Examining the effects of Theta Burst Stimulation on corticothalamic mediated inhibitory control and smoking relapse vulnerability

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BIOMEDICAL RESEARCH PROTOCOL
UNIVERSITY OF MISSOURI

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I. Research Objectives/Background

As of 2017, cigarette smoking remained the leading cause of preventable premature death worldwide and the CDC predicts tobacco use will result in more than 8 million deaths per year by 2030. Tobacco use disorder (TUD), like other drugs of abuse, is associated with deficits in prefrontal mediated inhibitory control (IC)—the ability to stop pre-potent behavioral responding. We recently reported findings in JAMA Psychiatry showing that IC task-based functional connectivity (tbFC) between the right inferior frontal gyrus (r.IFG) and thalamus (corticothalamic circuit) mediated the association between IC task performance (i.e. successful trial inhibition) and smoking relapse vulnerability: both in a smoking cessation study and delaying to initiate ad lib smoking in the laboratory. Those findings form the basis of our current work examining brain stimulation strategies to strengthen corticothalamic tbFC, improve IC and increase the odds of smoking abstinence among smokers.

Theta burst stimulation (TBS), a form of repetitive transcranial magnetic stimulation effecting areas stimulated and associated networks, is administered in two forms: a) Intermittent TBS (iTBS) which induces long-term potentiation—putatively strengthening network activity, and b) Continuous TBS (cTBS) which induces long-term depression—putatively dampening network activity. In our crossover pilot study with smokers (N=12), participants underwent a baseline fMRI visit while performing an IC task to obtain baseline performance and identify the IC-task related peak cluster in the r.IFG for stimulation in two subsequent TBS experimental visits: 1) iTBS and 2) cTBS (order counterbalance), each 15 min. prior to performing the IC task. A significant effect of condition on IC performance was observed ($F= 9.49$, $p<.03$) where, as compared to baseline, performance: a) improved by 14.4% following r.IFG iTBS (D =.81); b) worsened by -8.7% following r.IFG cTBS (D =.5); resulting in a total performance difference between the r.IFG TBS conditions of 25.3% (D = 1.56). These findings build on the extant literature on the role of the r.IFG in IC, demonstrate feasibility of using single-subject task-related brain activation to administer focal brain stimulation to modulate IC and provides the foundation for this proposal to examine whether: a) r.IFG TBS parametrically modulates corticothalamic tbFC (strengthen

following iTBS; weaken following cTBS); and b) iTBS-induced strengthening of corticothalamic tbFC reduces smoking lapse / relapse vulnerability. In the UG3 component, we will conduct an assessment of the acute effects of TBS on corticothalamic IC-tbFC, IC task performance and ad lib smoking in the laboratory.

The Specific aims and corresponding hypotheses of the UG3 proposal are as follows:

Aim 1. Examine the effects of TBS on IC neural circuitry and task performance. **Hypothesis 1:** As compared to baseline: r.IFG iTBS stimulation will strengthen corticothalamic tbFC and improve IC task performance; whereas r.IFG cTBS simulation will weaken corticothalamic tbFC and result in worse IC task performance.

Aim 2. Examine the effects of TBS on ad lib smoking. **Hypothesis 2:** As compared to baseline: r.IFG iTBS, will extend the duration of abstinence; whereas cTBS shorten the duration of abstinence during an ad lib smoking lapse paradigm in the lab.

Aim 3. Examine safety and tolerability of administering r.IFG TBS. **Hypothesis 3**: r.IFG TBS will have a similar overall adverse event profile in a TUD population as previously reported with dorsolateral prefrontal cortex TMS.

Exploratory Aims. Using MR Spectroscopy, the effects of TBS on r.IFG excitatory/inhibitory neurochemical balance (glutamate/GABA) will be examined. Effects of TBS on craving and mood will be explored.

If the milestone of the UG3 component are met, the subsequent UH3 project will be an adequately powered two-week trial with two-week follow-up to assess the efficacy of iTBS in TUD.

Overall rationale for our research approach. The research proposed here builds upon our novel findings demonstrating that corticothalamic tbFC mediates IC and smoking relapse vulnerability [1], and our preliminary data showing that targeting the corticothalamic circuit with TBS parametrically modulates IC in a biologically relevant, stimulation-dependent manner. The experimental design for the proposed research will address a number of important unanswered questions, including—*Does r.IFG TBS:*

a) Modulate corticothalamic circuitry in a dose-dependent manner? (Aim 1)
b) Modulate IC task performance in a dose-dependent manner? (Aim 1)
c) Attenuate lapse / relapse rates? (Aim 2)
d) Produce effects in neural mechanisms that correspond with effects in behavior (Aim 1 & 2)?
e) Have a good safety and tolerability profile? (Aim3)
f) Modulate r.IFG neurochemistry? (Exploratory Aim)
g) Modulate primary symptoms associated with TUD e.g. craving and mood? (Integrated throughout)

II. Drugs/Biologics/Devices

The MagPro X100 TMS Therapy System is indicated for the treatment of Major Depressive Disorder in adult patients who have failed to receive satisfactory improvement from prior

antidepressant medication in the current episode. It is a 510K/Pre-market Notification device (a device that is substantially equivalent to a legally marketed device), as described in 21 CFR 807 Subpart E.

III. Recruitment Process

Participants will include 40 adult nicotine dependent smokers (age 18-65; Female = 50%), recruited through the community and our advertising campaigns, individuals interested in participating will undergo a phone screen or complete a REDCap survey to determine eligibility and those who meet study criteria will be invited to our lab for a screening visit. Individuals will be screened by trained and certified research staff, asked to read and sign an IRB-approved informed consent and then be assessed on smoking and medical history and administered an MRI safety screening questionnaire. Participants meeting all selection criteria will be scheduled for subsequent sessions. *Full inclusion/exclusion criteria* are provided in the appropriate section below. Briefly, inclusion criteria include: participants will be 18-65 yrs. of age; smoke > 10 cigarettes/day for ≥ 2 yrs.; and have an expired carbon monoxide (CO) concentration of ≥ 10 ppm, using a handheld CO monitor (Vitalograph, Lenexa, KS). Briefly, exclusion criteria include: use of psychotropic (e.g. antiepileptic) medications in the last month; positive urine illicit drug screen; presence of an untreated medical illness; history of major neurological illness or head injury resulting in loss of consciousness; and any contraindication to MRI; and among females, positive urine pregnancy test. Participants meeting all selection criteria will be scheduled for subsequent sessions.

We will recruit participants from Columbia and the surrounding area in which MU is located. Participants will be recruited through IRB-approved advertisements in regional newspapers, flyers, and on internet sites affiliated with our laboratory and the university. These advertisements will briefly describe the study and ask interested individuals to contact the study coordinator or designated study staff. Following IRB approved procedures, the coordinator or designated study staff member will discuss the basics of the study and basic required principles of informed consent. If the potential participant remains interested, the coordinator or designated study staff member will ask focused screening questions using an IRB-approved phone script to see if the potential participant meets entry criteria. If they appear to be eligible, and continue to be interested in the study, he or she will be scheduled for an in-person screening visit. If not, they will be thanked for their interest. We will work within IRB requirements to assure Privacy Regulations are met.

IV. Consent Process

We will obtain formal written consent from all new participants enrolled in this project. Following policies of the MU IRB, written informed consent will be obtained and documented by the study's Research Coordinator or designated study staff before any study-related procedures are performed. Informed consent will be obtained in a private research office. A study coordinator or designated study staff member will review study procedures and the consent form with each potential participant. Each individual may take as much time as they like to decide if they do or do not wish to participate. A decision not to participate will not affect their participation in other studies at MU, nor will it affect their access to health care at MU.

V. Inclusion/Exclusion Criteria

Inclusion Criteria: Subjects will:

1. Be between the ages of 18 and 65.
2. Be in stable mental and physical health.
3. Be willing to provide informed consent.
4. Be able to comply with protocol requirements and likely to complete all study procedures.
5. Be a current nicotine dependent cigarette smoker (smoke ≥10 cigs/day) with a minimum smoking history of smoking an average of ≥ 10 cigs/day over the past two years.

Exclusion Criteria: Subjects with:

1. Contraindication to MRI (e.g., presence of metal in the skull, orbits or intracranial cavity, claustrophobia).
2. Contraindication to TMS (history of neurological disorder or seizure, increased intracranial pressure, brain surgery, or head trauma with loss of consciousness for > 15 minutes, implanted electronic device, metal in the head, or pregnancy, as indicated by a positive urine pregnancy test at screening).
3. Any use of substances that lower seizure threshold (such as thyroid medications or cocaine).
4. History of autoimmune, endocrine, viral, or vascular disorder affecting the brain.
5. History or MRI evidence of neurological disorder that would lead to local or diffuse brain lesions or significant physical impairment.
6. Unstable cardiac disease, uncontrolled hypertension, severe renal or liver insufficiency, or sleep apnea.
7. BAC greater than 0.0.
8. Any other condition or concern that in the Investigator's opinion would impact participant safety, compliance with study instructions, or potentially confound the interpretation of the study results.

As part of exclusion criteria, participants are specifically asked to not take part in any other research during their active participation in the protocol once eligibility at screening is made through the last visit day of the protocol.

VI. Number of Subjects

Human Subjects Involvement and Characteristics. We propose to enroll 40 participants that meet inclusion/exclusion criteria in order to obtain 30 complete usable datasets.

TARGETED/PLANNED ENROLLMENT: 40			
	Sex/Gender		
Ethnic Category	Females	Males	Total
Hispanic or Latino	2	2	4
Not Hispanic or Latino	18	18	36
Ethnic Category: Total of All Subjects*	40		
Racial Categories			
American Indian/Alaska Native	0	0	0
Asian	1	1	2
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	9	9	18
White	9	9	18
Other	1	1	2
Racial Categories: Total of All Subjects*	20	20	40

VII. Study Procedures and Research Design

The UG3 phase is a proof-of-concept study to test mechanistic hypotheses of the acute effects of TBS on corticothalamic inhibitory control (IC) task-based functional connectivity (tbFC), IC task performance and ad lib smoking in the laboratory while preliminarily evaluating safety and tolerability of TBS among individuals with TUD. Clearly defined milestones, focused on iTBS

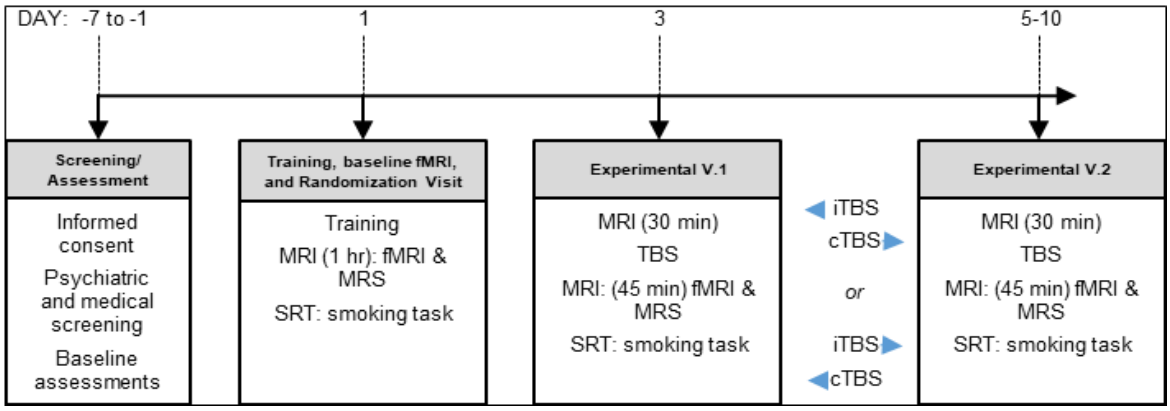

Figure 1. Overview of UG3 Study Protocol.

versus cTBS and baseline on IC circuitry, will be met before proceeding to Phase 2 (UH3), a 4-session (over 2-weeks) randomized iTBS versus cTBS trial to assess smoking behavior over two-weeks post-treatment period.

As shown in Fig. 1, individuals (N =30) with TUD will complete a baseline MRI visit and, using a crossover design, be randomly assigned to receive iTBS on one visit and cTBS on the other visit. Following TBS, they will repeat the protocol and then perform the SRT [4]. The effects of TBS on IC brain and behavior and smoking on the SRT, along with safety and tolerability, will be assessed.

Strategies to Ensure a Robust and Unbiased Approach. As detailed throughout this section, the proposed study will achieve robust and unbiased results via several design features including: explicit inclusion/exclusion criteria; randomization and blinding; use of validated measures; explicit hypotheses and well-validated analytic strategies; power estimates; and careful consideration of potential confounds. As standard practice in our laboratory, we will maximize participant retention and data collection compliance, while maximizing fidelity by providing participants with training on all task procedures prior to data collection.

Participant Retention and data collection compliance**.** We will minimize study drop-out and data loss due to participant burden by contacting participants 1 day prior to each session and provide session-specific details and reminders as needed. Over the study, participants will respond to digitally administered questionnaires regarding mood, craving and smoking over the past 24-hrs. This strategy has proven successful in characterizing self-report affect, craving and smoking behavior [50, 54]. In ongoing NIDA-funded smoking cessation studies by our group, response rates reach >95% following RA verification of compliance over a 4-week trial (R01DA038700); ~80% over 8-12 week trials (R34 DA042228, UG3 DA043231). The current proposed UG3 and UH3 studies both fall within a timeline where we have achieved greater than 95% participant compliance.

Training Session. Following consent, each eligible participant choosing to continue with the protocol will participate in an extensive training session to become familiar with all aspects of the study.

- *Inhibitory Control (IC) Task Training.* During training, participants will perform one complete 7.2 min. run of the IC task (see C.4.a.) and are required to achieve a minimum of 75% correct responses on "Go" trials and a minimum of 25% correct omissions on "NoGo" trials in order to demonstrate understanding of task instructions. If performance is sub-threshold, the participant will be provided feedback from the researcher and perform an additional practice run. Using this same training strategy in our prior studies with individuals with nicotine, cannabis, cocaine or opioid use disorder (N > 250), > 95% of participants have met performance criteria after the 1st training run and, and among those participants requiring additional training, 100% of participants met criteria after the 2nd training run.
- *Habituation to Scanner Environment.* Participants will practice the experimental task while inside of a mock scanner in order to reduce scan-related anxiety and improve scan data quality.
- *Practice ad lib smoking.* Participants will be introduced to, and practice smoking their preferred-brand cigarette using the Clinical Research Support System (CReSS) Lab smoking topography device to become familiar with smoking through the system and minimize effects of a novel smoking experience during testing.

Assessments, biomarkers, and self-report measures.

- Diagnostic Assessment. A medical history and fMRI safety screening questionnaire will be conducted to ensure that the individual is eligible to participate.
- Smoking history, behavior and dependence. The following validated measures of nicotine dependence and urge to smoke will be used at various times during the study: Shiffman/Jarvik Withdrawal scale [55], Fagerström Test of Nicotine Dependence [56] & Modified Cigarette Evaluation Questionnaire [57].
- Tobacco use biomarkers. Expired air CO concentrations will be measured at screening to establish eligibility (CO > 10) at baseline, and smoking or abstinence (CO < 6) status prior to each experimental session, using standard procedures [33, 34]**.** Urinary cotinine will be used to assess quantitative measures of smoking and abstinence (ABS <80 ng/ml).
- Smoking topography. Clinical Research Support System (CReSS) Lab smoking topography device (Plowshare Technologies) will be used to assess smoking topography. Measures to be evaluated include number of puffs, puff volume (milliliters), puff duration (milliseconds), puff velocity (milliliters/second) and inter-puff interval (seconds).
- Trait and State Measures. Impulsiveness will be measured with the BIS-11 [58].Mood state will be evaluated with the Profile of Mood States [59], which has been shown to reliably characterize the effects of abstinence on mood in dependent smokers [60, 61].
- EMA measures. Over the course of the study, participants will be prompted each day, via text messaging, to provide craving, mood ratings and quantify smoking (# cigs.) for the day through a link to a secure REDCap questionnaire and daily log of cigarettes smoked that day (see [50, 54]).
- Safety Assessment. Tolerability will be assessed using a sixteen-item review of systems (ROS) questionnaire [36] and reviewed with research team at weekly visits.

GoGo/NoGo Inhibitory control (IC) Task. The "GoGo/NoGo" task [4, 44] will serve as the experimental probe to evaluate the mechanistic underpinning of TBS on IC, determine whether or not to proceed from the UG3 mechanistic proof-of-concept project to the fully-powered UH3 phase. fMRI BOLD response will be collected from participants as they perform the task. Participants are instructed to press a button in response to common (gray colored circles: 75% of trials) and rare (yellow colored circles: 12.5% of trials) Go stimuli and inhibit responding to rare NoGo stimuli (blue colored circles: 12.5% of trials). The task provides errors of omission and reaction times during Go trials, controls for novelty detection during processing of rare Go trials (yellow circles) and errors of commission on NoGo trials (blue circles). *Behavioral data* will be processed and analyzed consistent with our prior work with this task [4]. Prior to analysis, NoGo performance will be corrected by scoring NoGo trials with null response as incorrect when the participant did not respond to the 'Go' trial preceding it, in order to control for lapses in attention. Data will then be analyzed in SPSS.

Smoking Relapse analog Task (SRT). The SRT was developed by the PI (Froeliger) and smoking behavior outcomes (time to lapse) shown to be predicted by IC corticothalamic neural-circuitry function [4]. In brief, the SRT is comprised of two phases. The first involves up to 10, six-minute

blocks of randomly presented trials of neutral, smoking and emotional images. Following each block, participants rate their craving on a scale from 1-10 and then choose to earn $1 for each additional block up to 60 min., or quit the task and smoke a cigarette using a pocket CReSS system in which topography measures are recorded.

Neuroimaging Protocol. Imaging will be performed on a 3T Siemens Prisma scanner: a high-resolution 3D MPRAGE anatomical sequence will be acquired (matrix = 256, flip angle = 9°, 166 slices, slice thickness = 1 mm); whole brain BOLD contrast sensitive images will be acquired using a multi-band (6) EPI sequence (60 slices, TR=800 ms, TE=30 ms, FOV=216, 2.4 mm^3 voxels). Prior to the acquisition of images, the anterior and posterior commissures will be identified in the mid-sagittal slice of a localizer series, followed by the acquisition of PA and AP field maps, and then followed the acquisition of functional images during the IC task.

fMRI IC task data preprocessing. Similar to our prior analytic strategy using this task [4], fMRI data will be preprocessed using SPM12 to remove noise and artifacts, motion corrected [51], temporally realigned using B-spline interpolation and smoothed with an 8mm FWHM Gaussian filter. Functional images for each participant will be processed in their native space.

IC Task Modeling. Preprocessed data will be entered into a first-level, whole-brain analysis using the General Linear Model to examine BOLD response to each of the 5 trials of interest: $NoGo_{correct}$ (successful inhibition), $NoGo_{incorrect}$ (error of commission), $RareGo_{correct}$ (novel-target detection), $RareGo_{incorrect}$ (novel-target error of ommission), and $Go_{incorrect}$ (error of omission). Each event will be modeled as a delta regressor (onset dur. = 0) and convolved with a canonical hemodynamic response function. Motion will be removed through rigid body rotation and translation and parameters included as covariates. A high-pass filter (128 seconds; .008 Hz) will be applied to remove slow signal drift. To idenitfy successful IC-BOLD response, controlling for novelty detection, a $NOGO_{correct}$–$RareGo_{correct}$ contrast image (IC-contrast) will be generated [4] and fed forward to ROI identification.

Region of Interest (ROI) Identification. For each subject, the IC-contrast image will be loaded into, and overlaid onto their coregistered T1, in MRIcroGL (http://www.mccauslandcenter.sc.edu/mricrogl/). The PI (Froeliger) will train a postdoctoral fellow on how to identify the peak cluster of activation in the r.IFG (e.g. neuroanatomical boundaries, software) and, once trained, work as a team to identify ROI's with >95% agreement. The IFG is comprised of three sub-divisions—Pars opercularis, Pars triangularis and Pars orbitalis—all of which have been demonstrated to play a role in inhibiotry control [52]. Across our work with TUD related disruptions in IFG activation [4, 32-35], activity in the posterior-ventral aspect of the Pars triangularis and/or posterior extent of the Pars opercularis—ventral to the inferior frontal sulcus, dorsal to lateral sulcus, anterior to the precentral sulcus—is the focal extent of the r.IFG node in the corticothalamic circuit that mediates IC and smoking relapse vulnerability [4], and is sensitive to TBS moudlating IC. Thus, we have clearly defined anatomical boundaries for confining the functionally defined peak cluster that will serves as the ROI for each participant. Each participants functionally defined ROI [as shown in Fig. 2]) will be used for neuronavigating TBS.

Neuronavigation Protocol. Following the baseline scan and ROI identification procedure, neuronavigation will be performed using the Rogue Research Inc. © Brainsight system. First, co-registered anatomical and functional ROI data will be entered into a participant's workflow profile, followed by skin and full-brain curvilinear reconstructions, 3-landmark identifications (bridge of nose, l.ear, r.ear), placing a pin on the ROI and coil alignment with the gyrus. The same setup parameters will be used across each of the two TBS visits and I/O BOX TTL triggers enabled to record the spatial parameters for each pulse: a) distance to target; b) target error; c) angular error; and c) twist error, for use in assessing quality control and entered as nuisance covariates in statistical analyses. In our pilot study (results shown in Fig. 1), the mean target error (i.e. shortest distance from the line projecting into the head along the coil's path) across all conditions was 2.6 mm (± 1.0)—within range of the manufactures recommended threshold (3mm)—for each participants ROI (samples in Fig. 2).

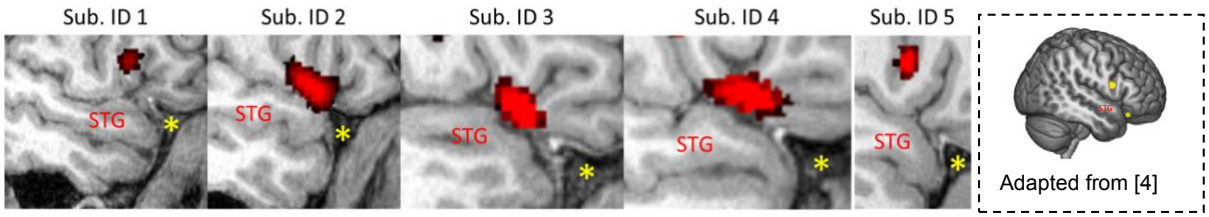

Figure 2. Individual variability in inhibitory control task-based r.IFG activation. Randomly selected cases (*n* = 5) from our pilot IC-task based fMRI-guided TBS study. Though all participants exhibited significant r.IFG activation in the IC-contrast image—similar to that reported in our prior published work [4: upper right excerpt], significant inter-subject variability in neuroanatomy along with variability in the foci of functional activation were identified above. Thus, an individual subject's functional activity in their native space provides greater spatial specificity for targeting stimulation, as compared to using group mean activity following normalizing to an average template. *Landmarks for reference: *= sinus; STG= Superior temporal gyrus.*

Theta Burst Stimulation Protocols. Following fMRI data preprocessing, modeling, ROI identification and verification (C.4.b) and setting up the neuronavigation protocol (C.4.c), participants will be randomized to receive TBS to the r.IFG on two separate experimental visits: iTBS on one visits; cTBS on one visit—counterbalanced across participants.

Determining Resting Motor Threshold. Standard procedures will be used to determine the participants resting motor threshold (RMT) using parameter estimation by sequential testing (PEST) procedures [53].

Intermittent Theta Burst Stimulation (iTBS) to the r.IFG. The total duration of the iTBS protocol [38] is 190 seconds. Participants will receive stimulation over the r.IFG (A series of 3-burst pulses presented at 5Hz,10 pulses/sec, 10 pulses/train, 20 trains, 10.0 sec intertrain interval; 80% RMT, MagPro) using a figure 8 coil (Coil Cool B65 A/P). This protocol has been shown to enhance IC performance as shown in Figure 1.

Continuous Theta Burst Stimulation (cTBS) to the r.IFG. The total duration of the cTBS protocol [41] is 34 seconds. Participants will receive stimulation over the r.IFG (An intermittent series of 3-burst pulses presented at 6Hz, 18 pulses/sec, 600 pulses/train, .1 sec intertrain interval; 80% RMT, MagPro) using a figure 8 coil (Coil Cool B65 A/P). This protocol has been shown to reduced IC performance as shown in Figure 1.

Randomization and Blinding. This study utilizes a double-blind, crossover design with a counterbalanced (latin-square) [62] TBS condition order. Given the short—~1hr duration of the

effects of a single session of TBS on neural function and behavior [38, 41], a crossover design with a minimum of a 2-day washout period between TBS session is appropriate for this study [63]. Both the participants and the research technicians, other than the technician administering TBS, will be blinded to the condition order. At the end of each visit, the participant and the researcher will independently complete a form indicating which TBS condition they believe was administered during the session and provide a confidence rating on a scale from 1-10.

Hypothesis Testing. All hypothesis testing will be conducted with $\alpha < .05$, unless otherwise noted. *Rationale.* Based upon the tenet that iTBS induces LTP and strengthens network activity, our prior published work demonstrating that corticothalamic tbFC mediates the association between successful IC task performance and inhibiting smoking [4], and our pilot data demonstrating r.IFG iTBS improves IC (Fig. 1), we hypothesize that r.IFG iTBS will strengthen corticothalamic tbFC, improve IC and attenuate smoking lapse/relapse.

Aim 1. Examine the effects of iTBS on IC neural circuitry and task performance. *Hypothesis 1:* As compared to baseline: r.IFG iTBS stimulation will strengthen corticothalamic tbFC and improve IC task performance; whereas r.IFG cTBS simulation will weaken corticothalamic tbFC and result in worse IC task performance. *Behavioral analysis plan:* Following procedures outlined in C.4.a, IC performance data will be analyzed with a repeated-measures ANOVA to examine the effects of Condition (Baseline, iTBS, cTBS). *fMRI data analysis plan:* Following procedures in C.4.b—C.4.d, IC corticothalamic tbFC will be processed and modeled using our validated strategy [4] and then Fisher-transformed correlation coefficients (rZ) scores for each participant during each condition will be fed forward and analyzed in SPSS using a repeated-measures ANOVA to examine the effect of Condition.

Aim 2. Examine the effects of iTBS on ad lib smoking. *Hypothesis 2:* As compared to baseline: r.IFG iTBS, will extend the duration of abstinence; whereas cTBS shorten the duration of abstinence during an ad lib smoking lapse paradigm in the lab. *Behavioral analysis plan:* As in [4], time to initiate ad lib smoking during the SRT (see C.4.a.2) will be analyzed in SPSS using a repeated-measures ANOVA to examine the effect of Condition. Main effects of Condition on secondary smoking topography variables (e.g. puff volume (ml) and number of puffs) will be explored.

Aim 3. Examine safety and tolerability of administering r.IFG TBS. *Rationale*. Though TBS has a good safety profile [36, 37], prior studies assessment of tolerability and adverse events (AE's) have largely confined those assessments to the day of study [37]. In addition, although the TBS stimulation parameters proposed herein are published standards, the location—r.IFG for ITBS is novel and thus both acute and protracted (24 hrs. post-stimulation) safety and tolerability profiles need to be assessed. *Hypothesis 3:* r.IFG TBS will have a similar overall adverse event profile in a TUD population as previously observed in dorsolateral prefrontal cortex TMS trials. *Assessment Plan.* Participants will be administered the ROS questionnaire (see C.9) at the beginning and end of each TBS visit, and 24hrs. post TBS. Dr. Froeliger will oversee participant compliance and, in the event of an AE, consult with Dr. Ithman who will serve as the Program Manager for AEs. Any AEs will be recorded in an AE log. If an AE is non-serious (self-limited with no intervention needed), no further action will be necessary. However, in the case of a serious, unresolved event, an AE follow-up log will be completed and subsequent safety procedures followed (see Human Subjects Section; DSMP). Treatment-emergent AEs for all participants and

comparisons between the number of participants with any adverse events will be conducted using Pearson's chi-squared test statistic.

Exploratory Aims. *MRS Rationale.* Converging evidence supports changes in glutamate release as a likely molecular mechanism associated with synaptic plasticity [64]. Glutamate receptors are necessary for the initiation and expression of LTP and LTD and GABA receptors are involved in the modulation of these phenomena. Consistent with animal findings [65], proton MRS studies have demonstrated TUD is associated with abnormal medial prefrontal [66] and thalamic [67] concentrations of glutamate along with evidence for medial prefrontal GABA disturbances being associated with attentional bias to drug cues [68]. Emerging evidence suggests that LTP-like TMS to l.dlPFC is associated with increases in localized glutamate concentrations in depressed [69, 70] and healthy [71] individuals. Recent research has also demonstrated that LTD-like cTBS to motor cortex is associated with increases in localized GABA concentrations [72]. However, to the best of our knowledge, no studies have investigated the impact of TBS on r.IFG GABA or glutamate concentrations or the impact of TBS on localized neurometabolites in TUD. We posit that r.IFG TBS induced neurochemical changes may subsequently correspond with changes in IC.

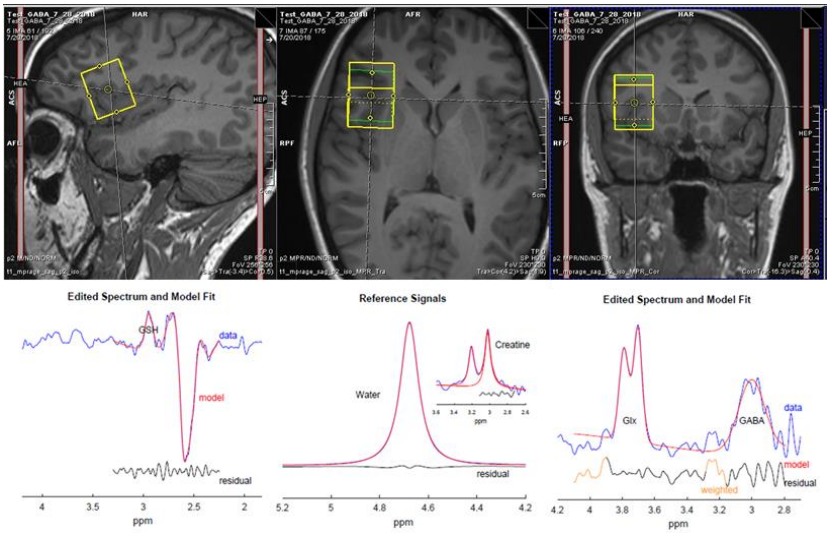

Figure 3. Sample r.IFG voxel placement (top panel), sample fitted GSH (bottom-left), GABA / Glx (bottom-right) and Water / Creatine (bottom-center) spectra acquired with HERMES.

Data acquisition: At each visit, the MRS acquisition voxel (25 x 25 x 30 mm^3) will be placed in the r.IFG to correspond to the brain tissue directly underneath the TMS coil (Fig. 3). Following placement of 6 saturation bands parallel to the voxel faces and auto-shimming, single-voxel water-suppressed. ^{1}H-MRS spectra will be acquired via HERMES (TR=2000ms; TE=80ms; number of averages=320) using editing pulse frequencies for GABA and GSH acquisition (1.9 [ON GABA], 4.56 [ON GSH], and 7.5 ppm [OFF] [73]; and a PRESS sequence maximally sensitive to glutamate (TR=2000ms; TE=40ms; number of averages=128 [74]. Unsuppressed water spectra will be acquired for each sequence. *Data analysis:* Skull stripping and whole brain tissue-type segmentation will be performed on MP-RAGE images using FMRIB software [75]. In-house MATLAB functions will be used to extract the 3D volume corresponding to the positioned MRS voxel to obtain within-voxel gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) tissue content for each subject. GM/WM ratios will be used as covariates in all statistical analyses. Eddy currents and residual water will be removed using in-house MATLAB functions. HERMES data will be analyzed using the Gannet MATLAB toolbox [76]. PRESS data will be analyzed using LCModel 6.3 [77]. Metabolites with fitting uncertainties <20% will be retained.

Water will be quantified from a Gaussian-Lorentzian fit to the non-water-suppressed data. Within-voxel tissue fractions of gray and white matter and cerebrospinal fluid will be calculated based on automated segmentation in SPM 12 using a volume mask generated in Gannet [78]. Metabolite concentrations will be normalized to unsuppressed water and corrected for within-voxel CSF fraction. Cramer-Rao Lower Bound (CRLB) values will be provided by the ProFit software and only estimates with CRLB values < 20% will be submitted to further analysis. Estimated metabolite peak areas will be normalized to the unsuppressed water signal. Finally, metabolite/water, along with metabolite/creatine, ratios will be corrected for within-voxel CSF fraction [79]. Glutamate, glutamine, and GABA ratios will be entered into HLM models with a priori contrasts to examine differences in metabolite concentrations following r.IFG iTBS vs cTBS and baseline. Associations between metabolite concentrations and corticothalamic tbFC, IC and smoking on the SRT will be examined.

Missing Data and Attrition. Missing data in repeated-measures study designs are problematic but can be minimized by effective communication between staff and participants and minimizing participant burden via maintaining a relatively compact study duration. The study team has significant experience with ensuring a robust and unbiased approach and minimizing missing data (See C.8)

Screening/Training Visit: During screening, all aspects of the study will be described to participants and informed consent will be acquired. Participants will provide demographic and employment history via self-report forms. Then, participants will be asked to provide breath samples to test 1) expired carbon monoxide (CO) and 2) an estimate of blood alcohol content (BAC). Additionally, urine samples will be obtained in order to assess pregnancy status and to screen for illicit drug use. Among females, the pregnancy screen will be conducted prior to the drug screen. If the result of the pregnancy test is positive, participants will not be screened for drug use or undergo any further study procedures. The results of the drug test will be recorded primarily for data analysis purposes and cocaine will be the only exclusionary illicit substance. After biological screening is complete, participants will complete medical and smoking history, MRI safety, and contact information forms and will be verbally guided through a timeline follow-back of cigarettes and other tobacco products. Participants who meet all selection criteria will be provided with the option to continue onto the training phase of the visit. During training, participants will learn and practice the inhibitory control (IC) task and fill out commonly used questionnaires that assess state and trait mood and cognitive processes (see Appendix).

Experimental Baseline Visit: At this visit, participants will first: provide an expired breath CO sample in order to assess recent smoking; and, among females, provide a urine sample in which the pregnancy test must be negative. Next, breath alcohol levels will be assessed with a handheld breathalyzer and participants must record a BAL of 0.0. If participants continue to be eligible after the biological samples, they will complete study questionnaires to assess mood and to characterize smoking-related behavior. After this, participants will move on to the first fMRI scan followed by the SRT task.

fMRI Scan: 1) a high-resolution anatomical scan; 2) a 7-minute run of the IC task (see Fig 1); and 3) a six minute resting state BOLD (blood-oxygen-level-dependent).

Randomization: This will be a single blind study where participants will be randomized 1:1 to receive one session of either intermittent TBS (iTBS) or continuous TBS (cTBS) following the first fMRI.

SRT: The Smoking Relapse task will be administered as described above.

Experimental Visits 1 & 2: At the beginning of each of these experimental visits, participants will first: provide an expired breath CO sample in order to assess recent smoking; and, among females, provide a urine sample in which the pregnancy test must be negative. Next, breath alcohol levels will be assessed with a handheld breathalyzer and participants must record a BAL of 0.0. If participants continue to be eligible after the biological samples, they will complete study questionnaires to assess mood and to characterize smoking-related behavior. After this, participants will move on to the first of two fMRI sessions followed by the TMS session and the second the fMRI and SRT task.

fMRI Scan 1: 1) a high-resolution anatomical scan; 2) a 7-minute run of the IC task (see Fig 1); and 3) a six minute resting state BOLD (blood-oxygen-level-dependent).

TMS: Upon randomization, participants will receive either iTBS or cTBS per the theta burst protocols described above.

fMRI Scan 2: 1) a high-resolution anatomical scan; 2) a 7-minute run of the IC task (see Fig 1); and 3) a six minute resting state BOLD (blood-oxygen-level-dependent). The second

SRT: The Smoking Relapse task will be administered as described above.

Analytic Strategy Behavioral and neural data analysis strategies are found in [1] and detailed below.

fMRI data processing. Pre-processing of functional images includes: slice-time correction and realignment[18]; motion outlier detection (framewise displacement > 4 mm (~ 1 acquisition voxel) www.nitrc.org/projects/artifact_detect) and correction (via nearest-neighbor interpolation); despiking at 4% of global mean (cibsr.stanford.edu/tools/human-brain-project/artrepair-software.html); coregistration of functional images to structural image; warping to MNI space using forward deformations, resampling to $(1.5mm)^3$ voxel size (i.e., 3.375μL) and smoothing with a $(10mm)^3$ FWHM Gaussian filter**.**

Task-based functional connectivity (tbFC) data processing. Pre-processed fMRI data will be uploaded into the conn14 toolbox (www.nitrc.org/projects/conn) for denoising and connectivity analyses. Using unsmoothed segmented tissue images, along with functionally-defined regions of interest (ROIs), significant clusters will be exported using MarsBaR (marsbar.sourceforge.net) from the $NoGo_{correct}$ - $RareGo_{correct}$ ANCOVA model. Mean time-courses from the unsmoothed BOLD signal from each ROI will be characterized with no additional principal components. Confounds (mean white matter, cerebrospinal fluid signal, and motion parameters) will be regressed out of the mean signal for each ROI. A high-pass filter of 0.008 Hz will be performed after confound regression (no detrending).

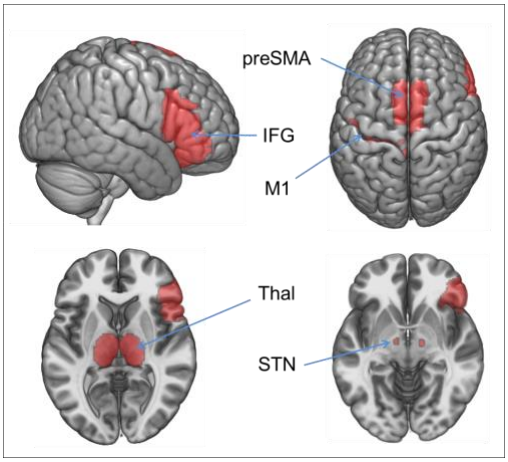

Figure 4: IC mask includes right inferior frontal gyrus (IFG), left precentral gyrus (BA4/M1), and bilateral thalamus (Thal), subthalamic nucleus (STN), and pre-supplementary motor area (preSMA).

Inhibitory Control Network (ICN) Mask. An ICN mask that was created in WFU Pickatlas (fmri.wfubmc.edu/software/pickatlas) for previous studies, including R.IFG, bilateral thalamus, STN, preSMA and left primary motor cortex (BA 4 / M1), will be used as an explicit mask in all analyses.

Statistical Analyses

General Statistical Considerations. Significance will be defined at $\alpha = .05$, with a cluster-determining threshold (CDT) of $p < .001$, as determined by Monte Carlo simulations (3dClustSim; afni.nimh.nih.gov/pub/dist/doc/program_help/3dClustSim.htm, May 2016). Specifically, 3dcalc will take the square root of the SPM model's error variance image (ResMS) and 3dFWHMx used to empirically determine the spatial smoothness of residual error in the model using the newly developed non-Gaussian autocorrelation function (ACF). Data from the NIH, replicating the Beijing datasets [19], show that these settings, combined with CDT of $p < .001$ and a $(10mm)^3$ smoothing kernel, maintain a true false-positive rate of 5% for regular event-related designs[20].

Experimental Inhibitory Control (IC) Task. Data from each fMRI scan will be entered into a 1st level, whole-brain analysis using the GLM[18] to examine BOLD response to each of the 5 trial types: $NoGo_{correct}$ (IC), $NoGo_{incorrect}$ (error of commission), $RareGo_{correct}$ (correct novel-target detection), $RareGo_{incorrect}$ (novel-target error of omission), and $Go_{incorrect}$ (error of omission). Each event type will be modeled as a delta regressor at the onset of the event and convolved with a canonical hemodynamic response function. Intra-run motion will be removed through rigid body rotation and translation and parameters included as covariates. A high-pass filter (.008 Hz) will be applied to remove slow signal drift. Finally, in order to examine successful inhibitory control (IC)-related BOLD response, controlling for novelty detection, a $NoGo_{correct}$ - $RareGo_{correct}$ contrast image will be generated (henceforth, IC) and used for hypothesis testing. This is to be distinguished from IC accuracy, which refers to the percent of correct 'NoGo' trials achieved. Main effects of condition (iTBS vs. cTBS) on IC will be assessed via ANCOVA. Next, mean percent signal change (PSC) from 1st level models during IC will be extracted (via MARSBAR) form each of ROI within the ICN mask (Fig 4).

Task-based Functional Connectivity (tbFC). Corticothalamic tbFC will be assessed at the 2nd level based on 1st level voxel-wise bivariate correlation maps (i.e., Fisher transformed correlation coefficient [rZ-value] maps) [21].

VIII. Potential Risks/Adverse Events

The main study procedures include completion of questionnaires, MRI, and TMS, all of which are generally considered minimal risk procedures. The primary risks are described below:

1. Magnetic Resonance Imaging. Because the MRI machine acts like a large magnet, it could move metallic objects in the MRI room during the examination, which could harmful to the participant. To prevent such an event from happening; loose metal objects, like pocket knives or key chains, are not allowed in the MRI room. If a participant has a piece of metal in their body, such as a fragment in their eye, aneurysm clips, ear implants, spinal nerve stimulators, or a pacemaker, they will not be allowed into the MRI room and cannot have a MRI. Having a MRI may be uncomfortable, particularly regarding feelings of claustrophobia and the loud banging noise during the scan. Participants will be asked to wear earplugs to avoid possible hearing impairment.

2. Transcranial Magnetic Stimulation:

   a. Investigational Device Exemption: Transcranial Magnetic Stimulation is an investigational device. It s a 510K/Pre-market Notification device (a device that is substantially equivalent to a legally marketed device), as described in 21 CFR 807 Subpart E.
   b. Potential Risks of TMS
      i. *Potential risk of a seizure:* The major risk using repetitive TMS subjects is the possibility of inducing a seizure. We have now studied and given rTMS to more than several hundred subjects over past 15 years. None of these patients has developed a seizure. We will exclude patients with a prior history of seizures.
      ii. *Potential for scalp discomfort and headaches:* Some people report mild discomfort when the magnetic pulses are applied over the scalp. A small number of people (~5%) report headache following TMS. However, the headaches are temporary and manageable with common over-the-counter pain remedies.
      iii. *Potential hearing loss:* The discharge of the rTMS coil generates a high-energy click that may cause cochlear damage. Foam earplugs can protect against these changes and will be worn by the subjects and the researchers present during TMS sessions.

3. Incidental Findings: Magnetic Resonance Imaging: Some MRI scans can detect medical conditions, such as cancer, brain injury, and abnormal blood vessels; however, this functional MRI is carried out purely for experimental purposes and we are not looking for brain disorders. Furthermore, the study researchers are not trained in diagnosing brain disorders; therefore, the researchers are not qualified to offer any medical opinions concerning the scan (good or bad). It is possible that the study researchers will notice something in a participant's scan that appears unusual and/or abnormal, if this occurs, the researchers will inform the participant of the finding and provide them with a copy of their scan, which they may take to

a medical expert for further review and diagnosis. Being told about such a finding may cause anxiety as well as suggest the need for additional tests and financial costs. Any costs associated with clinical follow-up(s) are the participant's and/or the participant's insurance carrier's responsibility. Participants who do not wish to be informed of such findings will be advised to not participate in this study.

4. Breach of confidentiality: There is the potential risk of breach of confidentiality of clinical ad laboratory information. Dr. Froeliger has experience as an investigator dealing with such sensitive information and has experience assuring that data is adequately protected. Safeguards to protect confidentiality include locked records and firewalls around password-protected electronic data, and all study data being coded, with the key linking the code with a participant's identity being kept on secure network storage as a password protected document. Similar safeguards are followed for storage and processing of MRI data. MRI data is stored on secure network storage maintained by MU, where the PI has dual appointment. The MRI scans are identified only by subject code, study code, and date of acquisition. Participants' initials will also be present on some questionnaires; however, the questionnaires containing the initials will be stored in a locked file cabinet.

ADEQUACY OF PROTECTION AGAINST RISKS

a. *Recruitment and Informed Consent.* Participants will be recruited from the general community through media advertising (print and online sources). The IRB approved Informed Consent (IC) will be obtained prior to the initial assessment. The consent will be explained orally and in the written form, and will be documented by the signature of the participant on the IC. Consent will be obtained in a private interview room so that the participant may ask any questions to the research staff.

b. *Protection against Risk.* Research staff will closely supervise participants throughout their enrollment in the study. Specific to the BAC assessment, if a participant's BAC is greater than 0.0 at any visit, the research staff will either a) ask the participant to remain in the laboratory until their BAC returns to 0.0, if they are driving, or b) work with the participant to ensure that they have alternative transportation home, including providing a taxi, if needed.

c. *Loss of confidentiality:* Paper-based information will be kept in on-site locked file cabinet(s) designated for study materials. Data collection instruments or forms containing participant names will be stored in separate secure locations from those instruments or forms containing subject identification (SID) numbers, and both will be stored separately from the master list linking the SID and names. Paper-based information will be accessible only to study personnel who need access to the information for study purposes. All electronic records will be stored on a password protected secure server with access limited only to study personnel who need access to the information for study purposes. All password protected hard-drive backups on will be stored in the PI's offices in a secure location. The results of drug and BAC testing will be stored in the same manner as other data (i.e. in separate location from any information containing participant PHI). The results will not be reported to any authority (e.g. employer or law enforcement) nor will they be available to them upon request.

IX. Anticipated Benefits

Participants will not directly benefit from this study.

X. Compensation

Participants will receive up to $285 total in cash over one screening/training visit and three experimental visits (total study visits = 4): $55 at the training visit, $50 for each of three fMRI session-visits ($150 total: baseline, TMS v1, TMS v2), $25 for each of two TMS session-visits ($50 total: TMS v1, TMS v2), up to $10 for performance on the SRT on each of three experimental visits ($30 total).

XI. Costs

The costs of this study will be covered by grant 5UG3DA048510-02 funded by NIH/NIDA. Materials and supplies will cost $1,725, fMRI scans will cost $58,500, advertising and participant recruitment will cost $9,305 and participant compensation will cost $8,550.

XII. Data Safety Monitoring Plan

This UG3 phase is a proof-of-concept study to test mechanistic hypotheses of the acute effects of theta bursts stimulation (TBS) on corticothalamic inhibitory control (IC) task-based functional connectivity (tbFC), IC task performance and ad lib smoking in the laboratory (smoking relapse task: SRT [4]), and while preliminarily evaluating safety and tolerability of TBS among individuals with TUD. Adults smokers meeting criteria for TUD (N =40) will complete a baseline MRI visit and, using a crossover design, be randomly assigned to receive iTBS on one visit and cTBS on the other visit. Following TBS, they will repeat the MRI protocol and then perform the SRT. The effects of TBS on IC brain and behavior and smoking on the SRT, along with safety and tolerability during each visit will be assessed.

XIII. References/Appendices

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