Protocol #:	**15-1016**
Project Title:	**Insular inhibitory neuromodulation to reduce cigarette craving and alter brain function in smokers**
Principal Investigator:	**Michael F. Regner, MD**
Version Date:	**Version 3 (August 22, 2018)**

I. Hypotheses and Specific Aims

The central hypothesis is that inhibitory insular TMS can be used to provide a non-invasive intervention to decrease nicotine craving, alter resting state brain connectivity, and reduce craving-associated insular activity in smokers. All experiments will be conducted in treatment-seeking smokers. These experiments are designed to evaluate the efficacy of insular neuromodulation using rTMS. Outcome measures include (1) cigarette craving measures, (2) resting-state fMRI connectivity measures, and (3) tasked-based fMRI insular activity measures. In a double-blinded two-arm (sham rTMS and inhibitory insular rTMS) randomized control trial with 20 smokers assigned to each arm, we will collect craving measurements, resting state fMRI, and task-based fMRI before and after a single session of rTMS treatment to investigate the following aims:

AIM 1. Determine if insular rTMS reduces cigarette craving.

H1 Compared to sham, insular rTMS will reduce craving in response to cigarette cues.

AIM 2. Determine if insular rTMS alters insular connectivity.

H2 Compared to sham, insular rTMS will reduce fMRI connectivity between the insula and brain regions associated with executive control, reward, and habit formation.

AIM 3. Determine if insular rTMS alters insular activity.

H3 Compared to sham, insular rTMS will reduce fMRI insular activity associated with cigarette cues.

II. Background and Significance

Nicotine dependence is a major health problem: Smoking is the single greatest preventable cause of mortality, morbidity, and unnecessary health care costs in the US.[1] It is estimated to cause 443,595 deaths annually and $193 billion per year in smoking-related health care costs and productivity losses.[1] Globally, tobacco results in over 6 million annual deaths and over half a trillion dollars in economic damage.[2]

Current treatments of nicotine dependence are inadequate: Nicotine dependence is notoriously difficult to treat, with relapse rates approximately 85% for counseling therapy and 78% for combined counseling and pharmacologic therapy.[3] Long-term abstinence from smoking is uncommon, with 6 month abstinence rates ranging between 19% and 33%.[4] High levels of craving have been repeatedly shown to be associated with high relapse rates.[5–7] Development of novel therapies to target craving could dramatically improve abstinence rates, decrease healthcare costs, and prolong lives.

The insula plays a key role in nicotine dependence: The insula is involved in interoception and salience. It integrates external information about stimuli and internal sensations including cognitive, homeostatic, and emotional states, to guide behavior.[8] The anterior insula has been shown to exhibit functional connectivity with the superior, middle, and inferior frontal gyri, bilateral temporoparietal junction, anterior cingulate cortex, precuneus, basal ganglia, nucleus accumbens, and thalamus.[8,9] Directed information flow from the insula to other brain regions, visible by fMRI, is increased in abstinent compared to satiated states in heavy smokers.[10] Moreover, the anterior insula shows increased connectivity during smoking cues compared to food cues with somatosensory cortex, amygdala, and basal ganglia, which increases as dependence severity increases.[11] A large meta-analysis demonstrated correlation between anterior insular cue-reactivity and subjective craving.[12] The anterior insula is also involved in aspects of nicotine dependence other than craving, such as delayed discounting,[13] valence,[14] and mitigating aversion to negative consequences of smoking.[15]

Several lines of evidence suggest involvement of the anterior insula in maintenance of nicotine addiction.[16,17] In a landmark study, Naqvi et al[16] reported that smokers with cerebrovascular damage to the insula were able to stop smoking easily without cravings or relapse, supporting a role of the insula and a broader interoception circuit in addiction. A prospective study over a one-year timespan replicated the conclusion that insular lesions were strongly associated with smokers spontaneously becoming non-smokers.[18] These findings have been corroborated in rat models of nicotine dependence.[19] Insular inactivation significantly reduced nicotine motivation, nicotine seeking-, and nicotine taking-behaviors, with no effect on food behaviors.[19] Results of bilateral insular deep brain stimulation lesions in animal models are also consistent with this hypothesis.[20] Given this evidence, inhibiting the anterior insula may be a useful strategy to reduce cigarette craving, as will be studied in **Aim #1**.

Sutherland et al.[21] recently proposed an elegant explanatory “toggle” hypothesis, applying the work by Seeley et al.[22] to nicotine dependence. Briefly, the hypothesis centers upon the salience network, comprised of the insula and anterior cingulate cortex. The insula is believed to “toggle” this network, directing brain function towards endogenous (i.e., default mode network) or exogenous (i.e., executive control network) stimuli. This insular function becomes usurped in nicotine addiction. The concept that the insula directs consciousness towards the most homeostatically relevant stimuli – internal or external – provides a neurobiological model to explain the cognitive deficits and functional connectivity findings of acute nicotine ingestion, nicotine satiety in dependence, and nicotine withdrawal.[21] It also provides a mechanism for the findings of spontaneous smoking cessation in patients with insular lesions. Importantly, the model affords the opportunity to investigate non-invasive perturbation of specific nodes (e.g., insular cortex) to reduce craving and hopefully improve abstinence rates. We plan to study this functional perturbation in **Aim #2** and **Aim #3**.

Neuromodulation in nicotine dependence shows promising results: Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive neuromodulatory technique in which a pulsatile magnetic field is created using an external solenoid to induce targeted depolarization of cortical neurons via electromagnetic induction. High frequency rTMS (> 3 Hz) transiently increases cortical excitability, while low frequency rTMS (1 Hz) reduces it.[23–25] TMS to the dorsolateral prefrontal cortex (DLPFC) has demonstrated good test-retest reliability in fMRI connectivity.[26]

Several studies have reported that excitatory rTMS targeting the DLPFC reduces cigarette craving and cigarette consumption.[27–29] Moreover, studies have demonstrated that a *single session* of excitatory DLPFC rTMS causes short-term reduction of cigarette craving,[30,31] cigarette consumption,[32] cocaine craving,[33] and craving induced by appetitive food.[34,35] These findings are consistent with the salience “toggling” hypothesis. Prefrontal cortex exerts top-down control to bias information in lower-level somatosensory cortices towards relevant input[36,37] and guide goal-directed behavior.[38,39] Enhancing prefrontal cortical activity through neuromodulation would be expected to shift the balance of neural circuitry away from the DMN and towards the ECN, biasing consciousness away from endogenous stimuli such as craving.

While previous work has reported that TMS targeting the dorsolateral prefrontal cortex (DLPFC) modulates craving responses;[28,29] human stroke and animal model literature suggests that the

insula may be more important in "toggling" away from DMN and toward ECN. To date only one paper describes rTMS specifically targeting the insula: a study of seven patients, reporting subjective changes in cold perception and tolerance of the intervention.[40] Dinur Klein et al.[41] applied high-frequency, low-frequency, and sham rTMS to the bilateral DLPFC with some magnetic field spread into the anterior insulae bilaterally. They reported high-frequency bilateral stimulation significantly reduced cigarette consumption and nicotine dependence, with significantly increased levels of abstinence both immediately after the treatment and at 6 months follow-up. No fMRI data were obtained. The major weakness of the Dinur-Klein et al study is that it primarily targeted the DLPFC, with minimal excitatory posterior treatment coverage of the insula. Given the salience toggling hypothesis, to optimally modulate craving therapies one should provide either excitatory stimulation to the DLPFC or inhibitory stimulation of the insula. *While most experts in this field acknowledge the critical role of the insula in nicotine dependence, there has been surprisingly little research exploring neuromodulation of the insula as a potential therapy.*

This proposal seeks to answer several questions: (1) Can inhibitory TMS be used to reduce cigarette craving in smokers? (2) Can inhibitory insular TMS alter insular connectivity and activity? And, (3) does inhibitory insular TMS alter brain-behavior relationships? The literature implicating the insula in maintenance of craving in smokers suggest rTMS targeting the insula may provide a more robust modulation of nicotine craving.

III. Preliminary Studies/Progress Report

Preliminary study of TMS targeting the insula: We measured resting-state fMRI connectivity in a healthy subject before and after inhibitory (1 Hz) targeting the anterior left insula (MNI coordinates: [-32, 18, 4])[9] at 80% motor threshold using a custom manufactured MagStim H-coil for deeper targeting and Brainsight frameless stereotactic TMS localization (Figure 1). This approach is identical to the proposed neuromodulation. The subject underwent a 6 minute resting state fMRI (RS-fMRI) exam with eyes closed prior to stimulation. The subject exited the magnet and was treated with a 15 minute continuous train of 1 Hz TMS targeting the left anterior insula using an H-coil. After stimulation, the subject underwent another 6 minute RS-fMRI exam. After standard preprocessing, a 5mm radius spherical ROI was centered at the TMS target coordinates, and connectivity measures with all other voxels in the brain were calculated. Results were thresholded at family-wise $p<0.05$ using with a voxel-level $p<0.005$ and a voxel cluster correction $q>23$. Results showed post-treatment increased insular connectivity to DMN regions (cingulate cortex and bilateral parietal lobes), middle temporal cortex, and inferior frontal cortex. Decreased insular connectivity was observed in amygdala, hippocampus, and dorsal attention areas. These results are consistent with our hypotheses and demonstrate (1) we are able to successfully administer TMS and measure fMRI connectivity changes, and (2) proof-of-feasibility that TMS targeting the insula can modulate neural functional connectivity in the resting state.

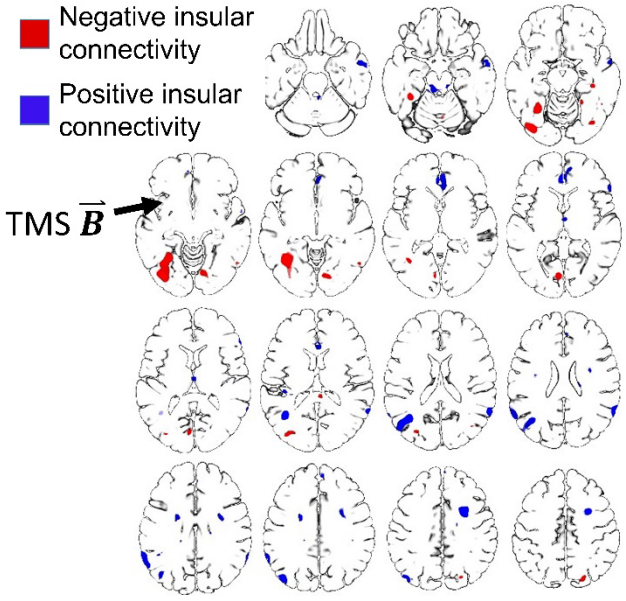


Figure 1. Increased (blue) and decreased (red) insular connectivity after inhibitory rTMS applied to a single subject. Figure rendered in neurologic space.

Preliminary study of functional connectivity changes in substance dependence: As part of Dr. Tanabe's (faculty sponsor) research on long term abstinent substance dependent individuals (SDI), I investigated RS-fMRI network connectivity between substance dependent individuals (SDI) and healthy controls (Figure 2). These SDI are unique in that they had prolonged remission of >1 year on average. Group independent component analysis was performed on 50 SDI and 50 healthy controls, and the number, direction, and strength of connections between components identified as standard resting-state networks were analyzed using Granger causality. Compared to controls, SDI showed greater Granger causal connectivity from right executive control network (RECN) to dorsal default mode network (dDMN) and from dDMN to basal ganglia network (BGN). Interestingly, the insula was incorporated in the BGN networks. We concluded that directed effective connectivity from the RECN to dDMN and dDMN to BGN in long term abstinent SDI may reflect higher top-down control from structures involved in executive function and behavioral monitoring over those involved in self-referential thought and reward. I presented these data at the International Neuropsychological Society 2015 Meeting. These results demonstrate our ability to conduct RS-fMRI network connectivity analyses which we plan to implement in secondary analyses, in addition to the primary seed-based methods shown above (Figure 2).

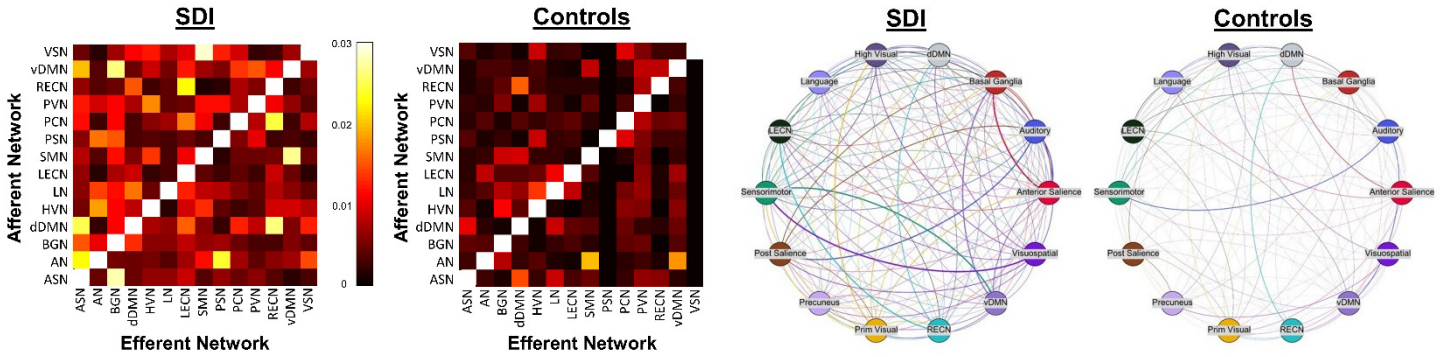


Figure 2. Left panels, directed Granger causal matrices for SDI (n=50) and controls (n=50). Colorbar corresponds to logarithm of F values. Right panels, network density topological graphs of SDI and controls. Thickness of each line corresponds to the Granger causal strength and color corresponds to the efferent network.

Preliminary study of neuronal response to appetitive food cues compared to neutral cues (Tregellas et al. 2013): Dr. Tregellas (co-sponsor) has extensive experience measuring the neuronal response to visual stimuli of foods of high hedonic value as compared to non-food stimuli,[42] demonstrating robust cue-induced activation in the insulae (Figure 3). I plan to use an identical visual cue paradigm, but will replace the food-cue images with images from the international smoking image series.[43]

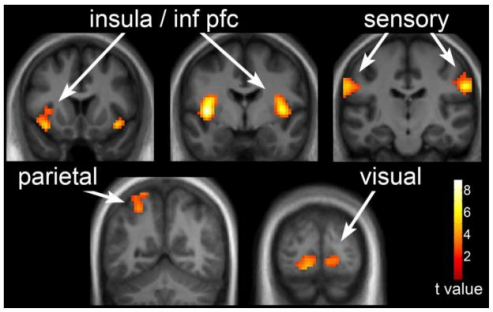
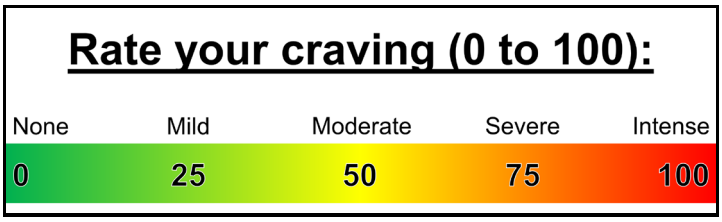


Figure 3. Neuronal response (n=25) to visual stimuli of foods of high hedonic value as compared to non-food stimuli, demonstrating robust cue-induced activation in the insulae.

IV. Research Methods

A. Outcome Measure(s): Outcome measures will include (1) cigarette craving measures, (2) resting-state fMRI connectivity measures, and (3) tasked-based fMRI insular activity measures.

A.1 Neuropsychological Testing: Our primary cognitive outcome will be the change after treatment compared to before treatment in the Questionnaire of Smoking Urges – Brief Version (QSU-brief), a measures craving. An additional important question is whether craving, smoking history, smoking characteristics, or personality traits are associated with specific changes in connectivity after treatment. Thus, we will perform the following assessments prior to treatment: (1) smoking history questionnaire, (2) Fagerstrom nicotine dependence scale (FNDS), (3) Wisconsin inventory of smoking dependence motives (WISDM-68), (4) behavioral inhibitory scale and behavioral activation scale (BIS/BAS), (5) Barratt impulsiveness scale (BIS-11). The QSU-brief will be administered before and after treatment. A visual analog scale will be used to quickly assess subjects' craving during the craving visual-cue task:



The craving data before and after treatment will be used to investigate **Aim #1**.

A.2. Magnetic Resonance Imaging. All MRI scans will be acquired using a 3.0 T Seimens Skyra MR system using a 20-channel head-neck coil. High-resolution 3D T1-weighted scans will be acquired before treatment for image registration and normalization. T2* weighted echo-planar imaging blood oxygen level dependent fMRI will be acquired with 3.4 x 3.4 x 3.4 mm voxels, TR 2100 ms, TE 30 ms, and flip angle 70°. Two fMRI sequences will be acquired before and after treatment: (1) a 10 minute RS-fMRI, in which subjects will be asked to lie quietly in the scanner and not think of anything in particular; and, (2) a 15 minute task-based fMRI exam during a cigarette craving cue task. fMRI data will be processed and analyzed as described in the methods section (see C.4 through C.8). Briefly, outcome measures will include insular functional connectivity (see **Aim #2**) and insular activity (see **Aim #3**) changes at the whole brain level after treatment compared to before treatment.

B. Description of Population to be Enrolled:

Sample Population Recruitment: Current smokers reporting motivation to quit will be recruited from the University of Colorado Behavioral Health & Wellness Program (BHWP). BHWP in conjunction with the University of Colorado Hospital provides consultation and treatment of tobacco dependence through their Tobacco Free Program. Subjects for this study will be recruited from the pool of patients considering enrolling in treatment through BHWP at the University of Colorado Hospital. Dr. Chad Morris is the director of BHWP; he or his clinical colleagues will ask each patient being seen by the Tobacco Cessation Team if they are interested in participating in a research study, and if so, they will be provided a flyer that includes basic information as well as Dr. Regner's contact information. The flyer will instruct interested prospective subjects to call or e-mail Dr. Regner to set up an appointment. This appointment will allow Dr. Regner to discuss the study with the participant in a private office maintained by the Department of Radiology, answer any questions the participant may have, and obtain informed consent. Public advertising for this study will also be performed using flyers with pull-off tabs (e.g., downtown public areas, convenience stores with permission of the owner) and newspaper/internet ads (e.g., Craig's List, Denver Post). All

advertising will comply with the *COMIRB Advertising Components Submission Form* without any deviation.

Inclusion criteria:

1) Age 18-55
2) English proficient
3) Ability to provide informed consent
4) Self-reported current average daily cigarette consumption >10/day for at least 1 year
5) Self-reported motivation to quit smoking

Exclusion criteria:

1) MRI/TMS[44,45] exclusions, including
    a. Claustrophobia
    b. Intracranial or spinal hardware
    c. Pacemakers
    d. MR-incompatible devices (Examples: pacemaker, deep brain stimulator, vagal nerve stimulator, cochlear implant, insulin pump, implanted medication pump, bone stimulator, implanted defibrillator)
    e. History of metal objects or fragments in the eye or skull, including shrapnel or metal plates
    f. History of stroke or other brain lesion
    g. History of attempted suicide or suicidal ideation
    h. Personal history of headaches, seizures, epilepsy, or status epilepticus
    i. Family history of epilepsy
    j. Medications known to lower seizure threshold (e.g., tricyclic antidepressants, antipsychotics, psychostimulants)
    k. Increased intracranial pressure, hydrocephalus, or pseudotumor cerebri
    l. Unstable coronary artery disease
    m. Current pregnancy or positive urine pregnancy test
2) Neurological illness
3) Prior neurosurgery
4) Schizophrenia
5) Bipolar disorder
6) Current (within the last two months) major depressive disorder
7) Substance dependence (except cannabis) or positive urinalysis for opiates, stimulants, or sedative on the day of testing
8) Alcohol dependence or positive breath test for alcohol on the day of testing
9) Use of tobacco products other than cigarettes.

Subjects will be randomized to one of two treatment groups: 1) sham TMS, or 2) inhibitory (1 Hz) TMS targeting the insula.

C. Study Design and Research Methods

Experimental Method for all Aims:

C.1 Enrollment. Prior to informed consent, the inclusion and exclusion criteria will be reviewed with each subject. Once eligibility has been determined, each subject will be provided with verbal and written informed consent. All questions will be answered.

Each subject will participate in a single session, during which inclusion and exclusion criteria will be reviewed, the subject will provide informed consent, women will be have urine pregnancy screens, urine and breath drug and alcohol tests will be administered, standardized psychological assessments will be performed, and then resting and task-based fMRI scans before and after rTMS. The following is the projected timeline for a given subject's study appointment:

Study Appointment Component	Duration (min)
1. Review of inclusion and exclusion criteria	10
2. Informed Consent	15
3. Urine and breath tests	5
4. Pre-treatment neurological examination	10
5. Initial standardized surveys	20
6. Pre-treatment MRI examination	49
High- definition structural T1W sequence	6
Resting-state fMRI sequence	10
Craving-cue task fMRI sequence/task	15
Magnetic Resonance Spectroscopy (MRS)	18
7. Walk to TMS Laboratory	5
8. rTMS Treatment	31
9. Walk to Brain Imaging Center	5
10. Post-treatment MRI examination	43
Resting-state fMRI sequence	10
Craving-cue task fMRI sequence/task	15
Magnetic Resonance Spectroscopy (MRS)	18
11. Post-treatment QSU-Brief	2
12. Post-treatment neurological examination	10
Total:	**205**

Subjects will be asked to remain abstinent from smoking for 3 hours prior to the study. Subjects will provide urine samples to screen for recent drug use and quantify urine cotinine levels. To ensure abstinence they must fill a questionnaire, test negative for alcohol by a breath screening test, and have a breath CO<10pp. Clinical assessment including a brief medical and psychiatric interview will be performed by Dr. Regner to determine eligibility. Subjects will undergo a brief (10 minute) neurological examination. Subjects will be educated on TMS and MR safety and will demonstrate their understanding.

C.2 Demographic, Psychological, Smoking, and Craving Assessments: Subjects will complete a basic demographic survey to document the date of birth, sex, ethnicity, education, handedness, and use of glasses. Age and sex must be determined because gray matter characteristics and brain function vary by age. Education and handedness must be documented because brain function is affected by these variables. The use of glasses must be documented because if subjects are unable to see the images during the craving cue task, it can introduce a bias in the data.

Medical history, current medications, and surgical/procedural history will be documented. Dr. Regner will review this information to ensure that the subject meets no exclusion criteria, and to ensure that the subject is taking no medications that lower the seizure threshold.

Subjects will complete a brief smoking history questionnaire. This will characterize the severity and nature of the subject's cigarette dependence, which may affect their responses to craving cues.

Subjects will complete the Fagerstrom Nicotine Dependence Scale (FNDS). This is a valid and reliable standardized survey to quantify the degree of cigarette dependence.[46,47]

Subjects will complete the Wisconsin Inventory of Smoking Dependence Motives (WISDM-68). This is a valid and reliable 68-item standardized survey designed to characterize the psychological constructs that motive an individual subject's smoking behaviors.[48]

Subjects will complete the Questionnaire on Smoking Urges – Brief (QSU-Brief). This is a valid and reliable standardized survey to quantify a subject's current intensity of craving.[49] This is the only standardized survey that in addition to being administered prior to rTMS treatment will also be administered after rTMS treatment.

Subjects will complete the Behavioral Inhibition Scale and Behavioral Activation Scale (BIS/BAS). The Behavioral Inhibition and Activation Scale is a 20-item self-reported questionnaire

used to measure responsiveness of motivational systems.[50,51] The first component is the Behavioral Inhibition Scale, which is used to quantify avoidance behaviors and reactions to the anticipation of punishment. The second component is the Behavioral Activation Scale, which is used to quantify positive affective and approach response tendencies to appetitive stimuli.

Lastly, subjects will complete the Barratt Impulsiveness Scale, version 11 (BIS-11). This is a 30-item self-reported questionnaire used to measure impulsivity; subjects rated whether phrases and words describing aspects of impulsivity are self-descriptive.[52]

C.2 MRI scan acquisition parameters: Prior to the MRI scan, subjects will be asked to complete the standard *UC Denver Brain Imaging Center MRI Research Subject Screening Form* to ensure they are safe to be scanned. Before and after rTMS, RS-fMRI and task-based fMRI examinations will be performed. This is a required component of any MRI examination. All scans will be performed on a research-dedicated 3.0 T Seimens Skyra MR system using a 20-channel head-neck coil. A VacFix head-confirming vacuum cushion will mitigate effects of head motion. High-resolution 3D T1-weighted scans will be acquired before treatment for image registration and normalization. Linear and second order shimming will be performed to reduce static field inhomogeneities at the skull base prior to fMRI scans. T2* weighted echo-planar imaging blood oxygen level dependent fMRI will be acquired with 3.4 x 3.4 x 3.4 mm voxels, TR 2100ms, TE 30ms, and flip angle 70°. Two fMRI exams will be acquired before and after treatment: (1) a 10 minute RS-fMRI, in which subjects will be asked to lay quietly in the scanner and not think of anything in particular; and, (2) a 15 minute task-based fMRI exam during a cigarette craving cue task.

C.3 1H-Magnetic Resonance Spectroscopy (MRS): Before and after rTMS, 1H-MRS examinations will be performed to quantify GABA, Glutamate, and Glutamine levels in the insula. 1H-MRS sequences will be used, as previously reported and used by colleagues in the Department of Radiology.[53] This MR sequence will be used for exploratory analysis.

C.3 rTMS Treatment: Subjects in the insular rTMS treatment arm will undergo a single 25 minute train of 1Hz rTMS targeting the right anterior insula[16,17,54] (MNI coordinates: [32, 18, 4])[9] at 90% motor threshold using the commercially available MagStim Rapid2 coil and Brainsight frameless stereotactic TMS localization system. Subjects in the sham rTMS treatment arm will have a sham coil placed in the same position. The sham coil creates sound, vibrations, and electrical stimulation similar to the active coil.[55] Subjects will undergo a brief (10 minute) neurological examination by Dr. Regner before and after rTMS treatment, which will include examination of the motor, sensory, language, abstract-thinking, memory, and spatial reasoning systems.

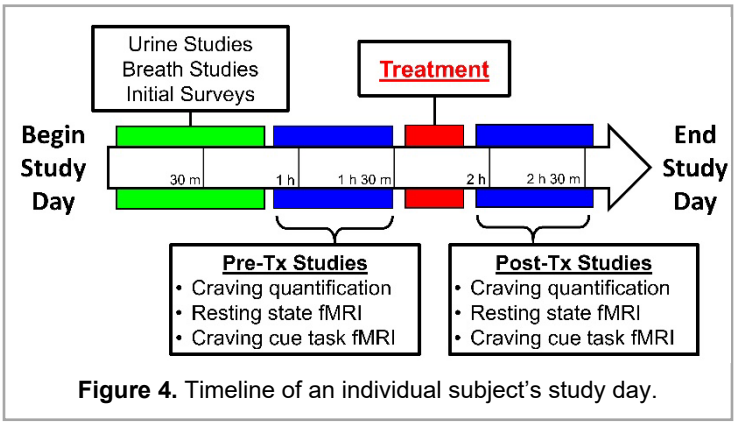


Figure 4. Timeline of an individual subject's study day.

The specific parameters of the rTMS proposed in this study are as follows:

Parameter	Value
Manufacturer	MagnaStim
Model Name	Rapid2
510(k) or Master File Number	
Amplitude (% of motor threshold)	90%
Frequency (pulses per second) [*a*]	1 Hz

Train duration [*b*]	1500 sec (25 min)
Number of trains per session [*c*]	1
Number of sessions in study [*d*]	1
Cumulative exposure (total # of pulses = *a* × *b* × *c* × *d*)	1500 pulses
Intertrain interval (time between trains)	N/A

The following table is the maximum safe duration (in seconds) of a single train of rTMS, reproduced without permission from Rossi et al.[45] Here, safety was defined as absence of seizure, spread of excitation or afterdischarge of EMG activity. Numbers preceded by > are the longest duration tested. This table was presented by consensus.[45] The parameters proposed in our study are highlighted in yellow and well within safe limits.

Freq (Hz)	Intensity (% of motor threshold)				
	90%	100%	110%	120%	130%
1	>1800	>1800	>1800	>360	>50
5	>10	>10	>10	>10	>10
10	>5	>5	>5	4.2	2.9
20	2.05	2.05	1.6	1.0	0.55
25	1.28	1.28	0.84	0.4	0.24

C.4 Comparison of craving responses to treatment: QSU and visual-analogue craving measures will be compared using a multivariate analysis of covariance (see C.4.1.1). These comparisons will provide results for **Aim #1**.

C.5 Image preprocessing: Raw BOLD data will be pre-processed using SPM8 (website: http://www.fil.ion.ucl.ac.uk). After discarding the first four volumes for saturation effects, motion correction will be applied with a 3D rigid body transformation. Subjects with head movement >2mm will be excluded. fMRI images will be spatially normalized to Montreal Neurological Institute (MNI) space by registering each subject's fMRI images to their high resolution T1W scan, which will be registered to MNI space using the DARTEL method.[56] Registered fMRI images will be spatially smoothed using a 4mm FWHM isotropic Gaussian kernel. Band-pass filtering (0.01Hz–0.08Hz) will be applied to reduce effects of low frequency drift and high-frequency physiologic noise. fMRI data will be corrected for movement parameters (3 rotational and 3 translational variables) as well as global, CSF, and white matter signal fluctuations.[9,57]

C.6 Seed Based Insular Functional Connectivity: Using customized MATLAB scripts, whole brain voxel-wise connectivity maps will be calculated according to Margulies et al.[58] from the 10 minute RS-fMRI exams using a seed-based approach with a 5mm radius sphere centered at the right anterior insular rTMS target. To compare insular connectivity, first we will apply a fixed-effects analysis to each subject's resultant whole-brain insular connectivity maps to investigate individual brain connectivity differences associated with treatment. Treatment and group comparisons will be performed using ANCOVA. These comparisons will provide results for **Aim #2**.

C.7 Craving cue task-based insular activity: After resting state fMRI examinations, all subjects will perform smoking cue/neutral cue craving tasks during a 15 minute fMRI using the aforementioned imaging parameters. We have previous experience using this task to elicit food craving.[42] We will use an identical visual cue paradigm, but will replace the food-cue images with the international smoking image series.[43] Each run will consist of pseudorandom presentations of different smoking-related and food-related images. Between images, participants will be asked to fixate on a cross image for variable duration of time to introduce jitter into the design and improve fit of the generalized linear model (GLM).

Task-based fMRI exams will be preprocessed as above. Data will be modeled in SPM8 using a GLM with customized square waveforms representing the condition (i.e., cigarette versus neutral stimulus) and duration of stimulus convolved with a canonical hemodynamic response function.

Contrast maps of smoking>neutral cues will be analyzed using second-level random-effects to compare these contrasts within and between treatment groups using ANCOVA. Two separate task-based analyses will be performed: (1) insular ROI analyses by extracting insular activity from the fMRI with a 5mm radius sphere centered at the insular TMS target, and (2) whole brain analysis. Both analyses will control for multiple comparisons using cluster-based correction for family-wise error $p<0.05$. These comparisons will provide results for **Aim #3**.

C.8 Resting state network analysis (secondary): One inherent limitation of the seed-based connectivity approach is that the resultant connectivity maps are highly dependent upon ROI selection. To circumvent the potential interference caused by seed selection, secondary investigation of the functional connectome will be performed using group independent component analysis[59] to make inferences using the GIFT toolbox (http://icatb.sourceforge.net) and using the same method and analysis pipeline as our preliminary data. Resultant resting state network connectivity maps will be compared by group to investigate differences in strength and direction of connectivity between salience, default mode, and executive control networks.

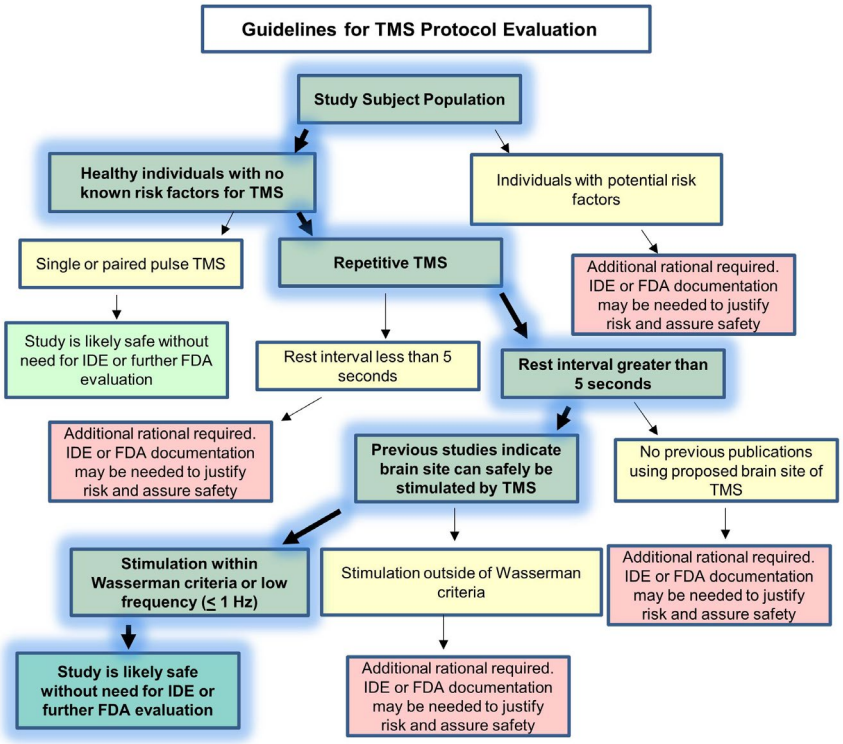
C.9 Brain-behavior relationships (secondary): Secondary analysis of brain-behavior relationships will be conducted using a GLM in SPM8 by regressing insular connectivity and activity (see C.3.6) against craving and dependence measures. This will characterize the relationship between behavior (**Aim #1**) and brain function (insular connectivity [**Aim #2**] and activity [**Aim #3**]).

C.10 Relative concentrations of GABA, glutamate, and glutamine after rTMS (exploratory): Exploratory analysis of the changes in GABA, glutamate, and glutamine concentrations induced by rTMS will be performed using spectroscopic peak detection methods and ANCOVA.

D. Description, Risks and Justification of Procedures and Data Collection Tools:

Risks Associated with TMS

1. **Headaches (common).** The risk of headaches or scalp discomfort is approximately 10%. This discomfort is self-limited and responds to over the counter analgesics (e.g., ibuprofen, aspirin, or acetaminophen).
2. **Seizures (rare)**. When used within parameters set by recent consensus statements, the risk of seizures is less than 1 in 1,000. Seizures reported have been self-limited. The lifetime incidence of seizures in the US population is 10%. Isolated self-limited seizures do not cause brain injury or other adverse effects to the subject, are painless, and do not have known adverse effects on health. There have been no reported cases of status epilepticus (uncontrollable seizure which can be disabling or life-threatening) or epilepsy (recurrent, unprovoked seizures) following TMS. When evaluating subject risk per the standardized COMIRB review procedure for TMS studies, studies with a low (<1%) risk of a single self-limited seizure should not be considered a serious risk to the health of a subject. In addition, according to the COMIRB Guidelines for TMS Protocol Evaluation, studies with stimulation parameters within the Wasserman criteria[60] and low frequency (≤1 Hz)[45] are likely safe without need for IDE or further FDA evaluation. Because this study will use a low frequency single train rTMS (1 Hz) within Wasserman criteria, ensure adequate hearing protection, administer neurological examinations before and after rTMS treatment, and monitor subjects by a medical doctor (Dr. Regner) during and for at least 60 minutes after rTMS treatment, we do not feel that an FDA IDE is required. This opinion is consistent with the aforementioned guidelines and COMIRB TMS review guidelines (our decision pathway is highlighted in blue):



Dr. Regner will be present throughout the subject's participation and for 60 minutes after the completion of the TMS treatment (during MRI scans). He will monitor for signs of seizure or muscle twitching. Dr. Regner is a physician trained in seizure management and will have ready access to life-support equipment (oxygen, suction, blood pressure monitoring, CPR equipment) and antiepileptic drugs.

3. **Hearing loss (rare)**. The risk of hearing loss is well below 1 in 10,000 and appears to be completely avoidable with appropriate hearing protection (ear plugs). All subjects are required to wear the standard-of-care hearing protection. Subjects are asked to report to study staff immediately if their hearing protection becomes loose or is otherwise inadequate. In addition, study staff will monitor all subjects to ensure compliance with hearing protection.
4. **Movement or heating of metallic objects (rare).** TMS may cause heating or movement of intracranial metal, such as in subjects with implantable electrodes, cerebrospinal fluid shunts, skull plates, or other intracranial metal. These subjects will be excluded.
5. **Damage to electronic medical devices (rare).** TMS may interfere with electronic medical devices such as pacemakers, implantable stimulators, or implantable pumps. These subjects will be excluded.
6. **Pregnancy (rare).** There is not sufficient data to assess the safety of TMS in pregnancy, although TMS has been performed in limited numbers of pregnant subjects without adverse effects. Pregnant subjects will be excluded.

<u>Risks Associated with MRI</u>

7. **Movement or heating of metallic objects** (rare, serious). The only known hazard associated with exposure to a static high magnetic field is that the magnet exerts a strong force on ferromagnetic objects. For this reason, ferromagnetic objects are excluded from the vicinity of the magnet so that they will not become projectiles. At the Brain Imaging Center, the research MRI for human use has a field strength of 3.0 T. Imaging at these field strengths is not considered a significant risk according to FDA guidelines. The scanning sequences applied are within the FDA guidelines for human MR scanning. In addition, every subject undergoes an extensive safety screening to determine whether

he/she has any implanted materials, objects, device, or dental retainers that may pose a risk. If there is any doubt about the nature of any implanted material, or any other contraindication to MR scanning, the subject will not be scanned.

8. **Damage to electronic medical devices** (rare, serious). See #7.
9. **Pregnancy** (rare, serious). Any woman who reports being pregnant or reports that they may be pregnant will be excluded from this study. Any woman of childbearing age who reports that she is not pregnant will undergo a urine pregnancy screen. Those who test positive will be excluded from participating in this study.
10. **Claustrophobia** (common). If patients are anxious they can be trained in the mock scanner located adjacent to the MR scanner to mitigate anxiety and help the subject adjust to the scanning environment. Subjects who want to quit immediately or who are otherwise not interested in participating in this study can do so at any time, and this option will be discussed with the subject during informed consent and at regular intervals thereafter.
11. **Nausea** (rare). Rarely, subjects may become nauseated as a result of the alternative magnetic fields or acoustic noise. Subjects are able to terminate participation in the study at any time.
12. **Irritation from loud sounds** (common). Loud sounds occur during functional MRI scanning. They are unpleasant, but not loud enough to be harmful. All subjects will wear standard-of-care ear protection while undergoing the MRI scans. Subjects are able to terminate participation in the study at any time.

General Risks Associated with Research Participation

13. **Potential risk of loss of confidentiality (rare).** This study includes drug screening, alcohol screening, and pregnancy test assessment. There is the potential that this information may not be kept confidential, such as in theft of study material.
14. **Fatigue (common).** Tiredness or boredom may occur during behavioral tasks and the MRI scan. Subjects are able to terminate participation in the study at any time.
15. **Frustration (common).** Subjects may become frustrated while performing the craving-cue task in the MRI scanner. Subjects are able to terminate participation in the study at any time.

E. Potential Scientific Problems:

I have experience conducting both structural and function neuroimaging experiments.[61,62] Nonetheless, this study has potential pitfalls. One limitation is that insular rTMS may also stimulate the lateral inferior frontal gyrus; by design, any rTMS treatment can also affect structures superficial to the target along the $\vec{B}$ trajectory. This potential pitfall can be easily addressed by correlating craving measures with (1) exploratory seed-based connectivity and (2) ROI based activity of all structures included in the rTMS field. Such exploration would determine if other structures showed altered connectivity/activity after insular rTMS, as well as determine if brain-behavior relationships in other structures were altered by insular rTMS. Another potential limitation is that the effect of insular rTMS may manifest only as changes in insular connectivity but not insular activity – we address this predicament by investigating both.

F. Data Analysis Plan:

F.1 Aim 1: Determine if insular TMS reduces cigarette craving:

F.1.1 Statistical Design: For a multivariate analysis of covariance model, we will fit a general linear multivariate model, with measures of craving before and after treatment as the outcomes. As predictors, we will fit indicator variables for treatment assignment, and FTND. We will use the Hotelling-Lawley test to assess treatment by time interaction, and the main effects of time and treatment.

F.1.2 Sample Size and Power Analysis: Li et al.[30] conducted an experiment similar to ours, measuring craving responses to DLPFC rTMS (Cohen's d = 3.0) or sham TMS (Cohen's d = 1.9).

Based on these estimates, we will need to recruit 15 subjects to each arm (total n=30) to detect similar differences with 80% power and 0.05 significance level. Our study should achieve similar power, even with up to 25% loss to follow-up in each arm. If our loss-to-follow up is only 10% per arm, we can detect an effect size as small as 0.962 with 80% power and 0.05 significance level. Our sample size is larger or similar to other studies using similar methods.[27,29,30,32,63]

F.2 Aim 2: Determine if insular TMS alters insular connectivity:

F.2.1 Statistical Design: A second-level random effects model will be used to compare insular connectivity by treatment and group using ANCOVA. Age will be controlled for as nuisance variables. A Monte Carlo cluster correction threshold will be applied to correct for multiple comparisons, using a voxel-wise $p<0.005$ and family-wise $p < 0.05$.

F.2.2 Sample Size and Power Analysis: Hanlon et al.[26] measured fMRI connectivity changes after rTMS to two separate prefrontal targets, resulting in Cohen's *d* values greater than 1.9. Based on these estimates, our target recruitment goal must be greater than 6 in each arm (total n=12) to detect significant differences with 80% power and 0.05 significance level. Our sample size n=32 (after 10% attrition of fMRI data, 10% attrition of TMS administration) is similar or larger compared to other studies using similar methods.[26,30,33,64,65] With a sample size of 32, we can detect a correlational effect size as small as 0.30 (f^2) with 80% power and 0.05 significance level.

F.3 Aim 3: Determine if insular TMS alters insular activity:

F.3.1 Statistical Design: Contrast maps of smoking>neutral cues will be analyzed using second-level random-effects to compare these contrasts within and between treatment groups using ANCOVA. Two separate task-based analyses will be performed: (1) insular ROI analyses by extracting insular activity from the fMRI with a 5mm radius sphere centered at the insular TMS target, and (2) whole brain analysis. Both analyses will control for multiple comparisons and nuisance variables as we will in Aim #2.

F.3.2 Sample Size and Power Analysis: Hanlon et al.[26] measured fMRI activity changes after rTMS to two separate prefrontal targets, resulting in Cohen's *d* values greater than 2.3. Based on these estimates, our target recruitment goal must be greater than 5 in each arm (total n=10) to detect significant differences with 80% power and 0.05 significance level. Our sample size n=32 (after 10% attrition of fMRI data, 10% attrition of TMS administration) is similar or larger compared to other studies using similar methods.[26,30,33,64,65] With a sample size of 32, we can detect a correlational effect size as small as 0.30 (f^2) with 80% power and 0.05 significance level.

G. Summarize Knowledge to be Gained:

Regardless of the results of our aims, this proposal will lead to significant future research directions. In the unexpected case that no differences in craving or fMRI are observed after insular rTMS, our interpretation will be that the insula is not as critical a neural node as the literature to date reports. This null result would direct our future efforts toward prefrontal targets, which has been previously demonstrated to be effective in craving mitigation. If we find that the insula is indeed an important neural node in craving behavior, these preliminary studies would pave the way for future, more sophisticated studies that compare and combine insular rTMS with prefrontal rTMS, characterize the laterality of the insula, and investigate the durability of craving mitigation with repeated rTMS as a potential long-term adjuvant treatment for nicotine dependence. Literature to date strongly support the latter conclusion, and we expect to find significant results. Regardless of outcome, this proposal will answer a critically important question for the millions of patients unable to stop smoking: how important is the insula in cigarette craving, and can insular rTMS reduce cigarette craving?

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