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Multi-modal assessment of GABA function in psychosis

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Table of Contents

	Page
Protocol Summary	iii
1.0 Introduction	1
2.0 Background and Significance	1
3.0 Specific Aims and Study Outcome Measures	3
4.0 Study Design.....	4
5.0 Study Participants and intervention.....	4
5.1 Number of Subjects	4
5.2 Participant Inclusion/Exclusion Criteria	5
5.3 Strategies for Recruitment and Retention	6
5.4 Description of Intervention	7
5.5 Intervention Assignment Procedures	7
6.0 Study Schedule and Assessments	8
6.1 Consenting procedures	8
6.2 Screening assessment visits.....	9
6.3 MRI sessions.....	9
6.4 Concomitant medications	11
6.5 Reimbursement of participants	11
6.6 Participant discontinuation/withdraw	11
7.0 Assessment of Safety and Regulatory Reporting	11
7.1 Data Safety and Management Plan	11
7.2 Event Windows, Missed Assessments, Missed Sessions and Protocol Deviation Reporting.....	13
8.0 Clinical Monitoring.....	14
9.0 Statistical Analysis	14
10.0 Source Documentation.....	16
11.0 Quality Control and Assurance.....	16
12.0 Ethics and Protectionn of Human Subjects.....	16
12.1 Ethical Standard and IRB Review	16
12.2 Subject Confidentiality	16
12.3 Potential Risks and Minimization of Risks.....	17
12.4 Potential Benefits and Justification of Risk.....	20
13.0 Data Handling and Record Keeping	20
14.0 References	21

Protocol summary

Considerable evidence implicates GABAergic dysfunction in the psychosis spectrum, which includes schizophrenia and bipolar disorder. GABAergic agents treat psychosis spectrum patients, often for anxiety, and the response of catatonic patients to GABA-enhancing benzodiazepines suggests a deeper link between GABA and psychosis. Post-mortem work strongly implicates GABAergic interneurons in these patients, but the links between these findings, clinical phenotype and therapeutics are far from clear. Accordingly, this proposal will undertake a transdiagnostic approach to elaborate linkages between GABA and the negative affective states (NA, e.g. anxiety, stress sensitivity) that are common across this psychosis spectrum. Using a multimodal neuroimaging strategy, magnetic resonance spectroscopy (MRS) will be combined with a pharmac-fMRI (phfMRI) challenge paradigm measuring blood oxygenation level dependent (BOLD) changes. MRS studies of GABA levels in the psychosis spectrum have yielded inconsistent findings, which this proposal will address by positing two processes: 1) Elevated GABA in early psychosis patients in the rostral medial frontal cortex (rMFC), and 2) Reduced GABA associated with more NA. The investigators will pursue preliminary data showing that NA may be linked to low GABA levels in the rMFC, and when controlling for NA, early psychosis patients have higher GABA in the rMFC.

Study Design: The study will employ a cross-over design to test hypotheses. In Aim 1, GABA MRS will be used to assess GABA levels in psychosis spectrum patients, comparing early episode patients (50% not taking antipsychotics) with healthy controls, as well as schizophrenia, schizoaffective and bipolar patients. By probing three distinct voxels in the medial frontal cortex, the investigators will show specific regional effects in the rMFC and demonstrate that antipsychotic medication is associated with reduced GABA levels. In Aim 2, the phfMRI challenge paradigm with a benzodiazepine will be used to measure dynamic GABA function. In published work, the investigators have demonstrated abnormal BOLD response (increased rather than decreased signal) in schizophrenia in the dorsomedial prefrontal cortex (dmPFC). This BOLD response to a GABAergic manipulation is correlated with NA in both patients and controls, consistent with a transdiagnostic dimension. The project will extend these findings across the psychosis spectrum. As the regulation of GABA function plays a critical role in the excitatory/inhibitory (E/I) balance, the phfMRI probe serves as a proxy measure of E/I balance, and it will be used to test the hypothesis that this dynamic measure is associated with NA and GABA concentration measured with MRS.

Population: Early psychosis patients (16-35 yrs), schizophrenia/schizoaffective patients (16-60 yrs), bipolar patients (16-60 yrs), healthy control subjects with no significant mental health problems.

Site: University of Michigan

Study Duration: 5 years for intervention

Subject Participation Duration: 4-6 weeks

Study Design: Double-blinded, cross-over

Number of subjects: Patients: 120 analyzable; Controls: 80 analyzable; Total enrolled: 232

Description of Intervention: Mechanistic, basic experimental study involving humans, one-time dose of lorazepam versus inactive placebo

Estimated Time to Complete Enrollment: 4 years

1.0 Introduction

Psychotic disorders -- schizophrenia, schizoaffective disorder and bipolar I disorder -- comprise some of the most debilitating psychiatric illnesses, accounting for more than \$150 billion (2016 adjusted) in direct and indirect costs in the United States.^{1,2} While current diagnostic nomenclatures place schizophrenia and bipolar disorders at the non-affective and affective poles of a Kraepelinian diagnostic spectrum, genetic, biological and clinical evidence indicates that the psychotic disorders share common neurobiological substrates,³⁻⁷ leading to calls to upgrade the 19th century nosology on which the current systems are based.^{8,9} This proposal will follow in this transdiagnostic approach, embodied in the Research Domain Criteria, by focusing on a molecular system (gamma-aminobutyric acid -- GABA), a common clinical phenotype (NA) and neurocircuitry implicated in affect regulation.

2.0 Background and Significance

Negative affect in the psychosis spectrum To begin, we consider the construct of negative affect(s). For simplicity, we will use the singular, affect, but we refer to a spectrum of related phenomena, such as anxiety, dysphoric states, depressed mood (not necessarily a major depressive episode), and sensitivity to minor, everyday stresses -- **clinical phenomena very familiar to any clinician working with psychosis patients**. This is not a new idea. An over-sensitivity to minor stress was noted by Bleuler,¹⁰ and Meehl described the phenomena of 'aversive drift' -- the tendency towards negative affective states that preceded the development of full-blown schizophrenia.¹¹ Schizophrenia patients report more negative experiences in daily life,¹²⁻¹⁵ and experience emotional stimuli in laboratory settings with a more negative bias.¹⁶⁻¹⁸ Bipolar disorder is also marked by significant experiences of negative affect between episodes.¹⁹⁻²¹ Depression is, of course, a diagnostic feature of bipolar disorder, and it occurs in up to 50 % of schizophrenia patients.^{22,23} Analysis of symptom profiles in schizophrenia consistently reveal independent factors of depression/anxiety, distinct from positive and negative symptoms.²⁴⁻²⁶ More recently, Dickenson and colleagues have identified a "distress" (NA) subtype in schizophrenia patients, distinct from deficit and 'low symptom' patients.²⁷ Our own work parallels Dickenson's, as we do not find a relationship between NA and negative symptoms,^{28,29} and NA is an independent predictor of functional outcome.²⁸ There is a relationship between NA and reality distortion,³⁰⁻³³ as one might expect, but longitudinal work has shown that negative cognitions and depressed mood lead to paranoia, but paranoia does not lead to NA³⁴, although other studies have noted a bi-directional influence.³⁵ The construct of NA is broad, but NA measures have significant prognostic value, predicting hospitalization at one year³⁶ and poor functional outcome in schizophrenia^{37,38} and bipolar disorder.¹⁹ In summary, **NA is effectively being re-discovered by the research community as a clinically significant phenomenon for schizophrenia and bipolar disorder**.

Treatment with GABAergic agents in schizophrenia and bipolar disorder Medications that potentiate GABA transmission, such as benzodiazepines (BDZ) and valproic acid, are used to treat patients in the psychosis spectrum, frequently for NA such as anxiety, but also as mood stabilizers for bipolar disorder.³⁹⁻⁴⁴ A recent Cochrane review found no support for BDZ as effective augmentation agents in schizophrenia,^{45,46} although the authors acknowledged the poor level of evidence and their inability to conclude that BDZ did *not* help. In fact, they acknowledged support for the use of BDZ for acute agitation and anxiety in schizophrenia. It is central to our proposal that anxiety is a type of NA, common in the psychosis spectrum.^{47,48} Social anxiety is particularly common in schizophrenia, occurring in 13 – 26% of patients⁴⁹⁻⁵¹, with up to 60% reporting some form of socially-triggered anxiety.⁵² Social anxiety is often difficult to separate from paranoia, and in the psychosis spectrum, the two clinical phenotypes may share a deeper connection, such as a GABAergic mechanism.

BDZ are not antipsychotics in the traditional sense, but diazepam has been shown to reduce psychotic relapse in schizophrenia patients,⁵³ and a iomazenil, a partial inverse agonist of the benzodiazepine receptor, increases psychotic symptoms in schizophrenia patients.⁵⁴ Another line of evidence linking GABA to the psychosis spectrum comes with catatonic states, occurring in affective and non-affective psychosis. Catatonia often responds dramatically and rapidly to benzodiazepines,^{55, 56} and reduced GABAergic binding has been identified in the right orbitofrontal cortex of catatonic patients.⁵⁷ Catatonia patients often show very strong emotional states, sometimes described as being 'immobilized by anxiety'.^{58, 59} However, most treatment with BDZs is not as successful as the treatment of catatonia, highlighting **the opportunity to exploit these important clues about GABAergic mechanisms and use this knowledge to develop better treatments.**

Evidence of GABAergic abnormalities from post-mortem studies Post-mortem studies of psychosis spectrum patients have demonstrated abnormalities in GABAergic systems, prominently implicating parvalbumin-positive interneurons (PVI), including reductions in parvalbumin expression and reductions in the 67-kDa isoform of the synthetic enzyme for GABA, glutamic acid decarboxylase (GAD67) in schizophrenia⁶⁰⁻⁶³ and bipolar patients.^{62, 64, 65} Abnormalities occur in pre- and post-synaptic terminals,⁶⁶ and the peri-neuronal nets surrounding PVIs are reduced.⁶⁷ These alterations have been observed throughout the cortex⁶⁸⁻⁷⁰ and hippocampus.^{71, 72} PVIs are fast-spiking neurons, particularly vulnerable to a variety of insults, as diverse animal models have shown,⁷³⁻⁷⁷ PVI damage may be a final common pathway of many serious mental illnesses, including bipolar disorder, autism, some depressions, and schizophrenia, mediated by converging influences of peri-natal stress, early life stress and inflammation, all triggering oxidative stress.⁷⁸ It is important to point out, however, that the clinical significance of PVI abnormalities is unknown, and there is no direct evidence linking PVI findings with the clinical response to GABAergic agents. Indirect arguments for a role in cognitive function highlight the role of PVIs in cortical gamma oscillations,⁷⁹⁻⁸¹ which are associated with integrative cortical functions such as attention and working memory,⁸²⁻⁸⁴ functions abnormal in schizophrenia.^{85, 86} Many other theories have been proposed,^{60, 61, 78, 87-90} and in some cases, PVI abnormalities are conceived of as secondary to other deficits in the psychosis brain. For example, it has been suggested that GABAergic interneurons might falsely sense reduced, excitatory pyramidal cell activity, and reduced GAD67 production may reflect the attempt to rebalance excitation/inhibition.^{61, 87} One of the challenges in the field has been sorting out primary from secondary and compensatory changes, but is clear that the nervous system is highly adaptive and simple measurements finding an enzyme or receptor up or down risk over-simplification. Although no single study can definitively untangle these issues, it is clear that multimodal approaches are needed.

Functional measures of GABA in the psychosis spectrum In vivo studies of GABA function are limited (see our review⁹¹), but some intriguing patterns have appeared. Overall, the density of GABA receptors (GABAR) in schizophrenia does not appear abnormal in radioligand studies,⁹²⁻⁹⁶ Magnetic resonance spectroscopy studies of schizophrenia patients measuring GABA concentration upon first glance appear somewhat contradictory, although closer examination reveals some important findings that bear follow-up studies. Findings are complicated by different voxel placements that have been lumped together in meta-analytic studies,^{97, 98} as well as confounds of age, stage of illness and medication status. We focus on voxels in the medial frontal cortex, since this region is associated with emotion and emotion regulation, relevant to NA.⁹⁹⁻¹⁰¹ Three separate groups have found elevated GABA concentrations in the rMFC (an area which includes overlying anterior cingulate cortex and overlying medial prefrontal cortex – see Figure 1) in early, unmedicated psychosis patients,¹⁰²⁻¹⁰⁴ while studies of older medicated patients have revealed no differences^{102, 105, 106} or reductions compared to healthy controls.¹⁰⁶ On the other hand, studies using pMFC voxels (usually including dorsal anterior cingulate cortex [dACC]) have found either reduced GABA levels,^{103, 107} or no difference from controls.¹⁰⁷⁻¹⁰⁹ A recent pre-post treatment study found that elevated rMFC activity was decreased with

antipsychotic treatment,¹⁰⁴ but a study of a pMFC voxel found no effect of treatment.¹⁰⁹ Some data suggests that age is associated with decreased signal, at least in the pMFC,^{103, 106, 109} although this was not born out in meta-analytic studies.^{97, 98, 110} However, these meta-analytic studies have suffered from lumping together disparate voxels within the MFC, so that only a handful (often 2 or 3) of studies overlap. Voxel placement may be critical, as pMFC and rMFC involve different functions, with the pMFC more involved in conflict/interference processing, as well as pain, while rMFC is more associated with reward, fear and social processing.¹¹¹ Studies of bipolar patients are even fewer in number (< 8 with MFC voxels) than schizophrenia, with mostly non-significant findings in the MFC,¹¹²⁻¹¹⁷ and similar confounds. Given the divergent findings and relatively small numbers of studies, it would appear that multiple factors influence GABA levels, such as medication and illness stage, and more data are needed to sort out the various processes. As we show in our preliminary data, rMFC GABA may be associated with NA, which leads to our Aim 1 to examine GABA levels in three voxels of the MFC, and in different illness stages.

Translational impact of linking GABAergic dysfunction to NA Linking NA to GABA activity will improve potential opportunities for exploiting GABAergic manipulations. We will show in our preliminary data that NA is a distinct dimension of the psychosis spectrum, which strongly predicts level of functioning, independent of psychosis, cognition, and negative symptoms, and is therefore an important clinical target. There is a growing recognition in the field that managing susceptibility to stress and reducing NA is a critical part of treating psychosis patients, particularly in the early phase of the illness and for people at high risk of psychosis.^{118, 119} **This proposal will provide a mechanistic grounding for this clinical observation which can guide the development of new therapies.**

This mechanistic grounding has been partially recognized in animal models of PVI deficits. For example, rats given prenatal infusion of methylazoxymethanol (MAM) exhibit heightened anxiety-related behavior as adults, increased firing of basolateral amygdala (BLA) neurons and larger increase in BLA theta power in response to conditioning stimuli. These behaviors are normalized by peripubertal administration of a BDZ.^{73, 120} GAD1 (the enzyme which produces the GAD67 protein) knock-down mouse models have shown impaired fear extinction¹²¹ and a GAD1 knock-down model developed by one of our consultants on this project, Dr. Kazu Nakazawa, has demonstrated impaired emotion regulation and BLA over-activation, but preserved working memory function.¹²² In spite of these animal models, theoretical work interpreting GABAergic deficits in psychosis has largely focused on cognitive deficits^{85, 123} and positive symptoms.⁸⁷ **Findings from the work proposed here would expand that phenotype to more explicitly include NA as a target for drug development and animal models.**

Pharmacologically, benzodiazepines are currently used in psychosis spectrum patients, but they have limited efficacy and many downsides, such as poor selectivity for GABAR subtypes and high liability for abuse. A study with an α -2/3 selective subtype for the GABAR initially showed promise,¹²⁴ only to fail in a larger, controlled trial.¹²⁵ However, relative to chlorodiazepoxide, this compound only has 11% efficacy at α -2 GABAR.¹²⁶ Alternative molecular targets include GABA α -5 specific agents, which may avoid sedative side effects and abuse potential,¹²⁷ or modulators of the Kv3.1b potassium channel which is specific to PVI,¹²⁸ Numerous subtype-selective agonists, antagonists, positive and negative allosteric modulators (including dual allosteric modulators) are currently in various stages of pre-clinical development.¹²⁹ While targeting GABA mechanisms in psychosis is not a new idea,^{126, 130-133}, the behavioral endpoints have not always been clear. **Results from the project should encourage additional research around manipulating the GABAergic system in order to develop new targets.**

3.0 Study aims and outcome measures

Specific Aim 1: Assess MFC GABA concentration in psychosis spectrum patients with MRS We will test the hypothesis that (H1.1) when controlling for NA, GABA in rMFC will be higher in EP, compared to HC, and a corollary hypothesis that (H1.2) rMFC GABA will be higher in unmedicated EP compared to medicated EP patients. On the other hand, in all groups, including HC, we predict that (H1.3) greater NA will be associated with lower rMFC and dmPFC GABA. We will also conduct exploratory analyses, comparing the three voxels and testing the hypotheses that pMFC and dmPFC GABA is lower in SCZ and BAD, compared to HC, and that illness stage is associated with higher GABA in rMFC.

Intervention: none (no clinical trial)

Primary outcome measure: GABA concentrations measured by MRS

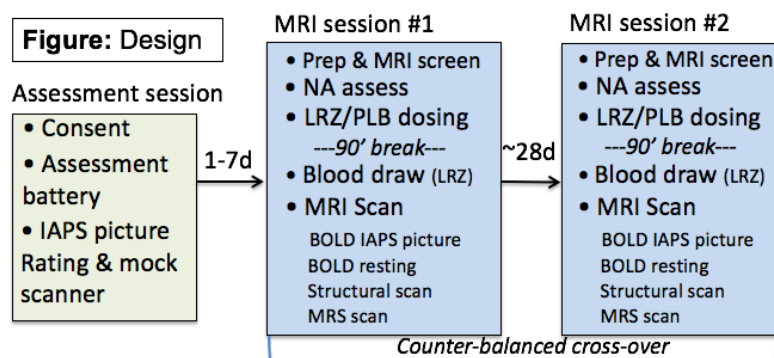
Specific Aim 2: Assess dynamic GABAergic function in psychosis spectrum patients with a phfMRI probe We will test hypotheses that (H2.1) while viewing affective stimuli, patient groups will show increased Blood oxygenation level dependent (BOLD) signal with phfMRI in dmPFC after LRZ challenge; and (H2.2) all groups will show a correlation between LRZ-induced BOLD change in the dmPFC and NA. To link GABA with our proxy measure of E/I balance, we predict that (H2.3) the LRZ-induced BOLD change will inversely correlate with dmPFC GABA. Secondary hypotheses will test for a dose-response effect for the preceding hypotheses.

Mechanistic intervention: One-time lorazepam challenge

Primary outcome measure: Blood oxygenation level dependent (BOLD) signal change in MFC after lorazepam (LRZ) challenge while viewing affective pictures.

4.0 Study design

Design: Placebo-controlled, randomized, double-blinded cross-over administration of a single oral dose of lorazepam, in two sessions (placebo or active) during MRI, approximately 28 days apart. The order of placebo versus lorazepam will be counterbalanced across subjects.



Duration of study: Approximately 4-6 weeks, depending upon scheduling, from initial screening to completion of study procedures. Note: The assessment session may be split over 2-3 days.

5.0 Study Participants

5.1 Number of Subjects

Three groups of psychosis spectrum patients, each with 40 analyzable participants, will be studied: 1) EP -- early psychosis patients, 2) SCZ -- schizophrenia or schizoaffective disorder patients, and 3) BAD -- bipolar disorder with histories of psychosis. EP patients, (≥ 16 and ≤ 35 yrs), will have a diagnosis of any primary psychosis (not caused by ingestions or general medical conditions), which could include schizophrenia, psychotic bipolar disorder, major depressive disorder with history of psychosis, schizophreniform disorder or other specified/unspecified psychotic disorder. Diagnosis will be determined by Structured Interview for Psychosis Syndromes/Scale of Psychosis-Risk Symptoms (SIPS/SOPS),¹³⁵

EP patients will also undergo the Structured Interview for Clinical Disorders (SCID),¹³⁶ if they do not have a clear diagnosis of schizophrenia or schizoaffective disorder. By SIPS criteria, subjects will have either 'Presence of Psychotic Symptoms' or 'Brief Intermittent Psychotic Syndrome (BIPS),' and onset of positive symptoms (>6 on any SOPS positive-symptom scale) within 2 years. Half of this sample will not be taking antipsychotic medications (past history of treatment allowed if with > 4 weeks past), while the other half will be permitted to take psychotropics (excluding those below).

We will stratify our recruitment into equal-sized early (2 years to 5 years duration of positive psychotic symptoms) and chronic cohorts (> 5 years duration), and we will also recruit similar proportions of gender between the EP, SCZ and BAD groups. Healthy comparison subjects will be recruited to match the age, gender, and highest parental education (HPE) level of the EPP groups and the SCZ+BAD groups, except that healthy control subjects will be ≥ 18 years of age (no minors). Control subjects will be without histories of lifetime or current psychiatric disorders (simple phobias permitted), after assessment by SCID interview.¹³⁶

A total of 232 subjects will be enrolled (48 SCZ, 48 BAD, 48 EP patients, 88 control subjects). Enrollment goals are inflated 20% over targets for patients and 10% over target for controls to account for dropouts and achieve a final count of 40 subjects for each patient group and 80 in the control group.

5.2 Participant inclusion/exclusion criteria

EP subjects, Inclusion/Exclusion criteria: (1) Meets DSM5 criteria for schizophrenia, schizophreniform disorder, schizoaffective disorder, bipolar disorder type 1, with history of psychosis, major depressive disorder, with history of psychosis, brief psychotic disorder, or other specified/unspecified psychotic disorder; or, meets SIPS criteria for Presence of Psychotic Symptoms or Brief Intermittent Psychotic Syndrome (BIPS) (2) age 16-35; (3) Positive symptom onset ≤ 2 years; (4) No history of active substance use disorder in the past 2 months; (5) Not currently on an involuntary treatment order; (6) Not taking chronic narcotics, barbiturates, benzodiazepines; (7) Absence of suicidal thoughts with plans or intentions, as assessed by C-SSRS; (8) No increases in psychotropic medication within the prior 4 weeks reflecting clinical instability and for approximately half of EP sample, or not taking antipsychotic medication within the prior 4 weeks. Patients may take as needed doses of benzodiazepines as clinically prescribed, as long as those doses are not required within 5 half-lives of an MRI session.

SCZ and BAD subjects, Inclusion/Exclusion criteria: (1) Meets DSM5 criteria for schizophrenia, schizoaffective disorder, or other specified/unspecified psychotic disorder (SCZ); or bipolar disorder type 1, with history of psychosis (BAD); (2) age 16-60; (3) Duration of positive symptom onset > 2 years; (4) No history of active substance use disorder in the past 2 months; (5) Not currently on an

involuntary treatment order; (6) Not taking chronic narcotics, barbiturates, benzodiazepines; (7) Absence of suicidal thoughts with plans or intentions, as assessed by C-SSRS; (8) No increases in psychotropic medication reflecting clinical instability within the prior 4 weeks. Patients may take as needed doses of benzodiazepines as clinically prescribed, as long as those doses are not required within 5 half-lives of an MRI session.

Healthy control subjects, inclusion/exclusion criteria: (1) No history of past or current mental illness (except for simple phobias), but prior h/o substance abuse OK if in remission for greater than 5 years; (2) Not taking any medication, prescription or non-prescription, with psychotropic effects (birth control medications allowed); (3) No first-degree family members with a history of a psychotic disorder (including bipolar disorder); (4) Age 18 – 60.

All subjects, inclusion/exclusion criteria: (1) Ability and willingness to give informed consent to participate; (2) If a woman of child bearing age, not pregnant or trying to become pregnant; (3) No history of serious neurological illness or current medical condition that could compromise brain function, such as liver failure; (4) No history of closed head injury, e. g., loss of consciousness > ~5 min, hospitalization, neurological sequela; (5) Ability to tolerate small, enclosed spaces without anxiety; (6) Vision equal to or better than 20/40 on a Snellen chart, with correction if necessary; (7) No metals, implants or metallic substances within or on the body that might cause adverse effects to the subject in a strong magnetic field, or interfere with image acquisition, e. g., aneurysm clips, retained particles (metal workers excluded), neurostimulators, foil-backed transdermal patches, carotid or cerebral stents, CSF shunts; magnetic dental implants, ferromagnetic ocular implants, pacemakers, automatic implantable defibrillators; (8) Size compatible with scanner gantry, e. g., men over 6 feet tall that weigh more than 250 lbs, men under 6 feet tall that weigh over 220 lbs, women over 5'11" tall that weigh more than 220 lbs, or women under 5'10" tall that weigh more than 200 lbs. Subjects of these weights or greater typically have difficult fitting into the fMRI scanner properly; (9) No subjects with hypersensitivity to benzodiazepines or to components of the formulation; (10) No subjects with disorders affected by benzodiazepines, such as compromised respiratory function, e.g., chronic obstructive pulmonary disease, or acute, narrow angle glaucoma.

5.3 Strategies for recruitment and retention

5.3.1 Recruitment of patients: Subjects will be recruited from several clinical sites and registries available to the PI. Allowing for dropouts and unusable data, we will recruit 20% over our target (48 patients for EP, SCZ & BAD groups). The PI directs the Program for Risk Evaluation and Prevention (PREP) clinic, which provides evaluations, research and treatment for those in the early stages of psychosis, the only program in southeastern Michigan focusing on early psychosis individuals, serving as a referral source for evaluations sought by primary care providers and other mental health practitioners and school-based counselors. The PREP program typically evaluates 1-2 patients/week.

Methods of recruitment: Two registries, in which patients have agreed to be contacted about possible research projects, will be utilized. Co-investigator McInnis oversees the Heinz C. Prechter Longitudinal Study of Bipolar Disorder (HUM0000606), which follows 514 patients with bipolar I disorder, and enrolls approximately 120 new participants/year. Dr. Taylor also maintains a research registry (HUM00123648) of nearly 100 SCZ patients, which is open to patients who have participated in studies, mostly involving neuroimaging by the PI and his collaborators. Recruitment will also occur through community advertisements (flyers, particularly in local clinics treating patients with psychotic disorders), and social media (e.g. Facebook/Instagram campaigns). Other means of reaching out to clinicians, such as talks, emails and fax-blasts will also be used. The study will also be listed on UMHealthresearch.org.

Michigan Medicine electronic medical records: To identify potential patients from this large patient pool, we will use the University's self-served data tools (DataDirect PHI, EMERSE) to query

electronic medical records and obtain IRB approval to contact potential participants. We will target patients aged 16 to 60 with chief complaints or diagnoses of a primary psychotic disorder (e.g., schizophrenia, schizoaffective, unspecified psychosis, etc.). Additionally, we will use EMERSE to perform searches in all text containing keywords such as “psychosis,” “delusions,” “hallucinations,” “mania,” and “paranoia”.

MyUofMHealth.org (also known as Patient Portal): This is designed for Michigan Medicine patients to access their medical records, and for communication between patients and providers/clinics. MyUofMHealth also provides the capability for consented patients to view the research studies they are enrolled in and to indicate interest in participating in a research study via targeted study invitations.

Recruitment processes: Pre-screening will occur when the study coordinator hears about a potential participant (through examining clinical records), or potential participant contacts the study team, via phone or email. If appropriate, e.g., for hospitalized patients, the study coordinator will make contact with clinical staff treating potentially eligible patients to discuss the suitability of a patient for the study. Clinical personnel will then ask potential subjects (usually in person, but a mailing may also be used) if they would like to discuss a research study. If the clinician happens to be a member of the research team, the same process will occur, although with special attention to avoid a coercive interaction by emphasizing the voluntary nature of research participation. The P. I. and his recruitment team will have regular meetings with clinicians, both to remind them of the study, and to inform them about the necessity of stressing the voluntary nature of participation. For all patients who are enrolled in the study, we will contact their clinician (psychiatrist or case manager), if not already done during pre-screening, notify them of their patient's interest and verify that substance abuse/dependence, or vulnerability to trauma, are not obstacles to the patient's participation. Pre-screening will also include contact with potential participants, usually by phone, but also by secure email if the potential participant prefers, to discuss a series of eligibility questions. Patients who appear eligible in the pre-screen will be invited for an initial assessment session.

Ethical considerations around recruitment: Recruiting patients with a psychotic illness requires careful attention to the needs and capacities of the patient, as well as scrupulous attention to ethical issues and potentials for coercion that exist in the clinical research setting. As early psychosis patients are often minors, or still dependent financially and emotionally on their parents, routine clinical practice in PREP involves family members, and this is often the case for a research study. While the risk for this type of MRI study is minimal, the procedures and setting can be stressful for psychosis patients. The voluntary nature of participation will be emphasized, differentiating it from routine clinical care. Patients are given ample time to examine the consent form and materials and encouraged to think about participation and discuss it with family members, if appropriate. Recruitment processes are divorced as much as possible from clinical care.

Assessment of capacity to consent/assent for the study: Patients with schizophrenia and bipolar disorder have cognitive impairments, but that does not mean they do not have decisional capacity to participate in a research study. In order to determine that our patient subjects are able to give consent/assent, a brief, 8-item questionnaire will be administered, modified from the University of San Diego Brief Assessment of Capacity to Consent (UBACC) to fit the particulars of this study.¹³⁷ After the informed consent is reviewed with the participant, they are given this questionnaire. Potential participants who cannot respond satisfactorily will not be permitted to enroll in the study. Per the published guidelines on assessing capacity, subjects will be required to score >12 out of 16 possible points on this scale. The published version of the scale recommends a cut-point of 14.5 out of 20, which corresponds to the cut-point of 12 on this adapted scale (two questions not included, per suggestions of Jeste et al,¹³⁷ to make the scale appropriate for this basic experimental study in humans).

5.3.2 Recruitment for Healthy Control Subjects: These subjects will be recruited by community advertisements, social media, and word of mouth. A website, maintained by the University of Michigan,

provides a central recruitment portal for all subjects in this study (UMHealthResearch.org). Potential study participants go to the website and search for protocols in which they can participate. The website also features an email notification system that will let potential participants know when a new study is recruiting subjects.

5.3.3 Retention of patients from screening through scanning With a psychotic disorder, there is some concern around patients worsening through the course of a study, although with the short duration of this study, the chance of symptom worsening is fairly low. The experience of the study team in the PREP clinic provides an appropriate framework in which to manage these patients. Because of diagnostic uncertainty in early psychosis patients, it is clinically appropriate to observe a patient over a several month period to ascertain the need for antipsychotic medication. The patients typically seen in PREP do not routinely require inpatient hospitalization; thus, they can be safely managed as outpatients. Many patients are not interested in taking antipsychotic medications. Therapists experienced in Cognitive Behavioral Therapy for Psychosis (CBTp) see the patients weekly, or more often if necessary. As always, the safety of our participants will over-ride the experimental protocol. If a patient is too unstable or impaired to participate, they will be withdrawn from the study.

Other steps to encourage retention: Reminder calls/emails/texts (depending upon preference) are provided to subjects before scheduled sessions. Because transportation can be a problem, cabs will be provided for participants to make sure that transportation is not a hindrance to continued participation.

5.4 Description of the intervention

5.4.1 Mechanistic intervention: Subjects will be randomized to receive either a dose of lorazepam (LRZ) or placebo (PLB) for the first MRI session, and then the other condition in the second MRI session. To study dose-dependent effects of LRZ, subjects will be assigned to one of two dose levels (*between subjects*: 0.01 mg/kg or 0.02 mg/kg). Subjects will receive oral solution containing either lorazepam or placebo. Subjects will sit quietly for approximately 90 minutes before entering the MRI scanner, to allow LRZ levels to attain their peak.

5.4.2 Regulatory status of intervention: Lorazepam is an FDA-approved medication, with specific indications for anxiety, insomnia, premedication for anesthetic procedures and status epileptics. The use of lorazepam in this study does not align with any of the approved indications; however, use for this study as a probe of brain physiology meets criteria for IND exemption according to 21 CFR 312.2(b):

- The drug product is lawfully marketed in the United States.
- The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication and there is no intent to use it to support any other significant change in the labeling of the drug.
- The investigation is not intended to support a significant change in the advertising for the drug.
- The investigation does not involve a route of administration, dose, patient population, or other factor that significantly increases the risk (or decreases the acceptability of the risk) associated with the use of the drug product (21 CFR 312.2(b)(1)(iii)).
- The investigation is conducted in compliance with the requirements for review by an IRB (21 CFR part 56) and with the requirements for informed consent (21 CFR part 50).
- The investigation is conducted in compliance with the requirements of § 312.7 (i.e., the investigation is not intended to promote or commercialize the drug product).

5.5 Intervention assignment procedures

5.5.1 Randomization and blinding: Subjects will be randomized to receive lorazepam first or placebo first. The randomization schedule will ensure an equal distribution across group (schizophrenia, bipolar, control), LRZ dosage and chronicity. Randomization lists will be generated by the study statistician and communicated to the Investigational Drug Service that will prepare dosages either as the oral formulation of lorazepam, or a placebo formulation. Participants and the study coordinator, who will be interacting with the participants, will be blind as to whether the participants receive LRZ or PLB. The data manager and study statistician will be aware of the intervention assignment. In the event of any adverse event requiring unblinding, the PI can be made immediately aware of intervention assignment.

5.5.2 Scheduling of MRI sessions: In order to reduce variability associated with menstrual cycles and GABA levels, both MRI sessions will be scheduled at the same time in their cycle. For participants with active cycles, the MRI sessions will be scheduled to occur from ~3-10 days after the beginning of menses or 21 days after menses start but before the next cycle begins. If the participant is taking contraceptives with 1 week of null pills/patches, the placebo MRI session will occur anytime except this week. If they are taking other forms of hormonal contraception (e.g., hormone-releasing IUD, depot injection), scheduling will be done as convenient. For participants who have undergone hysterectomy, or who are post-menopausal, sessions will be scheduled as convenient. In order to match the time period dictated by active cycles, we will schedule all participants with approximately 4 weeks between MRI sessions. However, given logistical challenges of enrolling psychosis subjects in this protocol, the 4-week interval may not always be possible. The PI will assess, on a case-by-case basis, whether or not added variance due to variations in GABA activity is offset by the importance of obtaining the data point from an additional subject.

6.0 Study Schedule and Assessments

Section 6.0 makes repeated references to remote visits, which will be conducted via an encrypted, HIPAA-compliant video conferencing platform (e.g., Blue Jeans, Zoom for Health, etc.) It will be standard practice to conduct said procedures remotely to better protect participants and staff against the risk of COVID-19. However, we will also provide the option for participants to have an in-clinic visit. In those cases, the study will follow all UM safety guidelines for entering and being in UM buildings (e.g., COVID screenings, PPE, social distancing, etc.).

Pre-Screening assessment preparations: Participants who screen-in from the phone screen will be scheduled for a remote video conference session or in clinic. They will be emailed a link, given instructions about the visit (e.g., how to open the link on their phone or computer), and sent a blank copy of the consent to review prior to their session.

Electronic Data Capture: Subjects will be asked to complete the online REDCap surveys prior to their screening assessment. Surveys are usually taken online within a web browser. REDCap has been approved for the University of Michigan research community, and individual investigators access the web interface through a University of Michigan REDCap portal.

Subjects will receive a coded link via email which will take them to the required surveys. The emailed link contains no identifiable information about the subjects, and once activated, establishes an https link with the REDCap server. Once completed, each survey is locked so that re-access is not permitted. We can also adapt the process for each subject (e.g., provide access on computers or tablets in the Department of Psychiatry for subjects who do not wish to or cannot use email). Some subjects may

request paper. Subjects who are currently entering responses on paper forms will continue to do so until they complete the protocol. A secure web-link to the REDCap site will be emailed along with the consent. For this, a waiver of documentation of informed consent will be sought. If subjects who complete these online surveys screen out, their REDCap data will be deleted. Responses entered by subjects will be available to the research assessors prior to the screening visits.

6.1 Consenting procedures

Informed consent will be obtained at the beginning of the visit (for minors, this will include a review of an oral assent script, assent from the adolescent, and signed consent from their parent/guardian). Electronic informed consent signatures will be collected from adult participants or, for minors only, a parent/guardian using SignNow. All procedures will adhere to the IRBMED Guidance for use of SignNow for Electronic Informed Consent Procedures.

If in-person visit required: The day before their scheduled visit, study staff will contact participants to administer the COVID pre-screen questionnaire, currently required for entry into the Rachel Upjohn Building. If cleared by the screener, participants will be scheduled to come to the Rachel Upjohn Building following current COVID protocols. Staff will escort them to a private interview room with a laptop or computer where a remote session will be established with the study team (in another room). Study staff will return to their office and provide informed consent procedures remotely. All signatures will be collected using SignNow.

6.2 Screening visits

The screening assessment visits will be separated into two parts: Part 1, which can be done remotely and Part 2, which will be done in-clinic at the Rachel Upjohn Building.

6.2.1 Part 1: Remote visit or in-clinic

fMRI Safety Assessments: Participants will answer questions from the fMRI Safety Screen form to ensure that there are no contraindications for fMRI. Participants of child-bearing age who think they may be pregnant will be given the option to take a urine pregnancy test in-clinic if eligible for Part 2.

Diagnostic assessments*: DSM5 diagnoses and Structured Interview for Clinical Disorders (SCID),¹³⁶ and Structured Interview for Psychosis Syndromes (SIPS),¹³⁵.

General assessments*:

- (1) *Hemispheric dominance* will be assessed with the Edinburgh Handedness inventory.¹⁴⁰
- (2) *Level of functioning* will be assessed with the self-rated Social Adjustment Scale (SAS).¹⁴²
- (3) *Assessments of potential moderators of NA* include a measure of childhood trauma, the Childhood Trauma Questionnaire (CTQ), a 30-item survey of adverse childhood experiences,¹⁴³ as well as Life Events Checklist,¹⁴⁴ for potentially stressful life events occurring in the last year (adolescent or adult versions as appropriate).
- (4) The *reward/hedonic dimension of emotion* (which may differentiate amongst the SCZ and BAD diagnostic groups), will assess hedonic drive and activation with the Behavioral Inhibition Scale/Behavioral Activation Scale (BIS-BAS),¹⁴⁵ a 20-item self-report scale, as well as the Temporal Experience of Pleasure Scale (TEPS),¹⁴⁶ an 18-item self-report scale which assess consummatory and anticipatory pleasure, which may have differential impairment in schizophrenia and bipolar disorders.^{147,}

¹⁴⁸

- (5) *Smoking status* will be assessed with the Fagerstrom scale.¹⁵⁰

(6) *General health status* will be assessed with the short-form 12.¹⁵¹ All of the instruments above have been validated in both adults and adolescents.

(7) *Substance Use History* will be assessed using the Substance Use History questionnaire derived from the Early Psychosis Intervention Network (EPINET) Core Assessment Battery (CAB).

(8) *Menstrual status*: In order to time scans with menstrual cycle, which affects measurement of MRS GABA levels, we will obtain date of last menses, or menstrual status, e.g. taking hormonal contraceptives, post-menopausal.

Symptom assessments*

Clinical state in the patient subjects will be assessed with the following:

- (1) Scale for the Assessment of Negative Symptoms (SANS);¹⁵²
- (2) Positive and Negative Symptom Scale for Schizophrenia (PANSS);¹⁵³
- (3) Columbia-Suicide Severity Rating Scale (C-SSRS);¹⁵⁴
- (4) Calgary Depression Scale for Schizophrenia (CDSS);¹⁵⁵ and
- (5) Young Mania Rating Scale (YMRS)¹⁵⁶
- (6) Abnormal Involuntary Movement Scale.¹⁵⁷

**We may repeat some assessments if too much time has lapsed between these assessments and MRI #1.*

Video/audio recording: We are seeking permission to have some assessment sessions recorded to obtain reliability/fidelity measures. Not all participants will be recorded; this will be determined by needs of the study. Unless further release of information is obtained, all recordings will be destroyed at the end of the study.

6.2.2 Part 2: In-clinic visit

Study staff and participants will be required to follow all departmental COVID-19 safety guidelines when in the Rachel Upjohn Building.

Neurocognitive functioning: A comprehensive assessment battery of dimensional measures will be performed in the initial visit, prior to the MRI, in order to perform post-hoc analyses and test alternative hypotheses. Neurocognitive functioning will be assessed with the MATRICS Cognitive Consensus Battery (MCCB)¹³⁸ which includes 10 tests covering 7 cognitive domains (including working memory), and the Reading subtest of the Wide Range Achievement Test (WRAT4)¹³⁹ as a measure of pre-morbid education attainment.

Pregnancy test: For participants who opted to have a urine pregnancy test based fMRI safety screening in Part 1.

Preparation for MRI scans: The subject will enter a "mock scanner" to practice the task. The mock scanner has a moving table that takes the subject inside a large tube, similar to the real MRI scanner. Study personnel can stay in the room as the subject learns and practices the task. Subjects find the mock scanner less intimidating, although the experience familiarizes them with the scanner environment and helps to de-sensitize them to the actual scanner. In the mock scanner, subjects will also be pre-exposed to the IAPS pictures, providing ratings of valence and arousal on a 7-point Likert scale.

6.3 MRI sessions

Staff and participants will follow the fMRI Lab COVID-19 safety guidelines.

6.3.1 Overview: MRI sessions will occur at the University of Michigan fMRI Laboratory. Each subject will undergo two MRI sessions, separated by ~4 weeks. Procedures will be identical for each session. Because subjects will receive a dose of LRZ during one of these sessions, they will be required to have a person with them to drive them home, or transportation will be provided for them via cab. Transportation will be required for both sessions, so as not to break the blind.

6.3.2 Urine drug and pregnancy tests: All participants of childbearing potential will be required to take a urine pregnancy test, which will be read immediately. Any who have positive results will be withdrawn from the study because of the potential for lorazepam to harm a developing embryo.

All subjects will be asked to provide a sample of urine for a urine drug screen, in order to exclude subjects who may conceal their use of substances of abuse, or prescribed medications. The screen (Accutest 8+1, JANT Pharmacal Corporation, Encino, CA) will test for amphetamines, barbiturates, methamphetamine, THC, cocaine, morphine, PCP, and benzodiazepines. At the time of recruitment, all subjects will be warned that they will undergo a drug screen. Results will be available within 5 minutes. Subjects who indicate the presence of an illicit drug will not undergo that day's fMRI scan. At the PI's discretion, the participant may be given the option to return at a later date to have the scan done, providing they have a clean UDS at that time. Those that are not given the option will be withdrawn. SCZ patients taking a benzodiazepine on as needed basis will not be excluded if their UDS is positive for benzodiazepines, since these substances may be detectable in the urine even if subjects do not take as needed medications within the proscribed windows.

6.3.3 Assessment of NA: Assessment of NA will be completed immediately after drug administration.

- 1) *The primary measure of NA will be the 9-item version of the PSI*,³⁶
- 2) As *secondary measures*, we will also administer the 8-item DES-neg,¹⁵⁸ and *BDI-II*,¹⁵⁹
- 3) The BDI-II has identical psychometric properties and nearly identical content as the BDI-1A except that it focuses on a 2-week time period to permit DSM diagnosis.

The DES-neg consists of the NA items from a larger 23-item scale designed to assess overall frequency of emotions in the past 2 weeks. Several other measures will permit us to assess and control for subjective experiences not relevant to the NA construct of interest.

- 4) As a tertiary measure for NA, we will use the MASQ62,¹⁶⁰ which is a 62-item self-report scale of symptoms occurring in depression and anxiety. It is based on a tripartite concept of symptoms: general NA, common to both anxiety and depression; anhedonia, unique to depression, and anxious arousal, unique to anxiety.

In order to rule out any contribution of medication side effects contributing to NA, we will also administer:

- 5) Glasgow Antipsychotic Side-effect scale (GASS),¹⁶¹
- 6) Simpson-Angus scale for extrapyramidal side effects (Simpson-Angus AE).¹⁶³

6.3.4 Steps to monitor intervention: Vital signs (blood pressure, respirations, heart rate) will be obtained prior to the dose of PLB/LRZ, and just prior to the blood draw for LRZ levels.

Subjects will be given the option to have their blood drawn for lorazepam levels, just prior to entering the scanner. Should they opt in, phlebotomy will be obtained by a trained phlebotomist, who will draw approximately 8 ml of blood for processing. Blood will also be obtained for inflammatory markers (e.g. TNF-alpha, CRP, IL-6), which have been associated with SCZ and BAD.

Just prior to entering the MRI scanner, subjects may have end-tidal PCO2 measured; data which may be used to process the arterial spin labeling scan. This procedure is not a required part of the protocol and may be suspended due to COVID, PI discretion, etc.

6.3.5 Imaging procedures: Scanning will be performed at the UM Functional Magnetic Resonance Imaging Laboratory (fMRI Lab) on a 3T MR 750 scanner (Waukesha, WI) using a 32-channel coil.

When we position a subject in the scanner, head movement will be minimized through: (a) instructions to the participant; and (b) packing the head inside the head coil with a system of foam padding and pillows that we have found is well-tolerated by the participants yet limits movement. Extra care will be taken to ensure that the participant's head is positioned as straight as possible since that eases the task of identifying landmarks used in positioning slices to be acquired in the fMRI scans. A mirror or MR-compatible goggle system projects the computer display output into the bore of the scanner. Stimuli are controlled and responses recorded either by E-Prime (PST, Inc., Pittsburgh, PA) or Presentation (Neurobehavioral Systems, Albany CA) lab software. Participants will wear a response claw on their hands with buttons in order to make button-press responses during cognitive tasks. The stimulus-presentation and response-collection systems have been tested to ensure that no artifacts are introduced and that there has not been an increase in the image-level noise. The goggle system may contain an eye tracking camera, which can permit the monitoring and possible analysis of eye movements during the scan. In addition, MR-compatible earphones may be used and physiological sensors may be attached. These sensors consist of a strain gauge, or strap that goes around the torso, to measure respiration, and plastic finger cuff, to measure heart rate by plethysmography (although latter may not be used if it is redundant with pulse oximeter). These non-invasive sensors are FDA-approved components of the scanner, and the fMRI Center has begun to incorporate the physiological information into the acquisition sequences in order to improve image reconstruction. Because this is an area of data analysis that remains under development, we may not employ these sensors in all scans. Once in the scanner, subjects can communicate via intercom with the technician running the scanner in the control room. A window separates the control room from scanner room, which is sealed off from the outside by a light vacuum seal. In addition to the intercom system, subjects are given a squeeze bulb. They are informed that should they need to exit the scanner immediately or gain the attention of the technicians, they can trigger an alarm in the control room by squeezing the bulb.

For the structural and BOLD acquisitions, we will leverage the University of Michigan experience with the Adolescent Brain and Cognitive Development study, ensuring state-of-the-art, high resolution data with multi-band acquisition. After localizer scans, subjects will undergo BOLD-weighted fMRI (EPI, TR=800 ms, FOV=23, slice=2.4mm, 96x96 matrix) for the IAPS task. They will then undergo two resting state scan runs (BOLD-weighted) for 10 minutes each to obtain data for functional connectivity analyses. Although resting state scans have typically been collected over 6-8 minutes, with a 2 sec TR yielding 180-240 frames, recent work has suggested that stable, reliable connectivity measurements are better obtained with 1500 frames collected over 20 minutes, and the 2 10-minute runs reduces subject fatigue.¹⁶⁴ Next they will undergo the pseudo-continuous arterial spin labeling (pCASL) Our implementation will use a 3D stack of spirals acquisition with 6 interleaves (TR=5000 ms, TE=4 ms, BW=125kHz, FOV=22 cm) preceded by a 1800 ms labeling period and an 1800 ms delay to allow the label spins to clear the arteries and enter the capillary/tissue compartment. We will acquire 20 pCASL control-label image pairs, which will be reconstructed to a 128x128 matrix. Next, we will acquire high-resolution structural scan (SPGR) for image normalization, as well as a T1 scan in the same prescription as the BOLD scans to facilitate normalization. The last scan in the sequence, separated by the structural scans to avoid spill-over from the IAPS activation, will be GABA edited MR spectra, acquired from three MFC voxels using a MEGA-PRESS sequence with the following parameters: TR = 1800 ms; TE = 68 ms (TE1 = 15ms, TE2 = 53ms); 256 transients (128 ON interleaved with 128 OFF) of 4,096 data points; spectral width = 5 kHz; frequency selective editing pulses (14 ms) applied at 1.9 ppm (ON) and 7.46 ppm (OFF); FOV = 240 x 240 mm; voxel size = 30 x 30 x 30 mm. Acquisition time for each voxel is approximately 8.5 minutes, and voxels will be placed as in Figure 5. Scan time will be kept to approximately 1.5 hour.

The acquisition parameters described here are representative of what will be employed in the actual acquisitions and may differ slightly. Power-monitoring software on the scanner will ensure total energy delivered to the subject will remain within FDA guidelines. Specifically, the specific absorption rate (SAR) will be not greater than: 1) 4 W/kg averaged over the whole body for any period of 15 minutes; 2) 3 W/kg averaged over the head for any period of 10 minutes; 3) 8 W/kg in any gram of tissue in the head or torso; 4) 12 W/kg in any gram of tissue in the extremities, for any period of 5 minutes.

6.3.6 IAPS task: Subjects will view emotional stimuli, consisting of aversive pictures (injured limbs, corpses), positive pictures (smiling children, cute animals, etc), and non-aversive pictures, selected from amongst the 800+ pictures in the IAPS.¹⁶⁵ Subjects will view 4 pictures (3 sec each) in 12 sec blocks of the same valence (36 unique pictures for each valence), and they will be instructed to 'Press the button when the picture changes and experience the feeling you get from each picture.' A robust block design will be used to ensure a strong signal amenable to manipulation in the phfMRI protocol. Each block will be separated by 12 sec of rest (fixation cross in the middle of a grey screen). Subjects will undergo 3 runs, of 9 blocks each, in pseudo-randomized order (11.4' total, including 6 acquisitions prior to the first block and 8 acquisitions after the last block to allow for the BOLD signal to establish a baseline). Although the primary analysis will contrast all pictures to the inter-block, implicit baseline, based on our preliminary findings, valenced conditions will be used to test secondary hypotheses about the reaction to aversive stimuli. Actual stimuli presentation times may differ slightly in implementation from that described here.

6.3.7 Monitoring subjects during MRI sessions: Subjects will be carefully monitored throughout the MRI sessions using subjective measures of emotional state and objective measures of physiology. Subjective measures include computerized VAS ratings of emotional state on five items (anxious/nervous, happy, drowsy, fearful, sad), from 'Not at all,' to 'Completely/totally.' All subjects will complete VAS scales at 4 points: 1) Outside the scanner, immediately after dose of LRZ/PLB; 2) Just after entering the scanner bore; 3) After the IAPS task; and 4) Just after exiting the scanner. Patient subjects will complete an additional VAS item, for the first and last VAS, in which they will rate the intensity of a target symptom that is most bothersome to them, e.g., auditory hallucinations, delusions of reference. In this way, we will assess whether or not symptoms are exacerbated by the scanning environment. Objective measures include continuous readout of heart rate and respirations while in the scanner, as well as online monitoring of responses made to the IAPS pictures and video monitoring to ensure that eyes remain open during the IAPS task and fMRI scans.

6.3.8 Debriefing (last session only): Subjects will be asked to guess in which session they think they received the active drug.

6.4 Concomitant medications

We will include patients on their clinically prescribed medications (stable doses for > 1 month), with a few exceptions. Any patient on chronic benzodiazepine therapy will be excluded in order to maximize the effect of the LRZ challenge. If patients are clinically prescribed as needed doses of benzodiazepines, they will still be eligible for participation as long as those doses are not required within 5 half-lives of an MRI session

6.5 Reimbursement of participants

In order to make participation worth the subjects' time, they will be reimbursed \$15/hour for the consent process and screening visits, and up to \$75 for each MRI (\$60 for the MRI + \$15 for the blood draw, if they opt in). Patients will be paid in full (either by gift card or check) when they complete the study. If they leave the study before completing all of the sessions, they will be paid for those sessions they have completed. If the participant is coming outside of the Green Cab radius, we will also provide round trip mileage reimbursement.

6. Participant discontinuation/withdraw

During the pre-screening process, subjects who are found to be non-eligible, before signing the consent form, will be considered *not enrolled*, and all data related to that subject will be destroyed. Subjects who consent and are found not eligible during the assessment and screening will be considered *screen failures*, and all research and identifying data will be destroyed, except for the consent form and limited information to document the screen failure (age, gender, diagnosis, specific reason for exclusion). Subjects will receive a subject number at the time of consenting. Subjects who pass screening will be considered *enrolled*. Subjects who leave the study after signing the informed consent, and after being deemed eligible/enrolled/randomized, will be considered *withdrawn*. Subjects may withdraw from the study at any time and for any reason. Subjects may be withdrawn by the investigator when continued participation would present an unacceptable risk to the subject. Based on previous experience, we expect that most subjects will be compliant with the study protocol. Subjects who experience severe symptom worsening, including suicidal thoughts or behaviors, will be withdrawn from the protocol.

7.0 Assessment of Safety and Regulatory Reporting

7.1 Data and Safety Management Plan

7.1.1 Overall framework of monitoring Study monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s)

7.1.2 Clinical documentation: All participants will have a record entered into their medical chart indicating participation in the protocol and drug dispensing.

7.1.3 Frequency and type of study monitoring

PD/PI: The PI or a co-investigator will be immediately available in person or by phone when study procedures are being conducted. The PI will meet with study staff on a weekly basis to monitor the progress of the study. During phases when subject recruitment is occurring, the PI will review screenings and scheduling of assessments, as well as strategies to improve recruitment yield. Issues around maintaining confidentiality and privacy of participants during screening, assessment and data collection will be reviewed. Any protocol deviations and adverse events will be reviewed at this weekly meeting. For serious adverse events, the PI will be notified as soon as the SAE comes to the attention of study staff.

Institutional Review Board monitoring: Approval of all procedures, advertisements and materials given to subjects will be secured from the University of Michigan IRBMED. Annual reviews will be conducted by IRBMED, including the number of subjects screened, enrolled and withdrawn. The IRB will also review protocol deviations, adverse events (according to the reporting timetable of IRBMED) and complaints that arise in connection with the study.

Data monitoring: Data entry will be audited by study staff, who were not involved in primary data entry. Patient questionnaire data will be entered largely through electronic data capture, e.g., Qualtrics

Independent Study Monitor: The Michigan Institute for Clinical and Health Research (MICHR) will provide independent study monitoring. Monitors will follow Standard Operating Procedures and detailed Monitoring Plans (written in conjunction with the Investigator) to provide a tailored approach for monitoring needs. MICHR monitors will serve as an independent source for unbiased Quality Control (QC). The study team will provide regular reports to the monitors on accrual, withdrawals, adverse events and protocol deviations.

Study Monitors will be responsible for the following:

- Development of a Monitoring Plan and tools to help facilitate the monitoring efforts
- Site Initiation Visits: This includes an onsite visit and monitoring report prior to study recruitment to ensure proper documentation is on file and study procedures/processes are in order.
- Interim Visits: Onsite or virtual visits to review subject consent, source documentation for protocol compliance and a review of the regulatory binder to ensure proper maintenance. This also includes preparation time for visits and report of findings after each visit. It is anticipated that the first interim visit will occur after enrollment of first subject, and then at approximately -3-month intervals thereafter. Frequency may vary according to enrollment and/or may be adjusted throughout the course of the study as conditions and needs evolve.
- Close-Out Visit: Onsite visit and monitoring report will occur when all subjects have completed the study; includes a final review of regulatory documentation and, if needed, subject data review.

Independent Safety Officer: A safety officer, a licensed physician with expertise in brain and behavior, who is not connected with the study will be appointed to review the study protocol and materials (consent form, study management document, DSMP) before the recruitment of the first participant. At regular intervals afterwards (after the first subject is enrolled, and then at 6 month intervals thereafter), the Safety officer will receive a de-identified report of study participants, as well as any adverse events or protocol deviations encountered. The report will include the subjective reactions to the study protocol, as recorded during data acquisition. In the event of a serious adverse event related to the study procedures or unanticipated problem, the safety officer will receive a report of this event/problem within 7 days of the study team becoming aware of it. At each reporting interval, the safety officer will provide written reports indicating whether the study is appropriate to continue. Appointment of the safety officer will be approved by the NIMH Program Office.

7.1.4 Adverse events arising in the course of participation:

7.1.4.1 Adverse Events Review: Monitoring will occur by the clinical study coordinator. The coordinator will then bring all possible AEs to the attention of the PI for evaluation, gradation and appropriate reporting. AE's will be assessed for severity (mild, moderate, severe), expectedness (expected, unexpected) and relatedness (definitely related, possibly related, unlikely related, not related). A log of all AE's will be maintained by the study coordinator.

7.1.4.2 Adverse Events Definitions and reporting timetable:

A Serious Adverse Event (SAE) in this study will be defined as an event that results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, or results in persistent or significant disability or incapacity. The degree of probable relation between study procedures and the SAE will be carefully evaluated and documented.

Serious adverse events will be reported to the IRB within 7 days of occurrence (or notification) of the event if they are definitely or possibly related to the study interventions and unexpected. If the SAE is expected, it will be reported within 14 days. Expected and unexpected SAEs, definitely or possibly related to the study, will be reported to the NIMH Program office within 7 days of becoming aware of the event. For SAEs that are not related to the study, reporting will occur at the time of scheduled continuation, or not at all if determined to be expected, per standard reporting timetable of the University of Michigan IRB.

Unexpected adverse events in this study will be defined as any adverse events for which the nature and severity are not consistent with expected or anticipated adverse events resulting from participation in the protocol.

If the event is: 1) unexpected in nature, frequency or severity; 2) related to the research, and 3) poses an increased risk of potential harm and/or actual harm than was previously known or recognized, the event will be identified as an Unanticipated Problem, and reported to the IRB as such.

Adverse events will be assessed as severe, moderate or mild. Moderate is defined as any non-serious event which causes discomfort and requires treatment, but does not pose any significant or permanent risk or harm to the subject or require in-patient hospitalization, including social or psychological trauma causing moderate or temporary distress, significant embarrassment, stigmatization of individual or community/ group, disruption of familial/social relationships or nontrivial emotional distress or upset. Moderate AEs will include evaluation in the emergency room not leading to hospitalization. Mild is defined as any non-serious event of less than moderate severity.

Moderate and mild AEs that are expected will not be reported to the IRB. Moderate/mild AEs that are unexpected and definitely or possibly related will be reported to the IRB at the time of scheduled continuing review. Moderate AEs that are unexpected and definitely not related will not be reported to the IRB.

Non-serious adverse events and unrelated serious adverse events will be reported in the annual progress report to the NIMH.

7.1.5 Quality assurance and quality control for data acquisition

Clinical assessments: Assessors will be trained on assessment instruments and established according to laboratory standards and good inter-rater reliability

MRI data: The fMRI lab maintains weekly schedule of calibration runs to assure consistent operation. Processing pipelines and standard operating procedures are in place ensure that data is processed in a uniform manner. Quality control at each step will be carried out by study staff to verify, through multiple metrics, image quality. Databases will track processing steps, in addition to automatically generated log files.

Medication safety: The Investigational Drug Service (IDS) maintains strict quality control oversight of storage and dispensing of drugs used in research studies. Detailed standard operating procedures exist and are regularly updated. Dispensing logs will be maintained by the PI and study staff, and medications will be kept in locked medication boxes, inside a locked medication room. Administration will be documented in the subject's medical record. Unused medication will be documented and returned to IDS.

7.1.6 Confidentiality and security of data

Confidentiality of data: Multiple steps are taken to ensure the confidentiality of sensitive information. Subjects are reminded that they do not have to answer questions that make them feel uncomfortable, which reinforces the voluntary nature of their participation. The confidentiality of all information gathered directly from subjects or obtained from the patient's medical records is assured by assigning records a coded research number and identifying all computer and paper files only by this code. A single tracking file will link the codes with subject identifiers, such as name and hospital number. In addition, consent forms and payment forms will be kept in a separate, locked room, apart from research data. All files will be kept in locked file drawers in locked rooms, to which only authorized research personnel have access. Electronic records will be kept on secure servers, and records with identifying information will be kept separate from records used to collect research data. Only staff members who have a need to know personal identifying information will have access to this information. All research staff are trained in research ethics and HIPAA regulations pertaining to protected health information. Screening forms for subjects who do not qualify for the study will be destroyed, except for anonymous information unlinkable to the subjects (such as age, gender and education). After the completion of data analysis, the record linking subjects to the research codes will be destroyed, thereby anonymizing the data.

7.2 Event Windows, Missed Assessments, Missed Sessions and Protocol Deviation Reporting:

Any safety-related assessments (C-SSRS, fMRI screening) which are missed will be reported to the IRB as a protocol deviation. For the other assessments, missing assessments will not be reported as protocol deviations unless they constitute > 10% of the total assessments. The time elapsed between the initial assessment and MRI sessions are intended to be less than 1 week, but scheduling logistics may mean this is not possible for all subjects. These deviations are expected to be minor and will not be reported, although they will be recorded. These allowances should affect neither the scientific integrity nor the safety monitoring provisions of the protocol.

8.0 Clinical Monitoring

Study staff will be trained in monitoring patient subjects and healthy participants through the study procedures. Initial assessment will occur at the Rachel Upjohn Building in the department of psychiatry, and a clinically trained, masters-level research clinician will assess all participants for their level of psychopathology. A highly trained study coordinator will take participants through the MRI session procedures, with back-up available from the masters-level research clinician and the PI, a board-certified psychiatrist who specializes in the treatment of psychotic patients. He will supervise all procedures for monitoring clinical status of participants and establish standard operating procedures.

9.0 Statistical Analysis

Data analysis for BOLD images: Data analysis will use validated and established routines implemented in our laboratory. For processing BOLD images, we will use well-tested routines from FSL 5.0.1 and SPM12 for realignment and spatial normalization (MNI-152) procedures implemented in VBM8. Strict quality control procedures are in place to evaluate movement. We will exclude runs where the standard deviation (summed across three rotational axes and translational directions) of realignment parameters is > 0.5 mm translation and > 0.2 deg rotation. Movement will be assessed using a framewise displacement (FD) measure,¹⁶⁶ which will permit analysis of movements within and between groups. First-level analysis will use the general framework of the modified General Linear Model,¹⁶⁷ implemented in SPM12 with temporal convolution. For the *IAPS BOLD data*, a design matrix will include regressors for 3 valences (aversive, non-aversive, positive) 2 sessions (LRZ, PLB), and 12 realignment parameters (including temporal derivatives of 6 realignment parameters). Second level analysis will utilize a flexible factorial design, with valence and scan session as within factors and group as between-subject factors. For statistical inference we will use topological false discovery rate (FDR)¹⁶⁸ providing corrected probabilities. For the *a priori* hypotheses about the dmPFC cortex, we will use a small volume correction over the anterior medial frontal cortex.

For the six MRS voxels, GABA concentrations will be quantified using the MEGA-PRESS difference spectra with Gannet 3.0 (<http://www.gabamrs.com/>),¹⁶⁹ which is specifically targeted for GABA edited MRS. Time domain data are frequency- and phase- corrected using spectral registration and filtered with a 3-Hz exponential line broadening and zero-filled by a factor of 16. Gannet models the GABA peak using a five-parameter Gaussian model between 2.19 and 3.55 pm, and the water peak using a Gaussian-Lorentzian function. In all analyses, metabolites values are scaled to water, and expressed in institutional units (IU), which we designate GABA, after they are corrected for the fraction of the voxel that is cerebrospinal fluid (CSF) and white matter (WM) as opposed to grey matter (GM) (using SPM12 segmentation), and for the different water relaxation times in CSF, WM, and GM¹⁷⁰. Although some previous MRS work has referenced GABA to Cr, which may reduce variability more than the water reference¹⁷¹, there have been recent reports of reduced Cr in schizophrenia subjects.¹⁷² We will examine creatine levels, and the GABA/Cr ratio, and alter our approach if Cr concentrations are not different between groups and GABA/Cr provides a more reliable signal (measured by Pearson r and coefficient of variation). Because we will have two GABA MRS sessions (LRZ & PLB), we will be able to assess comparative reliability (but the plan is to use only data from the PLB session for the primary analyses).

Primary Hypothesis testing: Aim 1: Assess MFC GABA concentration in psychosis spectrum patients with MRS The first set of hypotheses will focus on Process 1 and the EP patients, on & off antipsychotic medication (APM). (H1.1) *When controlling for NA, GABA in rMFC will be higher in EP, compared to HC.* For rMFC GABA, we will perform a 2-way ANCOVA, with group (EP, HC matched to the EP group) and gender as factors and NA as a co-variate. This will replicate results from our pilot data, which included medicated and unmedicated patients. A similar ANCOVA will test corollary hypotheses that (H1.2) *rMFC GABA will be higher in EP patients not on APM compared to EP patients taking APM.* Exploratory analyses will expand the ANCOVA to include 5 groups (EP-onAPM, EP-offAPM, SCZ, BAD, HC [all 80]), and post-hoc t-tests on PSI-adjusted values will test for a relationship: EP-onAPM $>$ EP-offAPM $>$ SCZ = BAD = HC ($\alpha = 0.05/3 = 0.017$). This series of exploratory tests will ascertain whether both medications and illness stage affect GABA, which will be verified if rMFC GABA in both EP-onAPM and EP-offAPM groups is greater than the SCZ & BAD groups. In both hypotheses, main tests for the primary outcome will be at $\alpha = 0.05$.

The third primary hypothesis will test for Process 2, that (H1.3) *greater NA will be associated with lower rMFC and dmPFC GABA, in all groups.* Since our overall hypothesis is that multiple processes may affect GABA, we will use multiple regression to predict GABA. We will evaluate models

for each voxel, with GABA as the dependent variable and the following independent variables: NA (from PCA of PSI, DES-neg & BDI-II), group-SCZ, group-BAD, group-EP, age, sex. Model significance level will be $\alpha = 0.05/2 = 0.025$. Two-way interactions will also be included in the model. This model will also allow us to test for group effects, and group X age interactions.^{103, 107} As a secondary analysis to test for the effect of illness stage (duration ill) on GABA, independent variables will be: NA, group-SCZ, group-BAD, duration ill, sex. Lastly, while we conceive of NA here as a trait measure, there have been reports that acute NA, induced by a threat of shock,¹⁷³ could reduce GABA, so we will substitute VAS scores of NA (anxious+fearful+sad) obtained in the MRI scanner for NA and compare predictive power. Exploratory analyses will examine differential effects in the three voxels, which tests the general hypothesis that functional heterogeneity in the MFC drives inconsistent findings in the literature. In distinction to the increased GABA signal found in the rMFC, findings in the pmPFC region for schizophrenia have been either equal to¹⁰⁷⁻¹⁰⁹ or less than^{103, 107} healthy comparison subjects, and age appears to drive GABA down, possibly more so in schizophrenia.^{103, 107} We will use a linear mixed model with voxel and subjects as random effects and group (EP, SCZ, BAD, HC) and gender as fixed effects, and age and PSI scores as co-variables. We will test for the presence of group X voxel interactions, as well as group X age X voxel interactions.

Primary Hypothesis testing: Aim 2, Assess dynamic GABAergic function in psychosis spectrum patients with a phfMRI probe *(H2.1) Patient groups will show increased BOLD signal with phfMRI in dmPFC while viewing affective stimuli, compared to the healthy subjects* We will test for group X session (LRZ vs PLB) interactions, which will reflect the aggregate effect of viewing salient pictures (across valence, per preliminary results). Post-hoc t-tests will demonstrate that the effect occurs in EP, SCZ and BAD groups, and that it appears in both early and chronic cohorts of patients. We will test for dose effects by extracting signal at foci with group X session interactions and main effects of session and testing for correlations between session effects and LRZ plasma level. We will also conduct exploratory, whole brain searches with stringent correction of type 1 errors. *H2.2) All groups will show a correlation between LRZ-induced BOLD change in the dmPFC and NA.* We will conduct a multiple regression analyses with NA (PCA component) as the dependent variable, with the following independent variables: delta-BOLD (LRZ-PLB), group-SCZ, group-BAD, group-EP, LRZ dose, positive symptoms (from PANSS), medication side effects (sum of scores from measurement of SAE, GASS and FIBSIR). Secondary analyses will be conducted for the effect of early versus late (SCZ & BAD groups only). *H2.3) LRZ-induced BOLD change will inversely correlate with dmPFC GABA.* This should be an inverse correlation, because our SCZ subjects had a positive delta-BOLD, in contrast to the negative delta-BOLD seen in the healthy controls. We predict that this Process 2 phenomenon is related to lower GABA levels. We will conduct a multiple regression analyses with dmPFC GABA as the dependent variable, with the following independent variables: delta-BOLD (LRZ-PLB), group-SCZ, group-BAD, group-EP. If we confirm the relationship of GABA with dmPFC, and with NA in H1.3, then we will conduct a mediation analysis¹⁷⁴ to test the model that GABA mediates the relationship between dmPFC delta-BOLD and NA. Secondary analyses will examine other voxels, such as the rMFC, where we found a small LRZ-PLB signal in our pilot data, below significance threshold (see Figure 2, focus @ -6, 47, -2, $Z=3.30, k=35, p_{FWE-corr}=0.20$).

Analyses described here are preliminary plans, which may differ slightly in implementation.

10.0 Source Documentation

Medical and research records will be maintained to ensure the protection of confidentiality of subjects (see section 12).

11.0 Quality Control and Assurance

A quality control process will be set up to ensure accuracy and consistency of data collection and upload to database files. Neuroimaging will undergo rigorous quality control assessments which are standard in the laboratory.

12.0 Ethics and Protection of Human Subjects

12.1 Ethical Standard and IRB review

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, as drafted by the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997) and the Declaration of Helsinki and Good Clinical Practice (GCP).

All protocol amendments must be IRB approved prior to implementing, except when change is for patient safety.

12.2 Subject Confidentiality

Measures to protect patient privacy: Study staff will make every effort to limit identifiable information on potential subjects during recruitment. Conversations in which a patient's name must be mentioned, e.g. to determine potential eligibility, will occur in private settings of the clinic. The minimum amount of information will be recorded, and staff are alerted to the dangers of printing, faxing and emailing sensitive information. Phone conversations with potential research subjects will occur behind closed doors, and staff will ask callers if they are in a location where sensitive information can be discussed without danger of revealing confidential information. Any information gathered on subjects who prove ineligible will be destroyed as soon as possible (a list of patients who have declined or screened out will be maintained through the recruitment phase in order to avoid contacting these subjects again).

Minimizing the potential for coercion of patients to participate: Because members of the research team are involved in clinical care and research, there is the potential that some patients of the study team may be recruited. It will be made clear to all potential subjects that participation is entirely optional and not a part of regular treatment. The bulk of the recruitment process will occur with the study coordinator, who does not treat patients in any of the clinical venues where recruitment will occur. Overall, the research team is experienced in clinical research and very mindful of the conflict between the dual roles of clinician and researcher. They firmly believe that the safety and desires of the patient take precedence over any research protocol and work hard to assure their patients that research participation is not required for treatment. In the case where a patient of a study team member is recruited, the consent process and signature on the informed consent document and will be obtained by the study coordinator.

Confidentiality and minors: The assessments in this protocol will ask participants about sensitive topics, such as mental illness, possible pregnancy, and substance use. We will inform the consenting parents of minor participants at the beginning of the process that we will respect the confidentiality of our teenage participants. It is important for us to let teenage participants know that they can be honest about sensitive topics without fear of reprisal from their parents. However, participants will also be informed about limits to confidentiality. If we learn that a subject has potential to harm him or herself, or another individual, we will break confidentiality and take steps to prevent

harm. Similarly, any evidence of potential child abuse will be reported to Child Protective Services. Since the assessment will occur along with clinical feedback to the minor participant and parents, we will need to discuss clinical situations that require an intervention, such as substance use disorders and substance use that interferes with treatment.

12.3 Potential Risks and Minimization of Risks

12.3.1 Risk of boredom or fatigue (likely, not serious). Participants may become tired or bored during the computerized cognitive testing.

To minimize risk: Participants will be allowed breaks in between testing sections.

12.3.2 Loss of confidentiality around sensitive information (rare, not serious).

To minimize risk: All subjects are assigned research numbers, and all research information collected is linked to the subject only by this number. Great care is taken to remove all identifying information from research records. A single tracking file contains links to the research records and subject codes. Recruitment tracking files are kept only for the duration of recruitment. Paper records are kept in locked file drawers in a locked room, to which only authorized research personnel have access. Paper records with identifying information (consent form, payment records) are kept in locked file cabinets, physically separate from the research records. Computer records with identifying information are kept on secure, password protected servers. Staff are trained to scrupulously protect the confidentiality of sensitive information and take care to limit the printing of documents with identifying information and to avoid unnecessary discussion of subject names. Screening forms for subjects who do not qualify for the study will be destroyed, except for anonymous information unlinkable to the subjects (such as age, gender, and education). However, if a subject may become eligible in the near future, e.g. they need to wait 1 month after a recent medication change, we will retain certain records so as not to repeat the assessments, with the assent of the potential participant.

12.3.3 Risks Associated with fMRI Scanning:

12.3.3.1 Discomfort or anxiety (occasional, not serious). There is a minor risk of discomfort or anxiety/panic from being in the confined space of the MRI scanner.

To minimize risk: The risks of discomfort and anxiety will be minimized by custom pads and pillows to make the subject as comfortable as possible. The subject can communicate with the machine operator via an intercom and may trigger an audible alarm at any time. Before the subject rolls into the bore of the magnetic, he or she is always reminded that they are free to stop the study at any time if they become uncomfortable.

12.3.3.2 Peripheral nerve stimulation (rare, not serious). Fast imaging sequences, such as those employed in this study, have the potential to induce peripheral nerve stimulation (PNS). PNS can be described as a light touching sensation on the skin surface and may cause mild discomfort but is not harmful to the subject.

To minimize risk: The MRI machine is operated within FDA guidelines so the potential for inducing PNS is low.

12.3.3.3 Slight dizziness, light-headedness or nausea (rare, not serious). Sometimes subjects report these symptoms during or immediately after the scanning session.

To minimize risk: If a subject feels light-headed, we will have them get up from the scanner bed very slowly, resting with the feet dangling for several seconds or more before attempting to stand.

12.3.3.4 Incidental finding: Magnetic resonance image will reveal a minor or significant lesion in the brain, e. g. a tumor, previously unknown to the subject, and requiring additional follow-up. (rare, serious).

To minimize risk: Subjects will be made aware of the risk of learning about an anomalous finding in the study that might require further evaluation with a clinical MRI study. We will also inform the subject that many abnormalities will not be picked up in this study, since the scanning sequences we use are not sensitive to many forms of brain pathology. However, in the event that we find something that is very obvious, such as a large tumor, the PI will personally inform the subject by phone call, or in person. A neuroradiologist is available through the fMRI laboratory to review suspicious findings and provide guidance about the urgency and advisability of clinical follow-up. No diagnosis will be offered, but the P.I. will recommend that the subject pursue a follow-up study with their primary care clinician. The PI and study team will facilitate referrals and may provide copies of the brain image if these would be deemed helpful.

12.3.3.5 Hearing damage - from loud, vibrating noises made by the scanner (very rare, serious).

To minimize risk: all subjects will wear earplugs and/or earphones throughout the procedure, which attenuate high decibel sounds, but still enable a subject to hear the intercom and respond to questions from the investigator while they are in the scanner.

12.3.3.6 Injury (very rare, serious). Because the strong electromagnetic fields can move metal objects and cause heating, there is a risk that loose objects (jewelry, keys) outside the body could be accelerated by the magnetic field, striking and injuring a subject. There is also a risk that the magnetic fields could disturb a metal fragment in the body, interfere with an implanted device, such as a pacemaker or neurostimulator, or cause metal (including foil-backed medication patches) on or in the body to heat up, causing harm.

To minimize risk: The MRI suite is kept clear of all objects that could be picked up by the magnetic field, and all subjects complete a comprehensive MR screening form prior to entering the scanner, which is reviewed by the MRI technologist (trained in clinical MRI) before scanning begins.

12.3.4 Risk from lorazepam

1) Common side effects from pharmacologic effects of LRZ (5 - 15 %): Drowsiness, ataxia, impaired concentration; risks could be compounded by interactions with other sedating drugs and/or alcohol.

2) Uncommon side effects of LRZ (<1%): Behavioral disinhibition – also known as ‘paradoxical reactions,’ involves increased agitation and lack of impulse control, and it may be more prevalent in children and elderly

3) Uncommon side effects of LRZ (< 0.1 %): Respiratory depression, confusion, dissociation, amnesia, acidosis, allergic reactions.

4) Risk to pregnancy: An increased risk of congenital malformations has been reported for participants taking minor tranquilizers.

To minimize risk: At the low dose selected for this study, we expect very few side effects beyond mild sedation. The selected doses of 0.01-0.02 mg/kg are significantly less than the dose given for sedation protocols (0.05 mg/kg), and even at these higher dosages, the drug is very safe. Screening procedures will ensure that patients taking drugs that could exacerbate sedating effects (narcotics, barbiturates) will be excluded, as will individuals with active substance use disorders. A review of basic health status will ensure that subjects have no conditions, such as liver failure, that would slow clearance of lorazepam. All participants will be carefully monitored throughout the study to ensure that

they do not experience sedation, confusion, behavioral disinhibition other mental status changes or respiratory depression. The majority of the time in the scanner, subjects will be required to make responses by pressing buttons and monitoring these responses will ensure that the subject maintains consciousness, inside the scanner. At the completion of the study, systematic assessments (VAS -- see above) will be obtained to assess sedation and mental status changes.

At the completion of the session, all subjects receiving lorazepam will be required to have another individual accompany them home after the scanning session, regardless of reported side effects. If they cannot find a person to accompany them, we will provide them with a cab directly to their home. If a subject were to have a more serious reaction, such as an acute confusional state or disinhibited behavior, we would arrange for urgent transportation to be evaluated in the UMHS Emergency room. Lastly, all subjects will be warned not to consume alcohol for 24 hours after the session, in order to avoid untoward effects from any use of alcohol after the study is done.

Risk to pregnancy: See above for MRI. Participants of child-bearing potential will be required to have a negative urine pregnancy on the day of each MRI session.

12.3.5 The risks associated with potentially disturbing material (IAPS pictures): There is a risk that subjects may become uncomfortable viewing the negative content of the IAPS pictures.

To minimize risk: The research team has had extensive experience with the IAPS picture set, in over 200 subjects, including persons with psychiatric diagnoses such as schizophrenia and post-traumatic stress disorder. This image set has been used at laboratories all over the world, and subjects tolerate the images without significant difficulty. The most aversive pictures consist of images that one would encounter in a "very gory R-rated movie," as we explain this to our subjects. When we inform our subjects about the study, we remind them they maintain control over their experience, and we encourage them to close their eyes for any image they find too intense. We also remind them that they can terminate the study, at any time. We have presented the images and discussed the protocol with members of the University of Michigan IRB, as well as community members at the local mental health agency. Mindful of potential psychological harm that some images could present for vulnerable psychiatric patients, we have excluded images suggestive of suicidal behavior from those we would present to our subjects. If any participant should become upset while viewing the pictures, the PI would be available to counsel the subjects and reinforce positive coping strategies to deal with the experience.

12.3.6 Risk of delay of treatment: Patients with psychosis who enter the study as unmedicated may experience a delay in treatment they would otherwise receive.

To minimize risk: The period of the study (4-6 weeks) is very short relative to the course of psychosis and the initiation of treatment in standard clinical settings. Because of diagnostic uncertainty in early psychosis patients, it is clinically appropriate to observe a patient over a several month period to ascertain the need for antipsychotic medication. The patients typically seen in the setting of the PREP clinic do not routinely require inpatient hospitalization; thus, they can be safely managed as outpatients. Many of the patients are not interested in taking antipsychotic medications. Therapists experienced in Cognitive Behavioral Therapy for Psychosis (CBTp) see the patients weekly, or more often if necessary. As needed doses of lorazepam will be provided (up to 3 days prior to an MRI scan). If a patient is too unstable or impaired to participate, they will be withdrawn from the study.

12.3.7 Risk of symptom worsening: Any patient study will entail the risk that symptoms may worsen, and that the stress of participation will exacerbate symptoms, including suicidal thoughts/behaviors.

Procedures for dealing with symptom worsening, suicidal thoughts, plans and intentions:

Clinically trained study staff will be alert for the emergence of symptom worsening amongst enrolled subjects during the course of participation. The fMRI protocol will include systematic monitoring before and after each session of basic emotions (fear, sadness, anxiety, nervousness), along with their prominent symptom, using a visual analogue scale. In the event of concerns about symptom worsening, the PI will be promptly notified, and plans will be formulated for additional emergency evaluation at the psychiatric emergency room (University of Michigan Psychiatric Emergency Services), if appropriate. Similarly, if the evaluation of a potential subject uncovers suicidal plans or intentions, the same procedures for emergency evaluation will be followed.

Suicide protocol: The suicide protocol begins with assessment of suicidal thoughts, plans, intentions, and behaviors with the C-SSRS (at initial screening). If assessment uncovers suicidal plans or intentions or recent behaviors, emergency evaluation will occur, and an appropriate plan of action will be followed, e. g. referral to psychiatric emergency room. The PI will be contacted and will join the decision-making process about how to manage the suicidal thoughts or behaviors.

Subjects who experience severe symptom worsening, including suicidal thoughts or behaviors, will be withdrawn from the protocol.

12.4 Potential Benefits and Justification of Risks:

There are no direct benefits for the participants in this research. However, the proposed experiments should reveal important information about the nature of mental processes involved in normal physiology as well as the pathophysiology of psychosis. Results from this study will inform future investigations into therapeutic agents that manipulate the GABAergic system, and a focus on the negative affect that occurs in person with schizophrenia, bipolar disorder and early psychosis. There is no other safer or less-invasive means of answering the questions posed by the experiments described above.

13.0 Data Handling and Record Keeping

13.1 Data handling

The investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents will be completed in a neat, legible manner to ensure accurate interpretation of data. Dark ink is required to ensure clarity of reproduced copies. When making changes or corrections, the original entry will be crossed with a single line, and initial and date the change. For some assessments, subjects may enter information directly onto a computer, rather than a paper form. Only research ID numbers will link these data with the subjects, and data will be stored in encrypted form on password-protected, secure computers. Systems that are only used to collect data, such as laptop or tablet computers, will also have research files securely deleted after upload to the central database.

Research data will be maintained on password protected computers, on a secure linux cluster maintained in the Psychiatry Affective Neuroimaging Laboratory (PANLab) or on secure UMHS-administered servers. Backup procedures include RAID drives with mirroring, and off-site backup.

Recruitment tracking files are kept only for the duration of recruitment. Paper records are kept in locked file drawers in a locked room, to which only authorized research personnel have access. Paper records with identifying information (consent form, payment records) are kept in locked file cabinets, physically separate from the research records. Computer records with identifying information (including electronic consents) are kept on secure, password protected servers. Electronic databases will be

used to store the coded data, and the key linking to identifiable subject information will be kept separate from the database with research data.

13.2 Plans to destroy links between research data and subjects.

At the point where additional data collection becomes unlikely, the identifying links between subject identity and the research data will be destroyed.

13.3 Data Sharing

The value of this research is significantly enhanced by making the data available for the widest possible use, and because of the rapidly changing world of data analysis, it is impossible to predict what future research will do with data collected for any particular project in the present. By keeping the data indefinitely, it will become available for projects, yet to be determined. These include projects run by the investigators, as well as those outside the institution through data sharing plans or central repositories. The NIMH, which funds this study, requires that data be uploaded into the NIMH Data Archive (NDA), which is a requirement of receiving funding.

When data are transferred to co-investigators and data repositories, de-identification will be performed, removing all HIPAA identifiers from the files. To prevent reconstruction of facial likenesses from structural image datasets, we will blur/obscure/strip the information from the MRI image around the face.

Subjects will be informed in the consent document that the data will be stored and that it may be shared with other investigators, both at the University and outside the University, through central databanks. The risk posed to subjects will be very, very small. Storing the data for an indefinite amount of time will follow strict protocols, outlined above, that will ensure the safety of the data and minimize the risk of accidental disclosure. Data that is shared or uploaded to central databanks will be de-identified and will be effectively anonymized, since investigators using these central databases will not have access to the keys linking individual subjects to the data. Furthermore, no data will be shared without an agreement by stewards of the central databank to require data use agreements and prevent investigators from even attempting to identify subjects. It is extremely unlikely that an investigator would even be able identify subjects from shared data, without already having access to a subjects' identifiers.

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