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A sham-controlled pilot trial of focused ultrasound modulation of the globus pallidus interna in schizophrenia

Principal Investigator:	**Donald Goff, MD** Department of Psychiatry, NYU Langone Medical Center One Park Avenue, 8th Floor New York, NY 10016 646 754-4843 donald.goff@nyulangone.org
Additional Investigators:	**Wei Qi, MD (Co-PI)** Department of Psychiatry, NYU Langone Medical Center One Park Avenue, 8th Floor New York, NY 10016 212 562-3460 wei.qi@nyulangone.org **Katlyn Nemani, MD** Department of Psychiatry, NYU Langone Medical Center One Park Avenue, 8th Floor New York, NY 10016 212 263-7419 katlyn.nemani@nyulangone.org **F. Xavier Castellanos, MD** Department of Child and Adolescent Psychiatry, NYU Langone Medical Center One Park Avenue, 7th Floor New York, NY 10016 646 754-5194 francisco.castellanos@nyulangone.org
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Statement of Compliance

This study will be conducted in accordance with the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), 21 CFR Parts 50, 56, 312, and 812 as applicable, any other applicable US government research regulations, and institutional research policies and procedures. The International Conference on Harmonisation ("ICH") Guideline for Good Clinical Practice ("GCP") (sometimes referred to as "ICH-GCP" or "E6") will be applied only to the extent that it is compatible with FDA and DHHS regulations. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

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List of Abbreviations

AE	Adverse Event
AHRS	Auditory Hallucinations Rating Scale
BOLD	Blood Oxygen Level Dependent
BPAQ	Buss-Perry Aggression Questionnaire
BPRS	Brief Psychiatric Rating Scale
CGI	Clinical Global Impression
CIMU	Conflict of Interest Management Unit
CRFs	Case Report Forms
CSPTC	Cortico-striato-pallido-thalamo-cortical
C-SSRS	Columbia Suicide Severity Rating Scale
DESA	Delusions Experience Sampling Assessment
DSM	Diagnostic and Statistical Manual
DSMB	Data Safety and Monitoring Board
EEG	Electroencephalogram
EHI	Edinburgh Handedness Inventory
ETC	Electrotechnical Technical Commission
FDA	Food and Drug Administration
fMRI	Functional Magnetic Resonance Imaging
FOV	Field of View
FUS	Focused Ultrasound
GPi	Globus pallidus interna
HIPAA	Health Insurance Portability and Accountability Act of 1996
MD thalamus	Mediodorsal thalamus
MoCA	Montreal Cognitive Assessment
MRI	Magnetic Resonance Imaging
NYUGSoM	NYU Grossman School of Medicine
PHI	Protected Health Information
PLIFUS	Pulsed Low-Intensity Focused Ultrasound
PSYRATS	Psychotic Symptoms Rating Scale
SAE	Serious Adverse Event
SAFTEE	Systematic Assessment for Treatment Emergent Side Effects
SCID	Structured Clinical Interview for DSM-V

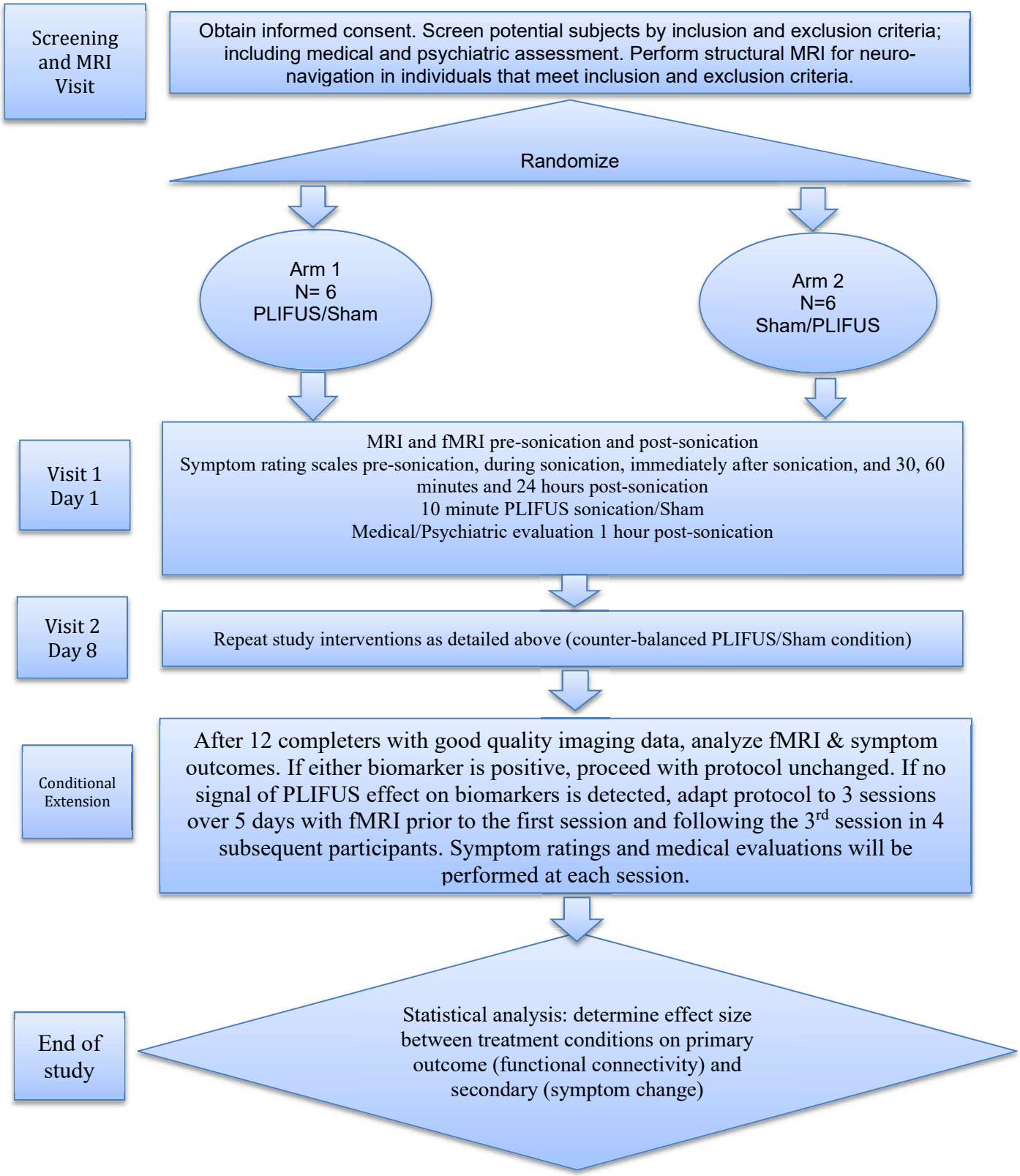
SNr	Substantia nigra pars reticulate
tDCS	Transcranial Direct Current Stimulation
TMS	Transcranial Magnetic Stimulation
UP	Unanticipated Problems

Protocol Summary

Title	A sham-controlled pilot trial of focused ultrasound modulation of the globus pallidus interna in schizophrenia
Short Title	Ultrasound modulation of the globus pallidus in schizophrenia
Brief Summary	The primary objectives of this study are to examine the tolerability of pulsed low-intensity focused ultrasound (PLIFUS) and its effects on brain function measured via fMRI functional connectivity and symptom response in individuals with schizophrenia. A neuronavigated single-element piezoelectric device will be utilized to noninvasively deliver transcranial PLIFUS sonication to the right globus pallidus interna, a brain region implicated in schizophrenia. We aim to enroll participants with schizophrenia in a random order, sham-controlled crossover trial of a single session of PLIFUS counterbalanced with a single sham sonication session, one week apart until we reach 12 completers. After the participant provides written informed consent, a medical and psychiatric evaluation will be performed to determine eligibility for inclusion in the study. The next visit will involve a structural MRI to guide neuro-navigation. The first intervention visit will include a 10 minute PLIFUS sonication or sham sonication, preceded and followed by MRI brain scans and an exit medical examination. Psychiatric symptoms will be assessed immediately prior to sonication/sham, during sonication/sham, and 5 minutes, 30 minutes, 60 minutes, 1 day and 7 days after sonication/sham. The second intervention visit will consist of sonication or sham preceded and followed by MRI brain scans, in addition to symptom assessments and post-sonication/sham medical evaluation. Participants will be closely monitored during and after procedures for tolerability and adverse effects, including 1 and 7 day follow-up visits. The goal of this pilot study is to establish feasibility and tolerability and to determine whether the sonication procedure produces effects on the imaging biomarker and on symptoms prior to proceeding to therapeutic trials using repeated administration. If PLIFUS effects on fMRI and symptom ratings are not detected in the first 12 completers with good quality imaging data, the trial will be changed to 3 sessions of PLIFUS or sham administered over 5 days for 4 subsequent participants.
Phase	Phase 2
Objectives	1. Assess safety and tolerability of PLIFUS of the right Gpi 2. Assess whether PLIFUS of the right Gpi reduces Gpi functional connectivity compared to sham 3. Assess whether PLIFUS of the right Gpi reduces psychosis (intensity of hallucinations and delusions) compared to sham. 4. Assess whether change in Gpi functional connectivity following PLIFUS correlates with change in psychosis 5. If a single session of PLIFUS does not reduce functional connectivity or symptoms in the first 12 completers with good quality imaging data compared to sham, assess whether 3 sessions of PLIFUS administered over 5 days reduces functional connectivity and symptoms in 4 subsequent participants.
Methodology	Double blind, random order, sham-controlled crossover trial of a single session of PLIFUS counterbalanced with a single sham sonication session.
Endpoint	Primary endpoint: Change in Gpi functional connectivity Secondary endpoints: Change in self-report Likert Scale scores of intensity of hallucinations and intensity of delusions
Study Duration	One year
Participant Duration	Three weeks
Duration of IP administration	Sonication will be delivered in a pulse pattern over 10 minutes

Population	12 individuals with schizophrenia, male or female, ages 18-55, with at least moderate, continuous hallucinations or delusions. Participants may be medicated or unmedicated, cannot be suicidal, cannot have a history of violence or suicidal behavior and cannot have unstable medical or neurological illness or active substance use disorder or contraindications to PLIFUS or MRI. We will continue enrollment until we obtain 12 completers with good quality imaging data. If a single session of PLIFUS does not reduce functional connectivity or symptoms in the first 12 completers with good quality imaging data compared to sham, 4 subsequent participants will be enrolled to assess whether 3 sessions of PLIFUS administered over 5 days reduces functional connectivity and symptoms.
Study Sites	Recruitment will occur at NYU Langone Health Manhattan, Brooklyn, and Bellevue Hospital. PLIFUS and neuroimaging will be performed at the NYU SoM research imaging center at 53rd and Lexington.
Number of participants	12 completers with a single session of PLIFUS and sham. Potentially, 4 additional participants will be enrolled if a single session of PLIFUS does not reduce functional connectivity or symptoms in the first 12 completers with good quality imaging data compared to sham, to assess whether 3 sessions of PLIFUS administered over 5 days reduces functional connectivity and symptoms.
Description of Study Agent/Procedure	The Sonicator-1000 system was developed in-house at the NYU Langone Health Tech4Health Institute. It is an MRI-guided neuronavigation single-element focused ultrasound platform, which has been modified from an earlier design that is currently being used in human research at Brigham and Women's Hospital. The system will non-invasively deliver ultrasound sonications transcranially that selectively target specific areas of the brain using a neuronavigation system and the subject's MRI brain scan. The device is hardwired to only emit ultrasound power levels that are much-below levels that might produce damaging effects to the brain like tissue heating, cavitation, or blood–brain barrier disruption. Extensive research has been conducted to determine non-thermal pulse parameters and acoustic intensity levels to safely transmit PLIFUS through the scalp–skull–meninges media while localizing the low ultrasound energy into the brain for inducing neuromodulation. This has also been validated through preclinical and clinical studies using histological methods. The sonication parameters follow FDA and Electrotechnical Technical Commission regulation standards for diagnostic and therapeutic ultrasound. Previous human studies with similar laboratory-built custom PLIFUS devices have safely induced temporary neuromodulation in healthy subjects with no serious adverse effects following a single session of sonication.
Reference Therapy	Sham PLIFUS (noise and patient experience are identical)
Key Procedures	PLIFUS
Statistical Analysis	Because this is a pilot feasibility study, we will examine results after the first 12 participants with good quality imaging data who have completed both phases, and will either continue the study unchanged or, if no signal is detected by fMRI or symptom change, we will switch to administering 3 sessions of PLIFUS or sham over 5 days. We will define our threshold for signal detection as an effect size of 0.5 or greater for the comparison between treatment groups (PLIFUS or sham) of change of biomarkers (fMRI and symptom severity) from baseline to post-treatment. All results will be considered exploratory. Formal statistical analyses will be performed using standard methods for a random-over crossover design; safety outcomes will be descriptive.

Schematic of Study Design



1 Key Roles

Donald Goff, MD
Department of Psychiatry, NYU Langone Medical Center
One Park Avenue, 8th Floor
New York, NY 10016
646 754-4843
donald.goff@nyulangone.org

Wei Qi, MD (Co-PI)
Department of Psychiatry, NYU Langone Medical Center
One Park Avenue, 8th Floor
New York, NY 10016
212 562-3460
wei.qi@nyulangone.org

Katlyn Nemani, MD
Department of Psychiatry, NYU Langone Medical Center
One Park Avenue, 8th Floor
New York, NY 10016
212 263-7419
katlyn.nemani@nyulangone.org

Michele Santacatterina, PhD, Statistician
Department of Population Health, NYU Langone Health
180 Madison Avenue, 2nd Floor
New York, NY 10016
646 501-3642
michele.santacatterina@nyulangone.org

F. Xavier Castellanos, MD
Department of Child and Adolescent Psychiatry, NYU Langone Medical Center
One Park Avenue, 7th Floor
New York, NY 10016
646 754-5194
francisco.castellanos@nyulangone.org

2 Introduction, Background Information and Scientific Rationale

2.1 Background Information and Relevant Literature

2.1.1 The Need for New Treatment Approaches in Schizophrenia

Schizophrenia is a disabling chronic illness characterized by psychosis (delusions and hallucinations), negative symptoms (apathy and reduced emotional expression) and cognitive deficits. Antipsychotic medications, which block dopamine transmission, substantially improves delusions and hallucinations in approximately 60% of patients, but side effects are poorly tolerated and relapse is common [1]. Psychosis can be very distressing for patients and their families, often results in hospitalization, and may contribute to violence. Currently there is no effective treatment for negative symptoms and cognitive deficits.

Over the past few decades, it has become clear that drug development targeting a single neurotransmitter is unlikely to provide improved efficacy due to the complexity and heterogeneity of the illness. Schizophrenia is most likely a neurodevelopmental disorder with complex genetics; well over 100 genes have been implicated, each conferring a very small risk [2]. Whereas the traditional molecular model of schizophrenia focused on dopamine transmission, current molecular models now focus on excitatory/inhibitory balance, regulation of glutamate (excitatory) and GABA (inhibitory) transmission and regulation of calcium influx. In parallel to new molecular models, circuit models have

emerged based on extensive neuroimaging and electrophysiologic evidence, implicating the salience network and the cortico/striatal/pallido/thalamic/cortical circuit. Pulsed low intensity focused ultrasound (PLIFUS) is an ideal tool to probe circuit and molecular models of schizophrenia since PLIFUS can target deep structures with high resolution and may alter calcium conductance, thereby altering excitatory/inhibitory balance. In addition to interrogating circuits in schizophrenia, PLIFUS may also represent a new therapeutic approach, since emerging evidence suggests that persistent effects on inhibitory/excitatory balance may be produced with repeated administration.

2.1.2 Pulsed Low Intensity Focused Ultrasound

PLIFUS utilizes ultrasound waves which are sound waves in the inaudible range of the frequency spectrum and can be transmitted through the skull to stimulate or inhibit brain regions with high spatial resolution. PLIFUS has important advantages over existing methods of neuromodulation. Unlike deep brain stimulation, it is noninvasive, safe, and inexpensive. Compared to transcranial magnetic stimulation, it has far greater resolution and can target deeper structures. PLIFUS has been extensively studied in animal models and in approximately 35 studies in humans, from which safety data have been obtained and parameters for safety are well established. It has not yet been studied in schizophrenia.

PLIFUS has not been FDA approved for any specific treatments but has demonstrated safety in animals and humans. PLIFUS is very different from high-intensity focused ultrasound that uses very high amplitudes of continuous wave ultrasound to create permanent lesions in the brain. PLIFUS uses extremely low intensities at the same power levels as standard ultrasound diagnostic imaging probes and is pulsed at low duty cycles and amplitudes yielding non-thermal effects on tissue. Studies in sheep have demonstrated modulation of regional brain activity with PLIFUS and the absence of any evidence of tissue injury based on histological analysis [3-5]. Other studies have demonstrated PLIFUS to be safe and effective at modulating neuronal excitability in pigs [6], rodents [7-13], and monkeys [3, 14].

In humans, PLIFUS has been applied to both cortical and sub-cortical structures demonstrating both safety and neuromodulatory effects [15]. PLIFUS has been applied to the primary [16, 17] and secondary [18] somatosensory cortex, primary motor cortex [17], primary visual cortex [19], and thalamus [20, 21] in human subjects. PLIFUS has been shown to affect the amplitude of evoked potentials [16, 17, 22], the dynamics of the electroencephalogram (EEG) [23], and the blood oxygen level dependent (BOLD) MRI signal [22, 24]. Behavioral modification was also demonstrated with use of PLIFUS; when targeting the primary somatosensory cortex, Legon et al. demonstrated enhanced performance on sensory discrimination tasks without affecting task attention or response bias [17].

In 2016, Monti et al. used PLIFUS in a “first-in-man” clinical trial assessing the safety and efficacy of thalamic PLIFUS in patients suffering from post-traumatic disorders of consciousness [21]. After sonication, the comatose patient demonstrated new motoric behaviors, improved recognition of language, reliable response to command, and ability to gesture for communication, representing an emergence from a minimally conscious state. In a follow up study, three patients with chronic disorders of consciousness (e.g., vegetative state or minimally conscious state) underwent two MRI-guided PLIFUS sessions targeting the thalamus [25]. The authors concluded that 1) MR-guided PLIFUS is feasible in outpatients, 2) the procedure is well-tolerated and safe without alterations in vital parameters, and 3) preliminary data demonstrated that two out of the three patients exhibited clinically significant increases in behavioral responses after exposure to each dose of PLIFUS compared to baseline. Despite the preliminary nature of these results, they provide evidence of the safety and neuromodulatory effects of transcranial PLIFUS in humans.

Safety data furthermore demonstrate that the bioeffects of PLIFUS in humans are likely due to mechanical forces rather than due to cavitational or thermal effects, given the low intensity and short duration of the sonications [15]. PLIFUS has not caused tissue damage in studies implementing these nonthermal bioeffects. Of note, the generation of standing waves can be successfully eliminated using a broadband composite tightly-focused ultrasound transducer and a reduced duty cycle with pulsed waves [26]. Studies have also demonstrated that the rate of tissue heating is often slower, and with a reduced chance of transient cavitation, when using pulsed waves versus continuous waves [26, 27]. Lastly, Legon et al [15] surveyed participants enrolled in a variety of ultrasound neuromodulation studies. None of the participants experienced serious adverse effects, and seven out of 64 participants experienced mild to moderate symptoms judged to be potentially related to the PLIFUS experiments. These symptoms included mild transient neck pain, anxiety, muscle twitches, and problems with attention. No new symptoms were reported upon follow up out to one month, and the authors concluded that the profile and incidence of symptoms appear to be similar to other forms of non-invasive brain stimulation such as transcranial magnetic

stimulation (TMS) and transcranial direct current stimulation (tDCS) which have a long-standing history of being safe for neuromodulation in humans.

Ultrasound for neuromodulation generally adheres to the safety guidelines of the Food and Drug Administration (FDA) for adult cephalic and obstetric diagnostic ultrasound applications [28]. These guidelines include derated limits of spatial peak pulse average intensity (I_{sppa}) of 190 W/cm^2, a spatial peak temporal average intensity (I_{spta}) of 720 mW/cm^2 (94 mW/cm^2 for adult cephalic), and a mechanical index of 1.9. Mechanical index is an indication of the ability to produce cavitation and related effects, therefore it has utility in predicting potential micromechanical damage [15]. There are no specific guidelines for energy deposition into the human brain, but many of the mentioned studies follow the IEC 60601 part 2 standard for therapeutic equipment, which sets the limit on acoustic intensity at I_{spta} = 3.0 W/cm^2 [29].

Past studies have determined the spatial error with neuronavigation-guided single-element piezoelectric transducers without skull aberration correction using bench-top testing and in preclinical studies was less than a 3 mm error in all directions when entering through side of the human skull [30]. Because the FUS will be entering through the flat acoustic temporal window to engage the Gpi in this study, we estimate minimal FUS beam distortion and the acoustic intensity will be attenuated by approximately 50%. Transmitting FUS through the top or back of the head has been shown to create larger shifts in beam direction, defocused beam focal dimensions, and significantly attenuated beam ultrasound pressure after the FUS beam passes through the skull and arrives at the brain target. Contours in the skull curvature and non-homogenous thickness produce these beam distortions and high attenuation effects which will be minimized in the proposed study. A particular benefit of FUS neuromodulation is its reversibility; any off-target effects are expected to diminish over time. Effects induced beyond the beam focus have been negligible in animal and human studies.

2.1.3 Brain Targets for Neuromodulation in Schizophrenia

Current pharmacologic treatments for schizophrenia target dopamine D2 receptors in the striatum, but these drugs are often ineffective and produce neurologic side effects [1]. A large body of evidence from neuroimaging studies has implicated the cortico-striato-pallido-thalamo-cortical (CSPTC) circuit in schizophrenia, which is modulated by dopamine. In a study of 71 medication-naïve first episode schizophrenia participants and 73 healthy controls we found increased functional connectivity between right anterior globus pallidus interna (Gpi) and several cortical and subcortical areas in patients compared to controls [31]. Furthermore, baseline functional connectivity between the right anterior Gpi and other brain areas, including the right dorsal anterior cingulate gyrus, correlated with severity of psychotic symptoms (all areas $p < 0.001$) and predicted response to antipsychotic treatment (adjusted R^2= 0.486, $p<0.001$). Consistent with our identification of the globus pallidus interna as a key node in circuits involved in psychosis, Cascella and colleagues [32] reported a single case in which they targeted bilateral substantia nigra pars reticulata (SNr) with deep brain stimulation in a woman with treatment-refractory psychosis. The patient experienced immediate relief of auditory hallucinations and gradual improvement of delusions which remained stable at 12 month follow-up. Auditory hallucinations returned when the current was briefly turned off. She experienced no adverse effects and demonstrated improvement in verbal fluency. Both the SNr and Gpi are primary outputs of the striatum and modulate the thalamus, serving as parallel nodes in the striato/pallido/thalamic circuit (**Fig. 1** [33]). Our finding of increased functional connectivity of the right GPI associated with psychosis is consistent with Gpi as a target for PLIFUS intervention since PLIFUS has been shown to decrease functional connectivity when targeting subcortical and deep cortical structures in monkeys [14].

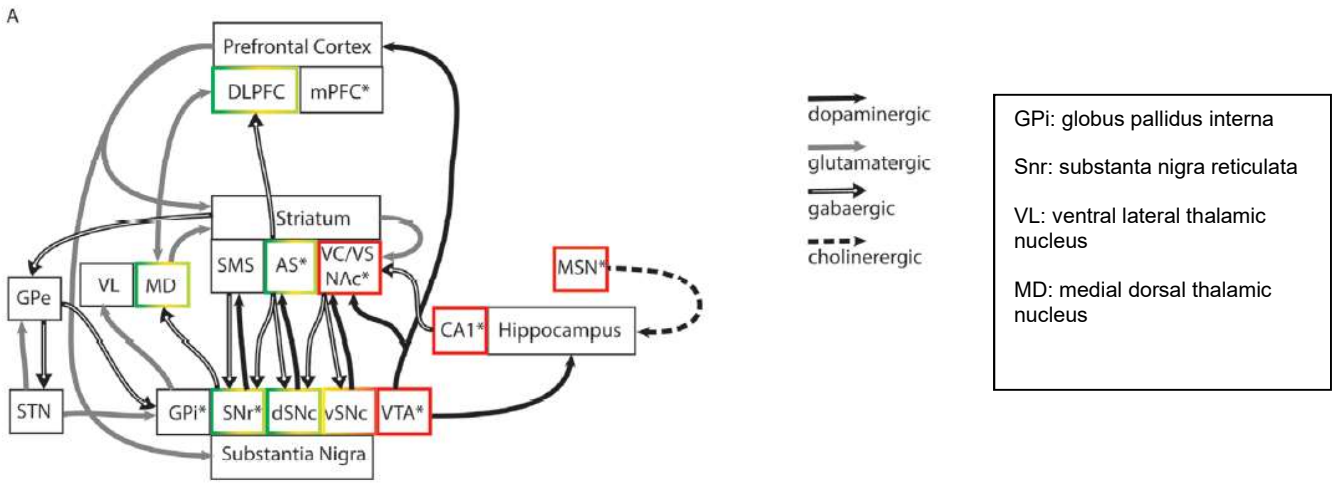


Fig. 1: Striato/pallido/thalamic circuit of the human brain.

The Gpi has been a standard target for surgical treatments for Parkinson's disease, dystonia, and Tourette's disorder; ablative surgery and deep brain stimulation of the Gpi are well-tolerated with rare complications. Deep brain stimulation of the Gpi in patients with Tourette's disorder is associated with improvements in tics, obsessive compulsive symptoms, and depression [34]. The Gpi is partitioned into a posterior section that contains sensory motor circuits and an anterior associative and limbic section, which contains circuits that modulate cognition and motivation [35-37]. Lesions of Gpi have produced both apathy and mania [37]. In addition to our evidence from fMRI implicating the right anterior Gpi in psychosis and in the therapeutic effects of antipsychotic medication, the Gpi is an ideal target for PLIFUS because of its position under the temporal portion of the skull where the bone is relatively thin and oriented in a perpendicular plane to the PLIFUS beam. This position minimizes ultrasound attenuation and refraction through the skull. A depiction of the proposed procedure of transmitting PLIFUS through the side of the head to sonicate the Gpi is shown in **Fig. 2**.

The GP has been the target of one previous study of PLIFUS in humans. Cain et al [38] sonicated left globus pallidus in healthy humans using an extremely low intensity 10 minute stimulation pattern which fell well below the safety threshold for cranial ultrasound established by the FDA. Functional MRI performed during sonication revealed a significant reduction in BOLD signal (blood oxygenation) in globus pallidus, thalamus and connected structures. Arterial spin labeling performed after sonication demonstrated a diffuse reduction in cortical blood flow. No side effects or behavioral effects were reported.

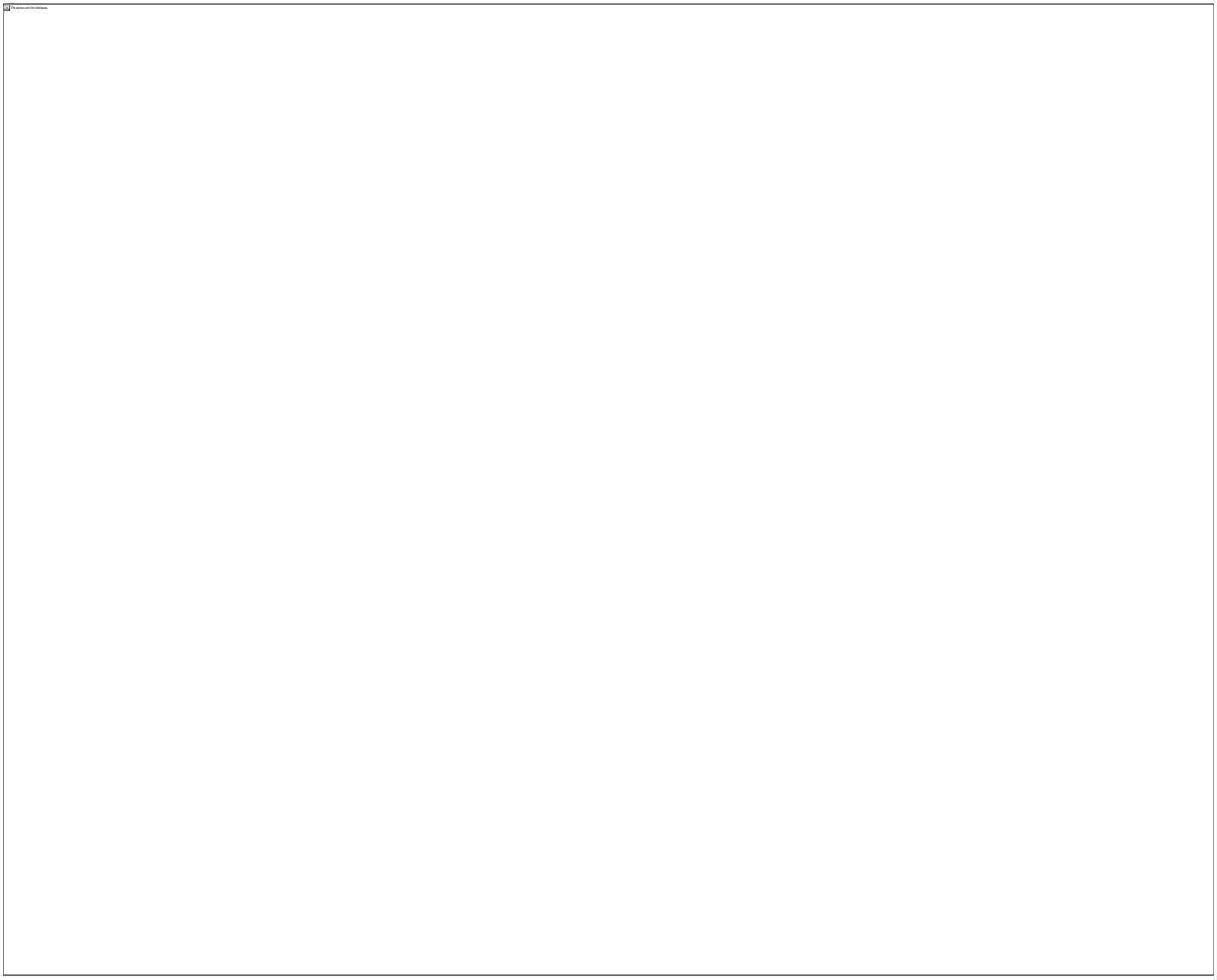


Fig. 2: Estimated ultrasound exposure to the brain during Gpi sonication and a mock demonstration photo of the FUS transducer coupled to the head via a water bag during PLIFUS. The Globus Pallidus and Thalamus are marked in the right hemisphere, as the ultrasound pressure field is superimposed over the brain in the targeted left Gpi.

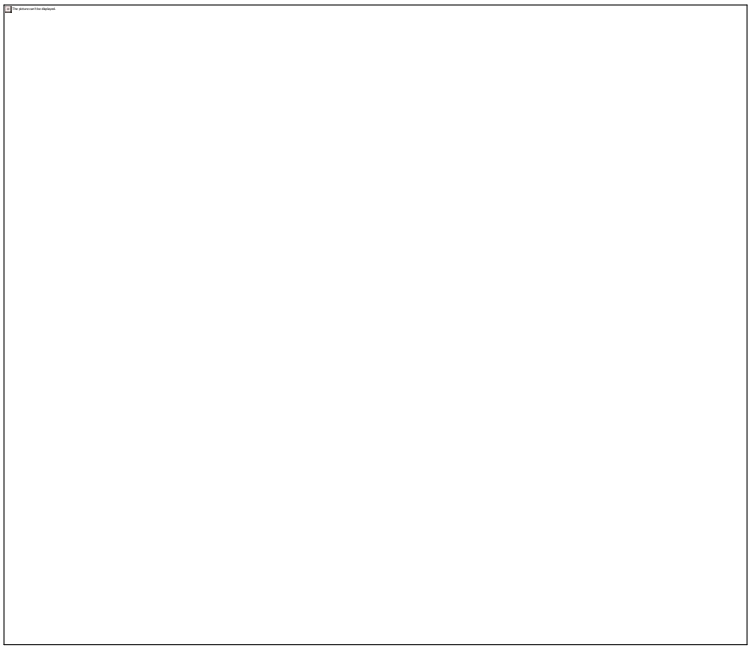


Fig. 3: Ultrasound pulse parameters to be used for Gpi sonication.

2.1.4 PLIFUS Parameters:

We will use the ultrasound pulse parameters used by Cain et al [39] for sonication of the globus pallidus (see previous paragraph) because their experiment demonstrated hemodynamic effects without adverse effects in healthy subjects and the stimulation parameters were very low-intensity and extremely low risk. If we do not see effects on functional connectivity or symptoms after the first 5 participants and if the single sessions are well-tolerated, we will switch to repeated dosing, applying three PLIFUS or sham sonications over 5 days since repeated exposures may be required to produce detectable effects. A single session of PLIUS will include 5 minutes net total dosage of PLIFUS. The exposure uses ten 30 sec blocks of PLIFUS sonication with 30 sec rests in-between spread over ten minutes. Sonication will be delivered (**Fig. 3**) in low-amplitude pulses using a carrier wave frequency: 361 kHz, pulse repetition frequency: 100 Hz, and duty cycle: 5 % yielding an I_{spta} 1.5 W/cm^2 at the Gpi. The acoustic intensity used is in the range of FDA and Electrotechnical Technical Commission (ETC) regulation standards for diagnostic and therapeutic ultrasound, respectively [28].

2.1.5 Assessments for Target Engagement and Potential Clinical Applicability

We will record self-report ratings of psychotic symptoms (hallucinations and delusions) prior to sonication, during sonication, and post-sonication. In addition we will perform fMRI at baseline and within 10 minutes after completion of sonication to examine effects on Gpi functional connectivity. We will use the same methods that we used in examining Gpi functional connectivity in medication-free schizophrenia patients before and after treatment [40]. As described above, Cain et al [39] observed hemodynamic changes during sonication and during imaging performed 30 minutes after sonication. Importantly, Folloni and colleagues [14] targeted the amygdala and the anterior cingulate cortex with a single 40 second train of PLIFUS in macaques and found decreased functional connectivity measured by fMRI 30-110 minutes after sonication with no adverse behavioral or histologic effects. We will utilize a sham-controlled, random-order crossover design, because expectancy may influence our measures, and intra-subject comparisons are far more precise than inter-subject comparisons given the heterogeneity of the illness.

2.2 Name and Description of the Investigational Agent

A detailed description of the device and its related procedures (photos, diagrams, and maintenance protocols) can be found in the DEVICE MANUAL document included in the IRB protocol packet. The Sonicator-1000 system was developed in-house at the NYU Langone Health Tech4Health Institute. It is a neuro-navigated single-element pulsed focused ultrasound platform. The system non-invasively delivers ultrasound sonications intracranially that selectively target specific areas of the brain. The device is hardwired to only emit ultrasound power levels that do not yield damaging effects to the brain like tissue heating, cavitation, and blood–brain barrier disruption, in order to

ensure safe preservation of tissue integrity. Extensive research has been conducted to determine non-thermal pulse parameters and acoustic intensity levels to safely transmit PLIFUS through the scalp–skull–meninges media while localizing the low ultrasound energy into the brain for inducing neuromodulation. This has also been validated through preclinical and clinical studies using histological methods. The device acoustic output limits follow FDA ETC regulation standards for diagnostic and therapeutic ultrasound [28]. Previous human studies with similar laboratory built custom PLIFUS devices have safely induced temporary neuromodulation in healthy subjects with no adverse effects following a single session of sonication.

This type of device has been implemented in several PLIFUS neuromodulation studies with healthy human and neurological disease based studies. Most systems were fabricated and designed in a university institutional lab. No FDA-approved and commercial neuro-navigated single-element pulsed focused ultrasound device platform is available on the market. The Sonicator-1000 neuronavigation system uses the 3Dslicer OpenIGTLink and Plus Toolkit standard network communication protocol used for image-guided interventions. Plus Toolkit and 3Dslicer interface a stereoscopic optical tracking camera (Polaris Spectra or Vicra, Northern Digital, Inc) used to track in real-time the spatial locations of objects (E.g. FUS transducer, patient's head location, navigation stylus, and ultrasound calibration components) within 0.5 mm.

The device control system uses a 2-channel wave generator and radio frequency amplifier to control the single-element focused ultrasound transducer. Transducers of various focal lengths can be interchanged in the device to achieve sonication at cortical or deep brain targets. A water membrane and ultrasound gel are used to couple the ultrasound device to the head yielding ultrasound transmission into the brain. Velcro straps and head cushion shims are used when needed to aid the connection of the water membrane to the head. The particular focal length used to target the Gpi is approximately 5.5 ± 0.5 cm. Transducers are fabricated in-house at Tech4Health via a "Primary Calibration" procedure. A transducer is spatially calibrated to the neuronavigation software by interrogating the focused ultrasound field with a hydrophone scanning system and then assigning a navigation crosshair reference point to the focal point location (peak acoustic intensity in the FUS beam). A certified hydrophone is used to calibrate the acoustic intensity in these calibrations in coordination with the electrical output power of the control system. Spatial and intensity calibration are verified for each transducer during their use on humans via a "Pre-Session Equipment Check". This process involves meausring the transducer function using a transducer power and verifying the transducer is still spatially calibrated to the neuronavigation system. The pre-session equipment check occurs proceeding all FUS sessions at the location of the FUS session. Calibration procedures are described in detail with images and templates for the "Primary Calibration", and "Pre-Session Equipment Check" log forms can be found in the DEVICE MANUAL.

The device operator will be able to monitor the position and power of the PLIFUS beam using the neuronavigation software and transducer power monitor, respectively. Multiple buttons located on the system controls and within reach of the operator can be used to instantly shutoff of the device and stop sonication. The device includes a user manual, calibration certificates, and device operation logs that will be included with the device at all times. All device subsystems are nested into a portable workstation and can be sealed for transport in a protected concert speaker box on wheels.

Operation of the device platform is closely monitored and the methods follow the latest guidleines set by the "International Transcranial Ultrasonic Stimulation Safety and Standards"[41, 42]. 1) Ultrasound output is continuously observed by the operator via transducer power monitor, verifying sonication is active. 2) The navigation software displays the location of the FUS beam focus in real-time during sonication exposure. 3) The power output of the device is displayed on the control system screen of the wave generator and radio frequency amplifier. 4) The transducer monitor continuously monitors the electrical power into the FUS transducer to verify predetermined acoustic intensity levels are reached. 5) Deive shutoff stop buttons are clearly visible and accessible on the control console. 6) The operation of the device requires completion of a strict Pre-Session Equipment Test.

2.2.1 Preclinical Data

2.2.2 Clinical Data to Date

Transcranial ultrasound has been studied in 704 human subjects in 35 trials, including studies in epilepsy, obsessive compulsive disorder, depression, Alzheimer's disease, Parkinson's disease, and chronic pain patients [43]. To our

knowledge, PLIFUS has not been studied in schizophrenia. No serious adverse events have been reported in human studies of PLIFUS; mild and transient side effects were reported in 3.3% of 704 subjects, including headache, neck stiffness, worsening mood, scalp heating, muscle twitches, cognitive effects (attention), itchiness, sleepiness, and anxiety [43]. In one prior study of sonication of the Gpi in 16 healthy volunteers, no adverse events were reported [38].

2.2.3 Dose Rationale

The PLIFUS dose (sonication parameters) was selected based on the study by Cain et al [38] in which the Gpi was the target of sonication and produced changes in hemodynamics (MRI BOLD) without adverse effects. This method involves a very brief (10 minute) pulsed sequence that delivers relatively low energy, which is much lower than most previous transcranial studies and follow FDA and ETC regulation standards for diagnostic and therapeutic ultrasound. An Ispta = 1.5 W/cm^2 is chosen as a conservative acoustic intensity compared to other FUS single-element device neuromodulation studies, where some studies reached up to I_{SPTA} = 4.4 W/cm^2 at the brain target yielding no adverse effects [18].

2.3 Rationale

PLIFUS is the first noninvasive technology that allows us to interrogate deep-seated brain circuits that have been implicated in schizophrenia. This study will be an exploratory step to link imaging biomarkers and patient self-report of symptom response to sonication of the right Gpi. We believe that PLIFUS may provide therapeutic benefit for two reasons. First, our previous work found that excessive functional connectivity of the Gpi was associated with psychosis, and that therapeutic response to antipsychotic medication was associated with decreased Gpi functional connectivity, whereas Folloni and colleagues[14] found that a single session of PLIFUS targeting the amygdala and anterior cingulate reduced functional connectivity. Second, the mechanism of action of PLIFUS appears to be an increase of neuronal calcium influx via mechanosensitive calcium channels [44]. Calcium channels have been strongly implicated by genetic studies in schizophrenia [45]; PLIFUS may produce compensatory changes in calcium regulation that correct a key molecular deficit in schizophrenia.

2.4 Potential Risks & Benefits

2.4.1 Known Potential Risks

Risks of PLIFUS:
Because ultrasound has been used extensively for many years as a diagnostic tool in other fields of medicine, FDA safety regulations are well-established and well-validated. Some investigations of brain sonication have exceeded these safety guidelines and have not produced serious adverse effects. Theoretical adverse effects at very high levels of energy, far in excess of what will be used in this trial, include injury to brain tissue (lesions, bleeding, disruption of the blood-brain barrier) due to excessive heating or cavitation. In 704 human subjects who have been studied in 35 trials of transcranial ultrasound, no serious adverse events have been reported. Mild and transient side effects have been reported in 3.3% of subjects, including headache, neck stiffness, worsening mood, scalp heating, muscle twitches, cognitive effects (impaired attention), itchiness, sleepiness and anxiety [43]. Rates of side effects associated with sham sonication have not been reported. The sonication parameters that will be employed adhere to FDA and ETC regulation standards for diagnostic and therapeutic ultrasound [28].

While PLIFUS is associated with only mild and transient side effects in healthy participants, it has not previously been studied in schizophrenia. It is possible that sonication of the Gpi might worsen symptoms of schizophrenia. If this happens, we expect it to be a transient effect, consistent with other behavioral effects. To minimize this risk, we will exclude from study individuals with a history of violence, aggression or suicidal behavior. We will be monitoring the severity of psychotic symptoms during sonication and will stop the procedure if symptoms worsen by more than 50% on AHRS and DESA or if the participants expresses clinically significant psychological distress or suicidal ideation. The study psychiatrist will remain with the participant during sonication and for 1 hour after completion of sonication to assess for worsening of psychosis and suicidality. The study psychiatrist will arrange for appropriate monitoring and further assessment after 1 hour if needed based on clinical judgement.

Risks of MRI:
A total of 7 MRIs will be performed using a Siemens 3T Prisma; one prior to the intervention visits and one preceding

and one immediately after each of the two sonication/sham visits. No investigational hardware or software will be used during acquisition of imaging data. There are no known radiation risks associated with MRI. However, individuals with metal implants, such as surgical clips or pacemakers should not have an MRI. Additionally, given that the MRI takes places in a confined space, and there are loud banging noises associated with the procedure, individuals may feel anxious during the procedures. Earplugs will be offered to subjects in an effort to help reduce the MRI related noise and subjects will be told that the MRI can be stopped at any time at his/her/their request. Some subjects may experience muscle twitches or tingling sensations and/or a slight increase in body temperature during some types of scan activity. These are very unlikely under current MR guidelines. The FDA has determined that the most common injury associated with MRIs were burns to the skin, most commonly caused by devices that conduct electrical energy, such as metallic objects, pulse oximeters, or EKG leads. While skin tattoos may, in theory, be conductive, the first systematic study of risks of tattoos in individuals undergoing MRI scans was recently published in the New England Journal of Medicine and found minimal risk [46]. The investigators studied a sample of 330 patients who had a total of 933 unique tattoos, including both professional and self-applied tattoos, and were imaged using a 3T MRI for a total of 585 imaging sessions. They excluded individuals with greater than 5% of their body surface covered by tattoos, with tattoos larger than 20 cm, or with tattoos on face, neck or genitals due to fear of adverse events. These criteria were arbitrarily determined by the investigators without strong evidence support. In this study, one participant reported a “tingling” sensation and one reported a sensation of warmth—no persistent adverse effects were observed. A more recent MRI safety study found these exclusionary criteria to be too stringent because 182 of the 376 participants with tattoos in their study (48.4%) would have been excluded [47]. They included all participants with tattoos regardless of size or location, including those with tattoos larger than 20cm (n = 60), tattoos on head, neck, or genitals (n = 125), and tattoos covering more that 5% of body surface (n = 28). None of these participants reported any adverse effects of MRI. In addition, the aforementioned study uses 3T MRI similar to our study. On the basis of this evidence, we will allow individuals with tattoos to participate regardless of size and location. Participants with tattoos will be instructed to notify the technician if they experience warmth in the location of a tattoo and the imaging session will be immediately terminated. All participants will be screened for metal objects and tattoos by both the research staff and the technicians at the imaging center to ensure that no unsafe conductive materials are present in or on the patient’s body.

In extremely rare cases, a magnet can lose its magnetism, in which case cooling fluids may be released noisily through escape valves and may collect in gas form in the scan room. The gas is not harmful in itself as long as fresh air is available. In this very remote event, participants will immediately be brought out of the magnet room.

Contrast will not be used during the MRI scans for this study.

Loss of confidentiality regarding psychiatric or medical information is a possible risk for which precautions will be taken. Participants will be assigned a study identification code that will be used for all study documents. All identifiers will be redacted from records from this study. Study documents collected in this study will be kept in a locked cabinet. Only research staff who are directly involved in this study will have access to that file.

Several measures have been taken to protect subjects against risks incurred by participation in this protocol. We will screen out potential subjects with medical vulnerabilities, including epilepsy or unstable medical illness, and will exclude individuals with a history of suicidality. Participants will be closely observed for 1 hour after their sonication/sham sessions and will be re-assessed after 24-48 hours and after one week.

2.4.2 Known Potential Benefits

No known potential benefits are associated with a single administration of PLIFUS in individuals with schizophrenia. Results of this study may lead to the development of a new therapeutic approach for psychotic symptoms that do not respond to currently available treatments.

3 Objectives and Purpose

The overall purpose of this study is to determine feasibility and tolerability of PLIFUS sonication of the right Gpi in individuals with schizophrenia and to determine whether a single session of PLIFUS sonication will decrease Gpi functional connectivity and decrease intensity of hallucinations and delusions compared to sham sonication. This pilot study will allow us to determine whether the proposed target and PLIFUS sonication parameters produce detectable changes in our imaging biomarkers in order to demonstrate target engagement and to determine whether

we can detect a signal suggestive of therapeutic efficacy to justify future therapeutic trials. If a single session of PLIFUS does not produce measurable effects in the first 12 completers with good quality imaging data, we will administer 3 sessions of PLIFUS and of sham, each over 5 days, in 4 additional participants.

3.1 Primary Objective
To evaluate tolerability, safety and evidence of target engagement (functional connectivity and symptom change) of a single session of PLIFUS targeting the right Gpi in individuals with schizophrenia compared to sham.

3.2 Secondary Objectives
If the primary objective is not successful, we will test the hypothesis that 3 sessions of PLIFUS targeting the right Gpi administered over 5 days will produce a decrease in Gpi functional connectivity and a reduction in symptoms compared to sham.

4 Study Design and Endpoints

4.1 Description of Study Design
This is a single-site, phase 2, sham-controlled random-order cross-over pilot trial of PLIFUS targeting the right Gpi in individuals with schizophrenia. Twelve individuals with schizophrenia who report continuous hallucinations or delusions of moderate or greater severity will receive one session of PLIFUS and one session of sham PLIFUS in random order, one week apart. If no effect of PLIFUS is detected on measures of functional connectivity or psychotic symptoms in the first 12 completers with good quality imaging data, the trial will be changed to 3 sessions of PLIFUS or sham administered over 5 days for 4 subsequent participants.

Potential participants will be enrolled at NYU Langone Manhattan, NYU Langone Brooklyn, and Bellevue Hospital. Recruitment-only procedures will occur at Manhattan Psychiatric Center (see section 5.5.3). Following informed consent, potential participants will be screened by a psychiatrist who will assess psychiatric and medical status. A Brief Psychiatric Rating Scale will also be administered to assess inclusionary status. Individuals who meet inclusionary and exclusionary criteria will then have a structural MRI performed at the MRI visit and with each fMRI scan at Intervention Visits 1 and 2 to guide neuronavigation of PLIFUS. The two sessions of PLIFUS/Sham will occur in random order, one week apart. Randomization schedule will be done using Matlab. Participants will first have an fMRI for measurement of right Gpi functional connectivity, preceded and followed by symptom rating scales. PLIFUS/sham will then be administered over 10 minutes, during which symptom ratings will again be recorded. Symptom ratings will be repeated 5, 30, and 60 minutes, 1 day and 7 days post-sonication/sham. fMRI will be repeated starting 10 minutes after sonication/sham. Safety assessments will be completed prior to discharge of participants 1 hour after each sonication/sham session and after 24-48 hours and 7 days.

4.2 Study Endpoints

4.2.1 Primary Study Endpoints
The primary study endpoint is the change in Gpi functional connectivity from baseline (pre-sonication) to post-sonication. The change in functional connectivity will be compared between PLIFUS and sham conditions. This measure was selected because of our prior finding that increased Gpi functional connectivity is highly correlated with psychosis severity in schizophrenia and because Fallon and colleagues [14] demonstrated that a single session of PLIFUS decreased functional connectivity in macaques.

4.2.2 Secondary Study Endpoints
Secondary study endpoints are measures of clinical symptoms, which are assessed using the following scales: AHRS, DESA, BPRS, and PSYRATS. Safety assessments will consist of the Columbia Suicide Severity Rating Scale (C-SSRS), MoCA, and SAFTEE and addendum assessment for side effects.

5 Study Enrollment and Withdrawal
Participants will be individuals with a diagnosis of schizophrenia who report continuous psychotic symptoms (auditory hallucinations or delusions) of at least moderate severity. Participants may be taking antipsychotic

medication or be medication-free, must be free of unstable psychiatric or medical illness, cannot have suicidal ideation, or a history of violence or of suicidal behavior (attempts), cannot have epilepsy or be taking anticonvulsant medication and must have no contraindication to MRI or sonication, including metal implants.

5.1 Inclusion Criteria

1. Males and females 18 to 55 years of age, inclusive, at time of informed consent
2. Must report current psychosis, as defined by a score of $\geq$ 4 on at least one of the following psychosis items on the Brief Psychiatric Rating Scale (BPRS): suspiciousness, hallucinations, unusual thought content, or grandiosity. Psychosis has to have persisted continuously for at least 4 weeks.
3. Must have a diagnosis of either schizophrenia or schizoaffective disorder as established by a Structured Clinical Interview for DSM-V (SCID)
4. If taking antipsychotic medication, the dose must be unchanged for at least 4 weeks prior to randomization. If not taking antipsychotic medication, must be intending not to start medication until after completion of the study (approximately 3 weeks)—this decision must be judged to be appropriate by the research psychiatrist and by the participant's clinician.
5. If assigned female at birth and of childbearing potential, patients must
    a. have a negative urine pregnancy test (all participants assigned female at birth regardless of childbearing potential will be required to submit a pregnancy test), and
    b. not be nursing or planning a pregnancy for the duration of the study through 30 days after the last sonication visit, and
    c. be abstinent or willing to use a reliable method of birth control from the Screening Visit and continue with the same method until termination from the study.

5.2 Exclusion Criteria

1. Current substance abuse / dependence for substances other than nicotine and THC, (i.e. alcohol, amphetamines, barbiturates, etc.)
   a. A positive urine toxicology screen (excluding THC, tricyclic antidepressants, or benzodiazepines (if prescribed).
   b. Moderate or severe cannabis use disorder.
2. Diagnosis of major mood disorder or other Axis I disorder other than schizophrenia or schizoaffective disorder as determined by a SCID
3. Current or recent suicidal ideation – suicidal ideation with intent or plan (indicated by affirmative answers to items 4 or 5 of the Suicidal Ideation section of the baseline C-SSRS) in the 6 months prior to screening or have a history of suicide attempt in the past 6 months or subjects who represent a significant risk of suicide in the opinion of the investigator.
4. Subjects who represent a significant risk of violence determined by the assessing clinician or PI, based on history, presentation, and the Buss-Perry Aggression Questionnaire [48] or a rating of $\geq$ 5 on the BPRS hostility item.
5. Pregnant or nursing or positive urine pregnancy test.
6. Significant medical or neurological illness by history or physical exam, including seizure disorder, history of loss of consciousness lasting more than 30 minutes related to head trauma or intellectual developmental disorder.
7. Metal implants, such as a cardiac pacemaker, intra-cardiac lines, neurostimulator, medication infusion device, jewelry/piercings that cannot be removed, and/or any other metal in the body that cannot be removed.
8. Claustrophobia
9. A history of brain surgery
10. History of syncopal episode within the past 6 months
11. Cochlear implants
12. Skin disease at intended stimulation sites
13. The consumption of more than four alcoholic units within 24 hours before Intervention Visit 1 and 2, and any recreational drugs within 48 hours before Intervention 1 and 2

5.3 Inclusion of Subjects Using Marijuana

The incidence of cannabis use psychosis patients in the US is estimated at 40% to 60% and a serious concern has been raised that excluding individuals who use cannabis may bias samples and make results less generalizable to clinical populations. There is no reason to expect a negative interaction between cannabis and PLIFUS. We will record past use of cannabis and urine toxicology screen results for cannabis at screening. Self-report of cannabis use will also be recorded at the beginning of each intervention visit. We will include this as a factor in our analysis to assess whether cannabis use influences response to PLIFUS. The determination of moderate or more severe cannabis use disorder will be based on the Diagnostic and Statistical Manual (DSM) 5 criteria.

5.4 Vulnerable Subjects

We will not study children because schizophrenia is an illness of adulthood and because PLIFUS safety has not been sufficiently studied in children. We will not enroll individuals with decisional incapacity. We will assess potential participants for their ability to understand the procedure, the risks and alternatives and will document this with a true-false test about key issues regarding participation for which we will require 100% correct answers.

5.4.1 Assessment of Capacity

Assessment of capacity will be done by licensed MDs or nurses with experience treating this patient population. The assessors are study team members that are identified in Research Navigator. They do not need to be independent of the study because they are thoroughly trained to make this assessment and will verify the assessment of capacity by use of a true-false quiz that includes questions to determine that the potential participant understands the procedures, the risks and alternatives to participation and that participation is voluntary .

The individual performing the assessment and/or monitoring ongoing capacity will complete our standardized Assessment of Capacity Form and will administer a written 10 question true/false quiz to establish that the potential participant understands procedures, risks and alternatives to participation.

The results of the capacity assessment will be placed in the subject's study record.

Prospective subjects will be informed of the results of the capacity assessment after it's conducted. If an individual is found not to have the capacity to consent, the assessor will explain this to the individual. The individual will not be enrolled in the study. The assessor will provide the necessary resources and referrals for further care and evaluation.

5.5 Strategies for Recruitment and Retention

Participants will be help-seeking males or females, ages 18-55, meeting diagnostic criteria for schizophrenia or schizoaffective disorder and currently exhibiting at least moderate, continuous psychotic symptoms defined by a score of 4 or greater on at least one psychosis item of the BPRS (suspiciousness, hallucinations, unusual thought content, grandiosity) and in the absence of psychotomimetic substance use or other potential organic etiologies, or major mood disorder, and in the absence of suicidal ideation, pregnancy, or significant medical illness (including epilepsy).

Referring clinicians will receive the "Information for Clinicians" document, which contains information about the study as well as contact information for the study team.

Patients will be recruited at NYU Langone Health medical centers in Manhattan and Brooklyn, as well as Bellevue Hospital and Manhattan Psychiatric Center. Clinicians will be asked to only refer individuals who they believe are appropriate for the study and who are capable of deciding to participate. Once referrals are given, the study team will access EPIC to determine the participant's initial eligibility. Dr. Goff and the study coordinator will maintain relationships with clinicians at these institutions in order to generate referrals.

As detailed in Dr. Mariana Lazar's protocol for s22-01421, Dr. Goff and his staff may also ask current or former participants in this study if they are interested in participating in s22-01421. For any individuals who are interested, their preferred contact information will be shared with Dr. Lazar's team for them to reach out. Of note, only former subjects who indicated that they are okay with being contacted by Dr. Goff's team in the future will be approached.

5.5.1 NYU Langone Hospital – Brooklyn and the Family Health Center

Subject recruitment will include collaboration with NYU Langone Hospital – Brooklyn and the Family Health Center at NYU Langone clinical leadership to help identify potential subjects. The clinical leadership team at FHC will utilize the inclusion/exclusion criteria developed by the study team to search the EPIC EMR and generate a list of potential subjects. This list will be screened to ensure that patients who have opted out of research recruiting are removed. The final list will be shared with the investigators who will then reach out to the patient's primary clinician, or another member of their clinical team, and see if they are willing to approach the patient and ask if they are interested in speaking to research staff about the research study. If the patient agrees, the member of the clinical team will connect the patient with the research team member.

5.5.2 Bellevue Hospital

Staff can refer patients from the Bellevue Adult Outpatient Psychiatry Clinic (OPC), Inpatient Psychiatry Units, and Comprehensive Psychiatry Emergency Program (CPEP) who are appropriate for study participation and willing to speak to an investigator, by reaching out to the study team with the MRN. The study team will then access EPIC to determine the patient's initial eligibility. If the patient seems eligible based on their chart, a psychiatrist/psychologist/nurse practitioner on the study team will assess the clinical appropriateness of the individual to participate in research and capacity to consent.

Consent and screening procedures can take place in the CPEP or Inpatient Psychiatry Units for those in which it is applicable. Of note, no screening laboratory procedures (urine drug screen, urine pregnancy screen) will take place for those who are screening in the CPEP and/or Inpatient Psychiatry Units. Following screening procedures, individuals who qualify, and who are judged by their clinicians to be safe and appropriate for participation, but still remain an admitted to the hospital, will continue in the study once discharged. Outpatients recruited from Bellevue can start screening at the OPC (including collection of urine laboratory specimens if the patient prefers to meet there instead of 1 Park Avenue or the 53rd St. Imaging Center), or can begin/finish screening either at 1 Park Avenue or the 53rd St. Imaging Center.

Of note, study staff may also come across potentially eligible patients at NYU Langone Manhattan, NYU Langone Brooklyn, and Bellevue Hospital via chart review or by participating in rounds. Study staff would first reach out to the patient's treating physician to inquire about appropriateness for participation and permission to contact the individual if deemed fit for screening.

The time required to complete recruitment and consenting will be variable depending on availability of clinicians. Potential participants will be allowed as much time as is needed to consider participation, including discussing this with family and their clinician. All interactions with potential participants will take place in private rooms to maintain confidentiality. Any identifiable data of screen failures will be destroyed immediately after the recruitment period has ended.

We will include individuals who speak Spanish as potential participants. In order to recruit Spanish speaking patients, our team will receive referrals from clinicians from our network of NYU-affiliated institutions and Bellevue Hospital. Clinicians who refer Spanish speaking patients will initially determine if the subject will be eligible to participate our study. If the patient qualifies and agrees to participate in our study, our team will provide a native Spanish speaking interpreter with training and understanding in medical terminology during the study visits. No Spanish-speaking participants will be enrolled until a modification is submitted to the IRB to provide the translated study documents.

5.5.3 Manhattan Psychiatric Center (OMH)

OMH MRNs will be shared by MPC clinicians with Dr. Qi via OMH internal emails. Dr. Qi will then review the charts of these patients for initial eligibility (check for MRI incompatibilities, violence, recent medication changes, etc.). Dr. Qi will obtain patient contact information either directly from the patient or via clinicians with the patient's verbal consent. Of note, the patient's consent for Dr. Qi to reach out will be documented in the OMH internal emails between her and the clinician. To clarify, the term "OMH" is used to describe the MRNs/charts/internal emailing system since OMH (Office of Mental Health) is the larger organization and MPC is the site we will be working with.

During the initial contact made with the patients, Dr. Qi will ask if it is okay to share a preferred method of contact with the NYU team, as well as provide the contact information for the NYU research team either verbally or through safe email (encrypted) with her NYU Langone email.

Of note, patients may also be introduced to the study team at their MPC clinic appointments, if the patient indicates this is okay with their clinician.

Since MPC clinicians are entirely in-person, one staff member at MPC may be used as a point person to speak to other clinicians about potential subjects (authorship can be offered to this individual).

5.5.4 Use of DataCore/Epic Information for Recruitment Purposes

We will use DataCore/EPIC for the recruitment of subjects via NYULangone MyChart. The study team will send a standardized recruitment message to the MyChart accounts of patients with a diagnosis of schizophrenia or schizoaffective disorder who are between 18 and 55 years of age. The recruitment message appears within the MyChart "Research Studies" activity tab and includes information about the study (i.e., the principal investigator, study coordinators, study name, and brief description). The tool enables patients to indicate whether or not they are interested in participating in the identified study, and this response is sent to the study team's Epic inbox. If an individual indicates that they are interested in participating, the study team will first reach out to their clinical team to determine the appropriateness of the individual to participate in research. After describing the study and providing the Information for Clinicians, research staff will ask: "Is the patient interested in participation in the study?" and "Is it clinically appropriate for this patient to participate in the study?". If the clinician reports that the patient is both interested and appropriate to participate, the study team will request the patient's preferred method of contact.

If the patient does not have an NYU and/or Bellevue affiliated clinician that they see regularly, the study team will contact them directly via MyChart.

Once contact is made, study staff will communicate to subjects the reason they are being contacted and will ask if they are interested in participating in this specific study. Should the potential subjects agree, the study team will provide the subjects with information regarding the next steps for participation.

We will also use DataCore/EPIC to generate and request two lists of patient names. Both requests will include patients that fit the following criteria for this study: diagnosis of schizophrenia or schizoaffective disorder, between 18 and 55 years of age, not deceased, not pregnant, and no request to opt out of research. One list will include patients who have visited the NYU Langone Hospital emergency departments (Manhattan and Brooklyn) and saw psychiatry hospital service in the past 6 months. The other list will include patients who have been admitted to the NYU Langone Inpatient Psychiatry Units (Tisch HCC10 and Brooklyn 5900) in the past 6 months. Study staff will request the standard SlicerDicer dataset with PHI including names and MRNs, as well as phone numbers and emails. Study staff will reach out to potentially eligible patients through this contact information. Subjects will be sent an IRB-approved REDCap screening survey or will be contacted on the phone using the IRB-approved phone recruitment script. All outreach efforts will abide to HIPPA policy. Of note, clinicians will not be contacted prior to reaching out to these patients as it is unlikely for them to have regular NYU and/or Bellevue clinicians since they are being identified from the ED and inpatient psych. If there is a clinician the patient sees regularly, this clinician will be reached out to, but if not, the patient will be contacted by the study team directly.

If a subject requests information regarding opting out of further recruitment for all research, subjects will be directed to contact research-contact-optout@nyumc.org or 1-855-777-7858.

5.5.5 Online Advertisements

We will recruit participants for treatment studies using a range of postings and online advertising strategies, including but not limited to Facebook, Instagram, Reddit, Craigslist, iConnect, etc. Online advertisements will use IRB approved messages. Interested potential participants will be able to express their interest through calling/emailing study team, or by filling out the IRB-approved REDCap screening survey. Since this REDCap screening survey features open-ended questions, there will not be an automated process enabled to inform these individuals of their eligibility; moreover, eligibility will be determined by study staff reviewing the information entered

into the form, and a prompt will not be used to inform potential patients they are not eligible. Eligible subjects will be contacted at the patient's preferred method of contact, as answered on the survey. For those who are deemed ineligible, the information entered into this REDCap survey will be discarded.

5.6 Duration of Study Participation

After informed consent, participants will complete a single screening session that will take approximately 2 hours. At the next visit, subjects will have a structural MRI performed which take approximately 20 minutes. They will then participate in two PLIFUS/sham sessions over 7 days that include structural and functional MRI scans as well as symptom rating scales and safety assessments. These symptom rating scales and safety assessments will be repeated 1 day after PLIFUS/sham and 7 days after the last PLIFUS/Sham session. We expect this process will take a minimum of 14 days and a maximum of 21 days to complete with a minimum of 3 in-person visits (if consenting, screening and MRI are performed at one visit and the final assessment is performed remotely) and a maximum of 5 in-person visits.

5.7 Total Number of Participants and Sites

The study will be performed at a single site (NYU Langone Manhattan) and participants will be enrolled in order to achieve 12 completers who complete both sonication/sham sessions. Potentially, 4 additional participants will be enrolled if a single session of PLIFUS does not reduce functional connectivity or symptoms in the first 12 completers with good quality imaging data compared to sham, to assess whether 3 sessions of PLIFUS administered over 5 days reduces functional connectivity and symptoms.

Screening and follow-up assessments can be performed at the Psychiatry Research Center at One Park Avenue or NYUGSoM Research Imaging Center at Lexington and 53rd St. MRIs and sonication/sham will always be performed at the NYUGSoM Research Imaging Center at Lexington and 53rd St. Participants will be recruited at NYU Langone Manhattan, NYU Langone Brooklyn, and Bellevue Hospital.

5.8 Participant Withdrawal or Termination

5.8.1 Reasons for Withdrawal or Termination

Participants are free to withdraw from participation in the study at any time upon request. An investigator may terminate participation in the study if:

- Any clinical Adverse Event (AE), or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

5.8.2 Handling of Participant Withdrawals or Termination

If participants withdraw or are terminated before completion of the study, we will attempt to conduct the safety assessments at 24-48 hours and 7 days if the participant experienced one session.

5.9 Premature Termination or Suspension of Study

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.
Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants

The study may resume once concerns about safety, protocol compliance, data quality are addressed and satisfy the IRB and DSMB.

6 Study Agent (Study drug, device, biologic, vaccine etc.) and/or Procedural Intervention

6.1 Study Agent(s) and Control Description

We believe the study will not require an FDA IDE because the PLIFUS parameters are within the FDA ETC safety standards for ultrasound devices. In addition, the PLIFUS sequence targeting globus pallidus used in this protocol has been previously utilized in human subjects without any adverse effects. However, if the IRB requests, we will contact the FDA about the IDE status of this study.

*PLEASE SEE "non-significant risk device assessment" document for detailed reasoning.

6.1.1 Acquisition

PLIFUS delivery into the brain will be verified via an acoustic receiver sensor strapped to the left side of the participant's head. Although the ultrasound energy is concentrated and focused on the GPi, an acoustic emission wave of almost negligible acoustic intensity can be detected from a receiver (ultrasound microphone) strapped to the opposite of the head. This faint signal will be used to confirm the transducer is transmitting into the head. The device operator can observe this signal with an oscilloscope contained in the control system console and it can be observed throughout the duration of the 10 minute sonication period. Sham sonication will not enable the detection of the acoustic emission wave, as there will be no ultrasound transmission into the head during sham.

6.1.2 Formulation, Appearance, Packaging, and Labeling

See the DEVICE MANUAL for device Appearance, Packing, and Labeling details.

6.1.3 Product Storage and Stability

The device platform and all its components are housed in a protective and portable concert instrument box with wheels. Contents can be locked in the box for security and storage.

6.1.4 Preparation

See DEVICE MANUAL for detailed device preparation and calibration procedures.

6.1.5 Dosing and Administration

Pulsed low-intensity focused ultrasound will be delivered over a total of 10 minutes using the following parameters: Pulse repetition frequency: 100 Hz, Pulse Width: 0.5 ms, Duty Cycle: 5%, Sonication Duration: 30s, Inter-Sonication Interval: 30 s. Total Time: 10 minutes. Ispta = 1.5 W/cm^2 (after passing through skull with estimated 50 % skull attenuation for all subjects). The sham condition will involve the device producing an identical sound as when the FUS is actively penetrating through the skull while an acoustic block is set in front of the transducer, situated under the water membrane. The team administering PLIFUS will consist of a technical expert (Alon Gilad, or other trained study team member), a research assistant and study psychiatrist. The research assistant, study psychiatrist and participant will be kept blind to treatment assignment (sham or sonication). The PLIFUS/sham administrator will be unblinded to treatment assignments.

6.1.6 Route of Administration

Transcranial guided by neuronavigation.

6.1.7 Starting Dose and Dose Escalation Schedule

The starting dose will be a single session following the parameter specified above (see Dosing and Administration). If an effect on functional connectivity or symptoms is not detected in the first 12 completers, the dose will be changed to three administrations spaced over 5 days using the same sonication parameters for 4 additional participants.

6.1.8 Dose Adjustments/Modifications/Delays

If change in GPi functional connectivity or change in symptoms is not greater following PLIFUS compared to sham with an effect size of at least 0.5 in the first 12 completers, the number of sessions (PLIFUS and sham) will be increased from 1 to 3 for 4 additional participants.

6.1.9 Duration of Therapy
2 sessions of PLIFUS/sham administration (delivered over a total of 10 minutes). An evaluable participant will be defined as having completed all sonication/sham sessions.

6.1.10 Tracking of Dose
A "Subject Dose Log" form will record the FUS exposure for each subject. This will include date and time of session, sonication delivery stamp, Active/Sham, transducer #, device operator, and all medical staff present during sessions. Only Alon Gilad will have access to this form to maintain the blind for *all* participants (e.g., another trained study team member may be unblinded to the treatment conditions for the particular subject(s) they are administering PLIFUS/sham for, but will not have access to the treatment conditions for other subjects).

6.1.11 Device Specific Considerations
See DEVICE MANUAL

6.2 Study Behavioral or Social Intervention(s)

6.2.1 Procedures for Training Interventionalists and Monitoring Intervention Fidelity

The training log for this study can be found in the regulatory binder.

An expert with extensive experience will be administering PLIFUS/sham. In the event that expert is unavailable to perform a subject's intervention visits, another trained study team member will administer PLIFUS/sham in his absence. In order to do so, this individual must be trained by the study's technical expert (Alon Gilad) and have both the DSMB's and technical expert's approval prior to PLIFUS/sham administration. Documentation of these approvals will be maintained in the regulatory binder in addition to the study training log. Of note, the same PLIFUS/sham administration will perform Intervention Visit 1 and 2 to maintain consistency. Those who administer PLIFUS/sham will be unable to rate outcome measures (AHRS, DESA, BPRS, and PSYRATS) for that subject.

Trained research assistants can administer the following scales: MoCA, CGI, BPRS, BPAQ, SAFTEE & addedum, AHRS, DESA, PSYRATS, and EHI. New research assistants will be trained before they may rate participants.

Only study team clincians can complete the following assessments: Assessment of Capacity, SCID, and C-SSRS. Of note, study team clinicians can also administer any of the research assistant designated scales mentioned above.

7 Study Procedures and Schedule

7.1 Study Procedures/Evaluations

7.1.1 Study Specific Procedures

Assessments:
Brief Psychiatric Rating Scale (BPRS) [49]*:* The BPRS is an 24-item scale that measures positive symptoms, negative symptoms, general psychopathology and affective symptoms. Individual items are scored on a seven point Likert scale. The BPRS has been extensively used in trials of antipsychotic agents and has been shown to be sensitive to change. Psychometric properties and the underlying factor structure are well-established. We will use a 1 week (7 day) lookback period for this clinical assessment.

Buss-Perry Aggression Questionnaire (BPAQ) [48]*:*
The Buss-Perry Aggression Questionnaire consists of 29 items and contains 4 subscales: Physical Aggression, Verbal Aggression, Anger, and Hostility. The BPAQ will be administered at screening to assess a history of violence.

Structured Clinical Interview for the DSM-5 (SCID): The SCID for the DSM-V (Modules A-E) will be used to assess the primary psychiatric diagnosis for patients. The other modules for the SCID will not be performed as they do not relate to the inclusion/exclusion criteria.

Edinburgh Handedness Inventory (EHI)[50]:
The EHI is a 10-item scale that measures laterality preferences when performing different tasks. Participants are asked to indicate which hand (right or left) they use when performing different actions. A laterality quotient is determined by calculating the percentage of activities that are completed solely with the right hand. A laterality score of 100 would reflect complete right-handedness. A score of 60% or greater will be used as the cutoff for right-handedness. Handedness will be recorded but is not an entry criterion.

Systematic Assessment for Treatment Emergent Side Effects (SAFTEE) [51]: The SAFTEE is a well-established and validated structured interview for assessment of side effects in intervention studies in schizophrenia. It consists of two components, an open-ended inquiry and a comprehensive review of all body systems.

An addendum to the SAFTEE will also be used to assess the following side effects that have been reported in other human subject ultrasound studies: mood deterioration, scalp heating, anxiety, neck pain, and pruritis (itching).

The Columbia Suicide Severity Rating Scale (C-SSRS) [52]: The C-SSRS is a clinical interview that measures the spectrum of suicidal ideation and behavior. It was developed in the National Institute of Mental Health Treatment of Adolescent Suicide Attempters Study to assess severity and track suicidal events through any treatment. It can be administered during any evaluation or risk assessment to identify the level and type of suicidality present and can also be used during treatment to monitor clinical worsening. It contains a 1-to-5 rating scale for suicidal ideation of increasing severity. It contains a Screening scale and a Since Last Visit scale. This assessment will not be reported as unblinded in future publications.

Clinical Global Impression (CGI):
The CGI consists of three global scales (items) that have been designed to measure the severity of illness, global improvement, and efficacy of treatment. Global Improvement is the 2nd scale in the CGI. Total overall improvement is rated on a 0-7 point weighted scale, ranging from very much improved (1) to very much worse (7) respectively.

MRI:
A structural MRI will be performed using a PRISMA 3T scanner for the purpose of guiding neuronavigation of PLIFUS at the MRI Visit (approximately 10 minutes) and with each fMRI resting state scan (either before or after, approximately 5 minutes, to help register fMRI scans) on Intervention Visits 1 and 2. The structural MRI will be a standard clinical sequence. Three-D structural images will be acquired using a T1-weighted MPRAGE sequence (TR/TE = 2300 ms/2.96 ms, FOV= 256 × 256 mm2, FOV phase=93.8%, acquisition matrix = 256 × 256, voxel size = 1 × 1 × 1 mm3, slice thickness = 1 mm, 192 sagittal slices).

fMRI:
Functional MRI will be performed before and after PLIFUS/Sham sessions to evaluate GPi functional connectivity. Data will be collected for 20 minutes using an echo-planar imaging sequence: repetition time/echo time (TR/TE) = 3000 ms/30 ms, field of view (FOV) = 216 × 216 mm2, FOV phase = 100%, voxel size = 3 × 3 × 3 mm3 , slice thickness = 3 mm, and 45 sagittal slices. Each functional run will contain 170 image volumes. All participants will be instructed to look at a fixation point on the screen and to not fall asleep during the resting-state data acquisition.

PLIFUS/Sham:
PLIFUS or sham will be performed in a procedure room adjacent to the MRI. Participants will be seated, gel will be applied to the right side of their head and the ultrasound transducer will be placed against the right side of their head. A water-filled membrane will occupy the space between the transducer and the participant's head. Sonication will be delivered in pulses over a 10 minute period. The sham procedure will be identical and will produce identical sounds so that neither the participant or the rater/psychiatrist will know which treatment modality is applied. Only the PLIFUS operator will be unblinded.

The Montreal Cognitive Assessment (MoCA) :

MoCA is a widely used rapid screening instrument for mild cognitive dysfunction. It assesses different cognitive domains: attention and concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation. It has little learning effect at one month and using alternative versions can decrease learning effect when used repeatedly[53, 54]. For this study, we will use MoCA 8.1, 8.2, and 8.3.

Psychotic Symptom Rating Scale (PSYRATS)[55]:
The PSYRATS is a well-validated 17 item, 5 point Likert scale which contains 6 items that characterize delusions according to 1.) Amount and duration of preoccupation, 2.) Conviction, 3.) Amount and intensity of distress, and 4.) Disruption to life. We selected the PSYRATS because it rates subjective distress and the degree to which delusions disrupt life. Factor analysis has revealed three dimensions: preoccupation, conviction and distress/life disruption, with modest correlation between preoccupation and conviction (r=0.28).

Auditory Hallucinations Rating Scale (AHRS):
A scale developed by Hoffman et al. measuring specific characteristics of auditory hallucinations (AHs) based on a 7-item scale[54]. It is a reliable tool that has been used in multiple TMS studies to assess changes of AHs[56].

Delusions Experience Sampling Assessment (DESA):
Items regarding delusions from a validated experience sampling assessment[57, 58] to assess moment-to-moment experience and detect more rapid changes of delusions. Participants will be asked to rate the same delusional belief at the moment on a 7-point Likert scale. It includes four items: conviction, distress, preoccupation, and disruption.

Communicating with Staff:
Study staff may use text messaging to communicate with subjects. This would only be for the coordination of study visits (i.e., if the subject is not available to do so over the phone and texting is easier at that moment), to ask the subject to give us a call back if they don't answer, or to confirm whether they are attending their study visit. Of note, the phone used by staff is an MCIT approved device, and phone number, as well as message history, will be deleted at subject's end of study/termination.

7.2 Study Schedule

7.2.1 Pre-Screening (Days -30 to -1)

- Review appropriateness for study with referring clinician
- Review medical and psychiatric history and current medications from the medical record (if available)

7.2.2 Screening Visit (Days -30 to -1)

- Complete quiz documenting the subject's understanding of study procedures, risks and alternatives.
- Complete Assessment of Capacity
- Obtain written informed consent.
- Complete Consent Checklist to document that informed consent procedures have been followed (i.e., Key Information Sheet provided, all questions were answered by study staff, consent given prior to any procedures being performed, etc.)
- Complete a medical and psychiatric evaluation by a study psychiatrist, including history and physical examination and recording of medications.
- Complete the Buss-Perry Violence Questionnaire by a research assistant
- Obtain urine sample for pregnancy test and urine toxic screen
- The psychiatrist will also complete a Structured Clinical Interview for DSM5 (SCID) to establish a diagnosis of schizophrenia or schizoaffective disorder and to assess exclusionary diagnoses, including substance use disorders
- The study psychiatrist will complete the Columbia Suicide Severity Rating Scale (C-SSRS), MoCA, and CGI.
- A research assistant will complete the Brief Psychiatric Rating Scale (BPRS) to determine whether the candidate meets symptom severity criteria.
- Determine eligibility based on inclusion/exclusion criteria

7.2.3 MRI Visit (Days -30 to -1)

- Obtain structural MRI scan for neuronavigation

7.2.4 Intervention Visits

Intervention Visit 1: (Day 0)

- Administer CGI, C-SSRS, SAFTEE, SAFTEE addendum, and PSYRATS
- Obtain structural MRI scan for fMRI registration (5 minutes)
- Obtain fMRI resting state scan (20 minutes)
- Administer AHRS and DESA prior to starting intervention
- Administer PLIFUS/sham over 10 minutes (re-administer AHRS and DESA at 5 minutes)
- Repeat AHRS and DESA immediately after PLIFUS/sham
- Obtain structural MRI scan for fMRI registration (5 minutes)
- Obtain fMRI resting state scan (20 minutes)
- AHRS and DESA repeated immediately after fMRI scan
- PSYRATS and CGI 60 minutes after PLIFUS/sham
- Psychiatric and medical assessment, including MoCA, SAFTEE, and SAFTEE addendum, 1 hour after PLIFUS/sham

Intervention Visit 1B: (Day 1-2) (may be remote)*

- AHRS and DESA
- SAFTEE & addendum
- C-SSRS

Intervention Visit 2 (Day 6-8)

- Administer CGI, C-SSRS, SAFTEE, SAFTEE addendum, and PSYRATS
- Obtain structural MRI scan for fMRI registration (5 minutes)
- Obtain fMRI resting state scan (20 minutes)
- Administer AHRS and DESA prior to starting intervention
- Administer PLIFUS/sham over 10 minutes (re-administer AHRS and DESA at 5 minutes)
- Repeat AHRS and DESA immediately after PLIFUS/sham
- Obtain structural MRI scan for fMRI registration (5 minutes)
- Obtain fMRI resting state scan (20 minutes)
- AHRS and DESA repeated immediately after fMRI scan
- PSYRATS and CGI 60 minutes after PLIFUS/sham
- Psychiatric and medical assessment, including MoCA, SAFTEE, and SAFTEE addendum, 1 hour after PLIFUS/sham

Intervention Visit 2B (Day 7-9) (may be remote)*:

- AHRS and DESA
- SAFTEE & addendum
- C-SSRS

7.2.5 Final Study Visit (Day 14-16) (may be remote)*

- AHRS and DESA
- SAFTEE & addendum
- C-SSRS
- BPRS

7.2.6 Withdrawal/Early Termination Visit*

If a participant withdraws are is terminate early, the following assessments will be completed:

- AHRS and DESA
- SAFTEE & addendum

- C-SSRS
- BPRS

*All remote visits will take place over WebEx, a secure video conferencing platform.

7.3 Concomitant Medications, Treatments, and Procedures

All concomitant medications will be allowed with the exception of anticonvulsants (including benzodiazepines). Concomitant prescription medications taken during study participation will be recorded on the case report forms (CRFs). For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the CRF are concomitant prescription medications, over-the-counter medications and non-prescription medications.

7.4 Justification for Sensitive Procedures

A sham sonication procedure will be included so that results are interpretable. Without a sham control, results are of very poor validity and the contribution of participants to the development of the science is compromised. Because we are employing a cross-over design, all participants will receive active PLIFUS.

7.4.1 Precautionary Medications, Treatments, and Procedures

7.5 Prohibited Medications, Treatments, and Procedures

Anticonvulsants, including benzodiazepines will be prohibited because they affect neuronal excitability and may mask the effect of PLIFUS.

7.6 Prophylactic Medications, Treatments, and Procedures

7.7 Rescue Medications, Treatments, and Procedures

We do not anticipate that rescue medication will be required.

7.8 Participant Access to Study Agent at Study Closure

PLIFUS is not currently available as a clinical procedure.

7.9 Assessment of Safety

7.10 Specification of Safety Parameters

Safety will be assessed by close monitoring by a study psychiatrist during and for 1 hour after each sonication/sham session by assessment of psychiatric symptoms and side effects (SAFTEE & addendum). SAFTEE, SAFTEE addendum, and CSSRS will be repeated one day and one week after each session. The difference between post-sonication and post-sham safety measures will be assessed for safety. In addition to serious adverse events that will be reported immediately, these data will be provided to the DSMB after the first 8 completers. If the dose is increased to 3 sessions, safety data will be provided to the DSMB after the 4 completers at the higher dose.

7.10.1 Definition of Adverse Events (AE)

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

7.10.2 Definition of Serious Adverse Events (SAE)

Serious Adverse Event

Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

7.10.3 Definition of Unanticipated Problems (UP)

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

This definition could include an unanticipated adverse device effect, any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (21 CFR 812.3(s)).

7.11 Classification of an Adverse Event

7.11.1 Severity of Event

For AEs not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.

7.11.2 Relationship to Study Agent

The research psychiatrist's assessment of an AE's relationship to sonication/sham is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study agent assessed. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- **Related** – The AE is known to occur with the study agent, there is a reasonable possibility that the study agent caused the AE, or there is a temporal relationship between the study agent and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study agent and the AE.
- **Not Related** – There is not a reasonable possibility that the administration of the study agent caused the event, there is no temporal relationship between the study agent and event onset, or an alternate etiology has been established.

For all collected AEs, the research psychiatrist who examines and evaluates the participant will determine the AE's causality based on temporal relationship and his/her/their clinical judgment. The degree of certainty about causality will be graded using the categories below.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Possibly Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related," as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to drug administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study drug administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

7.11.3 Expectedness

Dr. Goff will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study device.

7.12 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor. All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. UPs will be recorded in the data collection system throughout the study.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The PI will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

7.12.1 Reporting Procedures – Investigator notifying the IRB

Federal regulations require timely reporting by investigators to their local IRB of unanticipated problems posing risks to subjects or others. The following describes the NYULMC IRB reporting requirements.

Report promptly, but no later than 5 working days:
Researchers are required to submit reports of the following problems promptly but no later than 5 working days from the time the investigator becomes aware of the event:

- ***Unanticipated problems including adverse events*** **that are unexpected and related**
  - *Unexpected: An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.*
  - *Related to the research procedures: An event is related to the research procedures if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.*
  - *Harmful: either caused harm to subjects or others, or placed them at increased risk*

Other Reportable events:
The following events also require prompt reporting to the IRB, though ***no later than 5 working days***:

- ***Complaint of a research subject*** when the complaint indicates unexpected risks, or the complaint cannot be resolved by the research team.
- ***Protocol deviations or violations*** (includes intentional and accidental/unintentional deviations from the IRB approved protocol) for any of the following situations:
  - *one or more participants were placed at increased risk of harm*
  - *the event has the potential to occur again*
  - *the deviation was necessary to protect a subject from immediate harm*
- ***Breach of confidentiality***
- ***Incarceration of a participant*** when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- ***New Information indicating a change to the risks or potential benefits*** of the research, in terms of severity or frequency. (e.g. analysis indicates lower-than-expected response rate or a more severe or frequent side effect; Other research finds arm of study has no therapeutic value; FDA labeling change or withdrawal from market)

Reporting Process
The reportable events noted above will be reported to the IRB using the form: “Reportable Event Form” or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the study’s regulatory binder.

7.13 Serious Adverse Event Reporting
Any AE considered serious by the PI which meets the definition of an SAE included in Section 8.1.2, Definition of Serious Adverse Event will be submitted on an SAE form to the DCC. The DSMB will receive real-time notification of all SAEs.

We do not believe that this study requires FDA approval (IDE) because the sonication parameters that we are using fall well within the FDA ETC safety guidelines. However, if an IDE is required, we will report any adverse events within 10 calendar days if the information meets the following criteria:

(A) A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome);
(B) One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture);
(C) An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.”

In addition, if the study requires an IDE, we will notify the FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days of occurrence

7.14 Study Halting Rules
Administration of PLIFUS will be halted if three grade 3 AEs determined to be “probably related” are observed. Dr. Goff will inform the DSMB members within 24 hours of this occurrence and will provide the DSMB with AE listing reports. The DSMB will convene an ad hoc meeting by teleconference or in writing as soon as possible. The DSMB will provide recommendations for proceeding with the study. If the study is conducted under an IDE, Dr. Goff will inform the FDA of the temporary halt and the disposition of the study.

7.15 Safety Oversight
It is the responsibility of Dr. Goff to oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above. Medical monitoring will include a regular assessment of the number and type of serious adverse events. Dr. Goff will remain blinded to treatment condition (sonication versus sham) but the DSMB will have access to unblinded safety and efficacy data. Because this is a small pilot study, decisions regarding safety and efficacy, which may include increasing the frequency of treatment from one session to three sessions for 4 additional participants if a single session of PLIFUS does not produce measurable effects in the first 12 completers with good quality imaging data, or discontinuing the study due to adverse events, will be made by the DSMB without statistical tests since the sample size will be quite small (n=12).

Our existing data safety and monitoring board (DSMB) will monitor this study. Dr. Goff will be responsible for convening the DSMB, for providing information about enrollment, retention, efficacy, and side effects, and will be responsible for reporting minutes from the DSMB to the IRB. The DSMB has monitored five clinical studies over the past 9 years and is comprised of a statistician and two psychiatrists with extensive experience conducting clinical trials in patients with schizophrenia and in participating on DSMBs. If specific risks emerge additional members with expertise in the area of the potential safety issue will be added to the DSMB. The DSMB will approve the protocol prior to initiation of the study. The DSMB will review site performance, including recruitment, subject retention, protocol violations, and data quality reports. The DSMB will receive an unblinded report of all safety data (adverse events as defined above, including change scores on the AHRS and DESA, the SAFTEE assessments or findings upon clinician assessment) after completion of the first 5 subjects in addition to reports for bi-annual meetings. In

addition to all adverse events recorded by the SAFTEE assessments, the DSMB will receive a report of any additional participant complaints, instances of participant distress, or breaches of confidentiality. Because this is a pilot study with a very small sample size, formal statistical criteria are not identified in advance for a stopping rule. The DSMB may halt the study for reasons of safety—we are not aware of any significant safety concerns. The DSMB must approve the decision to switch from one session of PLIFUS to 3 sessions if no effect is detected after the first 12 completers with good quality imaging data. A failure to detect an effect size of 0.5 or greater compared to sham on the AHRS, DESA, and PSYRATS total score or on GPi functional connectivity after the first 12 participants will define "no effect" and trigger a switch to 3 treatment sessions over 5 days for phase II of the study which will involve an additional 4 participants. The DSMB will also be provided copies of all communications with the IRB. The DSMB will meet prior to initiation of the study and a minimum of every six months and will be provided data regarding enrollment and side effects and a study summary prior to each meeting. Dr. Goff and study staff will not attend the closed section of the DSMB meetings. Throughout the study, notification of any Serious Adverse Events (SAEs) as well as any proposed investigator-initiated changes in the protocol will be submitted to the DSMB. Based on its review of the revised protocol, the DSMB will identify the data parameters and format of the information to be regularly reported. All SAEs and adverse events will be tabulated and submitted to the IRB and DSMB in the bi-annual (every 6 months) data reports or at the time of continuing review. AEs will be reported to the IRB annually (at continuing review) if they are expected, related, and harmful. A summary of the outcomes of the safety reviews as prepared for the DSMB along with accumulated adverse events and deviations will be submitted to the IRB as part of an annual progress report at the time of the Continuing Review submission.

8 Clinical Monitoring

A study psychiatrist will be present during all sessions of PLIFUS and sham. Participants will be monitored during and after sonication until psychiatrically cleared by the study psychiatrist 1 hour after completion of each session. Participants will be contacted 24-48 hours and 7 days after each treatment period and symptoms, side effects and suicidality assessed.

9 Statistical Considerations

This is a pilot study intended to obtain preliminary data to guide future studies. The analysis will primarily be descriptive for this purpose and, due to the small sample size, effect sizes will be the primary signal of target engagement. Consistent with other studies in the field, we have selected an effect size of 0.5 as our threshold. In addition, there is a pre-planned review of results after completion of the first 5 participants; the administration of PLIFUS/sham will be increased from one session to three sessions over 5 days if an effect size of 0.5 or greater with PLIFUS is not detected in change from baseline in GPi functional connectivity or symptom response compared to sham.

The primary statistical analysis will be performed in completers without imputation of missing data and will be change in primary outcomes (GPi functional connectivity and symptom severity) from baseline to the first measure post-sonication or sham (immediately post-sonication for symptoms; 10-30 minutes for fMRI). PLIFUS and sham PLIFUS will be compared using a linear mixed effects model with baseline measures (GPI functional connectivity and severity of psychotic symptoms) as continuous variables; treatment (PLIFUS or sham) and sequence as categorical variables; and participant as a random effect. Similar secondary exploratory analyses will be performed at additional time points (symptom severity during sonication, 60 minutes, 1 day and 7 days after sonication). Secondary analyses of other outcome measures (BPRS total score, PSYRATS and CGI) will be analyzed following the same approach. Safety measures, including the SAFTEE and C-SSRS will be summarized descriptively. If the study design is adapted (sonication increased to 3 sessions) after the first 12 completers with good quality imaging data, results from the first 12 completers and subsequent 4 participants will be analyzed separately.

Sample Size

This is an unfunded pilot trial intended to assess feasibility and to collect preliminary data on safety and target engagement to support future grant proposals. Given these goals and fiscal constraints, we selected a total of 12 completers as an appropriate sample size to provide pilot data with reasonable reliability.

9.1 Measures to Minimize Bias

9.1.1 Enrollment/Randomization/Masking Procedures
Because this is a cross-over study, the primary concern with randomization imbalance is with an order effect. We will examine this possibility statistically. The sham condition consists of an identical procedure during which the sonication equipment produces an identical noise. We believe this will fully mask the treatment condition but will ask participants after each session which condition they believe they received and will analyze these results to verify the success of our masking.

9.1.2 Evaluation of Success of Blinding
Participants and symptom raters will be asked whether they believe the participant received PLIFUS or sham after each intervention session. The results of these queries will be analyzed and reported.

9.1.3 Breaking the Study Blind/Participant Code
The study blind will only be broken in the case of an unexpected serious adverse event. This decision will be made by Dr. Goff in an emergency; if not an emergency, it will first be discussed with the Chair of the DSMB, Dr. Buchanan.

10 Source Documents and Access to Source Data/Documents
Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial. It is acceptable to use CRFs as source documents.

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

Access to study records will be limited to IRB-approved members of the study team. The investigator will permit study-related monitoring, audits, and inspections by the IRB/EC, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

11 Quality Assurance and Quality Control
Dr. Goff will meet with the study team weekly to review procedures and documentation.

12 Ethics/Protection of Human Subjects

12.1 Ethical Standard
The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6.

12.2 Institutional Review Board
The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any

participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

12.3 Informed Consent Process

12.3.1 Consent/Assent and Other Informational Documents Provided to Participants

Consent forms describing in detail the study agent, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study product. The following consent materials are submitted with this protocol;

- Form for documenting capacity to sign informed consent
- 10 item quiz to document participant understanding of key aspects of participation
- Informed Consent Form
- Key Information Sheet

12.3.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. In addition, the potential subject must also achieve a perfect score on a ten-item true/false quiz that asks questions about the study procedures and potential risks. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the signed informed consent document and key information sheet will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

A copy of the signed informed consent document will be stored in the subject's research record. The consent process, including the name of the individual obtaining consent, will be thoroughly documented in the subject's research record. Any alteration to the standard consent process (e.g. use of a translator, consent from a legally authorized representative, consent document presented orally, etc.) and the justification for such alteration will likewise be documented.

For inpatients and outpatients, consent can take place remotely, in which a study team MD will review the consent form with the participant over WebEx. Participants will be provided an IRB-approved REDCap electronic consent form to sign, and a copy will be sent to their email.

12.4 Participant and Data Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

Participant confidentiality is strictly held in trust by the participating investigators and their staff. This confidentiality covers clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party.

The IRB may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital).

The study participant's contact information will be securely stored for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored at NYU Langone Medical Center. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. Data used for statistical analysis, including imaging analysis, will be de-identified. The study data entry will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the NYU Langone Medical Center.

13 Data Handling and Record Keeping

13.1 Data Collection and Management Responsibilities

Data will be collected on paper CRFs and an Excel database will be created and used to electronically store data. The Excel database will consist of separate Excel files for each study assessment, all of which will be stored in NYULH's secure research drive, individualy password protected, and accessible only to study team members with access granted to the folder (path: R:\gofflab\FUS\Database). These study team members may include, but are not limited to, the Principal Investigator, research coordinator(s), research assistant(s), study physicians, statisticians, and postdoctoral researchers. Only study IDs will be used throughout the database. Subject names, birthdays, and other personally identifying information will not be retained electronically. The creation of this database will aid in preliminary and future safety and other data analyses.

0f note, only data from subjects who completed **all** study procedures will be retained in this Excel database. Information from screen failures and subjects who terminated early will not be maintained electronically.

13.2 Study Records Retention

Study documents will be retained for the longer of 3 years after close-out or 5 years after final reporting/publication of results.

13.3 Protocol Deviations

Protocol deviations will be reported to the NYUGSoM IRB per their guidelines. Dr. Goff is responsible for adhering to the IRB requirements.

13.4 Publication and Data Sharing Policy

The study will be registered on Clinicaltrials.gov prior to initiation. The steering committee (Drs. Goff, Shoham) will oversee publications and presentations at meetings.

13.5 Study Finances

13.6 Funding Source

Funding for the study will be from the Doris Duke Charitable Foundation, Max Schlapp Fund, associated with the New York Community Trust, and from Dr. Goff's departmental research fund. No data and no identifying information will be shared with these funders.

13.7 Costs to the Participant
Participants will incur no costs and transportation will be reimbursed.

13.8 Participant Reimbursements or Payments
Consistent with our other studies involving individuals with schizophrenia, we will compensate participants for their time and pay for their transportation costs. We will pay the following:

Screening Visit (approx. 2 hours): $50
MRI Visit (20 minute MRI scan): $50
Intervention Visit 1 (3 hours): $150
Follow-up Visit 1 (20 minutes): $25
Intervention Visit 2 (3 hours): $150
Follow-up Visit 2 (20 minutes): $25
Follow-up Visit 3 (20 minutes): $50

If PLIFUS effects on fMRI and symptom ratings are not detected in the first 12 completers with good quality imaging data, the subsequent 4 participants will receive 3 sessions over 5 days. Participants will be paid $50 for each of these additional sonications.

14 Study Administration

14.1 Study Leadership
The Steering Committee will govern the conduct of the study. The Steering Committee will be composed of Dr. Goff (PI) and Dr. Shoham (senior neuromodulation expert). Dr. Goff and the technical FUS members will meet at least monthly.

15 Conflict of Interest Policy
The independence of this study from any actual or perceived influence, such as by device manufacturers, is critical. Therefore any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial.

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by the NYU Langone Conflict of Interest Management Unit (CIMU) with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All NYULMC investigators will follow the applicable conflict of interest policies.

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17 Attachments

These documents are relevant to the protocol, but they are not considered part of the protocol. They are stored and modified separately. As such, modifications to these documents do not require protocol amendments.

- Device Manual attached

18 Schedule of Events

Activity	**Screening Visit** [Day -30 to -1]	**MRI Visit** [Day -30 to -1]	**Intervention 1** [Day 0]	**Intervention 2** [Day 7, 6-8]	**Follow-up** [Day 14, 13 to 15]
Study team procedures					
Consent	X				
Medical History	X				
Physical Exam	X				
Vital Signs	X				
Psychiatric History	X				
Psychiatric diagnosis (SCID)	X				
Demographics	X				
BPRS	X				X
PSYRATS			X	X	
BPAQ	X				
AHRS			X	X	
MoCA	X		X	X	
DESA			X	X	

CGI	X		X	X	
Experimental intervention					
Randomization		X			
PLIFUS or sham			X	X	
Safety Assessments					
SAFTEE & addendum			X	X	X
C-SSRS	X		X	X	X
Medical/psychiatric assessment			X	X	X
Imaging Assessments					
Structural MRI		X	X (pre and post-sonication)	X (pre and post-sonication)	
Resting state fMRI			X (pre and post-sonication)	X (pre and post-sonication)	