Repetitive Transcranial Magnetic Stimulation as a Probe of Episodic Memory Neurocircuitry in Schizophrenia

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1.0 Background and Rationale

Schizophrenia is a chronic and disabling illness that is associated with impairments in independent living as well as social and vocational functioning[1]. Schizophrenia also represents an important societal burden; only 10% of individuals maintain employment, translating into annual lost wages of nearly $15 billion[2,3]. Cognitive dysfunction is a core facet of schizophrenia, contributing to profound social and vocational difficulties[4-6]. Episodic memory (EM) is an important cognitive domain that is commonly impaired in schizophrenia[7].

EM combines event-specific autobiographical experiences and information regarding the context in which events took place[8], aiding in making decisions and guiding actions in the present. EM deficits are associated with poor insight, poor treatment compliance, and significant social and occupational dysfunction[8-13]. There are no effective treatments for EM impairment, due in large part to a gap in knowledge regarding the neural mechanisms of EM dysfunction. In light of the significant functional disability and poor outcomes associated with deficits in EM there is a critical need for more effective therapeutic options for this important cognitive deficit.

Recently research has identified that dysconnectivity in key EM structures, such as the precuneus, may be associated with EM impairment, though the precise role of disordered cortical circuitry remains elusive[7,14-17]. The precuneus is believed necessary for autobiographical and episodic memory retrieval and mentalizing[18-22]. Cabeza et al. (2008) suggested that the precuneus is involved in supporting retrieval search, monitoring, and verification[14]. Altered function in the precuneus could be related to deficient EM functioning in various ways, including an impaired ability to make accurate assessments of familiarity[7]. Disrupted precuneus function may also impair attentional networks, interfering with the ability to retrieve previously encoded information[23]. The precuneus has reciprocal connections with other brain regions, including EM relevant structures such as the dorsolateral prefrontal cortex (DLPFC), the anterior cingulate cortex (ACC)[19], and the hippocampus[24]. Previous studies, including our own, have demonstrated that altered precuneus activity is associated with EM deficits in schizophrenia[7,14,16,17,25]. However, relative to other structures, the specific role of the precuneus in cognitive dysfunction in schizophrenia has not yet been determined. Clearly defining the role of structures, such as the precuneus, in EM dysfunction will enable investigators to develop more targeted therapeutic interventions for this important clinical phenomenon.

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive neuromodulation technique that received FDA clearance for use in treatment resistant major depressive disorder in 2008 and has become commonly used in clinical practice[26]. rTMS utilizes the application of a repetitively pulsed magnetic field over the scalp to induce an electric field within a discrete area of the cerebral cortex. This electric field results in altered ion flow across the neuronal cellular membrane and ultimately changes in neuronal polarization. rTMS modulates cortical activation depending on the stimulation parameters used[27,28]. Physiological studies have provided evidence that low-frequency (LF) rTMS produces an inhibitory effect on local cortical excitability[14,17-19,21]. In contrast, evidence suggests that high-frequency (HF) rTMS produces an increase in local cortical excitability[27-31]. Studies have also demonstrated that rTMS may increase or decrease functional connectivity between separate but related cortical structures, utilizing high and low frequency stimulation, respectively[32-35].

Work by our group and others have shown that rTMS is a viable option for investigating cognitive dysfunction in schizophrenia[33,35-37]. We recently completed a pilot-study investigating the effects of HF rTMS, compared to sham stimulation, on cognitive dysfunction in an early

phase psychosis (EPP) population. This study administered bilateral, sequential, double-blinded sham stimulation or HF (20 Hz) rTMS targeting the DLPFCs in twenty subjects with EPP. Ten sessions of rTMS or sham were administered over the course of two-weeks. Our pilot-study demonstrated not only a beneficial effect of rTMS on cognition but also that the intervention is safe and well tolerated in subjects with EPP. There were no significant adverse events during the course of this study and there were no significant differences in adverse events between the rTMS and sham group.

In spite of existing work studying rTMS as a treatment modality in schizophrenia, there are no studies that have examined the effects of precuneus directed rTMS on either EM deficits or the neurocircuitry subserving EM in schizophrenia. It is also important to note that the vast majority of studies using rTMS in schizophrenia have examined chronic populations where confounds associated with prolonged duration of illness may be present. EPP is a desirable population to study because these individuals tend to have fewer psychiatric and physical comorbidities and less antipsychotic drug exposure, all of which are factors that may confound investigations of new treatment interventions for this illness. In light of the significant unmet medical need associated with schizophrenia and the grave clinical effect of disrupted EM in the illness, rTMS modulating the precuneus, and potentially EM circuitry, represents an unexplored and potentially novel potential treatment option.

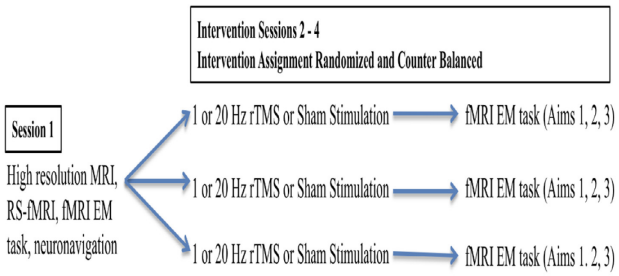
This study proposes to combine functional magnetic resonance imaging (fMRI) with inhibitory LF (1 Hz) and excitatory HF (20 Hz) rTMS protocols to interrogate the effects of rTMS targeting the precuneus on: 1) precuneus activation during EM task performance; 2) functional connectivity between the precuneus and key EM circuitry, specifically the DLPFC, ACC, and hippocampus and 3) performance during an in-scanner scene encoding and recognition EM task. This study will provide vital preliminary data on target engagement informing future clinical trials seeking to utilize rTMS to treat EM impairment in schizophrenia. This is an important population for study because if effective, rTMS may represent a novel treatment for EM deficits in schizophrenia. This study will also seek to refine the understanding of the brain circuitry that mediates the potential pro-EM effects of rTMS through the use of fMRI at baseline and following the course of rTMS administration.

2.0 Specific Aims and Hypothesis

The **first primary aim** is to demonstrate how targeting the precuneus with rTMS impacts functional activation (FA) in individuals with schizophrenia during EM tasks. We predict that, compared to sham stimulation, 1 Hz rTMS will result in decreased FA of the precuneus and 20 Hz rTMS will result in increased FA of the precuneus. The **second primary aim** is to demonstrate how targeting the precuneus with rTMS impacts precuneus functional connectivity (FC) in individuals with schizophrenia. We predict that compared to sham stimulation, FC between the precuneus, the DLPFC, the hippocampus, and the ACC will be decreased in response to 1 Hz rTMS and increased in response to 20 Hz rTMS. The **third primary aim** is to characterize the relationships between EM performance and changes in precuneus functional activation and connectivity. We predict that improved EM performance will be associated with increased precuneus functional activation and connectivity, while poorer EM performance will be associated with decreased precuneus functional activation and connectivity. The **secondary aim** is to characterize how targeting the precuneus with rTMS affects episodic memory performance in individuals with schizophrenia. We predict that compared to sham stimulation, 1 Hz rTMS will impair EM performance and that 20 Hz rTMS will improve EM performance.

3.0 Study Design

Fig 1. Study Outline



This will be a single site pilot study. 30 subjects with EPP, defined as medical record documentation of the onset of clinically significant psychotic symptoms within the past ten years, will be enrolled. Prior to randomization (Session 1), subjects will undergo fMRI during EM and RS paradigms. This baseline scan will also include a high-resolution structural sequence for neuronavigation purposes. Then on three separate days each occurring one-week apart, subjects will receive one session of inhibitory (1 Hz) rTMS, one session of excitatory (20 Hz) rTMS, and one sham stimulation session targeting the precuneus. The order of the three interventions will be randomized. Immediately following each rTMS or sham session, subjects will undergo repeat fMRI during EM and RS paradigms. We will also examine the effect of rTMS on EM performance.

rTMS Set up and Administration
rTMS will be delivered using the Magventure MagPro X100 Magnetic Stimulator (Magventure Inc., Alpharetta, Georgia). Motor threshold (MT) will be determined using single pulse stimulation over the left primary motor cortex, assessed as the lowest intensity producing five visible movements of the right abductor pollicis brevis out of ten stimulations. The Magventure MagPro X100 is equipped with a research-dedicated coil with combined active and sham stimulation capabilities. The active and sham functions share the same acoustic properties and sham mimics cutaneous stimulation, facilitating double-blinding. Study conditions (20 Hz, 1 Hz, sham) will be assigned a label, such as A, B, or C, by a non-blinded staff member who is not directly involved with the research team. Subjects will be assigned a sequence of blinded study conditions in randomized and counterbalanced fashion.

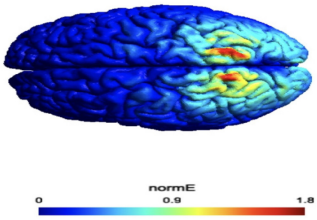
High Frequency rTMS: High frequency rTMS, or rTMS greater than 5 Hz, leads to faciliatory effects on brain excitability[27-31,38]. Previous studies have demonstrated that administration of as little as a single train or a small number of trains are able to produce an immediate increase in cortical excitability[31]. Additionally, earlier investigations have utilized high frequency rTMS in schizophrenia populations, demonstrating that the treatment is safe, well-tolerated and capable of altering cognitive performance[37,39]. Our group has employed 20 Hz rTMS at 110% of motor threshold in a stimulation protocol in-line with the current proposal to improve cognitive function in an EPP cohort[37]. High Frequency rTMS Protocol*:* Subjects will receive one session of high frequency rTMS within the following stimulation parameters: 20 Hz, at 120% of MT, 60 trains (1.0 second per train), 20 pulses per train, inter-train interval of 15 seconds, for a total of 1200 pulses over 16 minutes.

Low Frequency rTMS: Low frequency rTMS, or rTMS at 1 Hz or less, is able to produce an inhibitory effect on local brain activity. This has been observed in healthy subject motor corticospinal output[28]. Previous studies have demonstrated that a single session of 1 Hz rTMS is capable of inhibiting local brain activity and modulating distal but related circuitry[32]. This work is in-line with the use of 1 Hz stimulation proposed in this study. Low Frequency rTMS Protocol: Subjects will receive one session of low frequency rTMS within the following stimulation parameters: Continuous 20-minute train of 1 Hz rTMS, at 120% of MT, for a total of 1200 pulses. This protocol was shown by Chen et al. (2013) to produce an inhibitory effect on local cortical excitability as well as effects on connectivity of related circuitry[32].

Both high and low frequency stimulation protocols are within safety limits for rTMS[28,40,41].

Neuronavigation

Fig. 2 E-field precuneus modeling



Brainsight software (Rogue Research, Montreal Canada) will be used to reliably target the precuneus for stimulation. First, the subject's individual anatomy will be registered stereotactically using the acquired structural MPRAGE brain image and fiduciary points on the scalp visible with a Polaris vicar camera (Brainsight software). We will use the center of mass of the precuneus (derived via Freesurfer parcellation; Destrieux Atlas[42]; left/right combined) to identify the specific coordinates for stimulation. Stimulation site will be marked on a swim cap worn by the subjects at each stimulation session, consistent with prior TMS studies[32].

Electrical field modeling: The processed structural image will be used to generate E-fields based on realistic conductor head models using SimNIBS (2.1 or latest version). SimNIBS includes volume conductor modeling (following FreeSurfer segmentation), TMS coil specific magnetic dipole estimations, and finite element modeling of the electric field at each tetrahedral element of the subject's head mesh. In anticipation of actual TMS sessions with each patient, we will find the optimal TMS positioning to most strongly influence the precuneus, which will be the target for neuronavigated rTMS delivery.

rTMS administration monitoring

All subjects will be instructed to wear earplugs during each rTMS session and will be monitored by medically trained research staff throughout the entirety of each rTMS session.

Duration of Treatment

Subjects will complete three stimulation sessions (HF, LF, and sham) over three weeks, with one session occurring each week. Previous work has demonstrated that a single session of rTMS is sufficient to modulate target site functional activation as well as functional connectivity between the stimulation target and associated circuitry[32].

Sample Size

We plan to enroll 30 subjects and anticipate a 20% drop out rate, yielding 24 completers. This is consistent with our previous clinical trial efforts, including a study of adjunct rTMS for cognitive impairment[37,43]. For all planned analyses, a total of 24 subjects in a cross-over design will result in 0.8 power to detect an assumed treatment difference at medium effect size 0.6; using an alpha level of 0.05 and conservatively assumed a within-subject correlation of 0.5. As no precuneus rTMS pilot data was available, this standardized effect size of 0.6 is considered a conservative estimate to detect significant results.

Clinical Research Site

The IU Psychotic Disorders Program (IUPDP), which is directed by Dr. Alan Breier and is part of the IU Department of Psychiatry, is located in Indianapolis, Indiana. IUPDP research personnel will manage the day-to-day activities of conducting the trial, including subject recruitment, consenting and screening subjects, conducting study visits, and performing assessments. The IUPDP has 2 research psychiatrists, a fully dedicated study manager, a fully dedicated study coordinator, one dedicated subject recruiter, one dedicated research technician, and two raters (1 PhD, 1 Masters level) who have been trained and have extensive experience in conducting the assessments and cognitive tests used as outcome measures.

4.0 Study Population (Inclusion/Exclusion Criteria)

Inclusion criteria:

1. Between 18 and 40 years of age
2. Within 10.5 years (10 years, 6 months) of illness onset as defined by entry into treatment for psychotic symptoms
3. Able to give informed consent
4. Willing and able to adhere to the study schedule
5. Structured Clinical Interview for DSM-5 (SCID-5)[44] diagnosis of schizophrenia
6. Clinical stability defined by:
    a. Subjects must not have experienced an exacerbation of their illness within 4 weeks prior to randomization, leading to an intensification of psychiatric care in the opinion of the investigator. Examples of intensification of care include, but are not limited to: inpatient hospitalization, day/partial hospitalization, outpatient crisis management, or psychiatric treatment in an emergency room AND
    b. Antipsychotic treatment stability for at least 4 weeks prior to randomization (no change in antipsychotic dosing or addition of new antipsychotic medication)

Exclusion criteria:

1. Lifetime history of a seizure, excluding febrile seizures and those induced by substance withdrawal
2. First degree relative with idiopathic epilepsy or other seizure disorder
3. History of significant neurological illness
4. History of head trauma as defined by a loss of consciousness or a post-concussive syndrome
5. Pregnant or breast feeding
6. Known IQ < 70 based on subject report
7. Current acute, serious, or unstable medical conditions
8. Metallic objects planted in or near the head, including implanted pacemaker, medication pump, vagal stimulator, deep brain stimulator, TENS unit, ventriculoperitoneal shunt, or cochlear implants
9. Contraindications to MRI or otherwise unable to tolerate MRI procedures
10. History of electroconvulsive therapy
11. Subjects taking clozapine
12. Subjects who have participated in a clinical trial with any pharmacological treatment intervention for which they received study-related medication in the 4 weeks prior to randomization
13. Subjects considered a high risk for suicidal acts – active suicidal ideation as determined by clinical interview OR any suicide attempt in 90 days prior to screening
14. Current DSM-5 diagnosis of alcohol or drug use disorder (excluding nicotine or caffeine)
15. Subjects who require concomitant treatment with prohibited medication, as specified in Attachment 2

5.0 Subject Recruitment

Subjects will be recruited through referring community mental health centers, treatment providers, the IUPDP registry, and self-referrals through advertisement and word-of-mouth.

6.0 Clinical Assessments and Procedures

The following assessments will be administered according to the Study Procedures Table (Attachment 1). All assessments will be completed by study personnel trained to administer the instruments and will be based on interviews with the subject or questionnaires completed by the subject.

Columbia Suicide Severity Rating Scale (C-SSRS)
The C-SSRS captures the occurrence, severity, and frequency of suicide-related thoughts and behaviors during the assessment period. The definition of behavioral suicidal events used in this scale is based on those used in the Columbia Suicide History Form[45]. During the baseline assessment, questions are in relation to lifetime experiences and all subsequent questioning is in relation to the last assessment.

The Positive and Negative Syndrome Scale (PANSS)
The PANSS is the primary assessment instrument for psychopathology. The PANSS contains 30 items that assess symptoms of psychotic disorders including positive, negative and general psychopathology. The PANSS was chosen because of its widespread use in clinical studies of psychosis, and its demonstrated reliability in assessing psychopathology across diverse patient populations[46].

Diagnostic Interview
The Structured Clinical Interview for DSM-5 (SCID-5-RV) will be used to confirm the diagnosis of a psychotic disorder and/or rule out other diagnoses. The SCID-5-RV is a semi-structured interview designed to evaluate DSM-5 Axis I diagnoses[44].

Clinical Global Impressions Severity Scale (CGI-S)
The CGI-S is used for repeated evaluations of global psychopathology[47]. The CGI-S scale is widely used in schizophrenia research and is a single 7-point Likert scale rating severity of psychopathology on a scale of 1 (normal, not ill) to 7 (very severely ill).

Clinical Global Impressions Severity Improvement Scale (CGI-I)
The CGI-I is used to assess the clinical change as compared to symptoms at baseline using a 7-point Likert scale, ranging from very much improved (1) to very much worse (7)[47].

Abnormal Involuntary Movement Scale (AIMS)
The AIMS is a 12-item scale designed to record the occurrence of dyskinetic movements[48]. Items 1 to 10 are rated on a 5-point scale, with 0 being no dyskinetic movements and 4 being severe dyskinetic movements. Items 11 and 12 are yes/no questions regarding the dental condition of a subject.

Magnetic Resonance Imaging (MRI) Procedures
MRI will be performed at the IU Center for Neuroimaging using an integrated 45-minute exam, including structural and fMRI. Scans will be completed on a research-dedicated Siemens MAGNETOM Prisma 3T scanner. First, we will conduct a high-resolution 3D magnetization

prepared rapid gradient echo (MPRAGE) scan for individual anatomical reference, comprised of 160 sagittal slices with 1.0x1.0x1.2 mm voxel dimension. Subjects will practice tasks on a personal computer during Visit 1 and prior to the scan at subsequent visits. An MRI-compatible response box will be utilized for tasks, recording reaction time and accuracy. Functional scans will be acquired using T2*-weighted gradient spin echo-planar imaging (EPI) sequence with TR/TE 1200/29 ms for 54 axial slices and voxel size of 2.5x2.5x2.5 mm. fMRI will employ scene encoding and recognition EM paradigms, shown by our group and others to demonstrate reliable precuneus activation during cognitive functions of interest[49-59]. We have successfully implemented all tasks in multiple studies of patients with psychosis. A task-free resting state fMRI scan will also be acquired to measure functional connectivity between precuneus and associated structures and circuits.

Scene Encoding and Recognition Tasks: These tasks utilize stimuli and a task design modified from that used by Detre et al. in EM studies in temporal lobe epilepsy[49,50,55]. We will be using methods identical those in our previous work[7]. During encoding task, subjects view complex scenes one at a time and are instructed to remember each for later recognition testing **(Fig. 3).** Images are shown in a block design with interleaved 36-s image and control blocks. Image blocks consist of nine consecutive images, each displayed for 3.5 s, with an inter-stimulus interval (ISI) of 500 ms. A total of 36 scene images are shown. Control blocks consist of a retiled image, repeatedly displayed at the same rate. Subjects do not press buttons during the encoding phase. The recognition task is a separate event-related fMRI paradigm administered immediately after the encoding phase. During the recognition task, subjects are shown the 36 scenes from the encoding phase intermixed with 36 new scenes. These images will be displayed consecutively in a pseudorandomized manner, each for 3.5 s with an ISI of 500 ms. Subjects are instructed to indicate, via button press, whether each displayed image was previously seen (target) or new (foil). Button-press responses are recorded to assess reaction time and accuracy. Task length for each trial is 5:15. Different versions of the scene encoding and recognition EM tasks will be utilized, with order of administration randomized, to mitigate the potential impact of learning effects.

Figure 3. Scene Encoding and Recognition EM Task

Positioning and motion

Subjects are instructed to remain still during scanning and deformable foam cushioning is used to stabilize the head. Real time image reconstruction and processing are used for quality assurance at the time of scanning. For fMRI, minor subvoxel-level translation or rotation is adjusted during post-processing. Noise: Noise-attenuating headphones and ear stopples provide excellent noise reduction and permit adequate auditory perception.

Functional Imaging Stimulation and Physiological Monitoring Procedures

A comprehensive physiological monitoring system will be used with synchronized digital recording including pulse oximeter, respiratory belt, HR and BP measures, which will be available for analysis in relation to BOLD fMRI time series.

7.0 Safety Assessments

Vital Signs
A seated blood pressure and pulse will be assessed at study visits per Study Procedures Table (Attachment 1).

Medical History
The subject's lifetime medical history will be taken during the screening period. Medical history includes previous and current diseases.

Physical Examination
A physical examination including a neurological examination will be conducted as outlined in the Study Procedures Table.

8.0 Laboratory Assessments

A urine pregnancy test will be conducted in all female subjects, and a urine drug screen will be conducted in all subjects, as outlined in the Study Procedures Table.

9.0 Concomitant Medication

See Attachment 2.

10.0 Adverse Events

Adverse events (AEs), especially those for which the relationship to study treatment is not "unrelated," will be followed up until they have returned to baseline status or stabilized at the discretion of the PI. If after the follow-up period, return to baseline or stabilization cannot be established an explanation will be recorded in the source documentation.

11.0 Criteria for Repeat Assessments, Rescreening, and Discontinuation

Repeat Assessments
Screening assessments can be repeated within the screening window under the same screening number with the exception of eligibility criteria related rating scales/questionnaires. Subject diagnosis confirmation will not be repeated.

Rescreening
Subjects who screen fail may be rescreened one time, under the same screening number. If a subject is rescreened, all screening assessments (with the exception of the diagnosis confirmation) must be repeated and the stability criteria timelines must be met.

Discontinuation
Subjects will be discontinued under the following circumstances:

1. During the course of the study, subjects with an interruption of stimulation administration
2. Subjects unwilling or unable to complete study assessments or procedures
3. Subjects who experience seizure occurrence at any point during study participation
4. Subjects who require a change in dose of antipsychotic medication or the addition of a new antipsychotic medication

5. Subjects experiencing an exacerbation of illness requiring an increased level of care or one judged to be clinically significant by the PI.

If subjects discontinue from the study, completion of discontinuation assessments will be at the discretion of the PI. A subject may withdrawn from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the PI for safety, behavioral, or administrative reasons.

12.0 Data Safety Monitoring Board

The Indiana University Adult Psychiatry Data Safety Monitoring Board (DSMB) will be responsible for data and safety monitoring. The DSMB is responsible for reviewing study procedures, adverse events, safety mailings (if applicable), enrollment, active subjects, and ongoing conduct of the research. The DSMB members can ask questions and make comments and/or recommendations. The IRB is notified of significant findings by way of the DSMB meeting minutes at the time of continuing review. Due to the small sample size and single site design of this protocol, there is not sufficient justification for conducting interim analyses to examine trends. Data on the number of subjects enrolled and the number of adverse events will be reviewed by the DSMB every six months and more frequently if needed. The resultant report will be issued to the Indiana University IRB at least at the time of continuing review or more frequently by request. Any unanticipated events will be immediately directed to the PI who will follow the Indiana University IRB reporting procedures.

13.0 Statistical Considerations

Analyses

Power Analyses

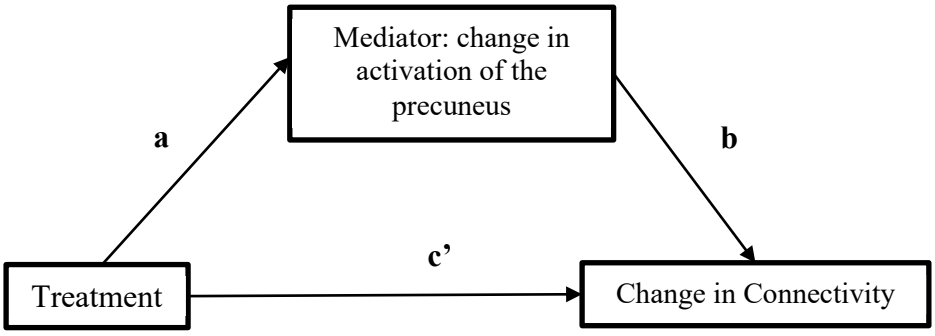
In terms of the power to detect a significant effect of precuneus directed rTMS on EM performance or neurocircuitry, there are no known calculations with EPP patients. We plan to enroll 30 subjects and anticipate a 20% drop out rate, thus yielding 24 completers. This is consistent with our previous clinical trial efforts in early phase psychosis populations, including a study of adjunct rTMS for cognitive impairment and negative symptoms[37,43]. For all planned analyses, a total of 24 subjects in a cross-over design will result in 0.8 power to detect an assumed treatment difference at medium effect size 0.6; using an alpha level of 0.05 and conservatively assumed a within-subject correlation of 0.5. As no previous precuneus rTMS pilot study was available, this standardized effect size of 0.6 is considered to a conservative estimate to detect the significant results.

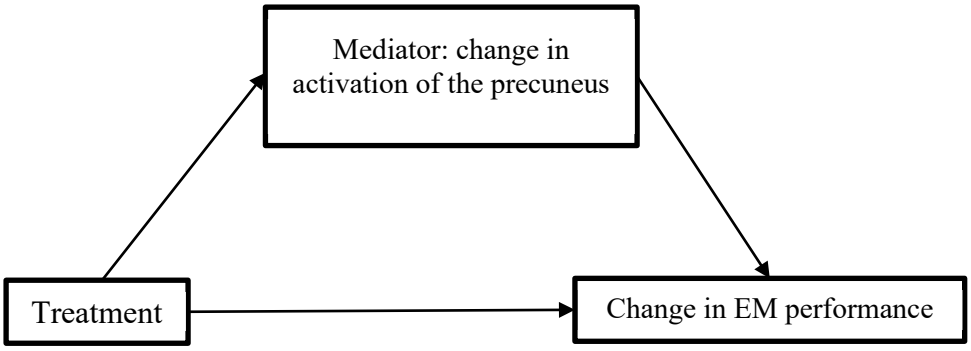
Statistical Analyses

Intent-to-treat (ITT) analysis will be conducted, and thus all randomized subjects will be included. ITT is the most conservative approach for detecting a treatment effect, because the inclusion of subjects who do not complete the intervention will serve to underestimate rather than overestimate any treatment effect. Sensitivity analyses including a subset analysis using a “completers only” analysis will be performed to test the robustness of the findings. Statistical tests will be performed based on two-sided test at the 5% level of significance.

Descriptive characteristics at baseline will be summarized for the whole sample. For continuous variables, the mean and standard deviation will be presented. For categorical variables, the number and percent will be reported.

Primary analyses: For each subject, change scores in activation of the precuneus (aim 1), functional connectivity (aim 2), and EM performance (aim 3) will be calculated at each treatment by using (post - baseline). We will employ a mixed model for repeated measures ANCOVA, of the general form for each measure: $change_{ijk} = \mu + \alpha_i + b_{ij} + \gamma_k + \beta X_{ijk} + \tau_d(i,k) + e_{ijk}$, where μ is overall mean; α_i is the effect of the ith treatment sequence ($i = 1, 2, \cdots, 6$); b_{ij} is the random effect with variance σ_b^2 for the jth subject ($j = 1, 2, \cdots, 24$) of the ith treatment sequence; γ_k is the period effect ($k = 1, 2, 3$); βX is the baseline value; $\tau_d(i,k)$ is the direct effect of the treatment administered in period k of sequence group i; and e_{ijk} is the random error with variance σ^2 for the subject in period k. Based on this model, we will compare the treatment difference in change score between 1 Hz and Sham as well as 20 Hz and Sham. Under the assumption of missing at random, this mixed effect model will provide the unbiased estimates[60].
Secondary analyses: We expect two mediation relations: (1) the treatment affects the connectivity through the activation of the precuneus; and (2) the treatment affects the EM performance through the activation of the precuneus. We use the first mediation as an example. The following 3 equations proposed by Baron and Kenny will be analyzed in order to establish mediation[61]: $change_{ijk} = \mu + \alpha_i + b_{ij} + \gamma_k + \beta X_{ijk} + \tau_d(i,k) + e_{ijk}$ (1); $change_{ijk} = \mu + \alpha_i + b_{ij} + \gamma_k + \beta X_{ijk} + \gamma M_{ijk} + \tau_d(i,k) + e_{ijk}$ (2); and $M_{ijk} = \mu + \alpha_i + b_{ij} + \gamma_k + \beta X_{ijk} + \tau_d(i,k) + e_{ijk}$ (3). Four steps are to be evaluated. First, a significant relation of treatment to the change in connectivity is required in (1). Second, a significant relation of treatment to the change in activation of the precuneus is required in (3). Third, mediator, the change in the activation of the precuneus, must be significantly associated with change in connectivity when both treatment and mediator are controlled as covariates. Fourth, the coefficients of treatment in (1) must be larger (in absolute value) than the coefficients of treatments in (2)[62]. If four steps are met, we will consider that the change in activation of the precuneus is mediator of the relationship between treatment and change in connectivity.





Brain Imaging Analyses: Aim 1. Data analysis of the blood-oxygen level-dependent (BOLD) time series will utilize standard preprocessing through the AFNI software package. Standard preprocessing includes motion correction, despiking, detrending, smoothing, and exclusion of signal and motion outlier time points. Each condition's timing will be convolved with a hemodynamic response function to model an ideal response for that condition. General linear modeling will provide a regression coefficient estimate (beta) of the BOLD response to each condition (Target correct; Foil correct; Target incorrect; Foil incorrect). We will also include regressors of no interest for motion and motion derivatives to reduce noise. For our primary analysis, we will create a region of interest (ROI) as a 10-mm radius sphere around our rTMS target in the precuneus. **fMRI Contrast:** Each event type will be modeled separately. Brain activity will be examined by contrasting the estimated beta coefficients for correct trials only (Target correct vs. Foil correct). We will **extract the mean response from this contrast from our precuneus ROI** and evaluate the aim via a mixed model for repeated measures. **Aim 2.** Beyond standard preprocessing, time-series data will be filtered to extract low frequency (< 0.08 hz) BOLD data. The ANATICOR approach (within AFNI) will be used[63] to reduce noise from physiological or hardware sources. ANATICOR utilizes tissue segmentation to control for spatially coherent fluctuations emanating from non-gray matter. Structural regions of interest (ROIs) will be defined on each side with Talairach-based atlases available via AFNI software for the ACC and bilateral middle frontal gyrus, in addition to the spherical precuneus ROI. Visual inspection of ROIs will ensure their correct position. The mean preprocessed time series will be extracted from each ROI, and time-series correlations between ROIs will be calculated. These correlations will be used as the dependent variable in a mixed model for repeated measures ANCOVA, with post-hoc tests as necessary. Supplementary analyses will examine connectivity differences of the precuneus ROI across the entire brain (corrected $p < .05$).

14.0 Data Management

Primary data will be collected via paper source documents, phone interviews, and direct data capture from clinical and symptom measurements. Data will be stored electronically in REDCap and paper source documents will be stored in a double locked and access controlled research records room. REDCap will be backed up automatically weekly. Imaging data will be stored in separate electronic files and merged with primary data as needed. The imaging data will automatically be stripped of all PHI prior to uploading to the database. Quality assurance steps will include: 1) Quality control data checks after each visit, 2) Single data entry by study staff, and 3) Data verification procedures throughout the study to ensure proper transfer of data from paper source to REDCap.

15.0 Privacy/Confidentiality Issues

Confidentiality will be protected by ensuring all research staff have been properly trained in confidentiality and human subject research procedures, coding all subject information when possible, and by securing subject files in a locked filing cabinet or on secured databases with access available only to the PI and research staff. Furthermore, data entered into a computer database will only use subject codes on secured computers that will be password protected with access available only to the PI and research staff. Any screening information obtained from potential research subjects who subsequently do not participate in the research study will be destroyed.

16.0 Record Retention

Paper copies of medical records and source documentation will be kept for seven years after the study is closed with the IRB. One year after study closure, the documents will be shipped to the Indiana University Department of Psychiatry long-term storage facility until destruction.

17.0 References

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ATTACHMENT 1: Study Procedures Table

Visit Number	1[c]	2	3	4	5[d]
Day[b]		0	7	14	28
Informed Consent	X				
Screening (Demographics, Inc/Exc, Medical & Psychiatric History of Self and Family)	X				
SCID V[a]	X				
Physical Exam	X				
CGI-S	X	X	X	X	
CGI-I		X	X	X	
Vitals	X	X	X	X	
Urine Drug Screen	X				
Pregnancy Test	X	X	X	X	
Motor Threshold Determination	X				
MRI – Baseline & Baseline EM Task	X				
Randomization (Order of Study Intervention)		X			
Neuro Navigation		X	X	X	
rTMS/sham Administration		X	X	X	
Post Stimulation MRI-EM Task		X	X	X	
PANSS	X				
AIMS	X	X	X	X	
C-SSRS	X	X	X	X	
Adverse Events	X	X	X	X	X
Concomitant Medication	X	X	X	X	X

[a]If subject received a SCID interview within 6 months of screening, it may not be repeated at the discretion of the PI

[b]Ideal time frame for administration will be on each Monday of three consecutive weeks. A total of 3 sessions. Visit window deviations will be granted at the discretion of the PI and will not be a protocol violation.

[c]Visit window for Visit 1 is 0-30 days

[d]Visit 5 may occur by phone and ideally will occur 14 days after Visit 4. Visit window deviations will be granted at the discretion of the PI and will not be a protocol violation.

Attachment 2: Concomitant Medication Table

Medication	Allowed	Notes
Amitriptyline	No	
Amphetamines et., methylphenidate, dextroamphetamine)	No	
Antiemetics (eg., metoclopramide, domperidone, others with dopamine blocking properties)	No	
Antiepileptic mood stabilizers	Yes	Stable dose, no changes or additions
Antihistamines, nonsedating (eg., loratidine, fexofenadine, cetirizine)	Yes	
Antihistamines, sedating (eg., diphenhydramine, hydroxyzine, meclizine, benztropine)	Yes-Episodic Use Only	No use within 24 hours of cognitive assessments
Antipsychotic medications	Yes	Stable dose over four weeks prior to randomization, no changes or additions during duration of trial
Barbiturates	No	
Benzodiazepines	Yes-Episodic Use Only	No use within 24 hours of cognitive assessments
Chlorpromazine	No	
Bupriopion	No	
Clozapine	No	
Decongestants (eg., pseudophedrine)	Yes-episodic use only	No use within 24 hours of cognitive assessments
Doxepine	No	
Dicyclomine	No	
Herbal medications or Over the Counter Medications w/ primary CNS activity	No	
Lithium	Yes	
MAOIs	Yes	
Methadone	No	
Mirtazepine	Yes	Stable dose, no changes or additions
Muscle Relaxants	Yes-Episodic Use Only	No use within 24 hours of cognitive assessments
Nicotine Replacement	Yes	
Nortriptyline	No	
Opiates	No	
Benzodiazepine derivative sleep agents (eg., Zolpidem)	Yes-Episodic Use Only	No use within 24 hours of cognitive assessments
SNRIs	Yes	
SSRIs	Yes	
Tricyclic antidepressants	Yes	
Trazodone	Yes-Episodic Use Only	