatric units within the Indiana University School of Medicine. It should be noted that the risk of antidepressant induced mania has been reported only in BD I subjects and there is controversy regarding whether antidepressants can do the same in BD II disorder let alone in HRUD subjects[73]. In any event, as discussed above, absent a firm diagnosis of BD based on past (hypo)manic symptoms clinically these patients cannot be started on lithium, anticonvulsants or atypical neuroleptics because of concern for side effects and the long-term nature of therapy. Therefore, HRUD subjects either get no treatment and needlessly continue to suffer from depression or more frequently they are treated with antidepressants in a clinical setting (as proposed in this application). However, close monitoring is advisable which the subjects will get in this study and which otherwise they may not get. We have elected to use selective serotonin reuptake inhibitors (SSRIs) or bupropion because there is some evidence that they are less likely to induce (hypo)mania.

E2b7. Treatment and Referral after the study: While the BD subjects is being screened and the fMRI scan being arranged and for the UD patients when they are coming to the end of the 24 months supervised treatment, we will start the process of identifying with the patients where they would like to be referred for treatment. A range of treatment options are available in the Indianapolis area including private university and other outpatient clinics and state hospital. We already have a handout available for all the addresses and phone numbers for patient to make an appointment which we routinely give to our research subjects after they finish the study with us. In this study, we will additionally call up the treater of the patient's choice and make sure that subjects will have an appointment. UD subjects will still be followed up every 3 months until the end of the study. As the patients included in this study will present as unmedicated because either they have never been diagnosed before or for some other reason elected not to take medication that was recommended (not an uncommon situation for many young patients) this process will help in patient complying and following through with treatment. In this way we will be able to get patients engaged in treatment which otherwise they are unlikely to get on their own.

E2b8. Loss of confidentiality: Documents which contain patient-identifying data will be kept in locked filing cabinets, with access restricted to authorized investigators participating in the research, and secretarial and administrative personnel who have been informed about the requirement to maintain confidentiality. Computer records that contain patient identifying data will be secured by passwords. Patients will not be identified in any reports or publications that may result from this study. University policies regarding HIPAA regulations for research subjects will be followed.

E2b9. Tolerability of fMRI procedures by psychiatric patients: Patients and healthy subjects may become uncomfortable or agitated during the scan. Subjects reporting symptoms of being uncomfortable in closed spaces will not be included in the study. The procedures for fMRI such as stimulus induced activation measurements have been used extensively in our radiology facilities. In the depression study and bipolar patients for which preliminary data has been provided patients tolerated the procedures very well. Other investigators at IUSM have used similar procedures with patients with schizophrenia and substance abuse disorders and the procedures have been well tolerated. The radiology imaging area is well staffed with personnel who have extensive experience in doing fMRI studies. A crash cart and other equipment and medical personnel for medical emergencies are also readily available. Throughout the procedures the patients comfort will be closely monitored and the study will be terminated if needed.

E2b10. Antidepressant treatment: Patients will be monitored and educated in detail for side effects of antidepressants. If a patient develops hypomania or mania they will be terminated from the

study and referred to the treater or their choice or in the worse case to an inpatient unit. The same will be done if the patient develops other unwanted side effects or desire treatment with another antidepressant or modality which cannot be provided in the study.

E2b11. Antipsychotic Treatment: Antipsychotics are generally well tolerated. All subjects will be monitored closely at each visit for any side effects. They will be given emergency phone numbers with which they can contact either the principal investigator or a psychiatrist on the research team who will be available 24 hours, 7 days a week.

E3. Potential benefits to subjects and others:

The risk/benefit ratio of the proposed study is favorable. The MRI is a routinely used medical procedure with little known harmful effects. In addition, the patients will have a thorough assessment for mood disorder and will have a better knowledge of how to get treatment for their illness. Subject's mental and physical state will be monitored closely and regular clinical treatment instituted immediately if subject's condition worsens and they cannot continue to participate in the study. Subjects will be compensated for their time to do the study and will be paid $25 for the screening visit and $75 for each MRI scan. UD subjects will also receive free treatment for the first 24 months of the study. They will also be paid $25 for the first six months of study visits and $50 for the remaining visits. The risk-benefit ratio of this study is quite low.

E4. Importance of knowledge to be gained

It is critical to be able to differentiate between unipolar depression (major depression or UD) and bipolar disorder depression (BDD). In particular, it is important to differentiate between bipolar II depression (hypomania without significant impairment of function and major depressive episodes) and UD. Understanding the neurobiology of why some patients only suffer from a depressive illness while others suffer from both (hypo)mania and depression is also critical in terms of understanding the etiology of these similar but at the same time very different illnesses. In addition, the ability to be able to make the correct diagnosis determines the type of treatment (mood stabilizers alone, antidepressants alone or a combination) indicated and has important implications in terms of long-term prognosis. Despite phenomenological and neurobiological research for past several decades, no single clinical or biological finding is available at present which can differentiate between the two illnesses. This study will investigate potential brain imaging markers for differentiating between the two groups. In the future, if reliable biomarkers are identified then it may be even be possible that a choice between antidepressants vs. mood stabilizers can be made at baseline and unwanted side effects and precipitation of mania can be avoided.

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