ADDICTION IS A BRAIN DISEASE:
COMPUTATIONAL AND FUNCTIONAL NEUROANATOMY OF SUBSTANCE USE DISORDERS
USING ADVANCED MRI AND NEUROSTIMULATION-INDUCED BRAIN LESIONS

by

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ABSTRACT

Substance use disorders (SUD) are a group of psychiatric diseases associated with significant mortality, morbidity, economic losses, social- and family-problems, and personal distress. Advances in the ability to image brain structure and function in vivo provide an opportunity to understand addictions. Novel techniques, such as transcranial magnetic stimulation, allow the ability to alter neurocircuitry function for potential diagnostic and therapeutic benefit. This dissertation sought to study SUD as diseases of neurocircuitry affecting reward, craving, and goal-oriented behavior. We conducted advanced neuroimaging experiments involving two different populations of SUD: long-term abstinent, severely-dependent cocaine and methamphetamine addicts (Chapters 2 and 3); and, acutely-abstinent, moderately-dependent cigarette smokers (Chapters 4 and 5 and Appendix 1).

Chapters 2 and 3 investigated structural and functional neuroimaging differences, respectively, in long-term abstinent cocaine and methamphetamine addicts compared to healthy controls. In Chapter 2, we observed that grey matter volumes in stimulant dependence differed compared to healthy controls (n=127), and that these changes differed by sex. We discuss the important implications with regards to sex-differences in the natural history of psychostimulant dependence. In Chapter 3, we investigated brain function as rest by functional connectivity changes amongst large-scale brain networks in a subset of the Chapter 2 population (n=100). We found that after long-term abstinence, large-scale brain networks...
thought to be involved in the pathogenesis of addiction followed a pattern of increased “top-down” cognitive control, which may reflect the necessary increased executive control over habit and reward systems to maintain disease remission. Stimulant dependence also demonstrated greater global efficiency and lower local efficiency amongst large-scale brain networks compared to healthy controls, suggesting abnormal brain organization despite long-term abstinence.

Chapters 4, 5, and Appendix 1 introduce and report results on our phase 1, sham-controlled, single-blinded, randomized clinical trial (www.ClinicalTrials.gov identifier: NCT 02590640) to investigate the effect of inhibitory insular transcranial magnetic stimulation (TMS) on cigarette cravings and brain function in acutely abstinent, moderately-dependent smokers. Compared to sham treatment, inhibitory TMS targeting the right insula reduced self-reported cigarette craving and decreased brain activity responses to visual cigarette cues in primary sensorimotor cortices, supplementary motor area, and right anterior insula. These findings provide proof-of-concept of a potential neuroanatomical target for smoking cessation therapy. While the results should be considered preliminary, they provide hope that TMS could be developed as a treatment strategy to help reduce the burden of cigarette addiction.

Overall, these neuroimaging studies in two different populations of substance use disorders provide evidence that addiction is a brain disease with endogenous (i.e., increased top-down control, Chapter 3) and exogenous (i.e., therapeutically imposed, Chapter 5) mechanisms of remission and treatment.

The form and content of this abstract are approved. I recommend its publication.

Approved: Jody L. Tanabe

Jason R. Tregellas
For Emilie –

who is my critic when I feel I have nothing left to perfect, and

who sings my praises when I feel I have nothing worthy of praise.
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Lastly, but most importantly, I would like to thank our patients and research volunteers. Addictions are often misguidedely considered a “moral failing,” conferring stigma and guilt that impairs awareness, treatment, and scientific progress. We must nevertheless persist and continue to shine light on these psychiatric diseases. Our field is indebted to those individuals who volunteered to walk into the light of scientific examination, if only for a few hours.
DECLARATION OF ORIGINAL WORK

I affirm that all work in this Doctoral Dissertation is my original work. Further, I confirm that all writing is my own writing. Work from others has been cited appropriately.

Michael Francis Regner
PhD Candidate

Date
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CHAPTER I
INTRODUCTION

Human use of psychoactive drugs is described in some of the earliest human historical records. Pathologic use of ingested substances was described in early Greek and Roman literature; for example, the Greek scientist Aristotle (384 BC – 322 BC) provided the first detailed description of alcohol withdrawal, while the Roman physician Celsus (25 BC – 50 AD) precociously described alcohol dependence as a systemic disease (Crocq, 2007). Historically, human use of psychoactive substances can be categorized into four major categories:

1. Spiritual or religious ceremonial use, such as imbibing the wine during Catholic mass;
2. Medical or therapeutic use, such as modern prescription opioids for pain control;
3. Socially acceptable use for pleasure and/or socialization, such as cigarette smoking amongst construction workers;
4. Abuse or dependence of a substance, in a manner discordant with pro-social norms and/or despite negative consequences (“addiction”).

This dissertation focuses on the fourth category of use, addiction, which by the Diagnostic Statistical Manuel-5th edition (DSM-5) is categorized under “substance use disorders.” Substance use disorders (SUD) in the Diagnostic Statistical Manuel-5th edition (DSM-5) are a heterogeneous set of psychiatric diseases characterized by physical dependence, diminished control over substance use despite negative consequences, and cravings to use the substance (American Psychiatric Association, 2013). SUD are characterized in severity by the number of symptoms, with 2 or 3 symptoms representing mild and greater than 6 symptoms representing severe SUD (American Psychiatric Association, 2013).
All drugs of abuse cause the release of dopamine into the nucleus accumbens (Di Chiara and Imperato, 1988; Koob, 1992), producing a rewarding "high" that positively reinforces consumption (Figure 1-1). The mesocorticolimbic reward circuit consists of dopaminergic neurons with cell bodies in the ventral tegmental area of the midbrain and terminal projections in the ventral striatum (nucleus accumbens), prefrontal cortex, and limbic/paralimbic regions. When this circuit is stimulated, dopamine is released into synaptic clefts in these terminal regions. Dopamine binds to postsynaptic receptors and is subsequently rapidly re-sequestered within the presynaptic axonal boutons by the dopamine transporter (DAT), terminating the reward signal. Cocaine (studied in Chapters 2 and 3), for example, artificially enhances dopamine signaling by inhibiting the dopamine transporter, thus prolonging dopaminergic activity. Nicotine (studied in Chapters 4 and 5 and Appendix 1) acts directly on dopaminergic

![Figure 1-1. Simplified scheme illustrating how common drugs of abuse modulate the reward circuit. Modified with permission from Nestler's Molecular Neuropharmacology (Nestler et al., 2015).](image-url)
reward neurons in the VTA as well as modulating interneurons. Over time, drug-use and drug-related stimuli become increasingly associated with the hedonic effects of drug induced dopamine transmission, and these stimuli serve as incentivized cues that trigger craving, anticipatory euphoria, or even symptoms of withdrawal (O’Brien et al., 1992). Drug dependence causes neurocircuitry adaptations that develop in response to repeated administration, as the brain’s effort to maintain overall neurotransmission homeostasis. Abrupt cessation of drug use after the development of addiction, in turn, unmasks withdrawal syndromes because the forces that keep these neurocircuitry alterations balanced are no longer present.

1.1. Neuroimaging in Substance Use Disorders

Neuroimaging has fostered a paradigm shift in the understanding of SUD over the past two decades. Although once considered a “moral failure,” in recent decades neuroimaging has demonstrated that SUD are brain diseases with predictable alterations in neurocircuitry underlying reward, craving, learning, and cognitive control (Tanabe et al., 2019).

Neuroimaging provides both structural and functional observations of altered neuroanatomy. For example, quantitative structural neuroimaging using voxel-based morphology (see Chapter 2) has demonstrated lower grey matter tissue volumes compared to healthy adults in the anterior cingulate, dorsolateral prefrontal, medial prefrontal, and parietal cortex in people dependent upon cocaine (Connolly et al., 2013; Regner et al., 2015), amphetamine (Daumann et al., 2011; Mackey and Paulus, 2013; Tanabe et al., 2009b), and nicotine (Brody et al., 2004; Pan et al., 2013).

In addition to brain structural changes, neuroimaging has furthered our understanding of the functional mechanisms in the brain that promote and maintain SUD. For instance, intravenously administered nicotine (Stein et al., 1998) and cocaine (Breiter et al., 1997) both acutely increase blood-oxygen-level-dependent (BOLD) fMRI signal in the striatum, amygdala, and prefrontal cortex. This pattern of reward-circuitry activation after acute drug
administration has been shown to be altered even in the absence of drug administration. A large meta-analysis investigated the brain changes associated with monetary reward anticipation and reward outcome in 643 individuals with addictive behaviors (including all common drugs of abuse, gambling disorder, and gaming disorder) compared to 609 healthy control individuals (Luijten et al., 2017). They observed that during reward anticipation, people with substance, gambling, and gaming addictions exhibited ventral striatum hyperactivation. During reward outcome, they exhibited decreased dorsal striatum hypoactivation. This suggests that brain processing of reward signals is perturbed in addiction, even for rewards unrelated to the addiction. Neuroimaging has also demonstrated significant long-term shifts in the cognitive function in SUD in the absence of drug administration. A parametric meta-analysis of fMRI cue-reactivity studies discovered that nicotine, alcohol, and cocaine individuals have abnormally increased brain responsivity to drug cues compared to neutral cues in the ventral striatum, anterior cingulate cortex, and amygdala – regions involved in reward and emotional regulation (Kühn and Gallinat, 2011). Although there are compelling common mechanisms of SUD across different substances and across individuals, significant heterogeneity exists.

1.2. Substance Use Disorders as a Class of Heterogeneous Neurocircuitry Diseases

Substance use disorders are understood to be chronic relapsing-remitting diseases that vary by drug, stage, and individual factors. First, SUD demonstrate brain and behavioral differences by drug and mode of use. For example, Hanlon et al. studied individuals dependent upon cocaine (n=55), alcohol (n=53), and nicotine (n=48) (Hanlon et al., 2018), and reported that each substance use disorder demonstrated overlapping yet anatomically distinct “hot spot” cue-induced craving fMRI brain response maps. This suggests that neuromodulatory efforts to therapeutically alter craving in SUD may need to be drug-specific. Second, there is significant evidence that each stage of addiction (Figure 1-2) is associated with distinct patterns of brain functional changes observed through neuroimaging compared to healthy controls (Koob and
Volkow, 2010). Third, and lastly, there are numerous individual factors that contribute to and modulate the pathology of SUD. These include genetic predispositions, socio-demographics, problems of inhibitory control, co-morbid diagnoses (e.g., conduct disorder), sibling use of substances, family history of addiction (i.e., upbringing independent of genetic inheritance), neighborhood poverty and disorganization, and even locoregional laws and norms (American Psychiatric Association, 2013; Beyers et al., 2004; Conger, 1997; Hawkins et al., 1992; Kendler et al., 2013; Samhsa, 2018; Scaramella and Keyes, 2001; Stone et al., 2012; von Sydow et al., 2002).

Figure 1-2. Simplified illustration of the natural history of substance use disorders. Active substance dependence (red) is characterized by sequential stages of binge/intoxication, withdrawal/negative affect, and craving/preoccupation (Koob and Volkow, 2010) that repeat a variable number of cycles, represented by m. Active disease episodes may be interrupted by periods of short- or long-term abstinence, represented by n. Relapse is indicated in blue. Note that at every stage of potential or active disease (top, in white) there is a possible avenue towards disease remission (bottom, in grey). Used with permission from Regner et al. (2016).
1.3. **Dissertation Narrative Outline**

The overarching technical theme heavily influencing each chapter is the signal processing technique for structural and function brain MRI, including statistical parametric mapping to make hypothesis-based inferences. For each analysis, T1-weighted images are segmented into tissue probability maps, including maps of grey matter, white matter, cerebrospinal fluid, bone, soft tissue, and air. These are non-linearly normalized into a standard space. Non-linear “warps” (forward and backward deformation vector fields across the field-of-view) are computed from native to standard space. Time-varying functional images are slice-time and motion-corrected, then forward-warped into standard space. Ultimately, generalized linear models are used to compute individual- and group-level contrast maps (T-statistic or F-statistic maps) for task-based fMRI, or alternatively connectivity maps for resting-state fMRI (either through spatiotemporal independent component analysis or seed-based whole-brain correlations).

The goal of this dissertation was to use advanced MRI-based neuroimaging and signal processing methods to address gaps in the literature regarding two different populations of SUD at different points along the spectrum of disease severity: long-term abstinent, severely-dependent cocaine and methamphetamine addicts (Chapters 2 and 3); and, acutely-abstinent, moderately-dependent cigarette smokers (Chapter 4 and 5 and Appendix 1). While these two populations differ in severity, they also differ in primary drug of abuse (psychostimulants versus nicotine) and stage of disease (chronic remission versus acute withdrawal).

Chapters 2 and 3 investigate structural and functional neuroimaging differences, respectively, in long-term abstinent psychostimulant (cocaine and methamphetamine) addicts compared to healthy controls. In Chapter 2, we report one of the largest samples (n=127) demonstrating that the differences in grey matter volumes in stimulant dependence compared to healthy controls after long-term abstinence differ by sex. Although limited by the cross-
sectional nature of the study, we discuss the important implications with regards to sex
differences in the natural history of psychostimulant dependence. In Chapter 3, we investigated
resting-state functional connectivity changes in stimulant dependence compared to healthy
controls in a subset of the population reported in Chapter 2 (n=100). We show that large-scale
brain networks defined by independent component analysis follow a pattern of increased top-
down cognitive control in long-term abstinent psychostimulant addicts compared to healthy
controls, which may reflect increased executive control over habit and reward systems
promoting remission. Stimulant dependence also demonstrated greater global efficiency and
lower local efficiency amongst large-scale brain networks, suggesting abnormal brain
organization despite long-term abstinence.

Chapters 4 and 5 and Appendix 1 report functional neuroimaging changes in acutely
abstinent smokers after a phase 1, sham-controlled, single-blinded, randomized clinical trial
(www.ClinicalTrials.gov identifier: NCT 02590640) designed to test the hypothesis that
inhibiting the right anterior insula will decrease cigarette cravings and alter brain activity and
connectivity. Chapter 4 serves is a review introducing and motivating our trial of inhibitory
TMS in acutely abstinent, moderate smokers. We specifically review compelling neuroimaging
and neuroscience evidence that the insula may serve as an important therapeutic target for
promoting smoking cessation, and we discuss possible mechanisms to pursue such
neuromodulation. We introduce a useful method of non-invasively modulating brain activity:
transcranial magnetic stimulation (TMS). Chapter 5 reports the main results of the clinical trial:
inhibiting the right anterior insula in smokers using low-frequency deep TMS results in
decreased cigarette craving and decreased brain fMRI responses to cigarette cues compared to
sham. Appendix 1 reports some but not all exploratory data collected during the trial, including
changes in resting-state connectivity after treatment compared to sham.
Each individual dissertation chapter addresses a specific yet important gap in the literature on the structural and functional computational neuroanatomy of SUD. Together, however, these neuroimaging studies at different points along the spectrum of substance use disorders provide support for a conclusion still controversial in the scientific literature (Lewis, 2018): addiction is a brain disease. We further extend this conclusion by investigating possible endogenous (i.e., increased “top-down” executive control, Chapter 3) and exogenous (i.e., therapeutically imposed, Chapter 5) mechanisms of disease remission and treatment. While these results span different subtypes of substance use disorders, they provide hope that both endogenous and exogenous treatment strategies are possible to help reduce the burden of addiction.
CHAPTER II
SEX DIFFERENCES IN GREY MATTER CHANGES AND BRAIN-BEHAVIOR RELATIONSHIPS IN STIMULANT DEPENDENCE

2.1. Abstract

This first chapter investigated whether sex modulates the effects of stimulant dependence on grey matter volumes (GMV) in long-term abstinence. We further sought to characterize how sex modulates brain-behavior relationships between GMV and specific behavioral measures, such as drug symptom count, behavioral approach, and impulsivity. In this prospective case-control study, 127 age- and sex-matched participants (68 controls [28F/40M] and 59 SDI [28F/31M]) underwent T1-weighted SPGR-IR brain MRIs on a 3T system. Images were segmented using voxel-based morphometry MATLAB-based software. After adjusting for age, education, and head size, sex by group interactions and main effects were analyzed over the whole brain using ANCOVA, thresholded at \( p < 0.05 \), corrected for multiple comparisons with family-wise cluster correction. Drug symptom count and behavioral measurements were correlated with whole brain GMV and five a priori regions-of-interest based on extant literature. Sex by group interactions on GMV were significant in numerous regions (\( p < 0.001 \)). Compared to female controls, female SDI had significantly less GMV in widespread brain regions (\( p < 0.001 \)). There were no significant GMV differences in male controls versus male SDI (\( p = 0.625 \)). Drug symptom count negatively correlated with nucleus accumbens GMV in women (left: \( r = -0.364, p = 0.047 \); right: \( r = -0.407, p = 0.031 \)) but not men (left: \( r = -0.063, p = 0.737 \); right: \( r = -0.174, p = 0.349 \)). Behavioral approach (\( p = 0.002 \)) and impulsivity (\( p = 0.013 \)) correlated negatively with frontal and temporal GMV changes in female SDI but not in other groups, demonstrating a sex by group interaction. Vast GMV changes in SDI were
observed in women but not men after prolonged abstinence. Sexual dimorphism in drug-related neuroanatomical changes and brain-behavior relationships may be a mechanism underlying the different clinical profiles of addiction in women compared to men. Future structural neuroimaging and clinical studies on substance use disorders should account for the modulatory effects of sex.


### 2.2. Introduction

Substance use disorders are common, with lifetime prevalence estimated to be 10.3% in the United States (Miller and Hendrie, 2009). Understanding the neurobiology of substance dependence is requisite to advancing treatments. Neuroanatomical changes in drug addiction have been studied extensively using voxel-based morphometry (Ersche et al., 2013). Structural changes have been observed in the orbitofrontal cortex (OFC), medial frontal gyrus (MedFG), anterior cingulate gyrus (ACG), insula, and nucleus accumbens in individuals who abuse stimulants (Ersche et al., 2013; Koob and Volkow, 2010). In the largest meta-analysis of stimulant dependence to date, Ersche et al. (Ersche et al., 2013) reported significant decreases in gray matter (GM) in the insula, ventromedial prefrontal cortex, inferior frontal gyrus, ACG, and anterior thalamus. GM changes have also been studied adult sibling pairs, of whom one sibling was dependent on stimulants and the other had no stimulant dependence history with an age- and sex-matched control group (Ersche et al., 2012). That study revealed changes in limbic and sensory areas in both members of the sibling pair compared to controls, suggesting that GM volume changes may predate addiction and could potentially be an endophenotype for substance use disorder.
Few previous studies have investigated the role of sex on changes in brain structure in stimulant dependence. This is surprising considering the well-characterized sex differences in clinical presentation and natural history of stimulant addiction (Becker et al., 2012; Perry et al., 2013). Women exhibit a telescoping clinical course compared to men in that they begin cocaine or amphetamine use at earlier ages (Becker et al., 2012; Griffin et al., 1989; Mendelson et al., 1991), show accelerated escalation of drug use (Brady and Randall, 1999; Greenfield et al., 2010; Lynch, 2006), report more difficulty quitting (Back et al., 2005; Lynch, 2006), and upon seeking treatment report using larger quantities of these drugs compared to men (Becker et al., 2012; Kosten et al., 1993). Neuroendocrine factors have been hypothesized to underlie an accelerated clinical course (Becker et al., 2012). Another hypothesis is that compared to men, women respond differently to stress which influences drug related behavior (Potenza et al., 2012). However, scant evidence exists for a neuroanatomical correlate of these clinical differences. Many studies recruit primarily men to exclude confounding sex effects (Barros-Loscertales et al., 2011; Fein et al., 2002; Franklin et al., 2002), while other studies do not include sex as a factor in their analyses of GM in stimulant dependence (Ersche et al., 2013; Sim et al., 2007; Tanabe et al., 2009a). In fact, only two studies have described structural differences between sexes in stimulant dependence (Rando et al., 2013; Tanabe et al., 2013). Rando et al. (Rando et al., 2013) reported lower GMV in the left inferior frontal gyrus, insula, superior temporal gyrus, and hippocampus in female SDI compared to female controls while male SDI exhibited less GMV in the precentral gyrus and mid cingulate gyrus compared to male controls. However, this study was significantly limited by the potential effects of recent alcohol use (mean 87 drinks in the month prior to scanning) and lack of long-term abstinence (mean 3 weeks of abstinence prior to scanning), allowing acute effect of substances to skew results. Tanabe et al. (Tanabe et al., 2013) reported differential effects of sex on insular volumes in SDI: female SDI had smaller insulae, whereas male SDI had larger insulae. This study was limited by
the small sample size (28 SDI) and rudimentary methodology (using FreeSurfer to estimate GMV). The paucity of large, prospective, well-controlled studies to investigate long-term sex differences associated with abstinent stimulant dependence is addressed by this study.

This study investigated whether sex modulates the effects of stimulant dependence on grey matter volumes (GMV) in long-term abstinence. We further sought to characterize how sex modulates brain-behavior relationships between GMV and specific behavioral measures, such as drug symptom count, behavioral approach, and impulsivity.

2.3. Materials and Methods

2.3.1. Subjects

One hundred twenty seven individuals including 68 controls (28F/40M) and 59 (28F/31M) SDI were prospectively recruited (Figure 2-1). Controls were similar to SDI on age and sex. Demographic information is reported in Table 2-1. SDI were recruited from a residential treatment program at the University of Colorado School of Medicine Addiction Research and Treatment Services. Inclusion criteria for SDI: lifetime dependence on stimulants Figure 2-1. Sample population inclusion and exclusion criteria.
Table 2-1. Demographic description of the sample population. Data are presented as mean ± SD where appropriate.

<table>
<thead>
<tr>
<th></th>
<th>Healthy Controls</th>
<th>SDI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>Sample Size*</td>
<td>40</td>
<td>28</td>
<td>31</td>
</tr>
<tr>
<td>Age (yr)‡</td>
<td>33.2 ± 9.9</td>
<td>31.9 ± 7.6</td>
<td>36.7 ± 7.6</td>
</tr>
<tr>
<td>Right-handedness*</td>
<td>92.5%</td>
<td>89.3%</td>
<td>96.7%</td>
</tr>
<tr>
<td>Education (yr)‡</td>
<td>14.0 ± 1.6</td>
<td>15.4 ± 1.1</td>
<td>12.6 ± 1.5</td>
</tr>
<tr>
<td>Years of Use (yr)</td>
<td>-</td>
<td>-</td>
<td>16.1 ± 7.0</td>
</tr>
<tr>
<td>Abstinence (mo)†</td>
<td>-</td>
<td>-</td>
<td>17.5 ± 15.6</td>
</tr>
<tr>
<td>Drug symptom count†</td>
<td>-</td>
<td>-</td>
<td>33.6 ± 14.3</td>
</tr>
<tr>
<td>Negative affect§</td>
<td>16.2 ± 6.7</td>
<td>14.3 ± 3.5</td>
<td>20.8 ± 9.0</td>
</tr>
<tr>
<td>Behavioral approach</td>
<td>39.3 ± 4.4</td>
<td>35.6 ± 8.3</td>
<td>42.1 ± 5.0</td>
</tr>
<tr>
<td>Impulsivity</td>
<td>59.1 ± 8.7</td>
<td>55.2 ± 7.1</td>
<td>69.6 ± 10.7</td>
</tr>
</tbody>
</table>

Percent Satisfying Dependence Criteria*:

- Stimulants - - - 100% 100% - NS -
- Cocaine - - 67% 41% - NS -
- Amphetamine - - 83% 89% - NS -
- Nicotine - - 73% 74% - NS -
- Alcohol - - 67% 67% - NS -
- Cannabis - - 50% 37% - NS -
- Opiates - - 37% 19% - NS -
- Club drugs - - 10% 7% - NS -
- Sedatives - - 10% 0% - NS -
- Hallucinogens - - 10% 4% - NS -

(methamphetamine, cocaine, or amphetamine-class substances) (DSM-IV). Control subjects were recruited from the community and excluded if dependent on alcohol or other drugs of abuse excluding tobacco. Exclusion criteria for all subjects: depression within the last two months, psychosis, neurological illness, prior head trauma resulting in greater than fifteen minutes loss of consciousness, prior neurosurgery, positive HIV status, diabetes, hepatitis C, bipolar disorder, other major medical illness, inability to tolerate MRI, IQ < 80, positive urine screen (AccuTest™), or positive saliva screen (AlcoScreen™). All participants provided written informed consent approved by the Colorado Multiple Institutional Review Board.
2.3.2. **Structured Interviews for Inclusions and Exclusions**

Composite International Diagnostic Interview-Substance Abuse Module (CIDI-SAM) is a computerized structured interview that assesses substance dependence diagnoses and symptoms for 11 different drugs of abuse (Cottler et al., 1989). All subjects were administered the CIDI-SAM to verify stimulant dependence in the SDI group and to exclude controls with abuse or dependence on substances other than tobacco.

Diagnostic Interview Schedule version IV (CDIS-IV) is a computerized structured interview used to screen for psychiatric disorders (Robins et al., 1995a). All subjects were administered the CDIS-IV to exclude those with lifetime psychoses, lifetime bipolar disorder, or major depressive disorder in the last two months.

The CIDI-SAM interview (Gelhorn et al., 2008) computes four abuse (i.e., legal problems due to drug) and seven dependence (i.e., uncontrolled substance use escalation) symptoms for each drug class. Drug use severity was calculated by adding abuse and dependence symptom counts. This approach is consistent with the single set of clinically relevant criteria in DSM-V, which was released after data collection for the current study.

The Barratt Impulsiveness Scale is a 30-item self-reported questionnaire used to quantify impulsiveness (Patton and Stanford, 1995). Participants rate whether phrases and words describing aspects of impulsivity are self-descriptive. The Behavioral Activation System (BAS) scale is a 13-item self-reported questionnaire used to measure responsiveness of motivational systems (Campbell-Sills et al., 2004; Carver and White, 1994); this quantifies positive affective and approach response tendencies to appetitive stimuli.

2.3.3. **MRI Examination**

Brain MRI was performed using a 3T MR scanner (General Electric, Milwaukee, Wisconsin) and standard quadrature head coil. High resolution T1-weighted SPGR-IR sequences were acquired for each subject using the following parameters: TR=45ms, TE=20ms,
flip angle=45°, 256x256 matrix, 240x240mm² field-of-view (0.9x0.9mm² in plane), 1.7mm slice thickness, and coronal plane acquisition. All images were evaluated by a board-certified neuroradiologist for structural abnormalities. No examinations were excluded on this basis.

2.3.4. Image Processing

T1-weighted brain MR images were processed using the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm8/) and SPM8 (http://www.fil.ion.ucl.ac.uk/spm/) software. Images were segmented into GM, WM, and CSF probability maps anatomically co-registered using Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) (Ashburner, 2007). Custom DARTEL templates were created for our sample population and used to register the images. Segmented images were non-linearly modulated after registration to preserve relative regional volume, after correcting for different brain sizes. Segmented GM probability maps of each subject were visually inspected for quality control by a radiologist; no images were excluded. Normalized, modulated images were smoothed with an 8mm³ full-width at half-maximum Gaussian kernel.

2.3.5. Whole brain analyses

Morphometric analysis

Whole-brain analyses on grey matter volumes (GMV) were performed using a two-way ANCOVA testing for sex by group interactions and main effects of group and sex. All analyses were adjusted for age, education, and head size measured by total intracranial volume. Age did not differ between groups but is an important covariate as it directly affects global GMV (Good et al., 2001). Education differed by group (Table 2-1). Significance levels were set at \( p<0.05 \) corrected for multiple comparisons with family-wise error (FWE) using AlphaSim Monte Carlo simulations (10,000 simulations) and voxel-wise threshold \( p<0.005 \). Cluster threshold corresponded to 1202 voxels (each voxel 3.375 mm³) or 4056.8 mm³. Whole-brain analysis
interpretation was restricted to the supratentorial space given reported methodological
difficulties of infratentorial space segmentation (Diedrichsen et al., 2009).

**Behavioral-morphometric analysis**

Within SDI, whole brain regression analyses examined GMV association with drug use
severity. In exploratory analyses, behavior by sex by group interactions were regressed against
whole brain GMV (M.F.R., radiology resident with two years’ experience studying brain
morphometric methods; J.T., neuroradiology professor with 20 years’ experience studying brain
morphometric methods). Significance was determined using the aforementioned cluster-based
FWE of \( p < 0.05 \) as well as threshold-free cluster enhancement (TFCE) with correction for
multiple comparisons using FWE of \( p < 0.05 \) (Smith and Nichols, 2009). TFCE was used to
evaluate small structures less than \( 4.05 \text{cm}^3 \) such as the nucleus accumbens, which would
otherwise be mathematically precluded from reaching significance (Radua et al., 2014; Smith
and Nichols, 2009).

**2.3.6. Regions of Interest (ROI)**

While whole brain analyses offers statistical robustness, cross-validation with pre-
defined ROIs using prior knowledge improves classification performance (Chu et al., 2012; Kerr
et al., 2014; Nieto-Castanon et al., 2003). Whole brain voxel-level analysis is data-driven
without regard to specific anatomy, while pre-defined ROI analysis is hypothesis driven for
specific neuroanatomical structures based on prior knowledge. To confirm results from whole
brain analyses, five a priori neuroanatomical structures were hypothesized to differ in SDI
compared to controls based on their involvement in reward, learning, executive control, and
affective processing, which are altered in SDI (Ersche et al., 2013; Koob and Volkow, 2010; Li
and Sinha, 2008): OFC, MedFG, ACG, insula, and nucleus accumbens. Masks for these structures
were created using the Automated Anatomic Labeling (AAL) atlas toolbox (Tzourio-Mazoyer et
al., 2002). Total GMV of each structure was calculated by summing voxels in the ROI-masked
structural GM map multiplied voxel-wise by the voxel modulation. GMV in each ROI was analyzed using a two-way ANCOVA on group, sex, and sex by group interactions, after adjusting for age, education, and head size. To correct for multiple comparisons, results were considered significant at FWE $p<0.05$ with Bonferroni correction for five ROIs (pairwise comparison $p<0.01$). In exploratory analyses, GMV in ROI were regressed against drug symptom count. Behavior by sex by group interactions were regressed against ROIs and considered significant with a Bonferroni correction for FWE across all structures ($p<0.05$; pairwise comparison $p<0.01$).

2.4. Results

2.4.1. Demographics, drug severity, and behavioral comparisons

SDI and controls were similar in age and sex (Table 2-1). There was no sex by group interaction on age. There was a sex by group interaction on education ($F_{1,127}=10.936, p<0.001$) and main effects of group ($F_{1,127}=74.914, p<0.001$). Female SDI had fewest years followed by male SDI, male controls, and female controls with the most years of education. SDI had mean 2.2 fewer years of education than control subjects.

Within SDI, there were sex differences in drug use severity ($p=0.015$) with men having greater drug symptom count. There were no sex differences in drug exposure, abstinence duration, or years of drug abuse.

There were significant sex by group interactions in behavioral approach and impulsivity: female SDI had highest approach and impulsivity, followed by male SDI, male controls, and female controls with the least approach and impulsivity.
2.4.2. Whole Brain Analysis

Sex by group interaction

A significant sex by group interaction on GMV was found in widespread areas of cerebral cortex, thalamus, and basal ganglia. To further characterize the interaction, the effect of group was investigated in each sex separately. No GMV differences were observed between control and SDI men. In contrast, large, widespread differences in GMV were observed between control women and SDI women (Figure 2-2). Compared to control women, SDI women had greater grey matter volumes in healthy control women compared to stimulant dependent women, after controlling for age and brain size ($p<0.001$).
significantly less GMV in frontal lobe (OFC, MedFG, superior frontal gyrus), limbic regions (insula, amygdala, cingulate gyrus), temporal lobe (temporal pole, uncus, parahippocampal gyrus, hippocampus, occipitotemporal gyri, superior temporal gyrus, middle temporal gyrus), and inferior parietal lobule. Greater GMV observed in control women compared to SDI women showed anatomical congruence to the sex by group interaction effect.

**Main effect of sex**

A significant main effect of sex was found (Figure 2-3), with women exhibiting comparatively greater regional GMV than men widely throughout cerebral cortex, thalamus, thalamus.

![Figure 2-3. T-value map illustrating statistically significant greater regional grey matter volumes in control women compared to control men, after controlling for age and brain size (p<0.001).](image-url)
and basal ganglia ($p<0.001$). Subgroup analysis demonstrated anatomically similar significant differences between healthy control men and women.

**Main effect of group**

A significant main effect of group was found throughout frontal, temporal, insular, and parietal regions ($p<0.001$). Controls had significantly greater GMV than SDI.

**2.4.3. Whole Brain Correlations with Drug Use Characteristics**

Significant negative correlations were found between drug use severity and bilateral nucleus accumbens GMV (Figure 2-4; left, 290 voxels, MNI coordinates [-9, 4, -2], $p(FWE-corr)<0.0001$; right, 271 voxels, MNI coordinates [8, 10, -6], $p(FWE-corr)<0.0001$). Years of substance use did not correlate with any GMV. Abstinence positively correlated with a small area of left superior frontal gyrus (171 voxels, MNI coordinates [-18, 38, 33], $p(FWE-corr)<0.0001$).
Neither years of substance use, abstinence, or drug use severity were found to have significant sex interactions on GMV.

### 2.4.4. Exploratory Whole Brain Group by Sex by Behavior Interactions

Significant three-way interactions between behavioral approach, group, and sex were seen in the bilateral middle frontal gyri (Figure 2-5, green; Table 2-2). In these areas, in female SDI behavioral approach correlated negatively with GMV, whereas female controls and both groups of men had positive correlation coefficients. Significant three-way interactions between impulsivity, group, and sex were seen in bilateral superior and middle temporal gyri, right insula, right superior temporal sulcus, and right inferior temporal gyrus (Figure 2-5, red; Table 2-2). In these areas in female SDI, impulsivity correlated negatively with GMV, whereas female controls and both groups of men had positive correlation coefficients.

![Figure 2-5. Sex by group by behavior interactions on GMV.](image)

*Left,* scatterplots illustrating the sex by group interaction on the correlation between approach and GMV in the bilateral middle frontal gyri (dorsolateral prefrontal cortex). *Right,* scatterplots illustrating the sex by group interaction on the correlation between impulsivity and GMV in the left superior temporal gyrus and left insula. *Middle,* clusters of whole-brain significance demonstrating sex by group by impulsivity (red) and sex by group by approach (green) interactions.

SDI behavioral approach correlated negatively with GMV, whereas female controls and both groups of men had positive correlation coefficients. Significant three-way interactions between impulsivity, group, and sex were seen in bilateral superior and middle temporal gyri, right insula, right superior temporal sulcus, and right inferior temporal gyrus (Figure 2-5, red; Table 2-2). In these areas in female SDI, impulsivity correlated negatively with GMV, whereas female controls and both groups of men had positive correlation coefficients.
2.4.5. ROI Analysis

Two-way ANCOVA revealed statistically significant sex by group interactions and main effects of group for all structures except nucleus accumbens (Figure 2-6). Post-hoc pairwise
comparisons revealed greater GMV in control women compared to SDI women in total volumes of each significant structure ($p<0.001$) but not between control men and SDI men, consistent with whole brain results.

### 2.4.6. ROI Correlations with Behavior

Drug use severity correlated negatively with nucleus accumbens GMV (Figure 2-4, right). This correlation was driven by SDI women; a steeper and significant negative correlation was seen in women (left: $r=-0.364$, $p=0.047$; right: $r=-0.407$, $p=0.031$) compared to men (left: $r=-0.063$, $p=0.737$; right: $r=-0.174$, $p=0.349$) in which the correlations were not significant. No other correlations between drug characteristics or behavioral metrics were significant. No behavior by sex by group or behavior by group interactions were significant in ROI structures.

### 2.5. Discussion

The current finding of significantly lower GMV in abstinent stimulant dependent women compared to healthy control women is striking for two reasons: (1) no group differences were observed in men, and (2) the involved regions are anatomically vast and overlap substantially with pathways implicated in reward, learning, executive control, and affective processing (Koob and Volkow, 2010). The widespread anatomical extent to which men and women differ in relation to abstinent substance dependence has not been reported. These differences in SDI women compared to men could reflect a greater neuroanatomical endophenotype that predisposes them to stimulant dependence or a vulnerability to morphologic changes that result from stimulant dependence more so in women than men. Decreased GMV in female SDI compared to female controls was most striking in limbic regions, particularly the insula, further suggesting a functional role of these structures in mediating the clinical phenotype.
We expanded upon these structural results with brain-behavioral correlations. Nucleus accumbens volume was negatively correlated with drug use severity, consistent with its role in reward and salience. Previous studies have shown that ventral striatal activity, including nucleus accumbens, correlates with the intensity of received rewards (Sescousse et al., 2013). Persons abusing and dependent upon stimulants undergo pathologic overstimulation of this nucleus and may exhibit compensatory down-regulation of neuronal synapses with reduced dendritic branching, number of axonal boutons, and degree of axon myelination leading to reduced GMV (Draganski and Kherif, 2013; Fields, 2013). The negative relationship observed in this study between drug use severity and nucleus accumbens volume was significant in women but not in men, despite men exhibiting greater drug use severity. This suggests women may demonstrate greater susceptibility to drug use severity changes, possibly through neuroendocrine mechanisms which will be discussed below. Behavioral approach and impulsivity both interacted with group and sex to significantly correlate with GMV (Figure 2-4, Table 2-2). Higher behavioral approach and impulsivity were associated with lower GMV in female SDI. Behavioral approach characterizes level of arousal and response to cues toward favorable outcomes and positive affective states; higher approach motivates behavior. Impulsivity describes decreased inhibitory control over potential actions leading to reward. Previous studies have reported significant sex differences between approach and impulsivity characteristics in SDI (Perry et al., 2013); however, this is the first study to report structural neuroanatomical correlates of these findings. Higher approach in female SDI was correlated with lower GMV in the bilateral DLPFC and may reflect a deficit in top-down control over approach behaviors toward drug cues. The current structural and brain-behavioral relationship differences by sex may result from neuroendocrine factors. For example, sex and ovarian hormones affect the number, density, and firing rate of dopaminergic neurons, with women
showing enhanced dopaminergic system engagement during initial drug exposure and exacerbated negative affective state during drug withdrawal (Becker et al., 2012).

Few studies have investigated sexual dimorphism GMV in stimulant dependence; in fact, only two studies report structural sex differences in SDI: Rando et al. (Rando et al., 2013) and Tanabe et al. (Tanabe et al., 2013). Consistent with our findings, Rando et al. (Rando et al., 2013) observed greater GMV in healthy compared to cocaine-dependent women in the left inferior frontal gyrus, left insula, left superior temporal gyrus, right temporo-occipital cortex, and left hippocampus. Tanabe et al. (Tanabe et al., 2013) observed a differential effect of sex on small regions of the insula. Consistent with our results, they reported that SDI women exhibited smaller insulae compared to controls. However, we report that the differences span nearly the entire insulae bilaterally. Both of these studies had modest patient sample sizes, 36 in Rando and 28 in Tanabe. A meta-analysis performed by Ersche et al. (Ersche et al., 2013) studied 494 stimulant dependent subjects (79% men) and 428 healthy control subjects (69% men) and reported smaller GMV in SDI compared to controls in the insulae, inferior frontal gyrus, ACG, and anterior thalamus; however, this study did not comment on any sex-effects. Other studies of drug effects on brain morphometry exclude women altogether (Barros-Loscertales et al., 2011; Fein et al., 2002; Franklin et al., 2002), pointing to the need for prospective studies to investigate effects of sex. Here we report significant neuroanatomical sexual dimorphism in the largest prospective sex by group sample of long-term abstinent stimulant dependence to date.

The lack of group differences in men was unexpected. Unlike our study, Rando et al. found small differences in men, with lower GMV in a small portion of the precentral and midcingulate gyrus in cocaine-dependent compared to healthy men. There are several possible explanations for this difference, such as recent large alcohol intake (mean 87 drinks in prior month), short length of abstinence (mean 3 weeks), and significantly older SDI than controls in
the Rando population. Our sample had much longer abstinence, mean 13.5 months. It has been reported that GMV “recovery” is associated with sustained abstinence (Connolly et al., 2013). For example, in the Ersche et al. (Ersche et al., 2013) meta-analysis of 494 stimulant dependent subjects, only four of the 13 studies included subjects abstinent for more than one month, with the majority of studies investigating active users. Thus, most studies examined acute drug effects. Because our study included subjects abstinent for at least 60 days, there may have been a “ceiling” effect. This hypothesis is consistent with results from Connolley et al. (Connolly et al., 2013) who found that in men, GMV positively correlated with early abstinence but tapered at 35 weeks to become equivalent to those of drug-naïve controls. Given the average 13.5 months abstinence in our study, GMV recovery may have already reached a steady-state in men by the time of recruitment.

One limitation of this study is the polysubstance use characteristics of the SDI population. While this precludes us from relating structural changes to a single drug, our sample has biological and ecological validity as it reflects an important, real-world, clinical population of SDI. Epidemiological data demonstrate that stimulant dependence does not often occur in isolation; instead most stimulant dependence individuals meet dependence criteria for other substances (Sara et al., 2012; Stinson et al., 2005). Importantly, the GMV differences observed here were not due to differences in drug exposure or symptom severity. Another limitation is that our sample was referred from the justice system and we cannot exclude the possibility that antisocial personality traits contributed to the findings. Third, SDI and controls differed in years of education. Although we statistically covaried for this confounding variable in all analyses it is possible that education could influence the observed differences.

2.6. Conclusion

Vast neuroanatomical changes observed in abstinent SDI were present in women but not in men. In particular, structures involved in reward, learning, executive control, and
affective processing pathways were affected: insula, OFC, ACC, MedFG, and nucleus accumbens. These changes correlate with drug use and behavioral measures and may help to explain differences in the clinical course of stimulant dependence in women compared to men.

2.7. **Acknowledgements**

Several co-authors contributed to this chapter: Manish Dalwani MS, Dorothy Yamamoto PhD, Robert Perry MD, Joseph Sakai MD, Justin Honce MD, and Jody Tanabe MD. This study was supported by the National Institute of Drug Abuse (NIDA) grants DA024104 (JT) and DA 027748 (JT).
CHAPTER III

TOP-DOWN NETWORK EFFECTIVE CONNECTIVITY IN ABSTINENT SUBSTANCE DEPENDENT INDIVIDUALS

3.1. Abstract

This chapter reports resting-state large-scale brain network connectivity changes in a subsample of the population presented in Chapter 2. We hypothesized that compared to healthy controls, long-term abstinent substance dependent individuals (SDI) will differ in their effective connectivity between large-scale brain networks and demonstrate increased directional information from executive control to interoception-, reward-, and habit-related networks. In addition, using graph theory to compare network efficiencies we predicted decreased small-worldness in SDI compared to controls. 50 SDI and 50 controls of similar sex and age completed psychological surveys and resting state fMRI. fMRI results were analyzed using group independent component analysis; 14 networks-of-interest (NOI) were selected using template matching to a canonical set of resting state networks. The number, direction, and strength of connections between NOI were analyzed with Granger Causality. Within-group thresholds were p<0.005 using a bootstrap permutation. Between group thresholds were p<0.05, FDR-corrected for multiple comparisons. NOI were correlated with behavioral measures, and group-level graph theory measures were compared. Compared to controls, SDI showed significantly greater Granger causal connectivity from right executive control network (RECN) to dorsal default mode network (dDMN) and from dDMN to basal ganglia network (BGN). RECN was negatively correlated with impulsivity, behavioral approach, and negative affect; dDMN was positively correlated with impulsivity. Among the 14 NOI, SDI showed greater bidirectional connectivity; controls showed more unidirectional connectivity. SDI demonstrated greater global efficiency
and lower local efficiency. Increased effective connectivity in long-term abstinent drug users may reflect improved cognitive control over habit and reward processes. Higher global and lower local efficiency across all networks in SDI compared to controls may reflect connectivity changes associated with drug dependence or remission and requires future, longitudinal studies to confirm.


3.2. Introduction

Substance dependence is a significant public health problem with an estimated 10.3% lifetime prevalence in the United States (Miller and Hendrie, 2009). Across substances of abuse, a generalizable pattern develops beginning with an initial stage of rewarding effects from occasional use and developing into a pathologic stage of loss of control, escalated use, compulsive drug seeking, and significant negative consequences (Wise and Koob, 2014). Individuals with substance dependence have been shown to exhibit higher levels of impulsivity, behavioral approach, and negative affect (Perry et al., 2013), and these differences have been associated with structural (Regner et al., 2015) and functional (Bell et al., 2014; Hyatt et al., 2012; Krmpotich et al., 2013; Wisner et al., 2013) brain differences compared to healthy controls. While task-based studies using fMRI and PET have contributed significantly to our understanding of functional brain changes in specific neuroanatomical areas (Jasinska et al., 2014), resting-state fMRI (rsfMRI) provides opportunity to explore large-scale networks and network interactions independent of task-specific neuropsychological constructs (Fox and Greicius, 2010). Advantages of rsfMRI include less confounding by differences in task paradigms, correlation of resting state networks (RSN) to specific tasks and neuropsychiatric
constructs (Smith et al., 2009), and reproducibility due to simplified experimental design and data acquisition (Chen et al., 2008).

Stimulant dependence is characterized by complex behaviors and, like other neuropsychiatric diseases, is thought to reflect pathology at the circuit-level rather than a single brain structure (Koob and Volkow, 2010). Moreover, activity and connectivity differences in stimulant dependence have been demonstrated using rsfMRI across disease stages and may explain the progressive behavioral phenotype changes across the natural history of the disorder (Sutherland et al., 2012). For example, active drug addiction stages include (I) binge/intoxication, (II) withdrawal/negative affect, and (III) preoccupation/anticipation (Koob and Volkow, 2010); involved circuits at these stages include (I) ventral tegmental area and striatum; (II) amygdala, bed nucleus of the stria terminalis, and ventral striatum; and (III) prefrontal cortex, hippocampus, basolateral amygdala, cingulate, and insula. Sequential cycling through these active disease stages is hypothesized to result in the neuroadaptive changes that give rise to compulsive drug-seeking and drug-taking (Figure 3-1).

Brain activity and connectivity at different disease stages have been correlated with individual differences in executive function, interoception, reward, and habit formation. For example, Gu et al. (Gu et al., 2010) observed decreased rsfMRI connectivity between nodes within the mesocorticolimbic reward pathway in active cocaine users compared to healthy controls. These findings are consistent with animal models, in which rats dependent upon and self-administering cocaine demonstrated decreased connectivity compared to control rats (Lu et al., 2014); affected pathways in this sample of rats included connections between the dorsolateral prefrontal cortex (PFC) and ventral striatum, as well as between the prelimbic cortex (homologous to anterior cingulate gyrus in humans) and entopeduncular nucleus (homologous to globus pallidus interna in humans) (Lu et al., 2014). These active disease findings stand in contrast to findings in disease remission. In short-term abstinent cocaine
dependence (≥3 days), Wilcox et al. (Wilcox et al., 2011) observed increased rsfMRI connectivity between the ventral striatum and ventromedial PFC. Camchong and colleagues (Camchong et al., 2014) measured resting state functional connectivity amongst reward processing regions in a cohort of stimulant dependent individuals at two time points, 5 weeks abstinence and 13 weeks abstinence, with comparison to a matched healthy control group. Abstinent stimulant dependent patients demonstrated increased functional connectivity compared to controls, consistent with prior studies in patients of 1.4 years of abstinence (Krpmotich et al., 2013) and 5.7 years of abstinence (Camchong et al., 2013). Although Camchong et al. (Camchong et al., 2014) found abstinent stimulant dependent patients demonstrated increased functional connectivity compared to controls, patients who relapsed between time points demonstrated decreased connectivity compared to patients who
maintained abstinence. The authors speculated that this reduction in functional connectivity from 5 to 13 weeks in relapsers compared to abstinent patients may be associated with these patients’ inability to maintain abstinence. These studies suggest that group differences in connectivity may be related to different stages of dependence/remission, possibly representing a transition from hypoconnectivity in limbic and subcortical regions during active dependence to increased top-down executive control in sustained abstinence.

Understanding differences in large-scale brain connectivity depends upon characterizing the relative activity within networks as well as between them. Two modes of functional interactions between brain regions include functional connectivity and effective connectivity. Functional connectivity is the simultaneous and temporally coherent activation of separate brain regions. Effective connectivity characterizes the directional flow of information. One method of characterizing effective connectivity is Granger causality (Seth, 2010), which is methodologically straightforward but requires careful application and interpretation.

To date, no study has investigated the effective connectivity differences in stimulant dependence. This is important because understanding the direction of information flow in large-scale brain networks may further elucidate mechanisms of abstinence and explain previously reported changes. To improve substance dependence treatments, a better understanding of the connectivity characteristics associated with long term remission are needed and may help to predict successful abstinence, evaluate treatment efficacy, and develop novel treatments. This study investigated the effective connectivity and graph theory characteristics of large scale networks in the resting brain in long-term abstinent SDI compared to healthy controls to provide holistic, organ-level measures of brain connectivity and organization for comparisons between groups. We hypothesized that compared to healthy controls, long-term abstinent SDI will demonstrate altered effective connectivity between large-
scale brain networks and increased directional information from executive control to
interoception-, reward-, and habit-related networks.

3.3. Materials and Methods

3.3.1. Sample Population

Fifty substance dependent individuals (SDI) and 50 healthy controls matched in age and
sex were prospectively recruited between October 2010 and June 2013. Demographic
information is reported in Table 3-1. SDI were recruited from a residential treatment program
at the University of

Colorado Denver Addiction Research Treatment Services. Inclusion criteria for SDI were
lifetime DSM-IV psychostimulant dependence (methamphetamine, cocaine, or amphetamine-
class substances) and abstinence from all drugs of abuse for a minimum 60 days, verified
through close supervision and random urine screens. Participants were permitted to have
previously met dependence criteria for substances other than psychostimulants due to the high
prevalence of polysubstance use in people dependent upon psychostimulants. Average
abstinence duration was $12.8 \pm 12.4$ months. Healthy controls were recruited from the
community and excluded if dependent on alcohol or other drugs of abuse except tobacco.
Exclusion criteria for all participants included major depression within the last two months,
psychosis, neurological illness, prior head trauma with loss of consciousness exceeding 15
minutes, prior neurosurgery, HIV, bipolar disorder, other major medical illness, inability to
tolerate MRI, positive urine or saliva screen (AccuTest™, AlcoScreen™), and IQ < 80. All
participants provided written informed consent approved by the Colorado Multiple
Institutional Review Board.
3.3.2. Structured Interviews and Questionnaires

All participants received structured interviews and behavioral measures. Drug dependence was assessed using the computerized Composite International Diagnostic Interview-Substance Abuse Module (CIDI-SAM) (Cottler et al., 1989). IQ was estimated with matrix and verbal reasoning Wechsler Abbreviated Scale of Intelligence subtests (WASI,
Psychological Corporation, 1999) and was recorded to exclude subjects with low scores (IQ < 80). The Diagnostic Interview Schedule version IV is a computerized structured interview used to screen for psychiatric disorders. Participants completed this interview to exclude those with a history of psychiatric disorders as described above. Substance dependence severity was operationalized as the number of total substance dependence and abuse symptoms, quantified by the Diagnostic Interview Schedule version IV (Gelhorn et al., 2008; Robins et al., 1995b).

The Behavioral Inhibition and Activation Scale is a 20-item self-reported questionnaire used to measure responsiveness of motivational systems (Campbell-Sills et al., 2004; Carver and White, 1994). Behavioral approach and inhibition were operationalized as the total Behavioral Activation and Inhibition Scales, respectively.

The Barratt Impulsiveness Scale (BIS-11) is a 30-item self-reported questionnaire used to measure impulsivity; participants rated whether phrases and words describing aspects of impulsivity were self-descriptive (Patton et al., 1995). Impulsiveness was operationalized as the total Barratt score.

Positive and Negative Affect Schedule–Expanded Form (PANAS-X) quantifies a participant’s positive and negative affect using a series of 60 words and phrases that are rated on a scale of self-description (Crawford and Henry, 2004). Positive and negative affect were operationalized as the total PANAS-X score for positive and negative attributes.

3.3.3. MRI Examination and Image Analysis

MRI Acquisition

Brain MRI was performed using a 3T MR scanner (General Electric, Milwaukee, Wisconsin) and standard quadrature head coil. Head motion was minimized using a VacFix head-conforming vacuum cushion (Par Scientific A/S, Odense, Denmark). Any subjects with ≥2 mm of head motion were excluded. High resolution T1-weighted SPGR-IR sequences (TR=45ms, TE=20ms, flip angle=70°, 256 × 256 matrix, 240 × 240mm² field-of-view (0.9 × 0.9mm² in
plane), 1.7mm slice thickness, and coronal plane acquisition) and resting-state functional scans (TR=2000ms, TE=30ms, flip angle=30°, axial acquisition, 64 × 64 matrix, 3.4 mm × 3.4 mm in-plane voxel size, 3mm slice thickness, 1mm gap, 150 volumes) were acquired. During fMRI acquisition, participants were instructed to close their eyes, not think of anything in particular, and not fall asleep.

**Image Preprocessing**

Resting fMRI images were processed using the SPM8 toolbox in MATLAB. The first four volumes of each examination were excluded to avoid saturation effects (Figure 3-2). Standard pre-processing steps included slice timing correction, rigid realignment and motion correction (motion >1 voxel/TR was censored), spatial normalization, and de-noising. Motion parameters (three rotation and three translation parameters) for censorship were calculated for each time-point using corresponding SPM realignment pre-processing values. Anatomical volumes were segmented into gray matter, white matter, and CSF tissue maps, and the resulting binary masks were eroded (1 isotropic voxel) to mitigate partial volume effects. CSF and white matter time
series were obtained using the mean signals from voxels based on eroded CSF and white matter SPM template masks. Mask erosion and time series extraction were performed using functions contained in the CONN toolbox (Whitfield-Gabrieli and Nieto-Castanon, 2012). After linear trends were removed, time series of the motion parameters, WM signal, and CSF signal were removed from the resting-state BOLD data using linear regression, and the resultant residual BOLD time series were band-pass filtered (0.008 Hz < f < 0.15 Hz) (Braun et al., 2012). The resultant filtered time series were spatially smoothed with a 6mm full width at half maximum Gaussian kernel.

Networks-of-Interest (NOI) Definition and Behavioral Correlations

Group independent component analysis (ICA) was performed using the GIFT toolbox as previously reported in the literature (Krmpotich et al., 2013; Tanabe et al., 2011; Tregellas et al., 2011a; Tregellas et al., 2011b) in order to define the networks-of-interest (NOI). For the purposes of this study, the term resting state networks (RSN) refers to the canonical spatial maps used to define the NOI. The term NOI refers to the independent components identified in our sample population and labeled by their corresponding RSN. The dimensionality of the data from each subject was first reduced to 100 components using principal component analysis. Subsequent group-level ICA yielded 34 components, the number of which was determined using the minimum description length (MDL) algorithm (Li et al., 2007). Fourteen canonical RSN templates (Table 3-2) were provided by Stanford’s Functional Imaging in Neuropsychiatric Disorders (FIND) Laboratory (Shirer et al., 2012). At the group level, the 34 identified components were spatially correlated with the canonical RSN templates. Components with the highest spatial correlation to the canonical template were labeled with the corresponding standard RSN label. These labelled components formed the set of NOI for subsequent graph analysis. All components were visually inspected by a neuroradiology fellow (N.S.) and radiology resident (M.R.) independently to confirm accuracy with the canonical RSN templates.
Concordance between inspectors was 100%. Subject-specific spatial maps and time courses were estimated using the GICA back-reconstruction function in GIFT.

For each subject, the strength (or coherence) of each NOI was operationalized as the mean beta value across spatial dimensions for that component in the mixing matrix. These values were regressed against impulsivity, approach, inhibition, and negative affect.

Regression between the NOI strength and subjects’ behavioral metrics were used to interpret the neuroimaging findings within the context of measurable behavioral characteristics.

**Effective Connectivity Analysis**

For each individual, the time courses corresponding to the NOI were obtained from the back-reconstruction function in group ICA. These NOI signals were linear trend removed, normalized to zero mean and unit variance, and band-pass filtered at 0.008-0.15 Hz. The

<table>
<thead>
<tr>
<th>Resting State Network</th>
<th>Symbol</th>
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<tbody>
<tr>
<td>Auditory Network</td>
<td>AN</td>
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<tr>
<td>Anterior Salience Network</td>
<td>aSN</td>
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<tr>
<td>Basal Ganglia Network</td>
<td>BGN</td>
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<tr>
<td>Dorsal Default Mode Network</td>
<td>dDMN</td>
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<tr>
<td>High Visual Network</td>
<td>HVN</td>
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<tr>
<td>Left Executive Control Network</td>
<td>LECN</td>
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<tr>
<td>Language Network</td>
<td>LN</td>
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<tr>
<td>Precuneus Network</td>
<td>PCN</td>
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<tr>
<td>Posterior Salience Network</td>
<td>pSN</td>
</tr>
<tr>
<td>Primary Visual Network</td>
<td>PVN</td>
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<tr>
<td>Right Executive Control Network</td>
<td>RECN</td>
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<tr>
<td>Sensorimotor Network</td>
<td>SMN</td>
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<tr>
<td>Ventral Default Mode Network</td>
<td>vDMN</td>
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<tr>
<td>Visuospatial Network</td>
<td>VSN</td>
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Table 3-2. Canonical RSN included in the analysis and their corresponding symbol abbreviations. Spatial maps were provided by Stanford’s Functional Imaging in Neuropsychiatric Disorders (FIND) Laboratory.
resultant NOI time courses were temporally concatenated across individuals into SDI and control groups (Deshpande et al., 2010a; Ding and Lee, 2013). Effective connectivity between all 14 NOI time courses in the SDI and control graphs were calculated using Granger causality (GC) analysis implemented in the Granger Causal Connectivity Analysis (GCCA) MATLAB toolbox (Seth, 2010). Significance was estimated by comparing observed group difference to a randomized null hypothesis distribution, and the test statistic was determined by the percentile position of the observed difference (i.e., the proportion of randomizations with values greater than or equal to the observed value). To determine null hypothesis distributions, subjects’ group labels were randomized and GC connectivity differences estimated for each randomization permutation until the aggregate randomization distribution achieved statistical stability. Significance level was \( \alpha = 0.05 \) using false discovery rate (FDR) \( q < 0.05 \) to correct for multiple comparisons.

**Global Network Measures**

To provide global network measures, graph theory measures were used to describe the topology of the graphs of NOI. The purpose was to provide holistic, organ-level measures of brain connectivity for comparisons between groups. These measures included total weighted network density, local efficiency (derived from clustering coefficients), and global efficiency (derived from path lengths). These measures’ derivations and their justification have been previously described in detail (Rubinov and Sporns, 2010).

To compute graph theory metrics the GC connectivity matrices were converted to a binary directed adjacency matrix, where connectivity strengths above and below a certain threshold cost level are set to 1 and 0, respectively (Ginestet et al., 2011). The cost threshold, \( K(G_x) \), was calculated by first sorting all elements other than the auto-correlating diagonals (identity axis) of the connectivity matrix in descending rank and keeping only the top \( x \)% (sub-
graph, $G_x$). Cost was then computed as the fraction of highest strength edges above the given threshold divided by the total number of edges:

$$K(G_x) \triangleq \frac{|\mathcal{E}(G_x)|}{|\mathcal{E}(G_{Sat})|}$$

where $G_{Sat}$ represents an edge-saturated network with the same $\mathcal{N}$ and the function $|\mathcal{E}(G)|$ represents the cardinality of $\mathcal{E}(G)$. Therefore, a low value reflects a sparse network. The Brain Connectivity Toolbox (Rubinov and Sporns, 2010) was used to calculate global and local efficiency at each cost level. Since there was no a priori reason to select a particular network cost threshold, connectivity metrics as a function of cost were computed and integrated across the cost domain $[0, 1]$ (Ginestet et al., 2011):

$$\int_0^1 K(G_x)dx$$

This approach abides by prior methodological recommendations to separate network cost from network topology (Ginestet et al., 2011). To test for significant differences between groups, a non-parametric randomized permutation test was performed. Subjects’ group labels were randomized and graph theory measures were calculated for each randomization permutation until the randomization distribution demonstrated statistical stability. These distributions formed the null hypothesis distributions representing no group differences for total weighted network density, integrated global efficiency, and integrated local efficiency. Significance of groups differences for each metric was determined by the percentile position within the null hypothesis distribution.

3.4. Results

3.4.1. Sample Population Demographics

There were no significant differences in age ($p=0.12$) or sex ($p=0.87$) between groups (Table 3-1). Years of education ($p<0.01$) and IQ ($p<0.01$) differed between groups. Controls had
higher IQ and more years of education than SDI. All SDI met DSM-IV dependence criteria for stimulants. Drug use characteristics are also summarized in Table 3-1. Eight controls met dependence criteria for tobacco. No controls met dependence criteria for drugs or alcohol.

3.4.2. Behavioral Metrics

Behavioral characteristics are summarized in Table 3-1. No group difference in BIS inhibition was observed ($p=0.43$). A significant group difference in behavioral approach was observed, with SDI exhibiting higher total BAS scores than controls ($p<0.001$). Further analysis of BAS subscales showed that SDI had higher scores on “drive” ($p<0.001$) and “fun-seeking” ($p<0.001$), but not “reward-responsiveness” ($p=0.42$). As expected, SDI reported higher impulsivity than controls ($p<0.001$) as well as significant differences in the motor, non-planning, and attentional subscales ($p<0.001$). No significant difference in positive affect was observed ($p=0.626$); however, a large difference in negative affect was observed ($p<0.001$), with SDI demonstrating greater negative affect scores than controls.
3.4.3. Network Analysis

Directed Connectivity Analysis

SDI network density was significantly greater compared to controls (p<0.001, Figure 3-3). This measure reflects increased overall mean GC causal connectivity strength between all 14 NOI compared to controls. Specifically, GC analysis results show that among 182 possible between-network pairs (Figure 3-4), only three pairs differed significantly across group in the FDR corrected data (Figure 3-5). Compared to controls, SDI showed stronger effective connectivity from the RECN to the dDMN and from the dDMN to the BGN (Figure 3-6). In addition, SDI showed stronger effective connectivity from the SMN to the VSN. SDI showed...
greater bidirectional connectivity (reciprocal GC connections) whereas controls showed more unidirectional connectivity among the 14 network components.

Figure 3-4. Directed GC matrices for SDI (left) and controls (right). Colorbar corresponds to logarithm of F values.

Figure 3-5. Effective connectivity matrix illustrating the FDR-corrected group differences between SDI and healthy controls. Colorbar corresponds to p-values. White arrows indicate Granger causal direction.
Network-Behavioral Correlations

The strength of the RECN correlated negatively with impulsivity (p<0.001), behavioral approach (p<0.001), and negative affect across the population (p=0.006) (Figure 3-7). In contrast, the strength of the dDMN correlated positively with impulsivity (p<0.001), but not behavioral approach (p=0.034) or negative affect (p=0.030) after correcting for multiple comparisons (Figure 3-7). No NOI correlated with positive affect or educational attainment in years.

Global Graph Measures

Group comparison results for local and global efficiency across the domain of cost functions are illustrated in Figure 3-8. Global efficiency was significantly higher in SDI than in
controls (p<0.01), suggesting greater global integration. Local efficiency was higher in controls (p<0.05), suggesting greater local specialization. These findings in conjunction suggest reduced small-worldness in SDI compared to healthy controls.

3.5. Discussion

This study revealed greater effective connectivity network patterns in abstinent substance dependent individuals compared to healthy controls. Specifically, in drug users who have been abstinent for on average over one year, effective connectivity analysis revealed increased information flow from the RECN to dDMN and dDMN to BGN compared to controls.

Figure 3-7. Correlations between mean beta value within the RECN and dDMN with impulsivity, approach, and negative affect metrics. Solid black lines indicate the linear regression, solid colored lines indicate the 95% confidence interval, and colored shaded regions indicate the prediction interval. Each point reflects a single participant’s mean beta within a given network, and their score on the given behavioral metric.
The areas of increased effective connectivity observed in our study correspond to regions involved in executive control (RECN), interoception (dDMN), reward (BGN), and habit (BGN). The mean strength of the RECN component correlated negatively with impulsivity, behavioral approach, and negative affect. In contrast, the mean strength of the dDMN component correlated positively with impulsivity and trended towards positive correlations with behavioral approach and negative affect. Given the prolonged abstinence of our SDI sample population, these findings are consistent with the hypothesis that successful long-term abstinence is associated with increased top-down cognitive control.

3.5.1. Increased Effective Connectivity from RECN to dDMN

The pattern of increased effective connectivity from RECN to dDMN is consistent with increased top-down executive control in long-term abstinence. Previous work has demonstrated both task-related and resting state hyperactivity within executive control and default mode cortices in abinent stimulant dependence associated with heightened behavioral
monitoring (Connolly et al., 2012; Mayer et al., 2013; Wilcox et al., 2011); however, this is the first study to suggest that these neural signals follow a directional flow of information from RECN to dDMN. Connolly et al. (Connolly et al., 2012) conducted a cognitive control task-based fMRI study of short- (2.4 ± 1.34 weeks) and long-term (69 ± 17.49 weeks) abstinent cocaine addicts. Abstinent cocaine users demonstrated increased activity in PFC, cingulate, and inferior frontal gyri compared to healthy controls. Moreover, short-term abstinent individuals showed right dorsolateral PFC (corresponding to RECN in our study) hyperactivity positively correlating with inhibitory control. Long-term abstinent individuals showed the same finding as well as anterior and mid cingulate (corresponding to part of the dDMN in our study) hyperactivity positively correlating with cognitive errors and heightened behavioral monitoring in abstinence. The present study showed that the RECN strength was negatively correlated with subjects’ impulsiveness, while dDMN strength was positively correlated with impulsiveness. Our results advance our understanding of neural network changes during substance use disorder remission: as abstinence progresses, cortices within the RECN and dDMN may become hyperactive to exert top-down executive control in a directed fashion; this neuroadaptive change may be associated with decreases in impulsivity and increases in inhibitory control.

However, the top-down cognitive control hypothesis is not straightforward because in addition to executive function and cognitive control, affect plays an important role. Albein-Urios et al. (Albein-Urios et al., 2014) showed that short-term abstinent (2.5 ± 5.5 months) cocaine dependent individuals had increased right dorsolateral PFC and bilateral temporoparietal cortex activation during negative emotion experiences without a concomitant increase in the subjective negative experience itself, suggesting an exaggerated neural response in these regions is required to produce normal levels of emotional salience. The regions reported closely resemble by visual comparison the RECN identified by our analysis. Albein-Urios et al. posited that these areas demonstrate increased sensitization toward negative emotions in SDI. If
increased RECN top-down control is a durable feature of long-term abstinence, the literature thus far suggests that its manifestations in human behavior are complex and not reducible to a single neuropsychological construct. Our finding that RECN strength is negatively correlated to negative affect while dDMN strength trended towards positive correlation with negative affect provides further evidence that the top-down control model may involve affective components as well, possibly through reciprocal connectivity with limbic areas.

Right-sided lateralization of our ECN findings is not unexpected given the asymmetric functional specialization of cerebral hemispheres in healthy humans. Cocaine dependent patients exhibit reduced resting state interhemispheric connectivity compared to healthy controls in prefrontal and parietal cortices (Meunier et al., 2012), suggesting increased lateralization of function. Connolly et al. (Connolly et al., 2012) reported that hyperactivity in the inferior frontal gyrus correlated with inhibitory control was greater in the left hemisphere in short-term abstinent individuals and greater in the right hemisphere in long-term abstinent individuals. They hypothesized that a shift from left to right inferior frontal gyrus for inhibitory control may reflect a transition from short-term to long-term abstinence. Our results of increased RECN effective connectivity in long-term abstinent stimulant dependence are consistent with this hypothesis, although future longitudinal studies are required for substantiation.

3.5.2. Increased Effective Connectivity from dDMN to BGN

Although the DMN is incompletely understood, growing evidence demonstrates its roles in internally directed tasks such as spontaneous cognition (Mantini and Vanduffel, 2013), self-referential (Vessel et al., 2013) and autobiographical thought (Buckner et al., 2008), and social understanding of others (Li et al., 2014). We demonstrated increased effective connectivity from the dDMN to BGN; however, this finding must be interpreted in the context of the structures within the NOI identified as BGN (Figure 3-4). This NOI included basal ganglia,
thalamus, amygdala, hippocampus, hypothalamus, midbrain, and pons. Thus, BGN included several key regions of the bottom-up mesocorticolimbic circuit including the ventral tegmental area, nucleus accumbens, amygdala, and striatum.

Prior studies have demonstrated hypoactivity in stimulant users in the dDMN and in BGN as well as decreased connectivity between these networks. In active cocaine users, Tomasi et al. (Tomasi et al., 2015) showed that cocaine cues disengaged fMRI activity in the ventral striatum, hypothalamus, and DMN in proportion to density of striatal dopamine receptors by PET. DMN activation has been shown to predict performance errors, is diminished in active cocaine dependence, and the extent of altered error-preceding activation has been reported to correlate with years of cocaine use (Bednarski et al., 2011). Gu et al. (Gu et al., 2010) used a seed-based fMRI paradigm in active cocaine users and found significantly decreased functional connectivity between multiple regions of the DMN and BGN. McHugh et al. (McHugh et al., 2014) showed that individuals successfully abstinent 30 days after detoxification had stronger functional connectivity between the amygdala, ventromedial PFC, and anterior cingulate cortex compared to those who had relapsed. By visual comparison, these regions correspond to structures within the NOI identified as dDMN and BGN in our study. Connolly et al. (Connolly et al., 2012) demonstrated increased anterior and mid cingulate activity in long-term abstinent compared to short-term abstinent individuals, activity which correlated with heightened behavioral monitoring. Together, these prior studies suggest that DMN activity may change during abstinence. Initial hypoactivation during early abstinence may transition to hyperactivation and increased connectivity with long-term abstinence. One interpretation is that findings of increased effective connectivity from dDMN to BGN in long-term abstinence may be a compensatory mechanism related to behavioral monitoring not seen in active users. However, longitudinal studies are needed to demonstrate this.
3.5.3. Increased Global and Decreased Local Integration

Our findings of increased bidirectional connectivity, increased global efficiency, and decreased local efficiency in long-term abstinent SDI compared to healthy controls suggests pathologically greater global integration and lower local integration in SDI; that is, a connectomic decrease in small-worldness. Similar findings in humans have only been reported using EEG data in 1-3 week abstinent methamphetamine dependent persons. Ahmadlou et al. (Ahmadlou et al., 2013) showed that these patients demonstrated a deviation from small-worldness and increased global hypersynchronization in the gamma frequency band, the EEG band most reactive to cognitive information processing. In contrast, active cocaine users demonstrated less global connectivity compared to healthy controls during a Stroop task; however, after adjusting for individual connectivity, cocaine dependent individuals showed greater intrinsic connectivity in the ventral striatum, putamen, inferior frontal gyrus, anterior insula, thalamus and substantia nigra (Mitchell et al., 2013).

Several animal studies provide important context for the interpretation of our findings. Schwarz et al. (Schwarz et al., 2012) used a pharmacological challenge design which revealed that rats under the acute effects of amphetamine compared to a saline vehicle exhibited less clustering (small-worldness) and increased connectedness within somatosensory, motor, cingulate, prefrontal, and insular cortices. In the rhesus monkey model, active cocaine self-administration was associated with decreased global functional connectivity that selectively affected top-down prefrontal circuits and control behavior while sparing limbic and striatal areas (Murnane et al., 2015). Interestingly, impaired connectivity between prefrontal and striatal areas during abstinence predicted cocaine intake when these monkeys were again provided access to cocaine (i.e., prediction of relapse), consistent with the connectivity pattern associated with relapse in humans as reported by Camchong et al. (Camchong et al., 2014).
Together these findings suggest there is globally decreased connectivity in active users and short-term abstinent with a transition to globally increased connectivity in long-term abstinent users. These findings may improve clinical management if global connectivity patterns can be used to predict abstinence success or trajectory in humans. Future longitudinal studies comparing global connectivity in active, short-term, and long-term abstinent drug users must be performed to address this question. Another approach could involve correlating abstinence duration with global connectivity across individuals, an approach we could not implement due to the group-level nature of our statistical design.

3.5.4. Limitations

Controversy Surrounding Granger Causality

While our study provides several important novel findings, it has limitations. Influences between specialized neural systems exist on a spectrum of temporal lag. Functional connectivity using temporal correlation reflect influences with causal latencies that are below the temporal resolution of the repetition time. These influences are not truly contemporaneous in vivo, but appear so by fMRI as a result of low temporal sampling and temporal blurring induced by the hemodynamic response function. Time-lag based measures such as Granger causality reflect slower influences with greater causal latencies that occur on the order of hundreds of milliseconds, which may provide greater power in predicting cause-effect relationships at the timescale of conscious thought (Tononi et al., 2016).

Neural signals between two nodes may have significantly different physiologic functions depending upon the directionality. As a result, segregating neural influences according to their directionality is necessary in order to properly examine brain function. Methods of examining effective connectivity using fMRI data include structural equation modelling (Buchel and Friston, 1997) and dynamic causal modelling (Friston et al., 2003). These methods require a priori hypotheses describing the theoretical connectivity structure and are limited to models
consisting of a small number of nodes. We used an alternative method, Granger causality, which is based on time-lag regressions and is more data-driven.

Granger causality is increasingly used in fMRI-based neuroscience (Chiong et al., 2013; Cohen Kadosh et al., 2016; Feng et al., 2016; Wen et al., 2013; Zhang et al., 2017) and has been previously applied specifically to independent component analysis as in our study (Demirci et al., 2009; Diez et al., 2015; Ding and Lee, 2013; Stevens et al., 2009; Zhong et al., 2012). However, criticisms of the application of Granger causality to fMRI data have included (Deshpande et al., 2010b): (1) lack of evidence that Granger causality in fMRI-level time series reflects causality in neuronal-level time series, (2) insufficient temporal sampling relative to the timescale of neuronal events, and (3) the possibility that spurious findings may result from systematic differences in hemodynamic response functions. Several recent developments have provided evidence that fMRI Granger causality reliably reflects neuronal causality (Deshpande and Hu, 2012; Deshpande et al., 2010b; Schippers et al., 2011; Wen et al., 2013). Seth and colleagues (Seth et al., 2013) demonstrated that Granger causality is reliably invariant to inter-regional differences in the hemodynamic response function, including the time-to-peak. However, they reported significant effects of temporal resolution on their results. Wen and colleagues (Wen et al., 2013) demonstrated that fMRI-based Granger causality is a monotonic function of neural Granger causality. Importantly, they showed that this relationship can be reliably detected using conventional fMRI temporal resolution and noise levels as was used here. However, they cautioned that differences in the hemodynamic response could lead to spurious results.

The impact of hemodynamic response variability is currently debated. Schippers and colleagues (Schippers et al., 2011) demonstrated that hemodynamic response variability was minimized by multisubject group inference. Statistically, this is intuitive because population averaging will augment systematic differences (e.g., true neuronal differences) while
suppressing random or pseudorandom differences (e.g., hemodynamic response variability). Some authors speculate that HRF variability could be systematic (Smith et al., 2012), and indeed this is a confound that by design exists in the majority of between-group fMRI studies using independent samples (D’Esposito et al., 2003; Hillman, 2014; Murphy et al., 2013). Accordingly, we cannot exclude that systematic differences in the neurovascular response to neural activity between groups may have contributed to our findings.

**Other Limitations**

Network resolution was limited by the manner in which independent component analysis identifies temporally coherent signals across the brain. For example, the network component identified as BGN included several non-basal ganglia structures, such as the thalamus, amygdala, hippocampus, and midbrain. Additionally, concatenation across individuals precluded correlation of individual psychological measures to resting state network Granger causality; as such, correlations between the strength of each NOI and behavioral metrics were used to provide psychological context for the findings. Lastly, polysubstance use and low educational attainment among psychostimulant users may be viewed as potential confounds or representation of real world clinical features. There is significant literature describing the correlation between drug use and low educational attainment; it is debated whether low educational attainment is the cause or result of drug use disorders (Fergusson et al., 2003; Swaim et al., 1997; Yamada et al., 1996). More recently, however, authors have reported that this correlation is due in part to shared genetic factors (Bergen et al., 2008) while others report that it is due to shared environmental or non-genetic familial risk factors (Grant et al., 2012; Verweij et al., 2013). These studies suggest that low educational attainment is a behavioral component of the pathology of substance use disorders. With regard to polysubstance use among SDI, while this limitation prevents our findings from being attributed to a single drug, it strengthens our results by providing biological and ecological validity.
Epidemiologic studies have demonstrated that psychostimulant dependence does not naturally occur in isolation; rather, most patients meet dependence criteria for other drugs of abuse (Sara et al., 2012; Stinson et al., 2005). Our sample population thus reflects the real-world, clinical population of patients with stimulant dependence.

3.6. Conclusion

Increased effective connectivity in long-term abstinent drug users may reflect improved cognitive control and behavioral monitoring (ECN) over self-referential thought (DMN), habit (BGN), and reward (BGN) processes in long-term abstinent drug users. Higher global and lower local efficiency across all networks in SDI compared to healthy controls may reflect connectivity changes associated with drug dependence or remission. Future, longitudinal studies are necessary to definitively characterize connectomic changes across the natural history of substance use disorders.

3.7. Acknowledgements

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4.1. Abstract

In this chapter, we review the literature and provide motivation for our randomized clinical trial of inhibitory TMS targeting the insula in smoker, presented in Chapter 5. Animal and human literature suggest that the insula is necessary for nicotine use disorders. Yet, much remains unknown about how insular function drives nicotine use. Insular subdivisions show distinct patterns of connectivity with large-scale brain networks, and each subdivision is associated with different functions and behaviors. Saliency, including "bottom-up" sensations and "top-down" sensitivity control mechanisms, is the common theme across insular functions. During acute withdrawal, the insula arbitrates bottom-up versus top-down saliency mechanisms to guide behavior – either to pursue smoking or to avoid relapse – and this arbitration is associated with craving and the nicotine withdrawal syndrome. The purpose of this narrative review article is to synthesize neuroimaging evidence of the insula's role in nicotine use disorder and present evidence for suitability as a neuromodulation target to promote cessation. Given the limited efficacy of standard-of-care treatments for nicotine use disorder, insular neuromodulation may contribute to the next generation of cessation treatments by offering what henceforth has not been available: a minimally-invasive, anatomically-driven approach to smoking cessation therapy.

This chapter is currently under review for publication in *Neuroscience & Biobehavioral Reviews*, pending revisions.
4.2. Introduction

Given the serious health problems caused by smoking, it is not surprising that the majority of smokers, nearly 7 in 10 in 2015, want to quit (Babb, 2017). Unfortunately, however, smoking cessation treatments are largely ineffective, with most abstinence attempts failing within the first 24 hours. Approximately 80% of patients relapse by six months, despite combined pharmacologic and behavioral therapies (Tobacco, 2008). Whereas only 1 patient out of 10 receiving behavioral therapy alone remains abstinent, only 2 patients out of 10 receiving pharmacological (e.g. nicotine replacement) and behavioral therapy remain abstinent after six months (Stead and Lancaster, 2012). These low success rates underscore the need for more effective interventions and improved prognostication of individuals most likely to benefit from a given intervention. To achieve these goals, a better understanding of the neural underpinnings of compulsive nicotine use is required.

A significant advance in our understanding of the neurobiology of smoking behavior was made in 2007, when Naqvi and colleagues observed that lesions to the insula disrupt cigarette smoking (Naqvi et al., 2007). These findings and subsequent studies that will be described in greater detail below convincingly demonstrated that the insula plays a critical role in smoking maintenance and cravings, and that the region may be a therapeutic target for smoking cessation. (Abdolahi et al., 2015a, b, 2017; Forget et al., 2010; Naqvi et al., 2007; Pushparaj et al., 2013; Suner-Soler et al., 2012).

The insula is involved in a wide array behaviors and functions, including salience, interoception, awareness, affect, anticipation, uncertainty, self-recognition, prediction error, perception, attention, and cognitive processing (Nieuwenhuys, 2012). Recent meta-analyses of functional MRI studies suggest that the insula contains two to seven functionally-distinct regions (Cauda et al., 2012; Chang et al., 2013; Deen et al., 2011; Kelly et al., 2012; Kurth et al., 2010). Current insula models suggest that the region plays a role in three broad categories of
function. (Deen et al., 2011; Uddin et al., 2014). The dorsal anterior insula is associated with cognitive control functions, such as attention, inhibitory control, and goal-directed cognitive tasks (Dosenbach et al., 2007). The ventral anterior insula is involved in with emotional-limbic functions, including peripheral physiological responses to emotional experiences, as measured by heart rate or galvanic skin response (Mutschler et al., 2009). Finally, the posterior insula mediates sensorimotor-interoceptive functions, and receives rich afferents from spinothalamocortical pathway carrying nociceptive, thermal, and other interoceptive information (Craig, 2002). Despite this functional diversity within insular subunits, several closely-related theories about the functional role of the insula in craving and smoking behaviors have emerged. While differing somewhat in their models and interpretations, the theories all point to involvement of the anterior insula in processing and balancing of “bottom-up” sensations versus “top-down” cognitive control processes (Figure 4-1). In this context, the

![Connectivity-based signal flow diagram of anterior insular control of bottom-up versus top-down mechanisms of salience.](image)

**Figure 4-1.** Connectivity-based signal flow diagram of anterior insular control of bottom-up versus top-down mechanisms of salience. The right dorsal anterior insula is involved in processing salience of externally-oriented stimuli and it is correlated with the executive control network (an externally-directed system). The right ventral anterior insula is involved in processing salience of internally-oriented stimuli and it is correlated with the default mode network (an internally-directed system).
insula may serve as a sensory signal bottleneck, such that insular lesions impaire cognitive processing of craving and nicotine withdrawal sensations.

The following sections of this review describe and discuss: (1) evidence of insular involvement in nicotine use disorder pathophysiology and (2) possible therapeutic strategies to target the region for smoking cessation using neuromodulation, a non-invasive technique capable of altering the function of specific brain regions and networks. Given the limited efficacy of standard-of-care treatments for nicotine use disorder, insular neuromodulation may contribute to cessation treatments by offering a non-invasive, anatomically-driven approach to smoking cessation therapy.

4.3. Insular Role in Nicotine Use Disorder

4.3.1. Introduction: Insular Lesions Disrupt Smoking Behaviors

Converging evidence strongly implicates the insula in the maintenance of smoking behaviors and cigarette craving. Naqvi and colleagues (Naqvi et al., 2007) reported that smokers with cerebrovascular damage to the right insula were able to stop smoking easily without cravings or relapse, supporting a role of the insula in addiction. A subsequent, large, prospective study over a one-year period also found that insular lesions in smokers were strongly associated with becoming a non-smoker (Suner-Soler et al., 2012). Abdolahi and colleagues (Abdolahi et al., 2015b) conducted a prospective cohort study with three-month follow-up in 156 smokers hospitalized for acute ischemic stroke, of which 38 were insular strokes. They reported insular damage was associated with increased odds of three-month continuous abstinence as well as cessation from all nicotine products at three months. Insular damage in the same cohort was also associated with fewer nicotine withdrawal symptoms and cravings compared to those with non-insular strokes (Abdolahi et al., 2015a, 2017). These findings have been corroborated in animal models of nicotine dependence. For example, insular inactivation in rat models significantly reduced nicotine motivation, nicotine seeking-, and
nicotine taking-behaviors, with no effect on food behaviors (Forget et al., 2010; Pushparaj et al., 2013). These findings will be discussed in the context of animal-model neuromodulation in Section 4.4: Implications of Insular Role in Nicotine Use Disorder on Neuromodulatory Therapeutic Development (page 71). These human and animal studies together demonstrate that insular lesions disrupt smoking behaviors and underscore the need to understand insular function in smokers in vivo.

4.3.2. Neuroimaging the Insula in Nicotine Use Disorder

Nicotine exposure causes two distinct sets of effects based on timing: acute states and chronic effects. Acute states refer to the short-term behavioral changes and altered brain function independent of dependency. Chronic effects refer to pharmacologic dependence and the associated neuroadaptations, independent of acute exposure to nicotine. Nicotine’s acute pharmacodynamics are distinguished from the durable neural changes caused by chronic use; that is, nicotine use disorder reflects chronic effects resulting from repeated acute exposure to nicotine. We synthesize the neuroimaging literature into four distinct stages of nicotine use disorder and recovery (Figure 4-2). First, we review studies of the acute effects of nicotine on neurobiology (page 60). Second, we review studies comparing chronic smokers to controls to understand dependence (page 62). Third, we review studies of nicotine-dependent individuals during acute abstinence to understand the mechanisms of the nicotine withdrawal syndrome and craving (page 65). Fourth, we review long-term abstinence as a model of neuroplastic recovery from nicotine use disorder (page 67). Long-term abstinence provides neuroimaging biomarkers of recovery may serve as useful indicators of treatment efficacy. Finally, we attempt to synthesize the findings from these four stages of the disease into a single model (Section 4.3.3: Putting it All Together: Unified Models of the Role of the Insula in Nicotine Use Disorder Pathogenesis, page 68). Our findings are summarized in Table 4-1.
Stage 1: Acute Nicotine Exposure (Neural Pharmacodynamics)

Like other drugs of abuse, nicotine acts on the brain’s reward circuit and induces dopamine release from ventral tegmental area neurons into the nucleus accumbens and prefrontal cortex (Volkow et al., 2012). Nicotine acts as a ligand at nicotinic acetylcholine receptors (nAChRs), a family of ligand-gated ion channels involved in three major circuits: (1) diffusely projecting cholinergic neurons from the brainstem’s ascending arousal system that synapse on dopaminergic neurons in the ventral tegmental area, (2) widely-projecting cholinergic neurons from the basal forebrain (nucleus basalis of Meynert) involved in attention, and (3) fast-acting excitatory post-synaptic potentials in autonomic ganglia associated with
autonomic and visceral sensations (Nestler et al., 2015). Through these molecular mechanisms, acute nicotine exposure affects circuits involved in arousal, reward, attention, and autonomic regulation. Neuroimaging allows scientists to image in vivo the downstream effects of nicotine-induced changes in these circuits.

Table 4-1. Summary of large-scale brain network neuroimaging findings and associated role of the insula across different stages of nicotine use disorder. The neuropharmacologic mechanisms associated with each disease stage are listed in italics.

<table>
<thead>
<tr>
<th>Disease Stage</th>
<th>Drug-Induced Mechanism</th>
<th>Neuroimaging Findings</th>
<th>Role of the Insula</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute Nicotine Exposure</strong></td>
<td><strong>Neural Pharmacodynamics</strong></td>
<td>▼ Default mode network activity (Tanabe 2011, Sutherland 2015) ▲ ECN activity (Sutherland 2015) ▲ SN-DMN-ECN connectivity (Lerman 2014) ▲ Anterior insula activity (Sutherland 2015)</td>
<td>• Mediating a higher-order representation of positive somatosensory, visceral, and interoceptive sensations associated with drug reward</td>
</tr>
<tr>
<td><strong>Chronic Nicotine Exposure</strong></td>
<td><strong>Pharmacologic Dependency</strong></td>
<td>▲ Insulo-cingulate connectivity • Positively associated with: FTND, successful future abstinence • Negatively associated with: incongruent errors on Stroop task, lifetime nicotine consumption (pack-years), future relapse (Janes et al., 2010; Lin et al., 2017)</td>
<td>• New homeostatic set point for visceral sensations associated with drug reward • Cigarette-related memory retrieval (Janes et al., 2015b)</td>
</tr>
<tr>
<td><strong>Acute Abstinence</strong></td>
<td><strong>Nicotine Withdrawal Syndrome</strong></td>
<td>▼ SN-DMN-ECN connectivity (Lerman 2014) ▲ Granger causality from insula to other brain regions (Ding and Lee, 2013) ▲ Right Anterior Insula – DMN connectivity associated with craving magnitude (Moran-Santa Maria et al., 2015)</td>
<td>• Representing the negative somatosensory, visceral, and interoceptive sensations associated with cravings (Abdolahi et al., 2015a, b, 2017)</td>
</tr>
<tr>
<td><strong>Chronic Abstinence</strong></td>
<td><strong>Neuroplastic Recovery</strong></td>
<td>▲ Right anterior insular activation in response to cue exposure, associated with lifetime nicotine consumption (Nestor et al., 2011; Zanchi et al., 2015) ▼ Salience Network Coherence (Zanchi et al., 2015)</td>
<td>• Representing the relative hyper-saliency of drug cues for further monitoring and decision-making • Hyper-saliency of drug cues is associated with insular activity and is durable up to 1 year</td>
</tr>
</tbody>
</table>
Acute nicotine exposure – the nicotine “high” – alters neural activation and connectivity patterns observed by fMRI in both healthy adults and individuals with nicotine use disorder. For example, nicotine administration relative to placebo in non-smokers led to decreased default mode network activity (Hahn et al., 2007; Tanabe et al., 2011) and significantly increased local efficiency of connectivity by whole-brain graph theory analysis, particularly in right-sided limbic and paralimbic areas (Wylie et al., 2012). Sutherland and colleagues (Sutherland et al., 2015) conducted a large Activation Likelihood Estimation (ALE) meta-analysis of acute effects of nicotinic agonists on brain activity changes in smokers as measured by fMRI or PET. The sample population included 796 participants spanning 77 different contrasts and experiments, including the resting state. Compared to placebo, nicotinic agonist administration was associated with decreased activity in the bilateral anterior insulae but mixed effects in the left middle insula. Nicotinic agonists also resulted in significantly decreased activity within default mode network regions and increased activity in executive control network regions. When comparing vehicles of nicotine administration (pure nicotine pharmacologic administration versus cigarette smoking), decreased left middle insula activity was common by conjunction analysis to both manipulations, while decreased right anterior insula activity was specific to cigarette smoking compared to pharmacologic administration. Importantly, this study did not evaluate or control for effects of satiation versus abstinence and heterogeneity of tasks (including resting state), which confound interpretation. Relative to placebo, however, acute nicotine exposure overall consistently leads to decreased anterior insular activity, decreased default mode network activity, and increased executive control network activity.

Stage 2: Chronic Nicotine Exposure in the Cigarette-Sated State (Pharmacologic Dependency)

Several studies have examined individuals with a nicotine use disorder during a resting state fMRI scan. One study found that reduced circuit strength between the insula and dorsal
anterior cingulate cortex, the two principal nodes of the salience network, was associated with increased addiction severity (Moran et al., 2012). These associations were observed when participants were scanned both after smoking or after acute abstinence, suggesting that decreased salience network coherence reflects a chronic effect of nicotine use disorder rather than an acute pharmacologic effect. Salience network coherence has been consistently associated with severity of nicotine use disorder across studies (Bi et al., 2017; Lin et al., 2017; Moran et al., 2012; Wilcox et al., 2017; Zhou et al., 2017). For example, Zhou and colleagues (Zhou et al., 2017) reported that reduced connectivity between the insula and anterior cingulate cortex was associated with increased nicotine use disorder severity. Li and colleagues (Lin et al., 2017) extended these findings, showing that reduced circuit strength between right insula and anterior cingulate cortex was associated with higher number of incongruent errors during a cognitive-control task, implicating this circuit in top-down cognitive control of saliency. More importantly, diminished circuit strength between these regions was associated with greater lifetime nicotine consumption. Overall, these studies provide converging evidence that reduced salience network coherence at rest is a marker of chronic nicotine use and reflects addiction severity.

Insular connectivity may also have prognostic importance related to vulnerability to relapse during future cessation attempts. For example, decreased connectivity between the insula and brain regions involved in cognitive control, including the dorsal anterior cingulate and dorsolateral prefrontal cortices, was associated with greater risk of future relapse after attempted cessation (Janes et al., 2010), possibly reflecting a mechanism of reduced top-down control. In another study, circuit strength between the insula and dorsal anterior cingulate cortex was significantly associated with enhanced smoking cue reactivity in areas involved in attention and motor planning, such as the right ventrolateral prefrontal cortex and dorsal striatum (Janes et al., 2015a). Interestingly, the authors reported that insular-anterior cingulate
connectivity in smokers was durable over a one-hour period and not associated with subjective craving or exhaled carbon monoxide, suggesting that increased salience network coherence may represent a chronic effect (i.e., a neural signature of hypersensitive cue reactivity in nicotine use disorder). Recent, larger studies have corroborated these findings that salience coherence is important in mediating chronic effects of nicotine use disorder. Wilcox and colleagues studied 144 individuals with nicotine use disorder during the resting state and reported that decreased circuit strength between the insula and dorsal anterior cingulate was significantly correlated with higher cigarette consumption (Wilcox et al., 2017). After controlling for addiction severity, increased circuit strength between these regions was associated with greater likelihood of successful abstinence. Similarly, a 10-week longitudinal study (Addicott et al., 2015) found that increased insular connectivity to executive control and striatal regions was seen in non-relapsers (i.e., successful abstainers) compared to relapsers. This suggests lower insular connectivity may be associated with relapse vulnerability. Together, these studies suggest that circuit strength between the insula and both (1) anterior cingulate, and (2) regions involved in cognitive control are not only markers of nicotine use disorder, but are also meaningful for prognosis, since it is associated with ability to quit smoking.

Insula activation during various tasks is also a potentially useful biomarker of nicotine use disorder. For example, Janes and colleagues. (Janes et al., 2017) studied 23 smokers during a cessation attempt, 10 of whom remained abstinent during a two-week follow up. Relative to successful abstainers, smokers who relapsed demonstrated increased right insular activation in response to cigarette cues, suggesting that this activation predicted likelihood of future use. In another study using a subsample of smokers from the Human Connectome Project dataset, individuals who smoked more cigarettes had greater right anterior insular activation in response to viewing faces expressing negative emotions such as anger (Dias et al., 2016). These studies suggest that greater insula activation in response to both smoking cues and emotional
cues may indicate a higher propensity for smoking and relapse. Neuroimaging smokers in nicotine withdrawal and experiencing cigarette cravings provides a possible mechanism for these observations.

*Stage 3: Acute Abstinence (Nicotine Withdrawal Syndrome)*

Acute abstinence in heavy smokers invariably causes the nicotine withdrawal syndrome, characterized by cigarette craving, hedonic dysregulation, cognitive difficulties, and increased negative affect (Jackson et al., 2015). Craving is a negatively reinforcing aspect of nicotine use disorder and is important for conferring relapse vulnerability (Ferguson and Shiffman, 2009). In a longitudinal study of smokers during abstinence, the strength of urges to smoke showed an exponential decline over 12 months of abstinence (Ussher et al., 2013). Six months after cessation, 13% of ex-smokers still reported “strong urges,” but after 12 months, no ex-smokers reported “strong urges,” although 34% reported “some urges.” Since the nicotine withdrawal syndrome and craving in particular are remarkably durable over time, the effects of acute abstinence on brain activity and connectivity could provide insights into nicotine use disorder and its refractoriness to treatment.

Several studies have examined how brain circuits are altered during acute withdrawal, suggesting altered large-scale brain network dynamics between salience, executive control, and default mode networks. For example, a within-subject study of the effect of 24-hour abstinence compared to satiation on resting-state connectivity in smokers demonstrated that abstinence compared to satiety was associated with weaker mutual inhibition between the default mode and salience networks (Lerman et al., 2014). Weaker between-network coupling predicted abstinence-induced cravings to smoke and less suppression of default mode network activity during a working memory task (Lerman et al., 2014). The insula specifically may be involved in causing the altered network connectivity observed in withdrawal. To investigate this, Ding and colleagues (Ding and Lee, 2013) studied 21 heavy smokers in cigarette-sated and abstinent
conditions. After smoking-replenishment, directed connectivity from salience network to default mode network was significantly reduced and directed connectivity from both executive control and default mode networks to the salience network was enhanced. Moreover, the insula showed significantly increased directed connectivity with salience, default mode, and executive control regions in cigarette abstinence compared to satiation. This suggests that directed information flow from the insula to other brain regions is increased in abstinent compared to sated heavy smokers, possibly reflecting increased signaling of withdrawal symptoms and craving. Moran-Santa Maria and colleagues (Moran-Santa Maria et al., 2015) studied acutely abstinent smokers using an fMRI visual craving-cue task. Psychophysiolologic interaction with a seed in the right anterior insula was used to infer directed connectivity. Results demonstrated significantly greater effective connectivity from the right anterior insula to the bilateral precuneus, a key node of the default mode network, during smoking compared to neutral cues. Insula-to-precuneus effective connectivity showed a significant positive correlation with craving magnitude, providing further evidence that this circuit between salience and default mode networks plays an important role in cue-induced craving.

Causal effects of cigarette cues on brain function during acute withdrawal were also investigated by Claus and colleagues (Claus et al., 2013). The investigators studied neural responses to cigarette cues in 116 smokers abstinent for ≥3 hr using a psychophysiological interaction centered on a left dorsal anterior insular seed. Results suggested that smoking cues compared to neutral cues caused stronger connectivity between the left insula and multiple nodes, including right insula (anterior and posterior), amygdala, somatosensory cortex, orbitofrontal cortex, and striatum. In addition, during smoking video exposure significant positive correlations were observed between insula activity and dependence severity; and again, salience network coherence was associated with dependence severity. The authors speculated that the anterior insula may contribute to the initial evaluation of cigarette cue
value, interoceptive processing of withdrawal symptoms, and engagement of motor circuits in preparation for drug-seeking behavior.

Drug expectancy, or prior beliefs about impending acute nicotine administration, has been shown to be a factor that modulates the effects of acute withdrawal. Gu and colleagues (Gu et al., 2016) studied 24 overnight abstinent smokers who performed a sequential reward learning task immediately after a cigarette-smoking intervention. Smokers received either a 0.6 mg nicotine cigarette or a de-nicotinized cigarette and were either told that the cigarette contained “nicotine” or “no nicotine”. All subjects completed all four intervention conditions. Only when smokers received a cigarette with nicotine and were told that it contained nicotine, significant activation in the ventral anterior insula was observed during a reward learning task, which was positively correlated with craving magnitude. This suggests that the anterior insula is not only involved in interoceptive processing, but that anterior insula processing of craving and reinforcement learning is modulated by drug expectancy, presumably through top-down cognitive influences.

*Stage 4: Chronic Abstinence (Neuroplastic Recovery)*

It is unclear whether neural function returns to healthy levels following long-term abstinence, or if the differences associated with nicotine use disorder are durable even after years of abstinence. Few studies have investigated the neuroimaging correlates of long-term abstinence in nicotine use disorder. This is unfortunate, because although chronic nicotine exposure results in upregulation of nicotinic acetylcholine receptors throughout the brain (Breese et al., 1997; Gentry and Lukas, 2002), former smokers exhibit nicotinic acetylcholine receptors concentrations similar to non-smokers (Breese et al., 1997), suggesting that pathologic upregulation is reversible. Similar evidence of neuroplastic recovery is suggested by behavioral changes during chronic abstinence. Measures of impulsivity have been shown to be abnormally elevated in active smokers, but former smokers show levels similar to never-
smokers (Bickel et al., 1999). Studies of ex-smokers thus may provide important insights into the successful maintenance of smoking cessation.

Despite limited literature, several small studies have examined insula connectivity dynamics in chronic abstinence. Zanchi and colleagues (Zanchi et al., 2015) studied non-smokers, active smokers, and ex-smokers during a craving-cue task fMRI scan and reported several findings supporting insular role in recovery. First, ex-smokers with greater right anterior insular activity in response to cigarette cues also had higher lifetime nicotine consumption. Second, ex-smokers demonstrated decreased circuit strength between the right anterior insula and anterior cingulate compared to non-smokers, but no significant difference was observed between ex-smokers and active smokers. This suggests that insular function may not completely recover in long-term abstinence, possibly reflecting a mechanism of persistent craving. Another fMRI study (Nestor et al., 2011) of smokers, ex-smokers, and healthy controls used an attentional bias paradigm with neutral cues, emotionally-evocative cues, and smoking cues. Across all cue conditions, ex-smokers exhibited significantly greater activation in the right anterior insula compared to active smokers and controls. In a separate experiment employing a go/no-go paradigm to investigate motor response inhibition and cognitive error monitoring, the ex-smokers had significantly greater error-related activation than both controls and smokers in the left insula. Taken together, these results suggest that heightened insular monitoring of cues and errors contribute to the successful maintenance of abstinence. Higher right anterior insular activity in ex-smokers compared to healthy controls may reflect a hypervigilance against smoking cues necessary for successful long-term abstinence.

4.3.3. Putting it All Together: Unified Models of the Role of the Insula in Nicotine Use Disorder Pathogenesis

Insular function in the context of nicotine use disorder illustrates this brain region’s important role in higher cognitive function in normal, non-addicted persons. One of the primary
functions of the anterior insula is salience detection (Seeley et al., 2007), such as identifying stimulus features that stand out or are of instinctual or learned importance. Saliency involves the selection of stimuli from a continuous stream of internal and external sensory inputs for additional processing. Another theory is that the anterior insula serves as the “apex of a predictive cortical hierarchy” that spans all sensory systems (Barrett and Simmons, 2015; Chanes and Barrett, 2016), selecting goal-relevant stimuli for attention and cognitive processing. The insula is unique amongst cortical areas in that it contains sequential yet overlapping maps from all exteroceptive and interoceptive senses (Craig, 2009, 2011). These higher-order maps are successively re-represented from posterior insula to middle insula to anterior insula, progressively acquiring additional sensory input maps, interoceptive signals, and reward signals along the way. The anterior insula then provides a single cortical representation of how an individual is feeling at a given time: the “global emotional moment” (Craig, 2009) or “cinemascopic awareness” (Craig, 2011). Despite these slightly differing models and interpretations, the evidence suggests broad involvement of the anterior insula in both polysensory processing and negotiating “bottom-up” sensations versus “top-down” sensitivity control mechanisms of salience. Normal insular physiology thus provides a framework for understanding insular pathophysiology in nicotine use disorders.

Several hypotheses have been proposed to explain the insula’s role in nicotine use disorder. One hypothesis centers on the salience network, comprised of the insula and anterior cingulate cortex. The insula is believed to serve as a toggle, directing brain function towards internal or external stimuli, in order to maintain homeostasis of cognitive resources and guide goal-directed behavior (Sutherland et al., 2012). Internal focus is reflected by greater default mode network (endogenous-oriented) activity, whereas external focus is reflected by greater
The insula's function of toggling between these two networks is hypothesized to be usurped in nicotine use disorder. The concept that the insula directs attention towards the most homeostatically relevant stimuli – internal or external – provides a neurobiological model to explain both cognitive changes and functional connectivity findings of acute nicotine ingestion, nicotine satiety in dependence, and nicotine withdrawal syndrome. This review of the evidence suggests that the critical role of the insula in maintaining nicotine use disorder is related to its function in providing conscious awareness of craving and withdrawal symptoms. Lerman and colleagues (Lerman et al., 2014) provided supporting evidence for this model, reporting decreased
between-network coherence amongst salience, default mode, and executive control networks in abstinence compared to satiety. They reported that weaker between-network coupling predicted abstinence-induced cravings and less suppression of default mode activity during performance of a subsequent working memory task, possibly reflecting a mechanism of cognitive and attentive impairments commonly observed during the nicotine withdrawal syndrome.

In summary, the evidence suggests that insular function is disrupted compared to healthy controls across all stages of nicotine use disorder (Table 4-1). Salience network coherence between insular and anterior cingulate nodes is particularly important for craving-induced behaviors, reflects disease severity, and has prognostic value. However, large-scale longitudinal studies are needed to understand the altered connectivity profiles of the insula with salience, default mode, and executive control regions at different disease stages. Although the evidence is still largely comprised of single-site, small-population studies, it provides a compelling neurobiological argument for future work. In the following section, we explore in detail the possibility of targeting the insula with neuromodulation as a therapeutic strategy for promoting abstinence.

4.4. Implications of Insular Role in Nicotine Use Disorder on Neuromodulatory Therapeutic Development

While pharmacotherapy can be used with some efficacy to diffusely modulate dysregulated brain circuits with the goal of promoting abstinence, new treatment strategies clearly are needed. One possibility is a targeted circuit-node approach, aligned with current understanding of the underlying pathology. Such a neurocircuit-based approach may improve successful cessation by intervening upon or modulating specific neuroanatomical structures that serve as key nodes within behaviorally relevant circuits, such as the insula in the case of nicotine use disorder. A promising candidate for this approach is neuromodulation.
4.4.1. **Therapeutic Neuromodulation in Animal Models of Nicotine Use Disorder**

Aside from systemic pharmacologic approaches, animal models have shown significant benefit of targeted neuromodulation. For example, one study (Forget et al., 2010) reported that insular inactivation via intracranial GABA agonist microinfusion in nicotine-dependent rats significantly reduced nicotine motivation, nicotine seeking-, and nicotine taking-behaviors, with no effect on food behaviors. These findings were further confirmed using an alternative lesioning method, bilateral insular deep brain stimulation, in a rat model of nicotine dependence (Pushparaj et al., 2013). Kutlu and colleagues (Kutlu et al., 2013) extended these findings by showing that locally infused D₁ but not D₂ antagonists into the rostral anterior insular cortex decreased rats’ nicotine self-administration acutely by more than 50%, with repeated D₁ antagonist infusions resulting in continued decreases in consumption without evidence of tolerance. The cause-and-effect relationship between decreased D₁ activity in the insula and decreased nicotine self-administration suggests that mesocorticolimbic dopaminergic afferents onto the anterior insula are critically involved in promoting and maintaining nicotine dependence (Kutlu et al., 2013). Disrupting this insular mechanism leads to diminished nicotine consumption, possibly through diminished interoception of reward (or lack of reward) signals.

4.4.2. **Therapeutic Neuromodulation in Humans with Nicotine Use Disorder**

Transcranial magnetic stimulation (TMS) is a neuromodulation technique that shows promise as a means to target insula function. TMS is a noninvasive intervention in which extracorporeal current-carrying electrical coils are used to induce rapid, transient, focal magnetic fields targeting a specific brain region. These transient magnetic field fluxes cause electromagnetic induction in underlying neural tissues that alter neural transmembrane potentials and in turn affect neural activity. Applying a sequence of TMS pulses causes long-term effects that either facilitate or inhibit neuronal excitability, depending upon multiple
factors, including pulse parameters and stimulation site. Based on studies of the corticospinal motor tract, low-frequency (≤ 1 Hz) repetitive TMS is inhibitory and high-frequency (≥ 5 Hz) repetitive TMS is facilitatory, with aftereffects closely paralleling long-term depression and long-term potentiation mechanisms of neuroplasticity (Hoogendam et al., 2010).

Since 2003, several studies in the English language literature have investigated the role of high- or low-frequency TMS targeting the dorsolateral prefrontal cortex in cigarette craving mitigation (Salling and Martinez, 2016). The focus in the literature on high-frequency TMS of the dorsolateral prefrontal cortex in smokers is likely related to its demonstrated efficacy in major depressive disorder (Brunoni et al., 2017; Milev et al., 2016) and relative accessibility of this region as a superficial target site compared to other, deeper structures. Overall, these studies demonstrate that both single-session and repeated-sessions of TMS to the dorsolateral prefrontal cortex reduces cigarette craving, although in some studies the cigarette consumption effects were mixed, highlighting the need for objective endpoints and response measures (as opposed to self-reported craving measures) in clinical trials.

Given the efficacy of dorsolateral prefrontal cortex neuromodulation in promoting smoking cessation, it stands to reason that other cortical areas involved in nicotine use disorder, such as the insula, may be useful therapeutic targets. Enhancing top-down control of craving-related behaviors has been shown effective. However, this is arguably an indirect method – by augmenting top-down control through excitation of dorsolateral prefrontal cortex, this approach is thought to enhance suppression of bottom-up craving urges. Alternatively, inhibiting the bottom-up craving urges from the cortical source itself may be a more direct and effective treatment. Human stroke and animal neuromodulation studies reviewed here implicate a crucial cause-and-effect role of the insula in maintaining bottom-up craving sensations and nicotine-consuming behaviors. In chapter 5, we report the main results of a randomized sham-controlled clinical trial (www.ClinicalTrials.gov identifier: NCT 02590640) in
active smokers to investigate the efficacy of insular inhibitory neuromodulation in directly reducing cigarette craving at the presumed neural source.

One of the major technical limitations in TMS is the limited spatial depth of electromagnetic induction and its inverse relationship with focality. Theoretical simulations have demonstrated that commercially available figure-of-eight coils can penetrate the cortex 1.0 to 3.5 cm within normal safety parameters (Deng et al., 2013). Across different coil geometries, stimulation of deeper brain targets necessitates greater electrical field spread (reduced focality). This tradeoff between electric field depth and focality poses important physical challenges to stimulating the anterior insula. Moreover, non-target brain stimulation further complicates investigation of behavioral or clinical outcomes associated with deep brain targeting, because adjacent and superficial areas are included in the treatment field and thus may confound observed associations. For example, one study applied continuous TMS to the right anterior insular cortex and control regions (occipital and somatosensory cortices) in healthy volunteers using a superficial (i.e., planar figure-of-eight) coil (Pollatos et al., 2016). Their results suggested that inhibiting the right anterior insula was associated with a significant decrease in cardiac and respiratory interoceptive accuracy (measured by a heartbeat counting task) as well as decreased perceptual confidence. There is debate about the targetability of the anterior insula using conventional superficial coils (Figure 4-4), however, with investigators noting that by using this approach the anterior insula receives about 25% of the maximum cortical energy deposition and greatest deposition in the overlying frontal and temporal opercula (Coll et al., 2017; Pollatos and Kammer, 2017).

Recently, a family of coil designs called Hesed (H) coils have been developed to achieve deep brain neuromodulation at the expense of a wide, relatively non-focal treatment field.
These coils provide near-complete stimulation of the frontal lobes. Dinur-Klein et al (Dinur-Klein et al., 2014) randomized a large sample of 115 heavy smokers to 13 daily treatments of high-frequency, low-frequency, or sham TMS using an H-coil designed to target the bilateral ventrolateral prefrontal cortex, dorsolateral prefrontal cortex, and insula. Smoking was measured by participants' self-report and urine cotinine levels. High-frequency TMS, but not low-frequency or sham TMS, during the presentation of visual smoking cues resulted in a 44% reduction in smoking at 3 months and 33% reduction at 6 months. Counter-intuitively, there was no significant difference in self-reported craving, suggesting that these effects may reflect
enhanced cognitive control rather than reduced incentive salience or reduced sensation of cigarette craving. This suggests that their findings of decreased cigarette consumption after high-frequency stimulation may reflect enhanced dorsolateral prefrontal cortex activity and top-down cognitive control. Malik S et al (Malik et al., 2018) applied excitatory and inhibitory TMS to the bilateral insula and surrounding cortical opercula using the H-coil in eight healthy participants in a within-subject, crossover, blinded, sham-controlled, proof-of-concept study. Synaptic effects were measured using PET with a dopamine agonist tracer. They demonstrated that inhibitory (1 Hz) TMS compared to sham and excitatory (10 Hz) TMS significantly decreased dopamine concentrations in the substantia nigra and sensorimotor striatum, with a trend towards significance in the associative striatum. In both these studies claiming to modulate the insula, the investigators could not definitively confirm that the insular cortex is indeed being stimulated, although future studies using fMRI could address this question.

Neuromodulatory methods not only include TMS, but also include transcranial direct current stimulation (tDCS) and deep brain stimulation (DBS). tDCS involves applying an electrical current to the brain between two electrodes, which affects neural tissues within the path of least electrical resistance. tDCS has been used to target the dorsolateral prefrontal cortex in smokers with reduction in cue-induced cravings; however, the non-focal nature of this method limits its utility in targeted, neuroanatomically-driven neuromodulation (Salling and Martinez, 2016). DBS, on the other hand, involves surgically implanting a stimulating electrode into target brain tissue. DBS targeting the ventral striatum in smokers has been reported in only one study, which reported higher rates of successful cessation compared to unaided smoking cessation in the general population (Kuhn et al., 2009). However, surgically-placed deep brain stimulation is invasive and practically limited by significant ethical considerations.

In summary, applications of non-invasive methods of brain stimulation in nicotine addiction are currently limited by their lack of spatial specificity and depth of targetability.
While dorsolateral prefrontal cortex neuromodulation has been shown to improve abstinence rates and mitigate cravings, other cortical areas such as the anterior insula have broader empirical support and may result in stronger treatment responses. Application of TMS to nicotine use disorder is promising but future studies are needed to define optimal targets, paradigms, and patient population. There is a clear clinical need for better smoking cessation treatments, and growing evidence specifically implicates the insula as a rational neuroanatomical target for investigational modulation therapies.

4.5. Conclusion

The insula is functionally heterogeneous, with distinct patterns of connectivity with large-scale brain networks associated with numerous functions and behaviors. Animal models and human lesion studies suggest that the insula is necessary for the maintenance of nicotine-seeking behaviors and nicotine-taking behaviors, likely through nicotine craving. Given the limited efficacy of standard-of-care treatments for nicotine use disorder, neuromodulation of this region may contribute to the next generation of cessation treatments by offering what henceforth has not been available: a targeted, neuroanatomically-driven approach to smoking cessation therapy. Moreover, because substance use disorders in general – including nicotine use disorder – are thought to be initiated and reinforced by maladaptive alterations in the dopamine reward system and associated corticolimbic and cognitive control circuits, such a targeted neuroanatomically-driven approach may advance treatments for other drug addictions as well. There is a clear clinical need for better smoking cessation treatments. Evidence strongly implicates the insula as a rational neuroanatomical target for modulation therapies. Neuromodulation of insula function has significant potential to improve smoking cessation rates amongst smokers, but continued technical developments and research are needed to overcome challenges in depth and specificity of targeting.
4.6. Acknowledgements

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5.1. Abstract

Cigarette addiction is a leading preventable cause of mortality, morbidity, and healthcare costs. Several lines of evidence suggest that the insula contributes to urges to smoke, and that inhibiting the insula can disrupt cigarette addiction. However, no studies to date have attempted therapeutic insular modulation in people. We hypothesized low-frequency repetitive transcranial magnetic stimulation (LF-rTMS) targeting the right anterior insula would decrease cigarette cravings and brain responses to cigarette cues. We conducted a randomized, single-blind, sham-controlled, phase I clinical trial in which active smokers (n=40) interested in quitting received a single session of either right anterior insula deep LF-rTMS (n=20) or sham treatment (n=20). Primary outcomes included craving measures and cigarette craving cue task fMRI (3T) brain activity responses, measured before and after treatment. Finite element model simulations of energy deposition intracranially informed a priori selection of regions of interest used to corroborate whole-brain results. Compared to sham treatment, right insula LF-rTMS reduced self-reported cigarette craving (p=0.033). Right insula LF-rTMS also decreased brain activity responses to visual cigarette cues at the whole-brain level in primary sensorimotor cortices, supplementary motor area, and right anterior insula (p < 0.001, pvoxel < 0.005, k > 464 voxels). There were no brain regions in which LF-rTMS caused increased activity response to cigarette cues. A single session of right anterior insula deep LF-rTMS reduced cigarette
cravings and brain activity in response to cigarette cues. These findings provide proof-of-concept of a potential neuroanatomical target for smoking cessation therapy.

5.2. Introduction

Cigarette addiction is a leading preventable cause of premature death, morbidity, and healthcare costs (Health and Services, 2014). Each year, approximately 480,000 people in the U.S. prematurely die from smoking-related illnesses. Overall, 1 in 5 U.S. deaths are caused by smoking (Health and Services, 2014). It is estimated that 46 million (~1 in 5) Americans smoke. About half of these smokers attempt to quit each year, with 70% of smokers wanting to quit (Babb, 2017). Unfortunately, standard-of-care smoking cessation treatments are largely ineffective. Approximately 80% of patients attempting to quit relapse within six months, despite combined pharmacologic and behavioral therapies (Tobacco, 2008). Craving is a defining feature of nicotine use disorders and predicts relapse (Potvin et al., 2015; Saunders and Robinson, 2013). For these reasons, exploring novel treatments to reduce craving remains an important objective.

Evidence has implicated the insula in the maintenance of cigarette craving and smoking behaviors. Naqvi and colleagues (Naqvi et al., 2007) reported that a significant proportion of patients with damage to the insula compared to other brain areas were able to stop smoking easily without cravings or relapse. Subsequent prospective studies confirmed that insular lesions in smokers strongly predicted spontaneous continuous abstinence, fewer nicotine withdrawal symptoms, and reduced cravings (Abdolahi et al., 2015a, b, 2017; Suner-Soler et al., 2012). This pattern was also observed in animal experiments using pharmacologic and electrical lesioning of the insula (Forget et al., 2010; Kutlu et al., 2013; Pushparaj et al., 2013). For example, insular lesioning via intracranial GABA-agonist microinfusion in nicotine-dependent rats significantly reduced nicotine motivation-, nicotine seeking-, and nicotine taking-behaviors, with no effect on food behaviors (Forget et al., 2010). However, to date no
studies have attempted to modulate smokers’ insular function in vivo to investigate possible therapeutic benefits.

One promising approach to modulate insular function is through transcranial magnetic stimulation (TMS). TMS is a non-invasive neuromodulation technique that has recently demonstrated early-phase success in promoting smoking cessation. Studies of the corticospinal motor tract have shown that low-frequency repetitive TMS (≤ 1 Hz, LF-rTMS) is inhibitory and high-frequency repetitive TMS (≥ 5 Hz, HF-rTMS) is facilitatory, with aftereffects closely paralleling long-term depression and long-term potentiation mechanisms of neuroplasticity, respectively (Hoogendam et al., 2010).

While no studies to date have investigated insular TMS in smokers, several studies have investigated effects of LF- and HF-rTMS targeting the dorsolateral prefrontal cortex (DLPFC) on cigarette craving and consumption (for reviews, see Kedzior et al. (2018); Makani et al. (2017); Salling and Martinez (2016); Song et al. (2018)). Multiple studies demonstrate that excitatory HF-rTMS to the DLPFC reduces cigarette craving. The mechanism is thought to involve augmenting “top-down” executive control, thereby enhancing cognitive suppression of “bottom-up” craving urges. There are currently no human studies, however, that investigate disrupting this system in smokers by inhibiting “bottom-up” craving urges from the presumed cortical source of craving itself. Similar bottom-up approach has been attempted in cocaine users by targeting cortical reward centers in the orbitofrontal cortex (Hanlon et al., 2017). Hanlon et al. reported that left frontopolar TMS delivered in a single day in cocaine addicts significantly decreased TMS-evoked BOLD signal in the orbitofrontal cortex and insula. Only one study attempted insular modulation in smokers – by targeting the bilateral DLPFC using an H-coil (Dinur-Klein et al., 2014), but the large treatment field precluded distinguishing effects of insular from overlying DLPFC stimulation. A small number of studies have attempted to modulate posterior-superior insular function in healthy participants, reporting changes in
thermal/pain sensation (Ciampi de Andrade et al., 2012; Lenoir et al., 2018) and interoceptive tasks such as a heartbeat counting (Pollatos et al., 2016), although the targetability of the insula is disputed (Coll et al., 2017). Spagnolo et al. targeted the bilateral prefrontal cortices and insulae using the H-coil in healthy individuals and reported no measurable effects on either a blink suppression task or a forced-choice risk-taking task (Spagnolo et al., 2018). No studies to date have selectively targeted the insula in smokers. We sought to address this gap in the literature with the current study.

We conducted a randomized, singled-blinded, sham-controlled, parallel-group phase I clinical trial (www.ClinicalTrials.gov identifier: NCT 02590640) to answer the question: does right insular inhibition in smokers reduce cigarette craving and brain responses to cigarette cues? This proof-of-concept study hypothesized that a single session of LF-rTMS (inhibitory) targeting the right anterior insula in active smokers would acutely decrease cigarette craving and alter brain activation in response to cigarette smoking cues.

5.3. Materials and Methods

This study was reviewed and approved by the Colorado Multiple Institutional Review Board in accordance with the Declaration of Helsinki. All participants provided written informed consent. Recruitment, enrollment, data collection, treatments, and MRI examinations were conducted at the University of Colorado Anschutz Medical Campus. All investigators except the physician administering treatments (MFR) were blinded to group assignment until completion of blinded analysis of primary outcomes, defined as self-reported craving and brain activity responses to cigarette cues.

5.3.1. Study Design

This was a randomized, singled-blinded, sham-controlled, parallel-group, clinical trial in which active smokers interested in quitting received either right anterior insula deep LF-rTMS
or sham treatment. Randomization was performed using a computerized random number generator (http://www.random.org) in blocks of two. A physician trained in TMS and seizure management administered all treatments. Participants were blinded to group assignment and asked if they believed they received real or sham treatment, with their responses recorded.

This study was subject to a protocol deviation. In four participants originally randomized to LF-rTMS, there was insufficient time to measure RMT and apply LF-rTMS between scheduled MRI exams. This timing issue was due to random factors unrelated to the participant’s smoking or physiology; it occurred when there was another research MRI scheduled immediately after the current study’s post-treatment MRI. Subsequent logistical changes to the scheduling of participants obviated this issue (i.e., participants were scheduled in evenings such that there were no exams for other research studies scheduled after the current study’s pre-treatment MRI, allowing for greater flexibility in timing).

5.3.2. Sample Population

Participants were recruited through internet advertisements, publicly-posted flyers, and tobacco cessation consultations at the University of Colorado Hospital Emergency Department and Inpatient Services. Ninety-one healthy, right-handed, treatment-seeking, smokers (≥ 10 cig/d for ≥1 year) between 18 and 55 years old were recruited; forty completed full study appointments (Figure 5-1). Telephone screening included brief medical, substance use, and psychiatric histories to determine eligibility before scheduling the study appointment. Exclusion criteria included [1] use of non-cigarette tobacco products; [2] current use of nicotine replacement therapy, bupropion, or varenicline; [3] major medical disorders; [4] current pregnancy or pregnancy-seeking; [5] active abuse or dependence of illicit substances; [6] MRI or TMS contraindications; and [7] self-reported major psychiatric disorder.

Eligible participants were invited for study appointments. Participants were instructed to maintain their normal smoking habits and not smoke for ≥3 h prior to their study
appointment, confirmed by exhaled carbon monoxide concentration ([CO]_{Exhaled}). Brief abstinence was imposed to establish a baseline state of craving and promote susceptibility to smoking-related visual cues.

At the beginning of the study appointment, each participant provided medical, surgical, and social histories and underwent a brief neurologic examination by a physician (Figure 5-2).

Figure 5-1. CONSORT enrollment diagram for this phase 1 human trial. Careful attention was paid to maintaining all participants’ blinding to group assignment throughout each study visit.

Subjects’ breath was tested for alcohol content to exclude participants acutely under the influence of alcohol. [CO]_{Exhaled} was measured with a MicroSmokelyzer (Bedfont Scientific; Kent, United Kingdom) to confirm acute abstinence ([CO]_{Exhaled} ≤ 10 ppm). A urine sample was
collected for semi-quantitative urine cotinine testing and urine toxicology screening for common drugs of abuse.

**5.3.3. Behavioral Measures**

Each participant completed standardized surveys including the Fagerström Test for Nicotine Dependence (FTND), Wisconsin Inventory of Smoking Dependence Motivations (WISDM), Barratt Impulsiveness Scale, and Behavioral Approach/Inhibition Scales (BAS/BIS). All subjects completed craving assessments using the Questionnaire of Smoking Urges-Brief (QSU-B, 10-items) immediately before the pre- and post-treatment MRI examinations.

**5.3.4. MRI Examination**

MRI exams were acquired before and after treatment at the University of Colorado Brain Imaging Center using a Siemens 3-Tesla Magnetom Skyra scanner (Siemens AG; Munich, Germany) and 20-channel neurovascular coil. Structural images included a T1-weighted 3D magnetization prepared rapid gradient multi-echo sequence (MPRAGE; sagittal plane acquisition; repetition time [TR] = 2300 ms; echo time [TE] = 2.24 ms; inversion time [TI] = 900
ms; echo train length [ETL] = 250 ms; flip angle = 8°; 1 mm slice thickness, 176 slices; FOV = 220 mm with 256 × 256 matrix; time = 5:21 min). Functional images were acquired with a T2*-
weighted echo-planar gradient-echo sequence (GE EPI FID; axial oblique plane acquisition; echo time [TE] = 28 ms; repetition time [TR] = 2000 ms; flip angle = 70°; slice thickness = 3mm with
1mm gap, 32 slices; FOV = 220 mm with 64 × 64 matrix; acquisition time = 6:04 min). Head
motion was minimized with a VacFix head-conforming vacuum cushion (Par Scientific A/S,
Odense, Denmark).

Pre- and post-treatment MRI exams also included resting-state fMRI, ASL, and MR
spectroscopy sequences, reported separately (Figure 5-2).

5.3.5. Cigarette Craving Cue fMRI Task

fMRIs were acquired while participants viewed visual stimuli using fiberoptic binocular
digital goggles. Four “dummy” scans were acquired at the beginning of each run to achieve spin-
history field homogeneity. Stimuli included pseudorandomized, temporally-jittered blocks of
smoking-related and neutral-related cues, separated by crosshair fixation to improve fitting of
generalized linear models. Smoking-cue images included the heads and mouths of people
smoking, people lighting a cigarette, hands holding a cigarette, and cigarettes in ashtrays or in
packs. Neutral images were matched to smoking images with regards to color, complexity, and
form; presence and number of faces and body parts; presence and type of places; and presence
and number of tools (cigarettes and cigarette packs were considered tools). This matching
controlled for activation of specialized cortical areas involved in cognitive processing of faces,
body parts, places, tools, and numbers/counting. Each block lasted 18 seconds and consisted of
four stimuli shown for 4.5 seconds each. Participants were asked to lie quietly while viewing
images and imagine themselves in the specific situations portrayed.
**5.3.6. Determining Resting Motor Threshold**

Resting motor threshold (RMT) was determined by single pulse TMS of the left paracentral lobule (lower extremity motor cortex). The left paracentral lobule was chosen as the motor target because of its greater depth compared to other primary motor cortex targets, comparable to the insula. The coil was positioned over the left paracentral lobule and adjusted the target location until each single pulse TMS triggered muscular contraction. TMS power was then decreased to define resting motor threshold (RMT) defined as the minimal amplitude required to generate at least 4 of 8 stimulations with motor activity with right tibialis anterior electromyography response amplitude greater than 0.5 mV. All participants, including those in the sham-TMS treatment arm, underwent RMT determination.

Four subjects in the treatment group and four subjects in the sham group did not have a reliably measured RMT due to insufficient time between scheduled MRI examinations. This timing issue was due to random factors unrelated to the participant’s smoking or physiology. It occurred only when there was another research MRI exam scheduled immediately after the current study’s post-treatment MRI. Other random factors unrelated to the participant’s physiology also contributed (e.g., participant using the restroom or phone after the first MRI, delay in T1W image transfer). Subsequent scheduling changes described above obviated this issue.

**5.3.7. Right Insular Deep Low-Frequency Repetitive Transcranial Magnetic Stimulation**

Participants’ T1-weighted images were loaded into the BrainSight computer system (Rogue Resolutions; Cardiff, United Kingdom) allowing for precise cortical targeting using real-time infrared-based stereotactic intracranial navigation. Scalp- and pial-surface digital reconstructions were created for each participant. Pointer and coil positions were visualized continuously in real-time relative to surface reconstructions and raw multiplanar T1-weighted images throughout the treatment (displayed on a monitor visible to participant and physician).
Participants and investigators, except the physician who applied treatments, were blinded to the treatment arm.

Right insula LF-rTMS was administered to the right anterior insular second gyrus brevus using a custom-built, 120°-angulated, 80 mm double figure-of-eight coil manufactured by Magstim Ltd (Whitland, Camarthenshire, UK). Targets were selected based on visual inspection of individual participants’ T1-weighted images by a radiologist, and individual target coordinates were recorded in MNI space. Target variability during the treatment was permitted up to ±3 mm in any direction. Treatment $\vec{B}$-field trajectories were orthogonal relative to participants’ scalp-surface. Due to uncomfortable jaw clenching in three subjects, the trajectory angle was manipulated up to 30° relative to the scalp surface orthogonal to mitigate treatment $\vec{B}$-field engagement of the temporalis muscle. LF-rTMS included a single 25 min train of 1500 total 1 Hz pulses at 90% of RMT using the described target and trajectory. All participants completed a post-treatment written survey to [1] document whether they believed they received real or sham treatment, and [2] obtain a post-treatment QSU-Brief craving assessment.

5.3.8. **Sham Treatment**

Sham TMS used a custom-built sham coil designed to look, sound, and feel identical to real treatment. To mimic LF-rTMS, cutaneous electrodes were placed on participants and connected to an electrical amplifier-controller. Cutaneous current was administered for 25 min at 1 Hz to trigger facial muscle contractions and skin sensations. All participants, including those in the LF-rTMS treatment arm, had skin electrodes placed on the scalp to ensure uniformity of participant experience; however, only those in the sham group received current. All participants completed post-treatment written surveys.

5.3.9. **Blinded Analysis**

Upon completion of study enrollment, blinded analysis was performed of craving and fMRI task results. Because only single-blinding could be applied during data collection (i.e., the
physician applying LF-rTMS versus sham was unblinded by necessity), a second blind was applied at the analysis stage to insulate results from potential investigator bias. Blinding of participant labels was applied by a biostatistician uninvolved in the study to this point (AJ); the blinding label key was stored on a secure server to which none of the co-investigators had access. Blinded analyses included: [1] two-way repeated-measures analysis of variance ([LF-rTMS > sham] × [post-treatment > pre-treatment]) performed on QSU-Brief craving measures, [2] visual inspection of motion- and nuisance-variance correction of fMRI on a subject-by-subject basis, [3] main effect of cigarette cues on fMRI activation across groups (cigarette cue > neutral cue contrasts, pre-treatment scans only, one-sample t-test), and [4] interaction effect of group by time on fMRI activity task contrasts ([cigarette cue > neutral cue] × [post-treatment > pre-treatment] × [blinded group A > blinded group B]). The results of these blinded analyses were reviewed with senior co-authors and documented (MFR, JRT, JLT) before unblinding group labels and analyzing all data.

5.3.10. General Data Analysis

After unblinding, all non-imaging data collected on participants was digitally stored in a secure database. Demographic, psychometric, and behavioral comparisons using Satterthwaite two-sample t-tests, Cochran–Mantel–Haenszel statistics, and Fisher’s exact tests were calculated using SAS-based JMP Pro 14.1.0. A two-way repeated-measures analysis of variances (sham versus LF-rTMS × pre- versus post-treatment) was performed on QSU-Brief craving measures. McNemar’s test was performed to assess concordance between participants’ beliefs regarding group assignment with actual group assignment.

Because this proof-of-concept clinical trial was intended to evaluate efficacy rather than effectiveness, we performed per protocol or as-treated analysis (i.e., answering the question “what is the effect of receiving treatment”) for primary outcomes. All analyses are per protocol unless explicitly stated otherwise. Secondary analyses included both intention-to-treat and
sensitivity analyses (i.e., answering the question, “what is the effect of assignment to a given treatment”). Detailed justification of this approach is provided in Section 5.8 - Supplement: Justification of Analysis Approach for Primary and Secondary Outcomes, page 109.

5.3.11. Neuroimaging Signal Pre-Processing

Structural and functional images were pre-processed using MATLAB 2017 and SPM12 software (Wellcome Trust Centre for Neuroimaging; London, UK). T1-weighted images were segmented and normalized to MNI space. BOLD pre-processing included: [1] removal of first four TRs (at acquisition), [2] slice-timing correction, [3] rigid realignment of BOLD images to the first TR, [4] motion scrubbing/censoring (Power et al., 2014), [5] non-neural noise correction using aCompCor (Behzadi et al., 2007; Muschelli et al., 2014), and [6] non-linear deformation into MNI space. Individual TRs with framewise displacement ≥0.3 mm were censored; binary censoring indicators and rigid realignment parameters were included as first-level covariates. Both total number of valid TRs (i.e., non-censored) and mean framewise displacement across each run were included as second-level nuisance covariates. Participants with ≥15% of all TRs censored were excluded; none met this criterion. Brain activation responses induced by cue blocks were modeled at the first level with a box-car function convolved with a double-gamma hemodynamic response function.

BOLD data were visually inspected by three investigators (MFR, JRT, JLT) on a subject-by-subject basis during blinded analysis to document agreement on quality control prior to analysis (Power et al., 2014). For both pre-treatment and post-treatment scans, this included: [1] line plots of realignment parameters, [2] line plots indicating censored TRs, and [3] BOLD signal greyplots before and after nuisance variance correction. Motion- and nuisance-correction appeared appropriate in all subjects; none were excluded on this basis.
5.3.12. Neuroimaging Data Analysis

The main effect of cigarette cues (cigarette cue > neutral cue) at the pre-treatment timepoint across groups (one sample t-test) was computed to inspect effects of visual cue exposure on brain activity.

Effect of treatment group was evaluated using a 2x2 mixed-independence factorial design. Time was the within-subjects factor (pre- versus post-treatment); group was the between-subjects factor (LF-rTMS versus sham). First-level SPM contrasts were constructed for each participant using paired t-tests (post-treatment [cigarette > neutral cues] > pre-treatment [cigarette > neutral cues]). Second-level SPM contrasts were constructed using a two-sample t-test comparing first-level contrasts by group. Images were masked at the second-level using a grey-matter mask with the cerebellum excluded. Whole-brain significance threshold was set at familywise p < 0.05 (AlphaSim-corrected assuming spatial auto-correlation; voxelwise p < 0.005; cluster extent k ≥ 464 voxels, or k ≥ 3712 mm³ ≈ 3.7 cm³) (Cox et al., 2017; Eklund et al., 2016).

5.3.13. Finite Element Method (FEM) Simulation and ROI Selection

ROI selection was based on a finite element method (FEM) head model of induced current density created using SimNIBS v2.1 (www.SimNIBS.org (Bicalho Saturnino et al., 2019; Thielscher et al., 2015; Wang and Eisenberg, 1994; Windhoff et al., 2013)). Finite element method (FEM) is a numerical method for solving differential equations (DEs), which is useful when analytic solutions to these problems are impractical or impossible. Our model treats the application of LF-rTMS to the head as a boundary-value problem governed by DEs describing the electromagnetic potential throughout a space with varying tissue conductivity. Several strengths relevant to neurostimulation simulation include realistic representation of complex geometries, tissue conductivity/anisotropy properties, and estimation of local effects (Opitz et al., 2011).
The mechanism of action of TMS upon neural function is electromagnetic induction through Ampere’s Law, which states that electrical current flowing through a closed-loop induces a magnetic field orthogonal to the flow of current according to the right-hand rule. Ampere’s Law is explicitly defined as:

\[
\mu_0 I_{enc} = \oint_C \mathbf{B} \cdot d\mathbf{l}
\]

where \(\mathbf{B}\) is the magnetic field induced by the applied current \((I_{enc})\), \(d\mathbf{l}\) is an infinitesimal differential vector of the curve \(C\) of applied current, \(\oint_C\) is the closed line integral around the current curve, and \(\mu_0\) is the permeability of free space. What this means is that if you take any imaginary closed-loop path surrounding a current-carrying wire (right side of above equation) and sum the magnetic field at all points along that path, that sum is directly proportional to the current flowing through the wire (left side of above equation), with the constant of proportionality being the permeability of free space.

The complex head and coil geometries, differing tissue conductivities, and voxel-wise differences in anisotropy make an analytic prediction of induced intracranial currents impossible. Thus, we used FEM to numerically estimate expected brain parenchymal currents induced by the TMS coil, and in turn, to estimate an expected spatial dose-response map.

Briefly, the standard five compartment head model (WM, GM, CSF, skull, and skin) was used using a canonical T1W image in MNI space. Tissue conductivities were set to \(\sigma_{\text{skin}} = 0.25\) S/m (average between outer skin and fat as given in Truong et al. (2013)), \(\sigma_{\text{skull}} = 0.01\) S/m (Dannhauer et al., 2011), \(\sigma_{\text{CSF}} = 1.79\) S/m, \(\sigma_{\text{GM}} = 0.276\) S/m, and \(\sigma_{\text{WM}} = 0.126\) S/m (Thielscher et al., 2011). Tissue anisotropy was estimated using a canonical DTI image in MNI space with 32-directionality basis. Final FEM meshes contained about \(5 \times 10^5\) nodes and \(3 \times 10^6\) tetrahedral elements; this discretization has shown satisfactory spatial convergence (Bicalho Saturnino et al., 2019).
The governing DEs for the electric field ($\vec{E}$) used in the FEM are (Bicalho Saturnino et al., 2019; Wang and Eisenberg, 1994):

$$\vec{E} = -\frac{\partial \vec{A}}{\partial t} - \nabla \phi$$  \hspace{1cm} \text{Eq. 2}

$$\nabla \cdot (\sigma_{\text{tissue}} \nabla \phi) = -\nabla \cdot \left( \sigma_{\text{tissue}} \frac{\partial \vec{A}}{\partial t} \right)$$  \hspace{1cm} \text{Eq. 3}

where $\vec{A}$ is the magnetic vector potential of the TMS coil (the curl of which results in the magnetic field proper: $\vec{B} = \nabla \times \vec{A}$) and $\phi$ is the electric potential. Magnetic vector potential ($\vec{A}$) can be conceptualized as momentum per unit charge (in units of V · s · m$^{-1}$) or potential energy per unit element of current (in units of J · A$^{-1}$ · m$^{-1}$). This is analogous to electric potential ($\phi$), conceptualized as potential energy per unit charge (in units of volts, joules per coulomb, or electronvolts per elementary charge). Standard vector calculus operators of dot product ($\cdot$), gradient ($\nabla$), curl ($\nabla \times$), and divergence ($\nabla \cdot$) apply. This model assumes a quasi-static approximation of Maxwell’s equations at low frequencies, meaning that the current as a function of time and the electric field as a function of space can be separated into their own homogenous equations of time and space, respectively (Bicalho Saturnino et al., 2019). This model also assumes homogenous Neumann boundary conditions, meaning that there is no current flow to the outside of the head. The vector potential of the standard 70mm figure-of-eight planar coil was pre-calculated as in (Thielscher and Kammer, 2004) and previously empirically validated (Bungert et al., 2017).

FEM simulation results of the predicted electromagnetic field produced by a standard planar transcranial magnetic stimulation 70mm figure-of-eight coil targeting the right anterior insula are presented below. Note that this energy field deposition estimate is considered conservative, as in our study we used a custom-built angulated coil which theoretically provides a deeper energy deposition field (Deng et al., 2013).
The above figure shows the results of the predicted magnetic potential (left, presented as time-derivative) and normalized absolute value of the electric field (right), illustrating the pattern of energy deposition. Maximum energy is deposited in the scalp, superficial soft tissues, and cerebrospinal fluid due to high tissue conductivities. This illustrates the difficulty in targeting deep structures, such as the insula or anterior cingulate cortex, using conventional coil designs. This is consistent with model results of insular targeting reported by Pollatos and colleagues (Pollatos and Kammer, 2017).

In any numerical method used to solve a system of partial differential equations, two critical questions must be answered: [1] “did we solve the equation?” (i.e., convergence analysis) and [2] “did we solve the correct equation?” (i.e., comparison with experimental observations) (Kheyfets, 2016). First, the FEM method used here has demonstrated good convergence with ≈3% error or less using the free parameters herein specified (Bicalho Saturnino et al., 2019). Second, this method as employed here has also shown good comparison with experimental observation; it has been recently validated against hand electrophysiologic responses in vivo (Bungert et al., 2017).
This model is subject to several limitations. First, we estimated TMS targeting the right anterior insula in a standardized neurological space using population averaged T1W images. Inter-individual differences in gyral anatomy would be predicted to influence the tissue energy deposition (Bicalho Saturnino et al., 2019), and thus subject-specific estimates of current induction would be more accurate. Second, we modelled the treatment using a planar coil with relatively shallow tissue energy deposition, whereas the coil used in this study was angulated and predicted (in spherical head models) to exhibit a deeper treatment field (Deng et al., 2013). Lastly, the FEM model is a numerical estimate. Although it has been validated against hand electrophysiologic responses in vivo (Bungert et al., 2017) and converges at or less than ~3% error with free parameters specified here (Bicalho Saturnino et al., 2019), it has not been validated in a rigorous fashion, such as through using three-dimensional printed head models with tissues of known conductivities or ex vivo in cadaveric brains with neurosurgically-placed depth electrodes.

5.4. Results

5.4.1. Participant Characteristics

Recruitment and randomization of the sample population are illustrated in Figure 5-1. Participant characteristics are provided in Table 5-1. Participant psychometric survey results are provided in Table 5-2.

Four (10% of the sample) participants originally assigned to LF-rTMS were administratively re-assigned (after randomization) due to study appointment timing issues described above. However, participant blinding was maintained. These four subjects reported that they believed they received “real treatment” in their post-treatment survey. There was no significant group difference in the number of subjects who believed that they received real TMS (p=0.205) and the majority believed they received treatment (Table 5-3).
5.4.2. Self-Reported Cigarette Craving

Insular LF-rTMS ($T_{19} = -5.323, p < 0.001$) but not sham ($T_{19} = -1.820, p = 0.084$) caused a significant decrease in cigarette craving. Compared to sham treatment, LF-rTMS of the right insula reduced self-reported cigarette craving ($F_{1,38} = 4.921, p = 0.033$, Figure 5-3).

5.4.3. Neural Activity Responses to Cigarette Cues: Whole Brain Analysis

Cigarette compared to neutral cue exposure caused significant, bidirectional changes in brain activity during the pre-treatment fMRI (Figure 5-4). Brain activity significantly increased in the bilateral dorsolateral prefrontal cortex, bilateral primary visual cortex, and bilateral higher-level visual cortex during cigarette cues compared to neutral cues. At an uncorrected level, brain activity in the bilateral insula was observed, consistent with prior studies (Claus et al., 2013). Brain activity significantly decreased in the bilateral posterior cingulate gyrus and precuneus during cigarette cues compared to neutral cues (Figure 5-4).

Compared to sham, LF-rTMS of the right insula decreased brain responses to visual cigarette cues at the whole-brain level (Figure 5-5). LF-rTMS compared to sham reduced brain
Table 5-1. Sample population demographic and smoking characteristics.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>LF-rTMS</th>
<th>Sham</th>
<th>Whole Sample</th>
<th>Group comparison p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37.5 ± 11.2</td>
<td>41.0 ± 8.7</td>
<td>39.3 ± 10.1</td>
<td>0.278</td>
</tr>
<tr>
<td>Sex by Birth (M/F)</td>
<td>13/7</td>
<td>11/9</td>
<td>24/16</td>
<td>0.518</td>
</tr>
<tr>
<td>Education (years)</td>
<td>13.3 ± 1.9</td>
<td>13.3 ± 1.8</td>
<td>13.3 ± 1.8</td>
<td>0.932</td>
</tr>
<tr>
<td>Ethnicity (self-reported)</td>
<td></td>
<td></td>
<td></td>
<td>0.394</td>
</tr>
<tr>
<td>White / Caucasian</td>
<td>17</td>
<td>14</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Other (Multiple Groups)</td>
<td>3</td>
<td>6</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Handedness (R/L)</td>
<td>18/2</td>
<td>18/2</td>
<td>36/4</td>
<td>1.000</td>
</tr>
<tr>
<td>EtOH (stand. drinks / week)</td>
<td>0.43 ± 1.21</td>
<td>1.25 ± 2.90</td>
<td>0.84 ± 2.23</td>
<td>0.251</td>
</tr>
<tr>
<td>Cannabis User (Y/N)</td>
<td>10/10</td>
<td>11/9</td>
<td>21/19</td>
<td>0.751</td>
</tr>
<tr>
<td>Cigarette Use Characteristics and History</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Last Cigarette (hours)</td>
<td>5.18 ± 3.42</td>
<td>5.1 ± 3.18</td>
<td>5.14 ± 3.26</td>
<td>0.943</td>
</tr>
<tr>
<td>Age of First Cig (years)</td>
<td>14.75 ± 3.35</td>
<td>14.05 ± 4.19</td>
<td>14.40 ± 3.76</td>
<td>0.563</td>
</tr>
<tr>
<td>Age of Daily Cig Use (years)</td>
<td>17.10 ± 2.73</td>
<td>16.68 ± 4.40</td>
<td>16.89 ± 3.62</td>
<td>0.716</td>
</tr>
<tr>
<td>Onset Enjoyment</td>
<td>6.13 ± 3.11</td>
<td>6.50 ± 3.36</td>
<td>6.31 ± 3.20</td>
<td>0.716</td>
</tr>
<tr>
<td>Dependence Duration (years)</td>
<td>19.50 ± 12.19</td>
<td>24.38 ± 10.28</td>
<td>21.94 ± 11.40</td>
<td>0.180</td>
</tr>
<tr>
<td>Current avg cig consumption (#cigs/day)</td>
<td>19.10 ± 6.69</td>
<td>18.70 ± 4.38</td>
<td>18.90 ± 5.58</td>
<td>0.824</td>
</tr>
<tr>
<td>Maximum cig consumption (#cigs/day)</td>
<td>36.60 ± 15.56</td>
<td>31.00 ± 9.68</td>
<td>33.80 ± 13.10</td>
<td>0.181</td>
</tr>
<tr>
<td># Lifetime Quit Attempts</td>
<td>2.50 ± 1.57</td>
<td>2.80 ± 1.91</td>
<td>2.65 ± 1.73</td>
<td>0.591</td>
</tr>
<tr>
<td>Longest Abstinence (days)</td>
<td>287.35 ± 467.13</td>
<td>161.50 ± 321.18</td>
<td>224.43 ± 400.78</td>
<td>0.328</td>
</tr>
<tr>
<td># Cigs Smoked in Last 24h</td>
<td>14.85 ± 7.77</td>
<td>13.00 ± 6.89</td>
<td>13.93 ± 7.31</td>
<td>0.431</td>
</tr>
<tr>
<td># Cigs Smoked in Last Week</td>
<td>122.95 ± 39.50</td>
<td>127.40 ± 29.31</td>
<td>125.18 ± 34.41</td>
<td>0.688</td>
</tr>
<tr>
<td>FTND1</td>
<td>5.15 ± 1.76</td>
<td>5.55 ± 2.26</td>
<td>5.35 ± 2.01</td>
<td>0.538</td>
</tr>
<tr>
<td>[CO]Exhaled2 (ppm)</td>
<td>5.00 ± 1.49</td>
<td>5.90 ± 1.41</td>
<td>5.14 ± 1.50</td>
<td>0.057</td>
</tr>
<tr>
<td>[Cotinine]Urine</td>
<td></td>
<td></td>
<td></td>
<td>0.485</td>
</tr>
<tr>
<td>30-100 ng/mL</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>100-200 ng/mL</td>
<td>5</td>
<td>8</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>200-500 ng/mL</td>
<td>8</td>
<td>6</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>500-1000 ng/mL</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>&gt;1000 ng/mL</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Is cigarette consumptions</td>
<td>13/7</td>
<td>14/6</td>
<td>27/13</td>
<td>0.736</td>
</tr>
<tr>
<td>decreasing in past week? (Y/N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consumption reduction in past</td>
<td>2.60 ± 4.03</td>
<td>1.90 ± 3.34</td>
<td>2.25 ± 3.67</td>
<td>0.553</td>
</tr>
<tr>
<td>week (#cigs/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5-2. Sample population psychometric survey results.

<table>
<thead>
<tr>
<th>Wisconsin Inventory of Smoking Dependence Motives (WISDM)</th>
<th>LF-rTMS Mean</th>
<th>SD</th>
<th>Sham Mean</th>
<th>SD</th>
<th>Whole Sample Mean</th>
<th>SD</th>
<th>Group comparison p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affiliative Attachment</td>
<td>4.04</td>
<td>1.06</td>
<td>4.37</td>
<td>1.03</td>
<td>4.21</td>
<td>1.04</td>
<td>0.323</td>
</tr>
<tr>
<td>Automaticity</td>
<td>4.27</td>
<td>0.96</td>
<td>4.15</td>
<td>1.23</td>
<td>4.21</td>
<td>1.09</td>
<td>0.733</td>
</tr>
<tr>
<td>Loss of Control</td>
<td>4.21</td>
<td>1.20</td>
<td>4.20</td>
<td>1.18</td>
<td>4.21</td>
<td>1.17</td>
<td>0.973</td>
</tr>
<tr>
<td>Behavioral Choice Mieloration</td>
<td>3.80</td>
<td>0.96</td>
<td>4.22</td>
<td>1.01</td>
<td>4.01</td>
<td>1.00</td>
<td>0.184</td>
</tr>
<tr>
<td>Cognitive Enhancement</td>
<td>3.94</td>
<td>1.20</td>
<td>4.03</td>
<td>1.03</td>
<td>3.99</td>
<td>1.10</td>
<td>0.800</td>
</tr>
<tr>
<td>Craving</td>
<td>4.34</td>
<td>1.22</td>
<td>4.30</td>
<td>1.28</td>
<td>4.32</td>
<td>1.24</td>
<td>0.925</td>
</tr>
<tr>
<td>Cue Exposure and Associative Processes</td>
<td>4.29</td>
<td>1.08</td>
<td>4.55</td>
<td>0.86</td>
<td>4.42</td>
<td>0.97</td>
<td>0.397</td>
</tr>
<tr>
<td>Negative Reinforcement</td>
<td>4.38</td>
<td>1.13</td>
<td>4.22</td>
<td>0.82</td>
<td>4.30</td>
<td>0.98</td>
<td>0.615</td>
</tr>
<tr>
<td>Positive Reinforcement</td>
<td>3.93</td>
<td>1.01</td>
<td>4.48</td>
<td>1.02</td>
<td>4.21</td>
<td>1.04</td>
<td>0.047</td>
</tr>
<tr>
<td>Social Environmental Goals</td>
<td>4.14</td>
<td>1.08</td>
<td>4.26</td>
<td>1.04</td>
<td>4.20</td>
<td>1.05</td>
<td>0.711</td>
</tr>
<tr>
<td>Taste Sensory Processes</td>
<td>4.47</td>
<td>1.05</td>
<td>4.33</td>
<td>1.05</td>
<td>4.40</td>
<td>1.04</td>
<td>0.672</td>
</tr>
<tr>
<td>Tolerance</td>
<td>4.17</td>
<td>0.78</td>
<td>4.06</td>
<td>1.21</td>
<td>4.12</td>
<td>1.01</td>
<td>0.735</td>
</tr>
<tr>
<td>Weight Control</td>
<td>4.12</td>
<td>0.95</td>
<td>4.29</td>
<td>1.20</td>
<td>4.21</td>
<td>1.08</td>
<td>0.624</td>
</tr>
<tr>
<td>Total Scaled Score</td>
<td>54.09</td>
<td>9.10</td>
<td>55.46</td>
<td>8.72</td>
<td>54.77</td>
<td>8.82</td>
<td>0.629</td>
</tr>
</tbody>
</table>

Behavioral Approach and Behavioral Inhibition Scales (BAS/BIS)

| BAS Drive                                                | 10.50          | 2.56 | 9.70       | 2.39 | 10.10             | 2.48 | 0.316                  |
| BAS Fun Seeking                                          | 9.50           | 2.54 | 10.15      | 2.62 | 9.83              | 2.57 | 0.431                  |
| BAS Reward Responsiveness                                | 15.20          | 1.91 | 16.00      | 3.03 | 15.60             | 2.53 | 0.324                  |
| BAS Total                                                | 35.20          | 5.18 | 35.85      | 4.50 | 35.53             | 4.80 | 0.674                  |
| BIS Total                                                | 17.95          | 3.25 | 19.20      | 2.48 | 18.58             | 2.93 | 0.180                  |

Barratt Impulsiveness Scale

| Attentional (2nd Order)                                  | 18.55          | 2.98 | 19.80      | 3.55 | 19.18             | 3.30 | 0.235                  |
| Motor (2nd Order)                                        | 27.10          | 4.61 | 28.15      | 4.85 | 27.63             | 4.70 | 0.487                  |
| Non-planning (2nd Order)                                 | 28.90          | 3.73 | 28.50      | 5.01 | 28.70             | 4.36 | 0.776                  |
| Total                                                   | 74.55          | 7.03 | 76.45      | 8.13 | 75.50             | 7.57 | 0.434                  |
Table 5-3. Sample population treatment characteristics, craving results, and beliefs regarding treatment.

<table>
<thead>
<tr>
<th>Treatment Characteristics</th>
<th>LF-rTMS</th>
<th>Sham</th>
<th>Whole Sample</th>
<th>Group diff p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tx Power (% MagStim)</td>
<td>69.95 ± 6.38</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Subject Belief Regarding Treatment</td>
<td></td>
<td></td>
<td></td>
<td>0.205</td>
</tr>
<tr>
<td>Received “real Tx”</td>
<td>18</td>
<td>15</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Received “sham Tx”</td>
<td>2</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

**Craving Scores (QSU-Brief) and Changes**

<table>
<thead>
<tr>
<th></th>
<th>LF-rTMS</th>
<th>Sham</th>
<th>Whole Sample</th>
<th>Group diff p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Treatment</td>
<td>57.70 ± 15.61</td>
<td>59.25 ± 9.18</td>
<td>58.48 ± 12.66</td>
<td>0.704</td>
</tr>
<tr>
<td>Post-Treatment</td>
<td>34.05 ± 13.82</td>
<td>50.30 ± 20.75</td>
<td>42.18 ± 19.25</td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td>Absolute Change (Post−Pre)</td>
<td>-23.65 ± 19.87</td>
<td>-8.95 ± 21.99</td>
<td>-16.30 ± 21.98</td>
<td><strong>0.033</strong></td>
</tr>
<tr>
<td>Relative Change ([Post−Pre]/Pre)</td>
<td>-37.24 ± 29.18</td>
<td>-13.37 ± 38.45</td>
<td>-25.31 ± 35.79</td>
<td><strong>0.034</strong></td>
</tr>
</tbody>
</table>

Figure 5-3. Self-reported cigarette craving (QSU-Brief) by group and time. LF-rTMS causally reduced self-reported craving compared to sham by per protocol two-way repeated-measures ANOVA of group × time (p = 0.033). No statistically significant difference was observed in craving after sham treatment, although there is a trend towards placebo effect (p = 0.084).
Figure 5-4. Pre-treatment main effects of cigarette cue exposure compared to neutral cues, collapsed across groups. Brain activity significantly increased in the bilateral dorsolateral prefrontal cortex, primary visual cortex, and higher-level visual cortex during cigarette cues compared to neutral cues (red color bar). Brain activity significantly decreased in the bilateral posterior cingulate gyrus and precuneus during cigarette cues compared to neutral cues (blue, $p < 0.001$). Results were corrected for multiple comparisons using familywise $p < 0.05$, voxelwise $p < 0.005$, cluster extent $k \geq 464$ voxels.
Figure 5-5. Whole-brain interaction effect using a per protocol 2x2 mixed factorial interaction effect of time (within-subjects: post-treatment – pre-treatment) × group (between subjects: LF-rTMS – sham). Significantly decreased cigarette cue brain response after LF-rTMS compared to sham was observed in the right primary sensorimotor cortex, bilateral supplementary motor cortex (premotor), and right dorsal anterior insula (blue, p < 0.001). No significant increased cigarette cue brain response was observed after LF-rTMS compared to sham. Results were corrected for multiple comparisons using familywise p < 0.05, voxelwise p < 0.005, cluster extent k ≥ 464 voxels.
activity in primary sensorimotor cortices, premotor cortices, and right anterior insula. There were no brain areas in which LF-rTMS compared to sham increased cigarette cue brain activity.

5.4.4. Neural Activity Responses to Cigarette Cues: FEM-Based ROI Analysis

Compared to sham, LF-rTMS of the right insula reduced brain activity responses to visual cigarette cues within the target stimulation ROI. No significant effects were observed in non-target stimulation and non-stimulated control ROIs (Figure 5-6). Brain activity responses to cigarette cues within the non-target stimulation ROI (right inferior frontal gyrus pars triangularis) did not statistically differ by group.

Figure 5-6. FEM-informed ROI analysis of brain activity responses using a per protocol analysis of covariance. ROI beta values and significance levels were extracted using the MarsBar toolbox after accounting for nuisance covariates, using the SPM12 design used for whole-brain analysis.
5.4.5. **Brain-Behavioral Relationships**

At the whole brain level, a significant association was found between change in craving (QSU-Brief) and brain activity responses to cigarette cues within the LF-rTMS group (Figure 5-7). Two clusters demonstrated significance: a large cluster spanning the bilateral perirolandic cortex and supplementary motor areas ($T_{\text{peak}} = 8.39$, $p_{\text{FWE}} < 0.001$) and another in the right anterior insula ($T_{\text{peak}} = 6.01$, $p_{\text{FWE}} < 0.001$). There was no correlation between change in craving and change in brain activity responses within ROIs.

![Figure 5-7. Correlation between absolute change in QSU-Brief self-reported craving (post-treatment $>$ pre-treatment) and change in brain activity responses to cigarette cues ([post-treatment $>$ pre-treatment] $\times$ (cigarette cues $>$ neutral cues)] within the LF-rTMS group defined per protocol. Results were corrected for multiple comparisons using familywise $p < 0.05$, voxelwise $p < 0.005$, cluster extent $k \geq 464$ voxels.](image)

5.5. **Discussion**

In this study, deep low frequency (LF)-rTMS to the right anterior insula in smokers resulted in: [1] decreased cigarette craving, [2] decreased brain responsivity to cigarette cues,

Per protocol analysis found that a single session of deep LF-rTMS targeting the right anterior insula significantly decreased cigarette cravings in the immediate post-treatment time period compared to sham. Evidence suggests that smoking relapse may be associated with decreased cognitive control over insular functions (Janes et al., 2010), and that the insula promotes smoking through increased cravings and attentional redirection towards cigarette-seeking behaviors (Naqvi et al., 2014; Noel et al., 2013). Our interpretation is that the observed craving changes reflect inhibition of “bottom-up” craving signaling from the insula. These results are in contrast to and extend prior studies which augmented “top-down” control over urges in the dorsolateral prefrontal cortex (Amiaz et al., 2009; Dieler et al., 2014; Dinur-Klein et al., 2014; Eichhammer et al., 2003; Huang et al., 2016; Johann et al., 2003; Kozak et al., 2018; Li et al., 2013; Li et al., 2017; Pripfl et al., 2014; Trojak et al., 2015; Wing et al., 2012). It is important to note that LF-rTMS effects on brain activity have primarily been studied from a mechanistic standpoint in the motor system; our findings provide support for the inhibitory aftereffects hypothesis of LF-rTMS action in cortical areas outside the motor system (i.e., associative cortex in addition to primary sensorimotor) (Chen et al., 1997; Miniussi et al., 2013). TMS mechanisms of action, however, remain largely speculative and multifactorial (Cirillo et al., 2017).

Most subjects in both treatment groups believed they received real treatment. This belief pattern did not differ between groups, strengthening our interpretation that the intervention decreased cravings above and beyond placebo effects. While most prior studies in nicotine use disorder and TMS used a blinded RCT design, none assessed participants’ post-treatment beliefs to confirm efficacy of the blinding.
We observed that LF-rTMS centered upon the right anterior insula in smokers reduced brain activity responses in the target stimulation region during the craving cue fMRI task. This supports the theory that the insula’s role in nicotine addiction is related to craving, although it is just possible that LF-rTMS to insula affected other withdrawal sensations involving interoceptive perception (Craig, 2002, 2009, 2011; Nieuwenhuys, 2012) not assessed by this study. While our whole-brain and ROI neuroimaging results provide supporting evidence for corticotopic specificity, the study remains confounded by this issue. Few TMS studies in smokers use fMRI or advanced neuroimaging to measure effects of neuromodulation on smoking-related brain activity. Here, fMRI changes using whole-brain and ROI approaches in conjunction with FEM predictions of the spatial dose-response curve provide evidence of a possible physical mechanism explaining the observed changes in craving.

Cigarette cues resulted in brain activity in bilateral dorsolateral prefrontal cortex, primary visual cortex, and higher-order visual cortex. Our results are consistent with a meta-analysis of craving cue fMRI studies that reported brain activity in the extended visual system in smokers, (Engelmann et al., 2012), a pattern thought to represent excessive incentive salience or increased allocation of attentional resources toward processing of appealing cue stimuli (Engelmann et al., 2012; Robinson and Berridge, 2008). This increased activation in response to cigarette cues was larger by meta-analysis in deprived smokers compared to sated – possibly suggesting effects of craving. Similar to our results, increased brain activity responses to cigarette cues have been reported in the prefrontal cortex, which may reflect a preparation to initiate drug-seeking behaviors (Claus et al., 2013; Engelmann et al., 2012). We also observed decreased main effects of cue in the bilateral posterior cingulate gyrus and precuneus, which appears in contrast with reported studies. We cannot confidently explain this result, although one possibility is that most previous studies compared cigarette cues to matched food cues,
which are presumably hedonically relevant, while we compared to matched neutral cues that contained no clear hedonic relevance.

Our observation of decreased brain activity responses in the right primary sensorimotor cortex and right premotor cortex after LF-rTMS was unexpected. Since these areas are anatomically distant from the stimulation field, it suggests a possible confounding stimulation effect related to facial sensations/muscular twitches or a corticocortical effect in the sensorimotor system secondary to insular changes. Participants undergoing LF-rTMS and sham both experienced mildly unpleasant “shocking” sensations in right facial soft tissues and involuntary right jaw clenching. However, we expect that if these sensations resulted in confounding brain activity, it would be represented in the contralateral cerebral hemisphere. Another possibility is that the right sensorimotor and premotor findings represent indirect corticocortical-association fiber effects from the ipsilateral posterior insula, which is known to be highly connected to sensorimotor areas (Craig, 2002, 2009, 2011; Nieuwenhuys, 2012). Alternatively, the finding may represent a decreased cognitive preparedness (supplementary motor area) or representation (primary sensorimotor) to act upon craving cues.

This study addresses a gap in the literature on the feasibility and efficacy of insular TMS targeting in smokers. This gap is surprising, given substantial evidence from animal (Forget et al., 2010; Kutlu et al., 2013; Pushparaj et al., 2013) and human (Abdolahi et al., 2015a, b, 2017; Naqvi et al., 2007; Suner-Soler et al., 2012) studies suggesting that the insula plays a major role in nicotine use disorder. One reason for this gap is the biophysical limitations of current TMS coil technology. We address this using a novel angled coil and calibrated the RMT to the paracentral lobule. We then transiently lesioned the anterior insula in smokers to reproduce the previously reported insula effects for possible therapeutic benefit.

This study has several strengths. There are several prospective, blinded, randomized controlled trials of DLPFC targeting in smokers. This RCT design, applied to a novel target,
theoretically allows for causal inference. Participant-blinding was maintained and demonstrably validated. Blinding of primary outcomes analysis by a biostatistician uninvolved in the study provided an additional safeguard against potential bias. Most participants believed they received “real treatment.” There were no significant group differences in this belief, demographics, smoking history, or psychometrics. Nevertheless, in caution and because our primary analysis was per protocol rather than intention-to-treat, we present our inferences as hypothesis-generating rather than hypothesis-testing.

This study is limited in several ways. First, four of 40 total participants (10%) were re-assigned post-randomization due to study appointment timing issues. This placed the study at risk for performance bias by Cochran criteria. It is possible that this biased the study, and thus the study must be replicated. For example, if the re-assigned participants had systematically higher RMTs, we would expect the treatment effects to be overestimated. If the re-assigned participants had systematically lower RMTs, we would expect the treatment effects to be underestimated. Second, whether LF-rTMS can directly modulate insular activation is controversial (Coll et al., 2017; Pollatos et al., 2016; Pollatos and Kammer, 2017). We believe that by selecting RMT within the deep paracentral lobule (foot) as opposed to the superficial “knob” precentral gyrus (hand) and by using a custom-built angled coil designed for deeper targeting, it is reasonable to conclude the insula received subthreshold energy deposition sufficient to affect neural function. Third, in the absence of a comparison cortical stimulation (e.g., dorsolateral prefrontal cortex or medial frontal cortex), the corticotopic specificity of our findings to the insula is unknown. It could be that decreased craving results from LF-rTMS to any brain area without regional specificity. Even if rTMS neural aftereffects are assumed to spatially match predicted energy deposition, we cannot prove that targeting right anterior insula specifically caused our observations. For example, the right inferior frontal gyrus, involved in inhibitory control, was subjected to greater energy deposition compared to insula
using our treatment paradigm. However, if we assume the aftereffects of LF-rTMS are inhibitory, we would expect the effects on a major inhibitory center to cause cognitive disinhibition, increased cigarette craving, and increased brain activity responses. Lastly, the small sample size limited statistical power. While the per-protocol effects were significant, the intent-to-treat effects was not, suggesting that the approach may work but requires replication in a larger sample.

5.6. Conclusion

In conclusion, per-protocol analysis showed that direct inhibition of the right anterior insula reduces craving, supporting previous evidence that insular lesions disrupt craving. We further demonstrated that LF-rTMS targeting the insula reduced insular activity responses to cigarette cues. It remains unclear if this would disrupt smoking behaviors and cigarette consumption with repeated treatments, which should be investigated. Future studies using a single-day appointment design must carefully consider the timing between pre- and post-treatment MRI examinations, if the study uses an MRI scanner shared between multiple research groups. This issue could be easily obviated in a longitudinal study, which we hope to pursue in a future grant-funded study. While these results should be considered preliminary, they provide hope that TMS could be developed as a treatment strategy to help reduce the burden of cigarette addiction.

5.7. Acknowledgements

This work was funded by NIH F32 DA041011 (MFR), RSNA RR-1620 (MFR), CCTSI M-15-81 (MFR), NIH R01 GM12108 (DHG), NIH R25 GM111901(DHG), and NIH R25 GM111901S1 (DHG). The authors would like to thank MRI technologists Deb Singel and Kendra Huber for their assistance with data collection. The authors would like to thank Dr. Isabelle Buard, Dr. Peter DeWitt, and Dr. Joshua Gowin for helpful discussion and support. Finally, the authors
would like to thank the anonymous study participants, who generously contributed to scientific research.

5.8. **Supplement: Justification of Analysis Approach for Primary and Secondary Outcomes**

<table>
<thead>
<tr>
<th>Sample Size (Tx/Sham)</th>
<th>Primary Analysis (As Treated)</th>
<th>Secondary Analysis (Intention-to-Treat)</th>
<th>Secondary Analysis (Sensitivity Analysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 40 (20/20)</td>
<td>n = 40 (24/16)</td>
<td>n = 36 (16/20)</td>
</tr>
<tr>
<td>Self-Reported Cigarette Craving (QSU-Brief)</td>
<td>( F_{1,38} = 4.921, p = 0.033 )</td>
<td>( F_{1,38} = 0.715, p = 0.403 )</td>
<td>( F_{1,38} = 2.837, p = 0.101 )</td>
</tr>
<tr>
<td>Whole-brain response to cigarette cues</td>
<td>( p &lt; 0.001 )</td>
<td>( p = 0.293 )</td>
<td>( p = 0.096 )</td>
</tr>
<tr>
<td>FEM-Informed ROI Analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right Insula</td>
<td>( T = 2.45, p = 0.029 )</td>
<td>( T = 1.15, p = 0.387 )</td>
<td>( T = 1.99; p = 0.081 )</td>
</tr>
<tr>
<td>Right IFG-PT</td>
<td>( T = 2.02, p = 0.074 )</td>
<td>( T = 0.83, p = 0.618 )</td>
<td>( T = 1.10, p = 0.363 )</td>
</tr>
<tr>
<td>Left SMG</td>
<td>( T = 0.15, p = 0.825 )</td>
<td>( T = -0.62, p = 0.809 )</td>
<td>( T = -0.39, p = 0.958 )</td>
</tr>
</tbody>
</table>

The National Institutes of Health Office of Extramural Research defines a clinical trial as Phase I if it “tests a new biomedical intervention in a small group of people (e.g., 20-80) for the first time to determine efficacy and evaluate safety.” (NIH) Because this proof-of-concept, phase I clinical trial was intended to evaluate efficacy rather than effectiveness, we performed an as-treated or per protocol analysis (i.e., answering the question “what is the effect of receiving treatment”) for primary outcomes (Spieth et al., 2016). In addition, we sought to provide meaningful inferences regarding the safety, tolerability, and dose-response characteristics of LF-rTMS targeting the insula in smokers. Secondary analyses included both intention-to-treat and sensitivity analyses (i.e., answering the question, “what is the effect of assignment to a given treatment”). In order to demonstrate proof-of-concept, we elected to focus on efficacy (i.e., an explanatory trial), evaluating whether the intervention produces the expected results under ideal circumstances. This is in contradistinction to effectiveness (i.e., a pragmatic trial), which evaluates the degree of beneficial effect under “real world” clinical settings (Gartlehner et al., 2006).
This study was exposed to a potential performance bias in group assignment, which occurs if there is insufficient adherence to the study protocol by either the participant or the investigator (Spieth et al., 2016). Four subjects in the treatment group and four subjects in the sham group did not have a reliably measured RMT due to insufficient time between scheduled MRI examinations. This timing issue was due to random factors unrelated to the participant’s smoking or physiology. It occurred only when there was another research MRI exam scheduled immediately after the current study’s post-treatment MRI. Other random factors unrelated to the participant’s physiology also contributed (e.g., participant using the restroom or phone after the first MRI, delay in T1W image transfer). Subsequent scheduling changes described above obviated this issue.

Because LF-rTMS could not safely be applied to four patients originally randomized to the treatment group prior to their post-treatment scan, they were administratively re-assigned post-randomization to the sham group by judgement of the study physician. Careful attention was paid to maintaining the subject blinding throughout the entire study appointment, and post-treatment surveys of participants’ beliefs regarding whether they received real or sham treatment provided interval validity of the blinding. It is possible that this re-assignment of four participants biased the study, and thus the study must be replicated. For example, if the re-assigned participants had systematically higher RMTs, we would expect the treatment effects to be overestimated. If the re-assigned participants had systematically lower RMTs, we would expect the treatment effects to be underestimated. Of note, group-differences comparison across demographic, smoking use/history, and psychometric characteristics suggest an absence of overall differences between groups.

We observed that in both secondary analyses (sensitivity analysis excluding subjects with post-randomization reassignment and intention-to-treat analysis labelling participant group labels by initial allocation rather than actual treatment) the directions of associations
were unchanged, although the statistical significance was no longer observed. This pattern may reflect small sample size and limited power. Because our primary analysis was “as-treated” or “per protocol,” it would be conservative to consider the results as hypothesis-generating as opposed to hypothesis testing. While we believe these results to be of interest to the scientific community, we recommend a larger sample confirmation study be undertaken to verify.
Primary Analysis Per Protocol (n=40) Results

Significance level for $p_{\text{family}} < 0.05$ is $k_E \geq 464$ (AlphaSim-corrected assuming spatial autocorrelation; voxelwise $p < 0.005$; cluster extent $k \geq 464$ voxels, which in real units is $k \geq 3712$ mm$^3 \approx 3.7$ cm$^3$) (Cox et al., 2017; Eklund et al., 2016).

<table>
<thead>
<tr>
<th>Anatomical Label</th>
<th>MNI Coordinates</th>
<th>$k_E$</th>
<th>$p_{\text{uncorr}}$</th>
<th>$p_{\text{FWE}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right inferior frontal gyrus</td>
<td>42  4  24</td>
<td>103</td>
<td>0.045</td>
<td>0.837</td>
</tr>
<tr>
<td>Right precentral gyrus / supplementary motor area</td>
<td>54 -14 50</td>
<td>996</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Right anterior insula</td>
<td>38  20  8</td>
<td>502</td>
<td>&lt;0.001</td>
<td>0.005</td>
</tr>
<tr>
<td>Left temporal operculum / Left posterior insula</td>
<td>-48 -10 -2</td>
<td>168</td>
<td>0.014</td>
<td>0.421</td>
</tr>
<tr>
<td>Right temporal operculum</td>
<td>54  2 -4</td>
<td>200</td>
<td>0.008</td>
<td>0.275</td>
</tr>
<tr>
<td>Left precentral gyrus</td>
<td>-34 -16 68</td>
<td>386</td>
<td>0.001</td>
<td>0.022</td>
</tr>
<tr>
<td>Right posterior insula</td>
<td>36 -12 -2</td>
<td>111</td>
<td>0.038</td>
<td>0.787</td>
</tr>
<tr>
<td>Left precentral gyrus</td>
<td>-18 -14 76</td>
<td>117</td>
<td>0.034</td>
<td>0.747</td>
</tr>
</tbody>
</table>
5.8.2. Secondary Analysis, Sensitivity Results (n=36)

Significance level for $p_{\text{family}} < 0.05$ is $k_E \geq 464$ (AlphaSim-corrected assuming spatial autocorrelation; voxelwise $p < 0.005$; cluster extent $k \geq 464$ voxels, which in real units is $k \geq 3712$ mm$^3 \approx 3.7$ cm$^3$) (Cox et al., 2017; Eklund et al., 2016).

<table>
<thead>
<tr>
<th>Anatomical Label</th>
<th>MNI Coordinates</th>
<th>$k_E$</th>
<th>$p_{\text{uncorr}}$</th>
<th>$p_{\text{FWE}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left posterior insula</td>
<td>-48  -8  -2</td>
<td>102</td>
<td>0.006</td>
<td>0.404</td>
</tr>
<tr>
<td>Right anterior insula</td>
<td>38   20   8</td>
<td>206</td>
<td>&lt;0.001</td>
<td>0.025</td>
</tr>
<tr>
<td>Right precentral gyrus</td>
<td>54  -14  50</td>
<td>161</td>
<td>0.001</td>
<td>0.083</td>
</tr>
<tr>
<td>Right temporal operculum, posterior</td>
<td>52   0    -8</td>
<td>120</td>
<td>0.004</td>
<td>0.252</td>
</tr>
</tbody>
</table>
CHAPTER VI
CONCLUSION

This dissertation sought to study substance use disorders (SUD) as brain diseases of altered neurocircuitry underlying reward, craving, and goal-oriented behaviors. We conducted advanced neuroimaging experiments involving two different populations of SUD: long-term abstinent, severely-dependent cocaine and methamphetamine addicts (Chapters 2 and 3); and, acutely-abstinent, moderately-dependent cigarette smokers (Chapter 4 and 5 and Appendix 1). While these two populations differ in overall disease severity, they also differ in primary drug of abuse (psychostimulants versus nicotine) and stage of disease (chronic remission versus acute withdrawal).

6.1. Specific Knowledge Gaps Addressed

In Chapter 2, we investigated differences in grey matter volumes and brain-behavior relationships in stimulant dependent individuals compared to healthy controls after long-term abstinence. We observed significant differences between stimulant dependent women and health control women in regional grey matter volumes, but no similar differences were observed amongst the men. We further observed that the relationships between grey matter volumes and behavioral metrics relevant to SUD differed by sex. This cross-sectional study was strong with regards to its sample size and signal processing technique; however, the results remain somewhat ambiguous. For instance, it is unclear to what extent these differences pre-dated the pathology (i.e., different endophenotypes), resulted from the pathology (i.e., different drug-induced neurotoxicity), or resulted from differences in recovery from the pathology (i.e., different neuroplasticity). Our results are consistent with the literature in that significant
structural neuroanatomical changes are associated with severe SUD, and highlight sex as an important variable in neuroimaging studies, despite being often ignored (Lind et al., 2017).

In Chapter 3, we investigated the resting-state functional connectivity changes in stimulant dependent individuals compared to healthy controls in a subset of the population reported in Chapter 2. We found that even after long-term abstinence (average 12.8 months), stimulant dependent persons exhibited a brain function pattern of increased “top-down” effective connectivity when compared to healthy controls. Compared to controls, stimulant dependent individuals showed significantly greater Granger causal connectivity from right executive control network to default mode network and from default mode network to basal ganglia network. Stimulant dependent individuals also demonstrated greater global efficiency and lower local efficiency, suggesting large-scale changes in brain connectivity despite long-term abstinence. These findings suggest that increased top-down effective connectivity in long-term abstinent drug users may reflect adaptive changes that foster successful remission, such as improved cognitive control over habit and reward processes.

Chapters 4, 5, and Appendix 1 introduce and report data from our phase 1, randomized, sham-controlled, single-blinded clinical trial investigating the effect of inhibitory insular transcranial magnetic stimulation (TMS) on cigarette cravings, brain responses to cigarette cues, and resting-state brain connectivity in acutely abstinent moderately-dependent smokers. Finite element model simulations of energy deposition intracranially allowed for estimation of a spatial dose-response across the brain. Compared to sham treatment, right insula inhibitory TMS reduced self-reported cigarette craving and decreased brain activity responses to visual cigarette cues in primary sensorimotor cortices, supplementary motor area, and right anterior insula. The fact that right anterior insular responses to cigarette cues were diminished after inhibitory TMS targeting the insula provides novel neuroimaging evidence of target
engagement; most prior studies targeted the dorsolateral prefrontal cortex. These findings provide proof-of-concept of a potential neuroanatomical target for smoking cessation therapy.

6.2. Limitations

This dissertation was subject to several limitations. First, a major limitation in studying long-term abstinence is the cross-sectional nature of the study designs. This limited our ability to determine if observed changes in brain structural and functional reflected endophenotypes that predated the pathology, changes associated with the disease course itself, or differences in neuroplastic recovery from the disease. Second, our randomized controlled trial was limited by the lack of a crossover and comparison target group. For example, we could not definitively determine if the changes we observed were secondary to stimulation of the right anterior insula or stimulation of the brain in general (i.e., we could not prove corticotopic specificity). In order to definitively prove causality, we would need to design the experiment using a within-subject counterbalanced crossover with at least three interventions (e.g., target insula, target prefrontal cortex, sham).

Lastly, our studies in both stimulant and cigarette addicts were confounded in part by polysubstance use/abuse and comorbid low educational attainment, albeit less so in the case of cigarette addicts (confounded only by marijuana use). While this precludes us from relating structural and functional changes to a single drug, our samples have biological and ecological validity as they reflect important, real-world, clinical populations of SDI. Epidemiological data, for example, demonstrate that stimulant dependence does not often occur in isolation; instead most stimulant dependent individuals meet dependence criteria for other substances (Sara et al., 2012; Stinson et al., 2005). There is significant literature describing the correlation between drug use and low educational attainment; it is debated whether low educational attainment is the cause or result of drug use disorders (Fergusson et al., 2003; Swaim et al., 1997; Yamada et al., 1996). More recently, however, authors have reported that this correlation is due in part to
shared genetic factors (Bergen et al., 2008) while others report that it is due to shared environmental or non-genetic familial risk factors (Grant et al., 2012; Verweij et al., 2013). These studies suggest that low educational attainment may be a pre-existing behavioral component of the pathology of substance use disorders.

6.3. Future Work

The immediate future work is currently underway. With regards to our randomized controlled trial, future work will investigate the changes induced by inhibitory TMS on measures of resting-state brain connectivity (Appendix 1), cerebral blood flow, and neurotransmitters thought to be involved in the mechanism of action of TMS, including glutamate, glutamine, and GABA. The FEM model stimulation presented will be compared to individual changes in cerebral blood flow to determine if this energy deposition map reflects an empirical spatial dose-response curve. The results from these analyses, including those presented in Chapter 5, will form the preliminary data for future scientific proposals that will extend and address limitations of the current clinical trial, including comparisons of different cortical targets for TMS.

This dissertation provides impetus for several strands of future work. First, long-term future work is needed to explore the effects and durability of repeated applications of TMS in smokers, including alternative/comparison cortical targets. Second, the mechanisms of TMS are still largely speculative, and future studies could potentially disentangle these mechanisms by optimizing TMS within the MRI scanner and neuroimaging TMS after different changes in target and pulse sequence. Lastly, substance use disorders span a complex disease spectrum and vary by drug, stage, and individual factors. Finding commonalities amongst these myriad presentations of addiction with regards to long-term remission is critically important. Neuroimaging long-term abstinent individuals, as well as individuals progressing through the abstinence process, has potential to improve our understanding of the neurocircuitry
mechanisms of successful abstinence. These three long-term areas of future work involving neuroimaging and neuromodulation could help understand addiction as a brain disease and potentially reduce the burdens associated with it.

6.4. Concluding Remarks

Each individual chapter addresses a specific yet important gap in the literature. Together, however, these neuroimaging studies in two different populations of substance use disorders provide evidence that: (1) addiction is a brain disease, and (2) addiction is a disease of neurocircuitry with endogenous (i.e., increased top-down control, Chapter 3) and exogenous (i.e., therapeutically imposed, Chapter 5) mechanisms of remission and treatment. Future work must continue to study mechanisms of and interventions that promote successful disease remission.
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NIH, NIH OER Definition of Phase I Clinical Trial. National Institutes of Health - Office of Extramural Research.


APPENDIX

INSULAR INHIBITORY NEUROMODULATION IN SMOKERS DECREASES CIGARETTE CRAVINGS AND ALTERS RESTING STATE BRAIN CONNECTIVITY

1.1. Materials and Methods

The methods of this section are identical to Chapter 5 unless explicitly stated otherwise.

1.1.1. MRI Examination

MRI exams were acquired before and after treatment at the University of Colorado Brain Imaging Center using a Siemens 3-Tesla Magnetom Skyra scanner (Siemens AG; Munich, Germany) and 20-channel neurovascular coil. Structural images included a T1-weighted 3D magnetization prepared rapid gradient multi-echo sequence (MPRAGE; sagittal plane acquisition; repetition time [TR] = 2300 ms; echo time [TE] = 2.24 ms; inversion time [TI] = 900 ms; echo train length [ETL] = 250 ms; flip angle = 8°; 1 mm slice thickness, 176 slices; FOV = 220 mm with 256 × 256 matrix; time = 5:21 min). Resting state functional images were acquired with a T2*-weighted echo-planar gradient-echo sequence (GE EPI FID; axial oblique plane acquisition; echo time [TE] = 28 ms; repetition time [TR] = 2000 ms; flip angle = 70°; slice thickness = 3mm with 1mm gap, 32 slices; FOV = 220 mm with 64 × 64 matrix; acquisition time = 10:00 min). Head motion was minimized with a VacFix head-conforming vacuum cushion (Par Scientific A/S, Odense, Denmark).

1.1.2. MRI Signal Pre-Processing

Structural and functional images were pre-processed using MATLAB 2017 and SPM12 software (Wellcome Trust Centre for Neuroimaging; London, UK). T1-weighted images were segmented and normalized to MNI space. BOLD pre-processing included: [1] removal of first four TRs (at acquisition), [2] slice-timing correction, [3] rigid realignment of BOLD images to the first TR, [4] motion scrubbing/censoring (Power et al., 2014), [5] non-neural noise...
correction using aCompCor (Behzadi et al., 2007; Muschelli et al., 2014), and [6] non-linear deformation into MNI space. Individual TRs with framewise displacement ≥0.3 mm were censored; binary censoring indicators and rigid realignment parameters were included as first-level covariates. Both total number of valid TRs (i.e., non-censored) and mean framewise displacement across each run were included as second-level nuisance covariates. Participants with ≥20% of all TRs censored were excluded; two met this criterion.

BOLD data were visually inspected by three investigators (MFR, JRT, JLT) on a subject-by-subject basis during blinded analysis to document agreement on quality control prior to analysis (Power et al., 2014). For both pre-treatment and post-treatment scans, this included: [1] line plots of realignment parameters, [2] line plots indicating censored TRs, and [3] BOLD signal greyplots before and after nuisance variance correction. Motion- and nuisance-correction appeared appropriate in all subjects; none were excluded on this basis.

1.1.3. Resting State fMRI Analyses

Seed-based whole brain connectivity maps were constructed for both the pre- and post-treatment timepoints. Seeds were selected according to the previously defined method (see Section 5.3.13, Finite Element Method (FEM) Simulation and ROI Selection, page 91).

Significance level for $p_{\text{family}} < 0.05$ is $k_E \geq 464$ (AlphaSim-corrected assuming spatial autocorrelation; voxelwise $p < 0.005$; cluster extent $k \geq 464$ voxels, which in real units is $k \geq 3712 \text{ mm}^3 \approx 3.7 \text{ cm}^3$) (Cox et al., 2017; Eklund et al., 2016).

Effect of treatment group was evaluated using a 2x2 mixed-independence factorial design. Time was the within-subjects factor (pre- versus post-treatment); group was the between-subjects factor (LF-rTMS versus sham). First-level SPM contrasts were constructed for each participant using paired t-tests (post-treatment [seed-based connectivity] > pre-treatment [seed-based connectivity]). Second-level SPM contrasts were constructed using a two-sample t-
test comparing first-level contrasts by group. Images were masked at the second-level using a grey-matter mask with the cerebellum excluded.

1.2. Resting State Connectivity Results

We observed statistically significant bidirectional seed-based whole brain connectivity changes after real treatment compared to sham for both the right inferior frontal gyrus, pars articularis as well as the right anterior insula. No statistically significant changes were observed in seed-based connectivity with the posterior middle temporal gyrus after real treatment compared to sham.