Background: There is converging evidence to suggest that kynurenine pathway disturbances may be related to the pathophysiology of schizophrenia. In particular, clinical, genetic, and post-mortem studies suggest that the disruption of key regulatory pathway enzymes results in increased CNS production of kynurenic acid (KYNA) (1-10); a known antagonist of α-7 nicotinic and N-Methyl-D-aspartate (NMDA) glutamate receptors (11,12). The KYNA antagonism of these receptors is hypothesized to be a critical mechanism in the development of the cognitive impairments observed in schizophrenia. In our previous work, we demonstrated that increased KYNA (following tryptophan (TRYP) challenge) impaired learning on verbal and visual memory tests in healthy controls. In addition, we found that increased KYNA decreased whole brain and frontal cortical gray matter cerebral blood flow (CBF) in people with schizophrenia. Previous studies have also shown reduced resting frontal CBF, assessed with ASL, in schizophrenia (13-16); importantly lower resting CBF is related to poorer cognitive function in schizophrenia (17,18). Furthermore, we identified a subgroup of people with schizophrenia with elevated serum KYNA levels, who were characterized by higher BPRS total, positive symptom, and thought disorder factor scores; and who exhibited a significant worsening on their performance of a sustained attention task following TRYP, but not placebo, administration. Finally, we recently reported that higher circulating KYNA correlates with lower brain glutamate in humans (19) and present preliminary evidence that higher brain KYNA is associated with lower white matter fractional anisotropy. The convergence of these results provides further support for the hypothesis that increased KYNA may be related to the pathophysiology of cognitive impairments in schizophrenia.

Of particular interest to the current project, we have recently demonstrated that high dose N-acetylcysteine (NAC) inhibits kynurenine aminotransferase (KAT) II activity, the enzyme that converts kynurenine to KYNA. Specifically, we have shown that NAC reduces basal extracellular KYNA levels, measured by microdialysis, in the prefrontal cortex of freely moving rats; and the *de novo* production of KYNA from its precursor. In addition, we have shown that the IC_{50} of NAC on KAT II activity in liver and brain tissue homogenates from mice, rats, pigs and humans is in the high μM to the low mM range. Finally, we tested the effect of NAC on pure human recombinant KAT II protein and found that NAC inhibits enzyme activity with a IC_{50} of ~500 μM. Taken together, these results indicate that NAC can reduce cerebral KYNA levels via KAT II inhibition.

Specific Aim: The purpose of the current study is to examine whether high dose N-acetylcysteine (NAC) blocks the adverse effects of increased kynurenic acid (KYNA) on selected measures of brain chemistry, function and behavior, through the inhibition of kynurenine aminotransferase (KAT) II, which converts kynurenine to KYNA. The study will be a double-blind, placebo-controlled, randomized cross-over challenge study, in which people with schizophrenia are pretreated with either high-dose NAC, 140 mg/kg up to a maximum of 15 g, or placebo, then receive tryptophan (TRYP), 6 gms. The tryptophan challenge method robustly increases peripheral measures of kynurenine and KYNA in humans and putatively increases brain KYNA levels, through the CNS conversion of kynurenine to KYNA; a process that is observed in both rodents and nonhuman primates. We will evaluate the ability of NAC to inhibit the conversion of kynurenine to KYNA with the following primary outcome measures: 1) we will measure serum kynurenine and KYNA before and after NAC/placebo pre-treatment and TRYP administration and examine whether NAC compared to placebo blocks the peripheral conversion of kynurenine to KYNA; 2) we will use the arterial spin labeling (ASL) technique to measure whole brain and frontal gray matter cerebral blood flow (CBF) before and after NAC/placebo pre-treatment and TRYP administration and examine whether NAC compared to placebo attenuates the effects of TRYP on ASL CBF measures; 3) we will use magnetic resonance spectroscopy (MRS) to measure glutamate and glutathione levels in the medial prefrontal cortex (mPFC) before and after NAC/placebo pre-treatment and TRYP administration and examine whether NAC compared to placebo increases MRS glutathione and glutamate measures; and 4) we will use diffusion tensor imaging (DTI) to measure white matter fractional anisotropy (FA) before and after NAC/placebo pre-treatment and TRYP administration and examine whether NAC compared to placebo increases white matter FA.

We will have two secondary endpoints. First, if we observe that NAC attenuates the effects of TRYP on ASL and/or increases mPFC glutamate levels or white matter DTI FA, then we will examine whether these effects are related to changes in cognitive measures of attention, verbal and visual memory, and working

memory. Second, we will examine the relationship of serum KYNA levels and kynurenine 3-monooxygenase (KMO) gene polymorphisms to the observed effects of NAC on our neuroimaging and cognitive outcome measures.

We hypothesize that NAC will inhibit KAT II, which will be reflected in the: 1) decreased peripheral conversion of kynurenine to KYNA; and 2) increased CBF, glutamate, and white matter fractional anisotropy (FA). In addition, we hypothesize that the NAC effects on the neuroimaging measures will be related to improved performance on cognitive measures of attention, verbal and visual memory and working memory. These observed effects of NAC will be greater than those seen with placebo. We further hypothesize that the NAC effects on ASL CBF, glutamate, and FA measures will be independent of NAC-induced changes in MRS glutathione, i.e., not due to the NAC oxidative stress mechanism, but, rather, will be correlated with NAC-induced reductions in the peripheral conversion of kynurenine to KYNA. Finally, we hypothesize that the observed effects of NAC on CBF, glutamate, and FA will be related to baseline serum KMO activity and KYNA levels. The demonstration that NAC reverses the adverse impact of increased KYNA levels will importantly support the development of KAT II inhibitors for the enhancement of cognition in schizophrenia.

Methods: The proposed study will be a double-blind, placebo-controlled, randomized cross-over challenge study, in which people with schizophrenia are pretreated with either high-dose NAC, 140 mg/kg up to a maximum of 15 g, or placebo, then receive TRYP, 6 gms. We will collect baseline and post-treatment KYNA and kynurenine; clinical; neuroimaging; and cognitive measures. Participants (n=100) will be of either sex and any race, between 18 - 55 years old, with a Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 (20) diagnosis of schizophrenia or schizophreniform or schizoaffective disorder. A best estimate diagnostic approach will be utilized in which information from the Structured Clinical Interview for DSM-5 (SCID; 21) is supplemented by information from family informants and medical records to generate a diagnosis. Participants will be judged by their clinician to be clinically stable. We will not restrict the type of antipsychotic with which the participant is treated or the use of concomitant medications; there is little evidence of a differential effect of first and second generation antipsychotics on KYNA levels (22). The inclusion and exclusion criteria are:

Inclusion Criteria:

1) Males and females
2) Age: 18 to 55 years
3) DSM-5 Criteria for schizophrenia, schizoaffective disorder or schizophreniform disorder (documented by SCID)
4) Prescription of antipsychotic medication for at least 60 days and constant dose for 7 days prior to study entry (either first or second generation antipsychotics permitted)
5) Female participants must agree to use a medically accepted means of contraception

Exclusion Criteria:

1) DSM-5 alcohol or substance use disorder in the last 3 months, other than nicotine and cannabis (documented by SCID). Only participants meeting a moderate to severe cannabis use disorder will be excluded.
2) History of an organic brain disorder; intellectual disability; or a medical condition, whose pathology or treatment could alter cognition or increase their safety risk of protocol participation
3) Active disorders that have been reported to affect tryptophan metabolism or interfere with absorption will be excluded (Acute Intermittent Porphyria, Celiac Disease, Crohn's Disease, Irritable Bowel Syndrome).
4) Excessive self-reported daily caffeine intake, defined as intake exceeding 1000mg or the equivalent of 10 cups of coffee
5) Pregnancy or lactation secondary to pregnancy
6) Presence of MRI contraindications (e.g. pacemakers)
7) Regularly scheduled use of monoamine oxidase inhibitors, migraine headache medications (triptans) and dextromethorphan. Excludes only daily use, prn permitted.

Participant Recruitment: Potential participants with SRDs will be recruited from three sources: the Maryland Psychiatric Research Center (MPRC) clinical programs: the Outpatient Research Program (ORP), the Treatment Research Program (TRP), and the First Episode Clinic (FEC); local outpatient mental health centers; and a standing recruitment database, from which we have enrolled participants in previous MPRC clinical trials. Potential MPRC and community participants will be identified primarily through chart review or nomination by their primary clinicians who are aware of study entry criteria and demands, and have been asked to identify clinically stable patients who may be interested in research participation. MPRC and community participants may also be self- or peer-referred for this study. Participants may also be recruited from internet based advertising (HP-00061828) that will refer interested participants to the MPRC web site

Study Assessments:

Clinical Assessments: The following rating scales will be used to characterize the baseline clinical state of the study sample and to assess whether there any changes in the clinical state of the study participants during their participation in each of the Challenge Phase visits: the Brief Psychiatric Rating Scale (BPRS; 23) total score will be used to measure global psychopathology. The four BPRS positive symptom items - conceptual disorganization, suspiciousness, hallucinatory behavior, and unusual thought content - will be used to measure positive psychotic symptoms. The Scale for the Assessment of Negative Symptoms (SANS; 24) total score will be used to measure negative symptoms; Calgary Depression Scale (CDS; 25) total score will be used to measure depressive symptoms; and Clinical Global Impression Scale (CGI; 26) severity of illness item will be used to confirm clinical stability prior to entry into the Challenge Phase and assess global changes during the Challenge Phase.

Cognitive Assessments: The following cognitive assessments will be used to assess the effects of NAC-induced KAT II inhibition on neuropsychological test performance. We will use the following 7 tests from the MATRICS Consensus Cognitive Battery (27): Brief Assessment of Cognition in Schizophrenia (BACS), Symbol-Coding, Hopkins Verbal Learning Test–Revised (HVLT-R), Brief Visuospatial Memory Test–Revised (BVMT-R), Continuous Performance Test-Identical Pairs (CPT-IP), Maryland Letter-Number Sequencing (LNS), and WMS®-III Spatial Span.

Clinical and Cognitive Assessment Training and Reliability: The MPRC provides resources for the conduct of training and interrater reliability procedures for the BPRS, SANS, CDS, and CGI. The clinical rater will be appropriately trained and meet required inter-rater reliability standards (ICC ≥ 0.80), before assignment as a rater. During the course of the study, interrater sessions for these assessments will be held every three months to maintain rater reliability and minimize rater drift. Dr. Gold will train the neuropsychological rater in the administration of the cognitive assessments.

Laboratory Assessments: The following laboratory assessments will be collected:

a) Kynurenine pathway metabolites: We will collect the following kynurenine pathway metabolites: kynurenine and KYNA. We will use high performance liquid chromatography to measure serum kynurenine and kynurenic acid levels. Perfusate samples will be diluted 1:2 for determination of both molecules. Plasma samples will be diluted 1:2 for kynurenine and 1:10 for KYNA determination. Twenty µL will then be subjected to HPLC analyses and analytes will be detected fluorimetrically.

b) We will collect ~8mL of whole blood which will be transferred into a 10mL Cryovial cryogenic tube and placed in -80 freezer until time of analysis. Single-Nucleotide Polymorphism (SNP) genotyping will be performed on gDNA isolated from blood with QIAamp DNA Maxi Kit (Qiagen) using TaqMan polymerase chain reaction (PCR) technology.c) Two aliquots of blood will be stored at -80 until analyzed for inflammatory markers, oxidative stress, and other related markers.

Neuroimaging Assessments and Procedures: All participants will undergo pre- and post-treatment structural, ASL, and MRS studies. MRI data will be acquired using a 3-T Siemens Prisma scanner equipped with a 64 channel head coil at the University of Maryland Center for Brain Imaging Research (CBIR), located at the MPRC. A T1-weighted structural image (MP-RAGE: 0.8 mm isotropic voxels, 256X256 mm FOV, TR/TE/TI = 2400/ 2.22/ 1000ms) will be acquired for spectroscopic voxel prescription and segmentation and anatomical reference.

Arterial Spin Labeling (ASL): We will use a Pseudo-continuous Arterial Spin Labeling (pCASL) sequence that provides full brain coverage with high spatial resolution and excellent WM signal-to-noise ratio (SNR) (SNR>15). Specifically, we will use a pCASL EPI with TE/TR = 16/4000ms, labeling duration = 2100ms, 24 contiguous slices with 5 mm thickness, matrix = 64 x 64, 3.4 x 3.4 x 5 mm resolution (FOV = 220 mm), labeling gradient of 0.6 G/cm, bandwidth=1594 Hz/pixel, labeling offset = 90 mm (32). A total of 68 alternating labeled and unlabeled image pairs will be collected. Equilibrium magnetization (M0) images will be collected using a long TR=10s protocol (33). ASL data will be processed using the pipeline described elsewhere (http://www.mccauslandcenter.sc.edu/CRNL/tools/asl). Absolute gray and white matter cerebral blood flow quantification will be calculated in native space from the mean perfusion images. Voxel-wise perfusion, in ml per 100 g per minute, will be calculated under the assumption that the post label delay is longer than the arterial arrival time (32), with labeling efficiency set at 0.85, and the mean transit time set to 0.7s, based on empirical data (34).

Magnetic Resonance Spectroscopy (MRS): Glutamate will be detected using very short TE phase rotation stimulated echo acquisition mode (STEAM: TR/TM/TE= 2000/10/6.5 ms, NEX=128, 2500-Hz spectral width, 2048 complex points, and $\Delta\varphi1=135°$, $\Delta\varphi2=22.5°$, $\Delta\varphi3=112.5°$, and $\Delta\varphi ADC=0°$) (35). Water suppression will be automated using a water suppression enhanced through T1 effects sequence (WET). Spectra will be analyzed using the fully automated, standard curve-fitting software, LCModel. Metabolites will be referenced to water and only metabolites of good fits (CRLBs< 20) will be included. Voxel will be segmented into CSF, gray, and white matter tissue using SPM8 and in house Matlab code; metabolites will be corrected according to Gasparovic et al (36). We will use the gold standard MEGA-PRESS sequence for GSH detection and quantification (TR/TE: 2000/120ms (37), NEX=256 (128 “on” and 128 “off”, on editing pulse frequency = 4.56ppm, refocusing pulse bandwidth=kHz) (38). GSH data will be analyzed using Gannet 3.0 and GSH/Water ratios with fit errors above15%, will be excluded. We have shown these sequences to be optimal for glutamate and GSH detection through rigorous phantom and simulation studies, and highly reproducible through test-retest studies in healthy control and schizophrenia samples (38-41).

Diffusion Tensor Imaging (DTI): Diffusion weighted data will be collected used a homologized DWI protocol, based on our on-going Amish Connectome Study, which consists of fifteen b-shells distributed equally between b=0-3500 s/mm2 with 32-directions/shell. The same analysis protocol is used in pigs and humans and combines diffusion tensor imaging (DTI), White Matter Tract Integrity (WMTI) and Permeability-Diffusivity (PD) modeling. DTI is a commonly used DWI approach, which describes the Gaussian properties of the diffusion displacement distribution of water in the brain by fitting a mono-exponential function to the weighted diffusion signal decay at a low “diffusion weighting” (b-value ≤ 1000 s/mm2). The choice of a lower b-value range makes DTI sensitive to water molecules with high diffusivities. DTI FA is a simple and empirical parameter; however, at higher b-values, DTI fails to approximate the non-mono-exponential decay of diffusion signal that is caused by the restriction of diffusion by cellular membranes. WMTI and PD models overcome DTI limitations; characterize the sources of the non-Gaussian distribution of diffusion signals; and provide additional characterization of WM integrity, which are sensitive to slow-diffusive water pool: Kurtosis Anisotropy (KA) and Permeability-Diffusivity Index (PDI). These two measures are sensitive to exchange between axonal and extra axonal water-pools and thus are sensitive to the functional component of axonal diffusivity (42-44).

Medical and Safety Assessments: In the 2-week Evaluation Phase, all participants will receive a complete medical history and physical examination, including vital signs (blood pressure, heart and respiratory

rates, height and weight), and a nicotine use questionnaire. We will also collect baseline EKG; CBC; complete metabolic panel, (including electrolytes, BUN/Creatinine, LFTs, lipid panel, and TSH); U/A; expired CO, and Toxicology screen assessments. Female participants will have a pregnancy test during the Evaluation Phase and on each challenge test day. The Side Effect Checklist (SEC) is designed to assess medication side effects commonly associated with pharmacological treatments. The SEC version for the proposed study rates 32 potential side effects, and comprehensively covers the side effects that have been previously reported with NAC and TRYP administration, i.e., nausea, vomiting, diarrhea, abdominal pain, dizziness, dysgeusia, rash with or without fever, and sedation. In addition, there are three "other" spaces for idiosyncratic participant complaints, which are not usually associated with medication treatment. We will use the self-rated Stanford Sleepiness Scale (SSS) to supplement our assessment of sedation (45). The SSS is a 7-point Likert scale.

Study Procedures: Study Visit 1 will last about 3 hours, Study Visit 2 will last about 2 hours, and Visits 3 and 4 each will each last about 7.75 hours for a total study participation time of 20.5 hours.

Evaluation Phase (Visits 1 and 2): There will be a 2-week Evaluation Phase, during which potential participants will undergo baseline assessments to determine whether they meet inclusion criteria. Participants will be clinically stable, with clinical stability defined as two consecutive CGI ratings with no change in score.

Challenge Phase (Visits 3 and 4): The Challenge Phase will utilize a double-blind, placebo-controlled, randomized cross-over design. A permuted block randomization system (block sizes 2 or 4) will be used to randomly assign participants to NAC, 140 mg/kg up to a maximum of 15 g, or placebo. There will be two Challenge Phase visits, which will be at least two weeks apart. On the day of each visit, participants will arrive at the MPRC Brief Stay Unit (BSU) at 7:15AM; all participants will have fasted since midnight. Participants are asked to avoid eating food of any kind after midnight the evening prior to visits 2 and 3. Participants are encouraged to drink water but to strictly avoid food, coffee, alcohol, and sodas. Participants who regularly use nicotine products will be given short breaks to use their products in order to avoid effects of nicotine withdrawal. The nicotine breaks will occur at least 60 minutes prior to the neuroimaging sessions in order to minimize the effect of nicotine on any of the neuroimaging measures. A heparin lock will be inserted for the repeated laboratory assessments and the initial clinical, cognitive, laboratory, and safety assessments will be conducted (including urine pregnancy test for female participants). Participants will then undergo their pre-treatment imaging studies. Upon completion of the imaging studies, participants will receive NAC, 140 mg/kg up to a maximum of 15 g, or placebo. They will receive the other study medication on the second visit (i.e., placebo or NAC). The proposed NAC dose has been used extensively to treat acetaminophen poisoning. We selected this high dose based on our preclinical studies showing high doses inhibit KAT II. There is extensive safety data available with this dose. We selected the effervescent formulation to minimize gastrointestinal and other side effects. In addition, the effervescent formulation is flavored, which helps to increase the tolerability and to mask the smell typically associated with NAC (46). Thirty minutes after NAC/placebo administration participants will receive TRYP, 6 grams in oral slurry form. The dose was chosen based on previous work showing cognitive effects to be associated with doses above 5 grams (47) and our the preliminary data from our previous Conte Center project, in which we demonstrated that TRYP (6 grams) produces a robust increase in KYNA and kynurenine and reduction in the ASL measure of CBF in participants with schizophrenia and related disorders.

Two hours after ingestion of NAC/placebo, participants will undergo the post-treatment imaging studies. We chose to begin scanning 120 minutes after NAC and 90 minutes after TRYP, because 1) our preclinical data show that NAC administered 30 minutes prior to TRYP administration attenuates KYNA production through KAT II inhibition; 2) our preliminary data suggests that peripheral kynurenine and KYNA levels are initially elevated 30 minutes after TRYP ingestion, and continue to increase over the next three hours and remain elevated for at least 3.5 hours; 3) NAC pharmacokinetic studies have demonstrated that peak serum levels occur within 90-150 minutes of administration and remain elevated for 4-6 hours (46,48); and 4) GSH, an indirect measure of NAC in the CNS, starts to elevate 50 minutes after NAC administration, reaches maximal elevation 90-110 minutes after oral NAC administration and remains elevated for at least 120 minutes (49). Thus, there should be sufficient time to complete the post-treatment MRI, clinical, laboratory, and cognitive assessments. A non-blind pharmacist will dispense all study medications. The blind will be broken

only if a medical emergency requires this information. If this occurs, the participant will be withdrawn from the study. The day after each challenge day, the RA will follow-up with the participant, via telephone, to evaluate whether there are any persistent effects from the receipt of the challenge medications. Any concerns of the participant will be reported immediately to the study physician.

Procedures/Assessments	**Evaluation Phase** **(2 weeks)**	**Challenge Visit #1:**	**Challenge visit #2:**	**Initials**
Informed Consent, Evaluation to Sign Consent	X			
Medical Screening: Medical History, physical exam, blood draw, urinalysis, toxicology screen, pregnancy test (females), EKG, medication use screening, caffeine use screening, Birth Control Selection (females)	X			
MRI screening	X			
Clinical Stability Determination (for participants with schizophrenia or a related disorder only): 2 unchanged CGIs	X			
Scheduling of challenge day visits	X			
Participant arrival at Brief Stay Unit (BSU) Review of Study Procedures		7:15 am	7:15 am	
Urine pregnancy (females); expired CO**; urine toxicology and breath alcohol testing		7:30 am	7:30 am	
Insertion of heparin lock		7:40 am	7:40 am	
Vital signs, SEC, SSS;		7:50 am	7:50 am	

Laboratory Assessments (1): kynurenine pathway metabolites, inflammatory markers, oxidative stress and other related markers				
Nicotine Break (1)* Protein Free Snack		7:55 am	7:55 am	
Cognitive Measures (1): MCCB measures: HVLT-R, BACS Symbol Coding, BVMT, CPT-IP, WMS®-III, LNS;		8:00 am	8:00 am	
Clinical Assessments (1): BPRS, SANS, CDS, CGI		8:45 am	8:45 am	
Neuroimaging Studies (1)		9:15 am	9:15 am	
Nicotine Break (2)		10:30 am	10:30 am	
Oral Administration of N-acetylcysteine (NAC) 140 mg/kg up to a maximum of 15gm or placebo		10:35 am	10:35 am	
Oral Administration of Tryptophan 6 grams		11:05 am	11:05 am	
Lunch		11:25 pm	11:25 pm	
Vital signs, SEC, SSS Laboratory Assessments (2)		12:05 pm	12:05 pm	
Neuroimaging Studies (2)		12:20 pm	12:20 pm	
Laboratory Assessments (3)		1:35pm	1:35pm	
Protein Free Snack Nicotine break (3)		1:40 pm	1:40 pm	
Cognitive Measures (2)		1:55 pm	1:55 pm	

Clinical Assessments (2)		2:40 pm	2:40 pm	
Vitals, SEC, SSS Laboratory Assessments (4)		3:05 pm	3:05 pm	
Participant discharged. If participant has not returned to pre-challenge baseline, they will be reassessed every 30 minutes until ready		3:15 pm	3:15 pm	

*Nicotine breaks are optional and subject to occur at different times as long as they do not occur within 60 minutes prior to the neuroimaging studies.

**Due to the COVID-19 pandemic, the expired CO breathalyzer assessment is an optional procedure.

Randomization and Blinding: This will be a double-blind, placebo-controlled, randomized cross-over challenge study. Participant randomization will use a permuted block randomization system (block sizes 2 or 4), in which treatment assignment order is random within each block, with an equal number of participants assigned to each treatment, to generate a list of treatment assignments. Thus, it will be difficult to ascertain the next treatment assignment, even if a participant becomes unblinded, while any imbalance in the number of participants between the treatment groups will be kept within tight limits. All raters, investigators and other staff will be blind to treatment assignment except for the research pharmacist. The research pharmacist does not participate in assessing any of the primary symptom or side effect dependent variables and conveys no information about treatment assignment to participants or staff except in a medical emergency.

Adherence: The NAC and placebo will be administered as dissolving tablets in a glass of 300 mL of water and the tryptophan will be given as a powder mixed with 150 mL of water. The participants will drink both mixtures in the presence of research staff.

Participant Payment: Participants will receive $20 per hour and $100 for the completion of each challenge day, for a total of approximately $620. Participants will be paid by check, which may take up to 4-6 weeks to receive payment.

Management of Data: Data collected for this study will be entered into the MPRC research data base using the usual procedures developed for MPRC clinical trials. This study will be reviewed UMB IRB and by a Data Safety Monitoring Board (DSMB).

Data Analysis: Statistical analyses will be conducted using intent-to-treat analytic strategies. The overarching goal of our statistical approach is to identify the NAC versus placebo effects on measurements collected from

various biomedical assays, including peripheral KYNA (Specific Aim #1), ASL-CBF (Specific Aim #2), DTI FA and MRS measures (Specific Aim #3). The multilevel mixed effect model will be used to examine the NAC effects, while accounting for the hierarchical random effects that 1) each participant will have two Challenge Phase visit days; and 2) on each day longitudinal and/or multivariate measurements will be recorded (e.g. KYNA will be measured at different time points); the multilevel mixed model is compatible with unbalanced measurements. Taking Specific Aim #1 for example, the model will be KYNA ~ Treatment + covariates + randomEff-day/time. Adjustments for multiple testing will be performed when necessary. When the variance and covariance matrix is difficult to compute, the hierarchical Bayesian model will be implemented for parameter estimation via Markov Chain Monte Carlo (MCMC) sampling. With a sample size of 100 participants and 20% attrition rate (i.e., 60 participants will complete the study), we will achieve power > 80% for all three specific aims. In Exploratory Aim 1, we will perform mediation analysis to examine the NAC effects on cognitive measures via the changes in the central nervous system (neuroimaging measures as mediators). In Exploratory Aim 2, we will evaluate whether the baseline KMO and/or KYNA levels can moderate the NAC effects on the measures described in Specific Aims 1-3 (50).

Specific Aim #1: Peripheral KYNA will be measured prior and after treatment on both Challenge Phase visit days (i.e., N-acetylcysteine (NAC) and placebo). We will denote the Challenge Phase visit day as d=1,2, and the multiple measurement times on each day by t=1,…, T for each participant i (i=1,…, 100). To test the hypothesis whether NAC compared to placebo blocks the peripheral conversion of kynurenine to KYNA, we will apply the multilevel mixed model to compare the changes of KYNA levels from baseline between the two treatment groups. The hierarchical random effects will include day and time points within a day. With a sample size of 100 participants and 20% attrition rate (i.e., 60 participants will complete the study), we will achieve power 80% to detect a small-medium effect size of Cohen's d 0.37 at the alpha level 0.05. In our previous study, the effect size of peripheral KYNA change (tryptophan vs. placebo) is d= 4.77. Thus, we will have ample power to detect the difference. The covariates including age, sex, and others will be examined and adjusted when necessary.

Specific Aim #2: To examine whether NAC compared to placebo attenuates the effects of tryptophan on ASL CBF measures, we will apply the mixed model to compare the changes of ASL CBF levels from baseline between treatment and placebo. The primary ASL outcome measure will be whole brain gray matter CBF. If significant, then we will perform additional analyses to examine whether specific cortical regions show group differences. The multiple comparison correction will be performed for the voxel level and cluster level inference using permutation test-based strategy (51). With a sample size of 100 participants and 20% attrition rate (i.e., 60 participants will complete the study), we will achieve power 80% to detect a small-medium effect size of 0.37 at the alpha level 0.05. In our previous study, the effect size of ASL whole brain gray matter CBF (tryptophan vs. placebo) was Cohen's d=0.48. Thus, we should have sufficient power to detect the difference. The covariates including age, sex, and others will be examined and adjusted when necessary.

Specific Aim #3: To examine whether NAC compared to placebo increases the whole brain white matter Kurtosis Anisotropy (KA) and Permeability-Diffusivity Index (PDI) and ERP interhemispheric transfer and MRS glutamate measures, we will apply the mixed model to compare the changes of FA, ERP interhemispheric transfer, and MRS glutamate levels from the baseline between treatment and placebo. With a sample size of size of 100 participants and 20% attrition rate (i.e., 60 participants will complete the study), we will achieve power 80% to detect a small-medium effect size of Cohen's d 0.44 at the alpha level 0.0125 with the Bonferroni correction. If the whole brain white matter KA and PDI are significant, we will perform follow-up analyses to examine whether specific cortical white matter regions show group difference. The multiple comparison correction will be performed for the voxel level and cluster level inference using permutation test-based strategy (51).

To synchronize all aims, we will perform mediation and moderation analysis and causal inference to systematically investigate i) whether the attenuation of the effects of elevated KYNA can alter performance on the cognitive measures via changing the function, chemistry and/or structure of the central nervous system (CNS); and ii) whether the complete or partial mediation pathways can be modified by the baseline KMO and KYNA levels. Importantly, the carefully designed challenge study will provide a unique opportunity to construct

the causal relationship that changing the dynamics of kynurenine-related pathways by NAC and TRYP =>changes in CNS=>altered cognitive functions in people with schizophrenia (52).

In Exploratory Aim 1, we will perform mediation analysis to examine the NAC effects on cognitive measures via the changes in the central nervous system (i.e., neuroimaging measures as mediators). The mediation analysis will be performed using the procedures in VanderWeele (50), since multiple mediators will be analyzed. The multiplicity will be adjusted, and variable selection steps will be performed to construct the most parsimonious mediation models. In addition, the multivariate CNS measurements may be correlated and the covariance/dependence structure will be taken into account for statistical inference (53). The model validation (e.g. bootstrap procedure) will be performed to ensure the robustness and stability of the mediation model.

In Exploratory Aim 2, we will evaluate whether the baseline KYNA and/or KMO activity levels can moderate the NAC effects in the Specific Aims 1-3 and Exploratory Aim 1. For the measurements in the Specific Aims 1-3, the interaction term between baseline KYNA and/or KMO activity and NAC treatment will be tested for the moderation effect. To examine whether baseline KYNA and/or KMO activity moderate the mediation pathways in Exploratory Aim 1, the nonparametric statistical inference (e.g. permutation tests and bootstrap inference) will be used due to relatively small sample size for the complex mediation and moderation model.

Potential Risks to the Participants:

Risks Related to N-acetylcysteine:

- Diarrhea. Somewhat likely and seriousness varies. If participants experience diarrhea they are free to end participation and may remain at the Research Center until they feel well enough to drive or someone can take them home. They will be instructed to drink fluids to remain hydrated.

- Flatulence. Somewhat likely and not serious. If participants experience flatulence they are free to end participation and may remain at the Research Center until they feel well enough to drive or someone can take them home.

- Nausea. Somewhat likely and not serious. If participants experience nausea they are free to end participation and may remain at the Research Center until they feel well enough to drive or someone can take them home.

- Vomiting. Somewhat likely and seriousness varies. If participants experience vomiting they are free to end participation and may remain at the Research Center until they feel well enough to drive or someone can take them home. They will be instructed to drink fluids to remain hydrated.

- Abdominal discomfort. Somewhat likely and not serious. If participants experience abdominal discomfort they are free to end participation and may remain at the Research Center until they feel well enough to drive or someone can take them home.

- Dysguesia. Not likely and not serious. If participants experience a change in their sense of taste they are free to end participation in the study and stop taking the study medication.

- Dizziness. Not likely and not serious. If participants experience dizziness they are free to end participation and may remain at the Research Center until they feel well enough to drive or someone can take them home.

- Rash (with or without fever). Not likely and not serious. If participants experience a rash, with or without fever, they are free to end participation and may remain at the Research Center until they feel well enough to drive or someone can take them home.

Risks Related to Tryptophan:

- Nausea, dyspepsia. Somewhat likely but not serious. In our previous experience with tryptophan, nausea was seen in 1 of 11 participants exposed to tryptophan. The participant vomited and then felt relief. If participants experience nausea they are free to end participation and may remain at the Research Center until they feel well enough to drive or someone can take them home.

- Vomiting. Somewhat likely and seriousness varies. If participants experience vomiting they are free to end participation and may remain at the Research Center until they feel well enough to drive or someone can take them home. They will be instructed to drink fluids to remain hydrated.

- Sleepiness. Somewhat likely and not serious. If participants experience sleepiness they are free to end participation and may remain at the Research Center until they feel well enough to drive or someone can take them home.

- Transient exacerbation of cognitive impairment and/or exacerbation of schizophrenia symptoms. Expected, not serious. We hypothesize a small, transient decrease in cognitive testing scores or worsening of schizophrenia after participants receive tryptophan. This impairment is on the order of magnitude one would expect when sleepy or distracted and will not impact any routine functioning. All participants are evaluated by trained medical staff prior to discharge to confirm a return to baseline. Any persisting symptoms or abnormal vitals will be reported to the study physician who will determine whether the participant is safe to be discharged. A return to baseline before discharge will be recorded in the participant's study documents.

Blood Draw:

- Infection or bruising at the blood draw site. Not likely, seriousness varied. The blood draw and IV catheter placement will be done by experienced nursing staff using universal precautions.

MR Scanning:

- Claustrophobia. Not likely, not serious. Participants will be screened prior to scanning. They can also abort the scanning procedure by squeezing a rubber signal ball. Research staff will be in communication with participants throughout the scanning.

- Thermal injury. Not likely, possibly serious. Participants will be screened prior to scanning for MR-incompatable materials.

- Backache. Somewhat likely, not serious. Participants who experience backache while lying in the scanner may abort the scanning procedure at any time.

- Temporary decrease in hearing after MRI. Not likely, not serious. The loud noises of the MRI machine may be loud enough to damage one's hearing. Participants will be fitted with ear plugs and/or headphones to reduce this risk. Participants may abort the scanning procedure at any time if they feel uncomfortable with the noise level.

- Nausea and vomiting. There may be a slight risk from taking the study compounds. If vomiting occurs inside the scanner, there is a risk of choking and that can be dangerous. Participants will be instructed to inform research staff if they feel nauseous before the scanning or anytime during the scan.

Miscellaneous:

- Boredom, Frustration. Somewhat likely, not serious. Participants may become bored or frustrated with interviews or testing. Participants may take breaks or end interviews/testing at any time- however, doing so may interfere with completion of the study and lead to early termination of participation.

- Loss of Confidentiality. Unlikely, potentially serious. All research participation carries the risk of loss of confidentiality. This is minimized by the research protocol's protections for participant data.

Adequacy of Protection against Risks: We will make every attempt to minimize all study-related risks. We will carefully monitor patients and psychiatric symptoms throughout the study

Confidentiality: Careful procedures will be used to protect the privacy of participants and the confidentiality of the data. Names will only appear on consent forms and on a master list that links them with study ID numbers (different from medical record numbers). This list will be stored in locked files in a separate location from the data. At the conclusion of the project, the list will be destroyed, unless continuation is planned and approved by the Institutional Review Board. All data (whether on forms or electronic data files) will be collected, analyzed, and reported according to the study ID number and will contain no names or other personal identifiers. Paper-based data will be stored in locked files. Electronic data files reside on desktop computers and are password protected.

Consenting: Potential participants will be identified through chart review or nomination by the treatment team. No participant will be approached for recruitment without approval of a primary clinician, who will determine suitability of the person for the protocol. A chart review will be completed for all nominated potential participants to reduce the likelihood that participants will be found ineligible after participating in more extensive assessment. The study interviewer will verify with the primary clinician that a potential participant is sufficiently stabilized to consider participation and has capacity to provide consent. This is done prior to the study interviewer approaching a potential participant. The study interviewer will be introduced to the potential participant and provide a brief overview of the project.

Research staff members are trained to recognize symptoms of severe mental illness and cognitive impairment that could undermine an individual's ability to provide informed consent. Interested participants will be provided study information and an informed consent form that contains all pertinent details of participation. As some potential participants will have poor reading skills, the consent form will be read aloud in tandem with their own silent reading of the document. Our research staff are carefully trained on obtaining consent from and interacting with people with seriously mentally illness and supervised by senior staff members. The individual securing consent will review any points about which the participant is unclear, and the participant will be invited to ask questions as needed. All participants who express willingness to provide consent will be queried about each paragraph of the agreement in order to ensure that they have adequate understanding of what they are agreeing to. Research staff are trained in strategies for interacting with people with severe mental illness, including speaking slowly and clearly, stopping to summarize frequently, and providing time for questions.

After reading the consent, and before obtaining a signature, a brief questionnaire, the Evaluation to Sign Consent (ESC), is administered to verify that the subject is competent to provide consent and has demonstrated comprehension of the consent document. The recruiter will also make a clinical judgment and not recruit participants who appear unable to grasp key aspects of the procedure. This approach, which requires a proactive demonstration on the part of the participant that they understand what is being requested,

has been used extensively at our sites. Per IRB regulations, a copy of the signed consent form is given to the participant, a copy is placed in the participant's medical record, and the original is kept in the laboratory. Research assistants obtaining informed consent will be experienced clinicians. They will receive detailed and standardized training as to how to obtain informed consent from people with severe mental illness. They will be observed obtaining informed consent from a potential participant by senior staff prior to being allowed to recruit on their own. Prior to signing the consent form, participants will be informed that participation in the study is contingent upon their meeting diagnostic criteria as determined in the clinical interview. All discussions with potential study participants will take place in a private room behind closed doors.

Potential Benefits of the Proposed Research to Participants and Others: By participating in this study there is no direct benefit to the study for the participant. However, we may improve understanding of illnesses that affect thinking and memory, and help physicians care for patients with such problems.

Importance of the Knowledge to be Gained: People with schizophrenia are characterized by marked cognitive impairments, which are major determinants of poor functional outcome in schizophrenia (54,55). Unfortunately, first and second generation antipsychotics have limited benefits for cognitive impairments (56,57), and studies of add-on pharmacological agents for the treatment of cognitive impairments have been largely disappointing to date (58-63). Cognitive remediation and other non-pharmacological strategies have shown some promise, but the observed beneficial effects rarely generalize beyond the measures included in the training exercises (64). In the absence of an effective treatment, cognitive impairments remain a critical unmet therapeutic need, and the demonstration that inhibition of KAT II leads to improved brain and cognitive function would represent a critically important development in the therapeutics of this disorder.

Our project is uniquely based on an emerging body of evidence, which suggests that disturbances in the kynurenine pathway, especially those that lead to increased levels of KYNA, may be of critical importance in the pathophysiology of cognitive impairments in schizophrenia. Second, the proposed study is the first experimental test of brain and behavioral effects of KAT II inhibition in human subjects. The results from the study would provide unique insights into the mechanisms underlying the role of KYNA in the pathophysiology of schizophrenia. Third, although the use of NAC is not innovative, the hypotheses that: i) NAC may work through its ability to inhibit KAT II; ii) there may be large effects in a subgroup of people with schizophrenia, i.e., those characterized by high serum KYNA levels or low KMO activity; and iii) the use of high NAC doses (>5 times other clinical studies) are innovative. Fourth, in order to provide a comprehensive evaluation of the effects of NAC on various KYNA related manifestations of the illness, we will use a multi-dimensional evaluation of the effects of NAC on KYNA production in schizophrenia, which will include behavioral, biochemical, neuroimaging, and phenomenological assessments. Fifth, the parallel assessments across the two preclinical studies and two clinical studies will provide a framework to bi-directionally inform the interpretation of the results of these studies. The ability to translate the results across each of the proposed studies is critically important for the proposed Center. Finally, the demonstration that NAC blocks the impact of increased KYNA levels in people with schizophrenia will importantly support the development of KAT II inhibitors for the enhancement of cognition in schizophrenia.

References:

1. Schwarcz, R., Rassoulpour, A., Wu, H. Q. Medoff, D., Tamminga, C. A., and Roberts, R. C. (2001) Increased cortical kynurenate content in schizophrenia. Biol. Psychiatry 50, 521-530. PMID: 11600105

2. Miller, C. L., Llenos, I. C., Dulay, J. R., and Weis, S. (2006) Upregulation of the initiating step of the kynurenine pathway in postmortem anterior cingulate cortex from individuals with schizophrenia and bipolar disorder. Brain Res. 1073-1074, 25-37. PMID: 16448631

3. Barry, S., Clarke, G., Scully, P., and Dinan, T. G. (2009) Kynurenine pathway in psychosis: Evidence of increased tryptophan degradation. J. Psychopharmacol. 23(3), 287-294. PMID: 18562404

4. Sathyasaikumar, K. V., Stachowski, E. K., Wonodi, I., Roberts, R. C., Rassoulpour, A., McMahon, R. P., and Schwarcz, R. (2011) Impaired kynurenine pathway metabolism in the prefrontal cortex of individuals with schizophrenia. Schizophr. Bull. 37(6), 1147-1156. PMCID: PMC3196941

5. Joseph, M. H., Baker, H. F., Crow, T. J., Riley, G. J., and Risby, D. (1979) Brain tryptophan metabolism in schizophrenia: A post mortem study of metabolites of the serotonin and kynurenine pathways in schizophrenic and control subjects. Psychopharmacology (Berl) 62(3), 279-285. PMID: 111294

6. Miller, C. L., Llenos, I. C., Dulay, J. R., Barillo, M. M., Yolken, R. H., and Weis, S. (2004) Expression of the kynurenine pathway enzyme tryptophan 2,3-dioxygenase is increased in the frontal cortex of individuals with schizophrenia. Neurobiol. Dis. 15(3), 618-629. PMID: 15056470

7. Linderholm, K. R., Skogh, E., Olsson, S. K., Dahl, M. L., Holtze, M., Engberg, G., Samuelsson, M., and Erhardt, S. l. (2012) Increased levels of kynurenine and kynurenic acid in the CSF of patients with schizophrenia. Schizophr. Bull. 38(3), 426-432. PMCID: PMC3329991

8. Wonodi, I.,Stine, O. C., Sathyasaikumar, K. V., Roberts, R. C., Mitchell, B. D., Hong, L. E., Kajii, Y., Thaker, G. K., and Schwarcz, R. (2011) Downregulated kynurenine 3-monooxygenase gene expression and enzyme activity in schizophrenia and genetic association with schizophrenia endophenotypes. Arch. Gen. Psychiatry 68(7), 665-674. PMCID: PMC3855543

9. Holtze, M., Saetre, P., Engberg, G., Schwieler, L., Werge, T., Andreassen, O. A., Hall, H., Terenius, L., Agartz, I., Jönsson, E. G., Schalling, M., and Erhardt, S. (2012) Kynurenine 3-monooxygenase polymorphisms: Relevance for kynurenic acid synthesis in patients with schizophrenia and healthy controls. J. Psychiatry Neurosci. 37(1), 53-57. PMCID: PMC3244499

10. Schwarcz, R., Bruno, J. P., Muchowski, P. J., Wu, H. Q. (2012) Kynurenines in the mammalian brain: When physiology meets pathology. Nat. Rev. Neurosci. 13(7), 465-477. PMCID: PMC3681811

11. Birch, P. J., Grossman, C. J., and Hayes, A. G. (1988) Kynurenic acid antagonizes responses to NMDA via an action at the strychnine-insensitive glycine receptor. Eur. J. Pharmacol. 154, 85-87. PMID: 2846328

12. Hilmas, C., Pereira, P. F., Alkondon, M., Rassoulpour, A., Schwarcz, R., and Albuquerque, E. X. (2001) The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: Physiopathological implications. J. Neurosci. 21, 7463-7473. PMID: 11567036

13. Pinkham, A., Loughead, J., Ruparel, K., Wu, W. C., Overton, E., Gur, R., and Gur, R. (2011) Resting quantitative cerebral blood flow in schizophrenia measured by pulsed arterial spin labeling perfusion MRI. Psychiatry Res. 194(1), 64-72. PMCID: PMC3185150

14. Ota, M., Ishikawa, M., Sato, N., Okazaki, M., Maikusa, N., Hori, H., Hattori, K., Teraishi, T., Ito, K., and Kunugi, H. (2014) Pseudo-continuous arterial spin labeling MRI study of schizophrenic patients. Schizophr. Res. 154(1-3), 113-118. PMID: 24581548

15. Kindler, J., Jann, K., Homan, P., Hauf, M., Walther, S., Strik, W., Dierks, T., and Hubl, D. (2015). Static and dynamic characteristics of cerebral blood flow during the resting state in schizophrenia. Schizophr. Bull. 41(1), 163-170. PMCID: PMC4266282

16. Oliveira, Í. A. F., Guimarães, T. M., Souza, R. M., Dos Santos, A. C., Machado-de-Sousa, J. P., Hallak, J. E. C., and Leoni, R. F. (2018) Brain functional and perfusional alterations in schizophrenia: An arterial spin labeling study. Psychiatry Res. Neuroimaging 272, 71-78. PMID: 29229240

17. Wright, S. N., Hong, L. E., Winkler, A. M., Chiappelli, J., Nugent, K., Muellerklein, F., Du, X., Rowland, L. M., Wang, D. J., and Kochunov, P. (2015) Perfusion shift from white to gray matter may account for processing speed deficits in schizophrenia. Hum. Brain Mapp. 36(10), 3793-3804. PMCID: PMC4714540

18. Wijtenburg, S. A., Wright, S. N., Korenic, S. A., Gaston, F. E., Ndubuizu, N., Chiappelli, J., McMahon, R. P., Chen, H., Savransky, A., Du, X., Wang, D. J., Kochunov, P., Hong, L. E., and Rowland, L. M. (2017) Altered glutamate and regional cerebral blood flow levels in schizophrenia: A 1H-MRS and pCASL study. Neuropsychopharmacology 42(2), 562-571. PMCID: PMC5399238

19. Chiapelli, J., Rowland, L. M., Notarangelo, F. M., Wijtenburg, S. A., Thomas, M. A. R., Pocivavsek, A., Jones, A., Wisner, K., Kochunov, P., Schwarcz, R., and Hong, L. E. (2018) Salivary kynurenic acid response to psychosocial stress: Inverse relationship to cortical glutamate in schizophrenia. Neuropsychopharmacology doi: 10.1038/s41386-018-0072-2 [Epub ahead of print]. PMID: 29728648

20. American Psychiatric Association (2013) *Diagnostic and Statistical Manual of Mental Disorders*. 5th Ed. Arlington, VA: American Psychiatric Publishing.

21. First, M. B., Williams, J. B. W., Karg, R. S., and Spitzer, R. L. (2015) *Structured Clinical Interview for DSM-5-Research Version (SCID-5 for DSM-5, Research Version; SCID-5-RV)*. Arlington, VA: American Psychiatric Association.

22. Ceresoli-Borroni, G., Rassoulpour, A., Wu, H. Q., Guidetti, P., and Schwarcz, R. (2006) Chronic neuroleptic treatment reduces endogenous kynurenic acid levels in the rat brain. J. Neural Transm. 113, 1355-1365. PMID: 16465454

23. Overall, J. E. and Gorham, D. R. (1962) *The Brief Psychiatric Rating Scale.* Psychol. Rep. 10(79), 812.

24. Andreasen, N. C. (1983) *The Scale for the Assessment ofNegative Symptoms (SANS).* University of Iowa, Iowa City.

25. Addington, D., Addington, J., and Matrick-Tyndale, E. (1993) Assessing depression in schizophrenia: The Calgary Depression Scale. Br. J. Psychiatry Suppl. 22, 39-44. PMID: 8110442

26. Guy, W., ed. (1976) Clinical Global Impressions. ECDEU Assessment Manual for Psychopharmacology. Rockville, MD: US Department of Health, Education and Welfare, DHEW Publication No. (ADM) 76-338.

27. Neuchterlein, K. H., Green, M. F., Kern, R. S., Baade, L. E., Barch, D. M., Cohen, J. D., Essock, S., Fenton, W. S., Frese, F. J. 3rd, Gold, J. M., Goldberg, T., Heaton, R. K., Keefe, R. S., Kraemer, H., Mesholam-

Gately, R., Seidman, L. J., Stover, E., Weinberger, D. R., Young, A. S., Zalcman, S., and Marder, S. R. (2008) The MATRICS Consensus Cognitive Battery, part 1: Test selection, reliability and validity. Am. J. Psychiatry 165, 203-213. PMID: 18172019

28. Otto, T., Wolf, D., and Walsh, T. J. (1997) Combined lesions of perirhinal and entorhinal cortex impair rats' performance in two versions of the spatially guided radial-arm maze. Neurobiol. Learn. Mem. 68(1), 21–31. PMID: 9195586

29. Liu, P. and Bilkey, D. K. (1999) The effect of excitotoxic lesions centered on the perirhinal cortex in two versions of the radial arm maze task. Behav. Neurosci. 113(4), 672–682. PMID: 10495076

30. Martin, S. J. and Clark, R. E. (2007) The rodent hippocampus and spatial memory: from synapses to systems. Cell Mol. Life Sci. 64(4), 401–431. PMID: 17256090

31. Spieker, E. A., Astur, R. S., West, J. T., Griego, J. A., and Rowland, L. M. (2011) Spatial memory deficits in a virtual reality eight-arm radial maze in schizophrenia. Schizophr. Res. 135(1-3), 84-89. PMCID: PMC3288352

32. Wang, J., Alsop, D. C., Li, L., Listerud, J., Gonzalez-At, J. B., Schnall, M. D., and Detre, J. A. (2002) Comparison of quantitative perfusion imaging using arterial spin labeling at 1.5 and 4.0 Tesla. Magn. Reson. Med. 48, 242-254. PMID: 12210932

33. Liu, C. K., Lai, C. L., Tai, C. T., Lin, R. T., Yen, Y. Y., and Howng, S. L. (1998) Incidence and subtypes of dementia in southern Taiwan: Impact of socio-demographic factors. Neurology 50, 1572-1579. PMID: 9633696

34. Wey, H. Y., Wang, D. J., and Duong, T. Q. (2011) Baseline CBF, and BOLD, CBF, and CMRO2 fMRI of visual and vibrotactile stimulations in baboons. J. Cereb. Blood Flow Metab. 31, 715-724. PMCID: PMC3049525

35. Rowland, L. M., Summerfelt, A., Wijtenburg, S. A., Du, X., Chiappelli, J. J., Krishna, N., West, J., Muellerklein, F., Kochunov, P., and Hong, L. E. (2016) Frontal glutamate and γ-aminobutyric acid levels and their associations with mismatch negativity and digit sequencing task performance in schizophrenia. JAMA Psychiatry 73(2), 166-74. PMCID: PMC4740214

36. Gasparovic, C., Neeb, H., Feis, D. L., Damaraju, E., Chen, H., Doty, M. J., South, D. M., Mullins, P.G., Bockholt, H. J., and Shah, N. J. (2009) Quantitative spectroscopic imaging with in situ measurements of tissue water T1, T2, and density. Magn. Reson. Med. 62(3), 583-590. PMID: 19526491

37. Chan, K.L., Puts, N. A., Snoussi, K., Harris, A. D., Barker, P. B., and Edden, R. A. (2017) Echo time optimization for J-difference editing of glutathione at 3T. Magn. Reson. Med. 77(2), 498-504. PMID: 26918659

38. Wijtenburg, S. A., Near, J., Korenic, S. A., Gaston, F. E., Chen, H., Mikkelsen, M., Chen, S., Kochunov, P., Hong, L. E., and Rowland, L. M. (2018) Comparing the reproducibility of commonly used magnetic resonance spectroscopy techniques to quantify cerebral glutathione. J. Magn. Reson. Imaging doi: 10.1002/jmri.26046. [Epub ahead of print] PMID: 29659065

39. Wijtenburg, S. A. and Knight-Scott, J. (2011) Very short echo time improves the precision of glutamate detection at 3T in 1H magnetic resonance spectroscopy. J. Magn. Reson. Imaging 34(3), 645-652. PMID: 21761460

40. Wijtenburg, S. A., Gaston, F. E., Spieker, E. A., Korenic, S. A., Kochunov, P., Hong, L. E., and Rowland, L. M. (2014) Reproducibility of phase rotation STEAM at 3T: Focus on glutathione. Magn. Reson. Med. 72(3), 603-609. PMCID: PMC3995860

41. Bustillo, J. R., Rediske, N., Jones, T., Rowland, L. M., Abbott, C., and Wijtenburg, S. A. (2016) Reproducibility of phase rotation stimulated echo acquisition mode at 3T in schizophrenia: Emphasis on glutamine. Magn. Reson. Med. 75(2), 498-502. PMCID: PMC4567519

42. Kochunov, P., Chiappelli, J., and Hong, L. E. (2013) Permeability-diffusivity modeling vs. fractional anisotropy on white matter integrity assessment and application in schizophrenia. NeuroImage: Clinical 3, 18-26. PMCID: PMC3791292

43. Kochunov, P., Fu, M., Nugent, K., Wright, S. N., Du, X., Muellerklein, F., Morrissey, M., Eskandar, G., Shukla, D. K., Jahanshad, N., Thompson, P. M., Patel, B., Postolache, T. T., Strauss, K. A., Shuldiner, A. R., Mitchell, B. D., and Hong, L. E. (2015) Heritability of complex white matter diffusion traits assessed in a population isolate. Hum. Brain Mapp. 37(2), 525-535. PMCID: PMC4718876

44. Kochunov, P., Rowland, L. M., Fiermans, E., Veraart, J., Jahanshad, N., Eskander, G., Du, X., Muellerklein, F., Savransky, A., Shukla, D., Sampath, H., Thompson, P. M., and Hong, L. E. (2016) Diffusion-weighted imaging uncovers likely sources of processing-speed deficits in schizophrenia. Proc. Natl. Acad. Sci. USA 113, 13504-13509. PMCID: PMC5127361

45. Hoddes, E., Zarcone, V., Smythe, H., Phillips, R., and Dement, W. C. (1973) Quantification of sleepiness: A new approach. Psychophysiology 10, 431-436. PMID: 4719486

46. Greene, S. C., Noonan, P. K., Sanabria, C., and Peacock, W. F. (2016) Effervescent N-acetylcysteine tablets versus oral solution N-acetylcysteine in fasting healthy adults: An open-label, randomized, single-dose, crossover, relative bioavailability study. Curr. Therapeutic Res. 83, 1-7. PMCID: PMC5024139

47. Silber, B. Y. and Schmitt, J. A. (2010) Effects of tryptophan loading on human cognition, mood, and sleep. Neurosci. Biobehav. Rev. 34(3), 387-407. PMID 19715722

48. Borgstrom, L., Kagedal, B., and Paulsen, O. (1986) Pharmacokinetics of N-acetylcysteine in man. Eur. J. Clin. Pharmacol. 31, 217-222. PMID: 3803419

49. Holmay, M. J., Terstra, M., Coles, L. D., Mishra, U., Ahlskog, M., Öz, G., Cloyd, J. C., and Tuite, P. J. (2013) N-Acetylcysteine boosts brain and blood glutathione in Gaucher and Parkinson diseases. Clin. Neuropharm. 36(4), 103-106. PMCID: PMC3934795

50. VanderWeele, T. (2015) Explanation in Causal Inference: Methods for Mediation and Interaction. Oxford University Press.

51. Eklund, A., Nichols, T. E., and Knutsson, H. (2016) Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates. PNAS 113(28), 7900-7905. PMID: 27357684

52. Valeri, L. and VanderWeele, T. J. (2013) Mediation analysis allowing for exposure–mediator interactions and causal interpretation: Theoretical assumptions and implementation with SAS and SPSS macros. Psychological Methods 18(2), 137-150. PMID: 23379553

53. Chen, S., Kang, J., Xing, Y., Zhao, Y., and Milton, D. Estimating large covariance matrix with network topology for high-dimensional biomedical data, computational statistics and data analysis, in process. doi.org/10.1016/j.csda.2018.05.008

54. Green, M. F. (1996) What are the functional consequences of neurocognitive deficits in schizophrenia? Am. J. Psychiatry 153, 321-330. PMID: 8610818

55. Green, M. F., Kern, R. S., and Heaton, R. K. (2004) Logitudinal studies of cognition and functional outcome in schizophrenia: Implications for MATRICS. Schizophr. Res. 72, 41-51. PMID: 15531406

56. Keefe, R. S. E., Bilder, R. M., Davis, S. M., Harvey, P. D., Palmer, B. W., Gold, J. M., Meltzer, H. Y., Green, M. F., Capuano, G., Stroup, T. S., McEvoy, J. P., Swartz, M. S., Rosenheck, R. A., Perkins, D. O., Davis, C. E., Hsiao, J. K., Lieberman, J. A., and CATIE Investigators; Neurocognitive Working Group (2007). Neurocognitive effects of antipsychotic medications in patients with chronic schizophrenia in the CATIE trial. Arch. Gen. Psychiatry 64, 633-647. PMID:17548746

57. Buchanan, R. W., Freedman, R., Javitt, D. C., Abi-Dargham, A., and Lieberman, J. A. (2007) Recent advances in the development of novel pharmacological agents for the treatment of cognitive impairments in schizophrenia. Schizophr. Bull. 33(5), 1120-1130. PMCID: PMC2632365

58. Choi, K. H., Wykes, T., and Kurtz, M. M. (2013). Adjunctive pharmacotherapy of cognitive deficits in schizophrenia: Meta-analytical investigation of efficacy. Br. J. Psychiatry 203(3, :172-178. PMCID: PMC3759029

59. Buchanan, R. W., Javitt, D. C., Marder, S. R., Schooler, N. R., Gold, J. M., McMahon, R. P., Heresco-Levy, U., and Carpenter, W. T. (2007). The Cognitive and Negative Symptoms in Schizophrenia Trial (CONSIST): The efficacy of glutatmatergic agents for negative symptoms and cognitive impairments. Am. J. Psychiatry 164(10),1593-1602. PMID: 17898352

60. Freedman, R., Olincy, A., Buchanan, R. W., Harris, J. G., Gold, J. M., Johnson, L., Allensworth, D., Guzman-Bonilla, A., Clement, B., Ball, M. P., Kutnick, J., Pender, V., Martin, L. F., Stevens, K. E., Wagner, B. D., Zerbe, G. O., Soti, F., Kem, W. R. (2008) Initial phase 2 trial of a nicotinic agonist in schizophrenia. Am. J. Psychiatry 165(8), 1040-1047. PMCID: PMC3746983

61. Winterer, G., Gallinat, J., Brinkmeyer, J., Musso, F., Kornhuber, J., Thuerauf, N., Rujescu, D., Favis, R., Sun, Y., Franc, M. A., Ouwerkerk-Mahadevan, S., Janssens, L., Timmers, M., and Streffer, J. R. (2013) Allosteric alpha-7 nicotinic receptor modulation and P50 sensory gating in schizophrenia: A proof-of-mechanism study. Neuropharmacology 64, 197-204. PMID: 22766391

62(28). Lieberman, J. A., Dunbar, G., Segreti, A. C., Girgis, R. R., Seoane, F., Beaver, J. S., Duan, N., and Hosford, D. A. (2013) A randomized exploratory trial of an α-7 nicotinic receptor agonist (TC-5619) for cognitive enhancement in schizophrenia. Neuropsychopharmacology 38(6), 968-975. PMCID: PMC3629385

63. Umbricht, D., Keefe, R. S., Murray, S., Lowe, D. A., Porter, R., Garibaldi, G., and Santarelli, L. (2014) A randomized, placebo-controlled study investigating the nicotinic α7 agonist, RG3487, for cognitive deficits in schizophrenia. Neuropsychopharmacology 39(7), 1568-1577. PMCID: PMC4023143

64. Dixon, L. B., Dickerson, F., Bellack, A. S., Bennett, M., Dickinson, D., Goldberg, R. W., Lehman, A., Tenhula, W. N., Calmes, C., Pasillas, R. M., Peer, J., Kreyenbuhl, J., and Schizophrenia Patient Outcomes Research Team (PORT) (2010) The 2009 schizophrenia PORT psychosocial treatment recommendations and summary statements. Schizophr. Bull. 36(1), 48-70. PMCID: PMC2800143