Protocol 12-DA-N474: Identifying neurological mechanisms that underlie acute nicotine withdrawal and drive early relapse in smokers.

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Protocol Title: Identifying neurobiological mechanisms that underlie acute nicotine withdrawal and drive early relapse in smokers.

Abbreviated Title: Neurobiology of nicotine withdrawal
Protocol Number*: 12-DA-N474*
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Principal Investigator:

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Total requested accrual:
Total Enrollees: 577 **Completers**: 500

Main Study:
120 Treatment-seeking smokers (enrollees), 85 Completers
42 non-treatment seeking smokers (enrollees), 35 completers.

Motivational Interviewing Arm:
300 people interested in quitting smoking, but not yet ready to set a quit date.

Transcranial Direct Current Stimulation (tDCS) arm:
60 non-treatment seeking smokers (enrollees), 35 completers
55 nonsmokers (enrollees), 45 completers. Up to the first 25 of these enrollees will complete the tDCS without MRI (Behavioral Pilot)

Project Uses Ionizing Radiation:	✠ No	☐ Yes
IND/IDE	✠ No	☐Yes
Durable Power of Attorney	✠ No	☐ Yes
Multi-institutional Project	✠ No	☐ Yes
NIDA is the coordinating site		
Data and Safety Monitoring Board	✠ No	☐ Yes

Technology Transfer Agreement
☐ No ✠ Yes
MTA with LSU for plasma samples. Expires December 12, 2022.

Samples are being stored ☐ No ✠ Yes (temporarily, plasma samples acquired for measurement of cortisol and ACTH will be destroyed once valid assays have been obtained.

Flesch-Kincaid reading level of consent form: 7.4
(exclude boilerplate in assessing reading level)

Medical Coverage Level:
Level 5 - The MAI (or designated clinician) is available by beeper or phone.

**
Level 1 - The MAI administers all drugs and remains physically present in the experimental room during all or part of study session as determined by the Clinical Director.
Level 2 - The MAI must be physically present in the experimental room during all or part of study session as determined by the Clinical Director.
Level 3 - The MAI must be present within the study building.
Level 4 - The MAI must be on the study campus.
Level 5 - The MAI is available by beeper or phone.

Précis:

Objective

The primary objective of the current protocol is to gain a greater understanding of the neurobiological mechanisms underlying acute nicotine withdrawal and contributing to the maintenance of, or return to smoking behavior among nicotine-dependent individuals, in the service of developing future smoking cessation treatments. The Nicotine Withdrawal Syndrome is a major cause of failed quit attempts in smokers, and targeting this time period for intervention may help improve smoking cessation outcomes.

Study Population

We will recruit treatment seeking and non-treatment seeking smokers, as well as matched non-smoker control participants.

Design

There are 3 arms included in this protocol, each of which aims to understand the neurobiology of the Nicotine Withdrawal Syndrome during the initial quit period, with the broader goal of increasing quit success rate in the future.

> **Main Study:** To understand (1) the acute neurobiological effects of nicotine withdrawal on treatment-seeking and non-treatment seeking smokers, (2) the long term neurobiological outcomes of varenicline treatment and smoking cessation counseling at 1, 6, and 12 months. We will recruit 85 treatment seeking and 35 non-treatment seeking smokers for a within (nicotine deprivation), between (treatment-seeking status) subjects randomized, double blind, placebo controlled study.
>
> **Motivational Interviewing Arm:** (1) To increase motivation and preparation for smoking cessation treatment among individuals who express an interest in quitting smoking but are not currently ready to enter treatment, in the service of increasing quit success rate and (2) to understand the neurobiological basis of motivation to quit smoking, and the interaction between motivation to quit and mechanisms that underlie acute nicotine withdrawal. We will recruit 300 current smokers interested in quitting smoking, but not yet ready to set a quit date.

Transcranial Direct Current Stimulation (tDCS) Arm: To understand the acute effect of tDCS on 3 large-scale brain networks dysregulated in nicotine addiction and withdrawal, the Default Mode Network, the Executive Control Network, and the Salience Network. We will enroll 60 non-treatment seeking smokers, with the expectation of 35 completers; and enroll 55 non-smoking controls, with the expectation of 45 completers, for a double blind, sham controlled, randomized crossover study. Smokers will be studied in nicotine abstinence and nicotine sated conditions, as in the Main Study design.

Outcome measures

Primary outcome measures:

1) Change in BOLD signal and FC related to task parameters, between drug (or tDCS) condition.
2) Behavioral performance on each of the tasks assessing inhibitory control processes, reward responsiveness, amygdala, striatal, BNST reactivity, impulsive decision making, cue reactivity and working memory (e.g., reaction time, error rate, hit rate, reward bias).
3) Self-reported craving, withdrawal symptoms and mood/affect
4) Smoking abstinence as determined by self-reported tobacco use, urine cotinine, and breath CO.

Secondary outcome measures:

1) MRS for glutamate concentration.
2) Plasma ACTH and cortisol.
3) Resting state CBF from ASL.
4) ERP and EEG measures.
5) Ratings and scores on self-report characterization measures.
6) Structural MRI and DTI data.
7) Resting state FC at 1, 6 and 12 months post-treatment.

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List of Abbreviations

AChRs = acetylcholinergic receptors
ACTH = Adrenocorticotropic hormone
BLA = Basolateral Amygdala
BNST = bed nucleus of the stria terminalis
BOLD = blood oxygen level dependent
CBF = cerebral blood flow
CeA = Central Amygdala
CRF = corticotrophin releasing factor
dACC = dorsal anterior cingulate cortex
dlPFC = dorsolateral prefrontal cortex
DMN = Default Mode Network
ECN = Executive Control Network
ERN = error-related negativity
ERP = event-related potential
NAcc = Nucleus Accumbens
PCC = posterior cingulate cortex
Pe = Error Positivity
PFC = prefrontal cortex
PPC = posterior parietal cortex.
rACC = rostral anterior cingulate cortex
rsFC = resting state functional connectivity
RT = reaction time
SN = Salience network
tDCS = Transcranial Direct Current Stimulation
vmPFC = ventromedial prefrontal cortex
VS = ventral striatal
VTA = ventral tegmental area
WM = Working memory

1. Introduction/Scientific Rationale

Introduction

In a review addressing smoking relapse rates following unassisted quitting attempts, Hughes, Keely and Naud (2004) found that while there is a decline in abstinence with time, ***49% to 76% of smokers relapse within the first week***. Thus, acute withdrawal processes appear to be the largest barrier to improving abstinence outcomes among nicotine-dependent individuals.

Acute nicotine withdrawal is associated with reports of dysphoric or depressed mood, increased negative affect (anxiety, irritability, anger) and craving for cigarettes (American Psychiatric Association, 1994). Laboratory studies are consistent with these subjective reports, showing reduced reward and pleasure responsiveness, (Powell, Dawkins, & Davis, 2002), increased startle reactivity (Hogle, Kaye, & Curtin, 2010) and increased craving to drug cues (Powell, Dawkins, & Davis, 2002; Wang et al., 2007). In addition, acute nicotine withdrawal has been consistently shown to impair inhibitory control (McClernon, Kozink, Lutz, & Rose, 2009b; Pettiford et al., 2007; Powell et al., 2002) and increase impulsive decision making (Field, Santarcangelo, Sumnall, Goudie, & Cole, 2006a; Pettiford et al., 2007; Powell et al., 2002). This reduced responsiveness to natural rewards coupled with enhanced negative affect, impulsive decision making and poor inhibitory control are likely to significantly contribute to, and perhaps interact to compound, the risk of early relapse. Animal models point to neuroadaptations within the striatum, Ventral Tegmental Area (VTA) and extended amygdala as a possible neurobiological basis for acute withdrawal effects. However, to date, few studies have addressed the neurobiological mechanisms that underlie these processes, and drive early relapse in humans.

The primary goal of the current research is to gain a greater understanding of the neurobiological processes underlying acute nicotine withdrawal and contributing to the maintenance of, or return to smoking behavior among nicotine-dependent individuals, in the service of developing future smoking cessation treatments.. We will address this goal by a 3-pronged approach.

First, the Main Study of this protocol will examine the neurobiological correlates of affective and behavioral changes that occur during acute nicotine withdrawal and the extent to which these processes predict markers of risk for early relapse. A secondary consequence of this analysis will be the identification of neurobiological processes which underlie responsivity to nicotine replacement. The outcome of such an analysis is two-fold. Firstly, studying the neurobiological basis of acute nicotine withdrawal in humans may lead to the identification of novel treatment targets which could attenuate early relapse rates over and above that achieved by current therapeutic interventions. Secondly, by examining individual differences in responsivity to nicotine replacement we may be able to identify early biomarkers of poor treatment response which could guide clinicians in the selection of alternative interventions. To meet these goals, we will examine inhibitory control processes, impulsive decision making, extended amygdala and striatal reactivity, as well as drug cue and stress reactivity in nicotine dependent individuals under a non-deprived state and following 36 hrs of nicotine abstinence.

Second, in the Motivational Interviewing Arm *(Section 1.5)*, we will examine the effect of individual differences in motivation to quit smoking on the neurobiological outcome measures of the Main Study arm. Smokers vary widely in their motivation and preparation for smoking cessation treatment, and this may account for a portion of the discrepancy between the number of

smokers who say they want to quit (which is high), and the number of smokers who successfully quit (which is low).

Third, we will assess a new technology that has the potential to be used as a novel smoking cessation aid, Transcranial Direct Current Stimulation (tDCS). tDCS is a promising technique for treatment of cigarette smoking because of its ability to modify large scale brain networks that are dysregulated in nicotine addiction and withdrawal (see below, *Sections 1.3, 1.4, 1.6)*.

The following section provides background for our current understanding of the nicotine withdrawal syndrome, and how the 3 approaches described above together will bring further insight into the causes of quit attempt failures, and how the research community can develop treatments that will prevent those failures.

Following this Introduction, and as per previous IRB requests, we have included all aspects of the tDCS arm in a discrete section of the protocol (see *Section 24*)

1.1. Anhedonia during acute nicotine withdrawal: neurobiological mechanisms

During acute withdrawal from nicotine, smokers typically report a state of anhedonia, defined as a reduced ability to experience pleasure from naturally rewarding activities/stimuli (Leventhal, Ramsey, Brown, LaChance, & Kahler, 2008). This is consistent with laboratory studies showing reduced anticipation of pleasure from everyday rewarding activities (Powell et al., 2002) as well as reduced reward responsiveness across a variety of tasks involving monetary incentives (Al-Adawi & Powell, 1997; Dawkins, Powell, West, Powell, & Pickering, 2006; Powell et al., 2002; Powell, Dawkins, West, Powell, & Pickering, 2010). High-levels of trait anhedonia and other depressive symptoms measured prior to quitting are in turn associated with poorer cessation outcomes and a greater number of previous quit attempts, in particular, greater relapse during the first 24 hours (Leventhal et al., 2008; Leventhal, Waters, Kahler, Ray, & Sussman, 2009).

Preclinical studies show that acute withdrawal from nicotine results in reduced dopamine release at the nucleus accumbens (NAcc) and an increase in brain-reward thresholds (Hildebrand, Nomikos, Hertel, Schilström, & Svensson, 1998; Hildebrand, Panagis, Svensson, & Nomikos, 1999). Studies suggest a role of corticotrophin releasing factor (CRF), in these effects (Bruijnzeel, Prado, & Isaac, 2009). During acute nicotine withdrawal, CRF increases in the brain and appears to act at the NAcc (Marcinkiewcz et al., 2009) to partially mediate elevations in brain-reward threshold.

Neuroadaptations within glutamatergic systems have also been implicated in the anhedonic aspects of acute nicotine withdrawal. Nicotine acts at nicotinic acetylcholinergic receptors (AChRs) on glutamatergic terminals across a range of brain sites, including the VTA, NAcc, prefrontal cortex (PFC) and hippocampus (Kenny & Markou, 2001). Chronic exposure to nicotine leads to upregulation of inhibitory Glu2/3 receptors, producing a negative feedback effect on glutamatergic neurotransmission, particularly within the VTA (Kenny & Markou, 2001). Upregulation of inhibitory mGlu2/3 receptors during the development of nicotine dependence thus results in a reduction in glutamatergic neurotransmission during acute withdrawal, which has in turn been linked to an increased reward threshold during this period (Kenny & Markou, 2001). There is also evidence of reduced glutamatergic transmission from the PFC to NAcc during acute nicotine withdrawal that may contribute further to these reward deficits (Markou, 2008).

Despite the link between depressive symptoms and early relapse to smoking, to date no study has examined the relationship between anhedonia produced by acute nicotine withdrawal and relapse outcomes. Furthermore, no study has examined the neurobiological basis of acute withdrawal induced anhedonia in smokers. Preclinical work implicates a CRF mediated reduction

in the release of dopamine at the NAcc as well as reduced glutamatergic neurotransmission, particularly at the VTA and between the PFC and NAcc. These preclinical findings provide a starting point from which to identify the neurobiological mechanisms that underlie anhedonia following acute nicotine withdrawal in humans. Findings may also provide insight into the manner and extent to which these mechanisms contribute to the high rate of early relapse among smokers.

1.2. Stress reactivity and negative affect during acute nicotine withdrawal: neurobiological mechanisms

During acute withdrawal from drugs of abuse, neuroadaptations occur in the extended amygdala (the central nucleus of the amygdala [CeA], bed nucleus of the stria terminalis [BNST], and a transitional zone in the shell of the NAcc) which have been tied to the increase in negative affect and stress reactivity during this period (Koob and Le Moal, 2008). High negative affect and stress reactivity are in turn linked to increased risk for relapse, particularly in response to stress (Fisher et al., 1998; Willinger et al., 2002; Bottlender and Soyka, 2005; Cosci et al., 2009; Back et al., 2010). For example, mice bred for heightened stress reactivity show heightened reinstatement of nicotine seeking following extinction (Bilkei-Gorzo et al., 2008).

Acute nicotine-withdrawal produces a substantial elevation in extracellular CRF at the CeA as well as an increase in anxiety-like behavior (e.g., defensive burying) and nicotine self-administration (SA) following abstinence (George et al., 2007). Systemic injection of a CRF_1 receptor antagonist has been found to block these nicotine-withdrawal related anxiogenic effects and dose-dependently reduce abstinence induced nicotine SA (George et al., 2007). In the same study, direct infusion of CRF into the CeA produced the same anxiogenic effects in naïve animals. These findings suggest that the anxiogenic effects of nicotine withdrawal are at least partly mediated by the actions of CRF at CRF_1 receptors and may be specifically related to an increase in CRF at the CeA.

Whether CRF is elevated at other brain regions during nicotine-withdrawal is unknown. Previous studies have shown in increase in extracellular CRF at the BNST during withdrawal from alcohol (Olive, Koenig, Nannini, & Hodge, 2002). While action of CRF at the BNST does not appear to mediate the increased reward threshold during nicotine withdrawal (Marcinkiewcz et al., 2009), studies with other drugs of abuse suggest a role for the BNST in negative affective aspects of acute nicotine withdrawal. For example, studies with alcohol and cocaine dependent rats implicate the BNST in stress-induced reinstatement of cocaine/alcohol seeking (Leri et al., 2002; Wang et al., 2006). A role of the BNST is also suggested by recent study showing enhancement of startle reflex under the threat of unpredictable but not predictable shock during nicotine withdrawal (Hogle et al., 2010). Threat of unpredictable shock produces a state of sustained anxiety mediated by the BNST in rodents (Davis et al., 2010) and shown to activate the BNST and CeA in humans (Alvarez et al., 2011).

Individual differences in stress reactivity and trait anxiety in humans have been tied to amygdala reactivity in response to conscious and unconsciously processed emotional stimuli/stressors (Etkin et al., 2004; Gianaros et al., 2008; Monk et al., 2008). Amygdala reactivity in part reflects the degree of top-down regulation by rostral anterior cingulate regions (rACC) (Etkin, Egner, & Kalisch, 2010). Functional and structural connectivity between the rACC and amygdala have also been linked to trait anxiety/stress reactivity (Kim & Whalen, 2009; Pezawas et al., 2005). Both amygdala reactivity and amygdala-rACC connectivity are modulated by a common polymorphism in the human serotonin transporter gene (5-HTTLPR) (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010; Pezawas et al., 2005). Serotonin is also heavily involved in the development of neural pathways that support emotion processing and regulation (Caspi et al., 2010). Individuals with the short allele of the 5-HTTLPR gene show lower expression of 5-HTT and enhanced

reactivity to stress and risk for depression, effects which appear to be mediated by enhanced amygdala reactivity and amygdala-rACC connectivity (Caspi et al., 2010; Pezawas et al., 2005). Amygdala reactivity and regulation of amygdala output by the rACC could thus play a role in the negative affective aspects of acute nicotine withdrawal.

To summarize, acute nicotine withdrawal is associated with enhanced reactivity to stress and a general increase in negative affect. At present, the neurobiological mechanisms underlying these effects in humans are relatively unknown. Preclinical work suggests that neuroadaptations within the extended amygdala may play a key role. As well, increases in central CRF during acute nicotine withdrawal and in particular the action of CRF at the CeA and BNST present potential mechanisms of interest, particularly in relation to stress-induced relapse. In humans, stress reactivity has been tied to enhanced amygdala reactivity and reduced top-down regulation of the amygdala by the rACC. These effects are both in turn modulated by a common polymorphism in the 5-HTTLPR gene. In the current study we thus aim to draw upon this body of research to identify key mechanisms underlying enhanced reactivity to stress and negative affect during acute nicotine withdrawal. We will utilize validated methods to probe reactivity in the amygdala and BNST as a function of acute nicotine withdrawal and self-reported negative affective symptoms, and also examine the extent to which these effects are mediated or modulated by the presence of the short allele of the 5-HTTLPR gene and a marker of central CRF.

1.3. The Brain's Default Mode Network: Does it play a role in acute nicotine withdrawal?

As reflected above, current understanding of the neurobiological mechanisms underlying acute nicotine withdrawal is based almost exclusively on preclinical research. Such preclinical work directs focus to discrete neural regions where neuroadaptations may be driving withdrawal processes. However, more recently human neuroimaging has seen an explosion of interest in intrinsic functional networks that show state and trait alterations across a range of clinical populations (Broyd et al., 2009; Sakoğlu et al., 2011). In terms of acute nicotine withdrawal, emerging evidence points to a central role of a set of functionally connected structures collectively termed the default mode network (DMN). Regions included in the DMN typically include the ventromedial PFC, posterior cingulate cortex (PCC), inferior frontal lobe, dorsomedial PFC, parahippocampal gyrus and hippocampal formation (Buckner, Andrews-Hanna, & Schacter, 2008). DMN regions were originally identified because they were consistently shown to deactivate (relative to resting baseline) during goal-directed tasks (Buckner et al., 2008; Greicius, Krasnow, Reiss, & Menon, 2003; D. A. Gusnard & Raichle, 2001). These regions also exhibit robust functional connectivity at rest (Buckner et al., 2008; Greicius et al., 2003) and during task (Sridharan, Levitin, & Menon, 2008).

The function of the brain's DMN is still in question, however, most evidence points to a role in mediating internal thought related to mental simulation (e.g., rumination on past and future events, others thoughts, moral dilemmas) and self-reflection (Amodio & Frith, 2006; Buckner et al., 2008; Gusnard, Akbudak, Shulman, & M E Raichle, 2001; Schacter, Addis, & Buckner, 2007). Interestingly, resting connectivity within, and cerebral blood flow to, the DMN have been shown to increase as a function of nicotine withdrawal symptoms and cigarette cravings (Cole et al., 2010; Wang et al., 2007). Moreover, independent of withdrawal, increased activity in default mode regions is consistently observed as a function of craving and/or reactivity to drug cues (e.g., Franklin et al., 2011; Goudriaan, De Ruiter, Van Den Brink, Oosterlaan, & Veltman, 2010; Li, Chiang-Shan et al., 2008; Park et al., 2007; Sinha & C. S. R. Li, 2007; Weinstein et al., 2010; Wilcox, Teshiba, Merideth, Ling, & Mayer, 2011).

Taken together, this evidence suggests that craving and negative affective symptoms associated with acute nicotine withdrawal may produce a state of rumination/self-reflection mediated by the DMN. This view is consistent with enhanced resting connectivity and reduced task-related deactivation in default mode regions seen among clinically anxious and depressed populations (Gentili et al., 2009; Lanius, Bluhm, Coupland, Hegadoren, & Rowe, 2010; Sheline, Price, Yan, & Mintun, 2010; Zhou et al., 2010) as well as with the substantial overlap in DSM-IV symptoms of major depressive disorder (MDD) and acute nicotine withdrawal (APA, 1994).

Increased engagement of the DMN may in turn contribute to the cognitive deficits observed during acute nicotine withdrawal (e.g., Myers, Taylor, Moolchan, & Heishman, 2008). During goal-directed tasks and at rest the DMN is negatively coupled with a functionally distinct executive control network (ECN), including dorsal frontal and parietal regions typically engaged during goal-directed tasks and problem solving (Buckner et al., 2008; Fox et al., 2005; Fransson, 2005; Hampson, Driesen, Roth, Gore, & Constable, 2010). Consequently, reduced task-related deactivation in default mode regions predicts subsequent performance errors as well as reduced activity in ECN regions (Eichele et al., 2008; Li, Yan, Bergquist, & Sinha, 2007).

To summarize, the brain's DMN appears to mediate processes associated with self-reflection and rumination on past and future events. Acute nicotine withdrawal is likely to produce an increase in rumination/self-reflection associated with cravings to smoke and negative affective processes. This will in turn produce enhanced connectivity/activity within the DMN at rest, reduced deactivation in default mode regions during task, and reduced engagement of the negatively coupled ECN during goal-directed task performance. This latter process may underlie observed deficits in sustained attention, performance monitoring and inhibitory control during acute nicotine withdrawal. According to this view, which is summarized in Figure 1, increased default mode activity/connectivity may reflect an intermediate endophenotype, driven by craving and negative affective processes during acute withdrawal, and resulting in impairments in cognitive functions and inhibitory control as well as increased risk of relapse.

1.4. Inhibitory control and impulsive decision making during acute nicotine withdrawal: potential neurobiological mechanisms

Deficits in inhibitory control mechanisms and impulsive decision making are considered central to drug addiction, which is characterized by compulsive use of drugs in the face of negative consequences (e.g., negative health outcomes). In the laboratory, inhibitory control deficits are revealed among nicotine-addicted individuals on tasks that require the inhibition of a prepotent response (Hester & Garavan, 2004). Nicotine-addicted individuals also tend to exhibit more impulsive decision making, characterized by sharper discounting of reward value (e.g., hypothetical money or cigarettes) as a function of time to delivery (Bickel, Odum, & Madden, 1999). More impulsive decision-making prior to quitting, as well as higher scores on trait impulsivity measures have in turn been linked to higher rates of relapse (Doran, Spring, McChargue, Pergadia, & Richmond, 2004; Kahler, Spillane, Metrik, Leventhal, & Monti, 2009; MacKillop & Kahler, 2009; Powell et al., 2010; Yoon et al., 2007).

As outlined previously, inhibitory control deficits and impulsive decision making are exacerbated during acute nicotine withdrawal (Field et al., 2006a; McClernon et al., 2008a; Mitchell, 2004a; Pettiford et al., 2007; Powell et al., 2002), which likely contributes to the high rate of early relapse among smokers. However, to date, there has been no research addressing relapse outcomes as a function of acute-withdrawal induced changes in inhibitory control and impulsive decision making. Moreover, there is minimal understanding of the neurobiological processes driving these changes. Research addressing the increase in inhibitory control deficits and impulsive decision making during acute nicotine withdrawal, as well as the neurobiological basis of these

effects, may serve to identify a novel means of combating the high rates of early relapse among nicotine-addicted individuals.

Inhibitory control processes. Breakdowns in inhibitory control may arise from poor monitoring for events (i.e., errors, goal-conflict) which signal the need to stop and adjust on-going behavior. They may also result from difficulty inhibiting/adjusting behavior in response to errors/conflict. It appears that these two components of inhibitory control are mediated by separable neurobiological mechanisms. The first, performance monitoring component, appears to be mediated by a network of structures centered around the dorsal region of the anterior cingulate cortex (dACC) and anterior insular cortex (Menon & Uddin, 2010; Seeley et al., 2007; Ullsperger, Harsay, Wessel, & Ridderinkhof, 2010). The two structures form part of an intrinsically connected "salience network" (SN) which is functionally distinct from other intrinsic brain networks including the DMN and ECN reviewed above. Within this network, the dACC monitors ongoing goal-directed behavior for salient events that require attention, while the anterior insula integrates high-level sensory, limbic, interoceptive and autonomic signals to generate subjective "feelings" which underlie awareness of salient intrinsic or extrinsic events (Menon & Uddin, 2010; Ullsperger et al., 2010).

While the SN plays a critical role in monitoring on-going behavior for salient intrinsic or extrinsic events that require shifts in attention or cognitive/behavioral resources, it is not involved in later adjustment and conflict-resolution processes. When tasks are cognitive, the SN engages ECN structures, in particular the dlPFC, which function to resolve conflict/adjust behavior in response to conflict/errors (Botvinick, Nystrom, Fissell, Carter, & Cohen, 1999; Kerns et al., 2004; Marco-Pallarés, Camara, Münte, & Rodríguez-Fornells, 2008). In contrast, when tasks involve errors/conflict of an emotional nature, the salience network engages rACC regions of the DMN (Egner, Etkin, Gale, & Hirsch, 2008; Etkin, Egner, Peraza, Kandel, & Hirsch, 2006; Ochsner, Hughes, Robertson, Cooper, & Gabrieli, 2010). Interestingly, recent evidence suggests that the SN, in particular the anterior insula, functions as a switch between the negatively coupled DMN and ECN (Sridharan et al., 2008).

The anterior insula is also thought to play a key role in conscious urges, such as cigarette craving (Naqvi & Bechara, 2009). This view was compounded by reports of spontaneous smoking cessation and a striking loss of craving following damage to the insula (Naqvi, Rudrauf, Damasio, & Bechara, 2007). Imaging studies also consistently report insula activation as a correlate of subjective drug craving (Franklin et al., 2011; Naqvi & Bechara, 2009; Wang et al., 2007).

Together, the above findings raise several potential mechanisms through which acute nicotine withdrawal may impair inhibitory control processes. Firstly, increased engagement of the DMN by craving/negative affective symptoms could reduce recruitment of the negatively coupled ECN during resolution of conflict. Secondly, previous findings (Eichele et al., 2008) suggest that reduced task-related DMN deactivation (e.g., during acute nicotine withdrawal) will result in reduced dACC engagement and hence impaired performance monitoring. Thirdly, if craving/negative affective symptoms functionally hijack the DMN, the capacity of these regions to effectively mediate conflict resolution/behavioral adjustment processes may also be altered. Finally, postulated involvement of the anterior insula in both craving and network switching raises the possibility that this region may play a role in enhanced DMN connectivity/activity during acute nicotine withdrawal.

Evoked-potentials underlying inhibitory control processes. Further insight into the neural mechanisms which underlie breakdowns in inhibitory control may arise from the study of scalp-recorded event-related potentials (ERPs) measured with electroencephalography (EEG). Much of the work reviewed above is based upon functional magnetic resonance imaging (fMRI) which produces a measure of blood oxygen level dependent (BOLD) activity in the brain. Although fMRI provides excellent spatial resolution, it is an indirect measure of underlying neuronal activity, with limited temporal resolution (typically sampling at 1Hz or slower). In contrast, ERPs are produced by the synchronous firing of neurons, and so provide a direct, real-time, measure of underlying

neuronal activity. The measurement of ERPs is thus an important tool for dissociating processes following stimulus or response onset which would be otherwise indistinguishable within the BOLD response.

Growing evidence indicates a dissociation between early negative (e.g., error-related negativity, ERN; N200) and late positive (error positivity, Pe; P300) ERP responses to errors and other salient events, respectively (Enriquez-Geppert, Konrad, Pantev, & Huster, 2010; Huster, Westerhausen, Pantev, & Konrad, 2010; Overbeek, Nieuwenhuis, & Ridderinkhof, 2005; Ullsperger et al., 2010). For example, children and young people with ADHD show normal ERN but reduced error positivity (Groom et al., 2010). They also show a reduction in late but not early evoked theta power in response to errors (Groom et al., 2010). Ullsperger et al. (2010) suggest that error negativity reflects error detection mediated by the dACC whereas error positivity reflects error awareness mediated by the anterior insula. Similarly, the N200 is thought to reflect early detection of conflict between expected and actual outcomes whereas later P300 responses are thought to reflect attentional allocation and evaluation of the salient event (Friedman, Cycowicz, & Gaeta, 2001).

There is also evidence that synchronization in frontal scalp recorded theta oscillations may provide a correlate of increased connectivity between dACC and areas that mediate later control/conflict resolution processes (e.g., dlPFC). For example, (Cavanagh, Cohen, & Allen, 2009) observed reduced theta power source localized to the dACC just prior to an error and increased theta power following an error. They also observed increased synchronization between medial frontal and lateral frontal electrode sites on error trials, which robustly predicted the degree of theta power on error trials. Furthermore, both theta synchronization and theta power predicted the degree of post-error reaction slowing (behavioral adjustment).

There is now extensive evidence showing that each of the above negative and positive event-related components is sensitive to inhibitory control deficits observed in substance dependent populations and their high-risk offspring (e.g., H. L. Cohen, Porjesz, Begleiter, & W. Wang, 1997; Franken, van Strien, & Kuijpers, 2010; Luijten, van Meel, & Franken, 2011; Porjesz et al., 1998). In contrast, the impact of acute withdrawal on these ERP correlates is relatively unknown. By measuring concurrent ERP and fMRI correlates of performance monitoring and conflict resolution/behavior adjustment processes in the current study, we hope to gain further insight into the neurobiological basis of inhibitory control deficits observed during acute nicotine withdrawal.

Impulsive decision making. Nicotine-addicted individuals tend to exhibit more impulsive decision making, characterized by sharper discounting of reward value (e.g., hypothetical money or cigarettes) as a function of time to delivery. More impulsive decision-making prior to quitting, as well as higher scores on trait impulsivity measures have in turn been linked to higher rates of relapse (MacKillop & Kahler, 2009; Yoon et al., 2007). Importantly, impulsive decision making is exacerbated during acute nicotine withdrawal (Field et al., 2006a; Pettiford et al., 2007; Powell et al., 2002). This in turn likely contributes to the high rate of early relapse among smokers. However, to date, there has been no research addressing relapse outcomes as a function of acute-withdrawal induced changes in impulsive decision making. Moreover, there is minimal understanding of the neurobiological processes driving these changes. Previous studies have shown that more impulsive decision making (steeper discounting as a function of delay to reward) is associated with enhanced striatal reactivity (Hariri et al., 2006) and reduced recruitment of default mode regions (Peters & Büchel, 2010). As outlined earlier, it is expected that acute nicotine withdrawal will be associated with reduced striatal reactivity. Given that more impulsive decision making results from enhanced striatal reactivity, we would expect reduced striatal reactivity to produce less impulsive decision making. Consequently, striatal changes are not expected to underlie impulsive decision making observed during acute nicotine withdrawal. Instead, it is expected that enhanced impulsive decision making during acute nicotine withdrawal will result from reduced recruitment of DMN regions,

because these regions are otherwise occupied with rumination related to craving and affective symptoms.

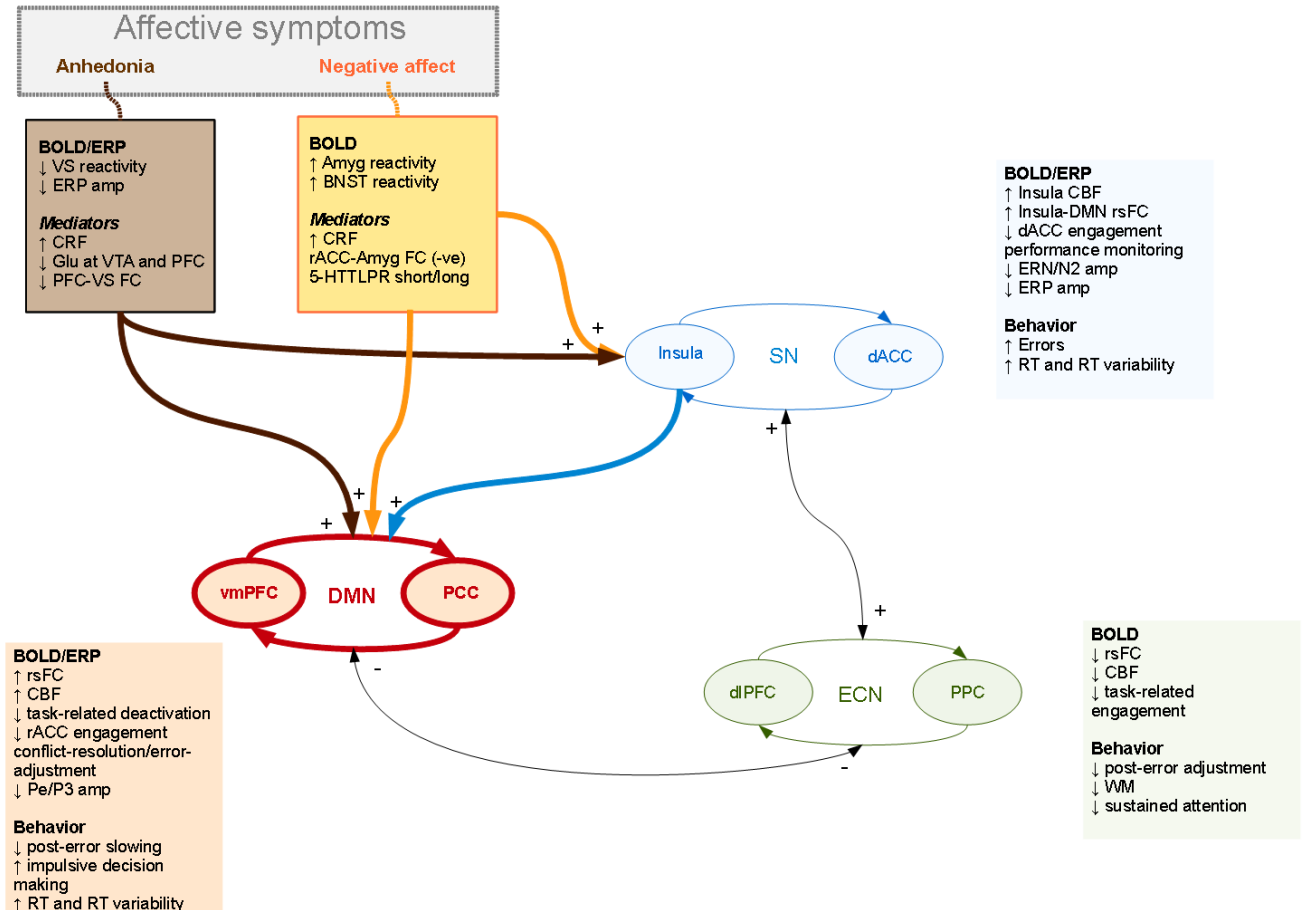


Figure 1. Summary of hypothesized neurobiological mechanisms underlying anhedonia, negative affect inhibitory control and impulsive decision making deficits experienced during acute nicotine withdrawal. The figure also outlines the proposed shift in resting state network (RSN) dynamics with thicker arrows illustrating increased input and connectivity during acute nicotine withdrawal relative to nicotine satiety. We also working memory (WM) and sustained attention deficits in our list of effects resulting from reduced engagement of the Executive Control Network (ECN) as these will be measured in the current protocol. DMN = Default Mode Network; SN = Salience network; VS = ventral striatal; BNST = bed nucleus of the stria terminalis; Amyg = amygdala; CRF = corticotrophin releasing factor; rACC = rostral anterior cingulate cortex; dACC = dorsal anterior cingulate cortex; rsFC = resting state functional connectivity; CBF = cerebral blood flow; BOLD = blood oxygen level dependent; ERP = event-related potential; RT = reaction time; ERN = error-related negativity; Glu = glutamate; VTA = ventral tegmental area; PCC = posterior cingulate cortex; vmPFC = ventromedial prefrontal cortex; dlPFC = dorsolateral prefrontal cortex; PPC = posterior parietal cortex.

1.5. Motivation to quit

Motivation to quit smoking can have a profound effect on neural activation and craving elicited to drug cues (for reviews Wertz & Sayette, 2001; Wilson, Sayette, & Fiez, 2004b). For example, Wilson and colleagues (2004) observed that engagement of prefrontal cortical regions (OFC, dlPFC, dACC) in response to drug-related cues is primarily observed in non-treatment seeking smokers. When treatment-seeking smokers are examined, studies are much less likely to observe cue-induced engagement of prefrontal regions (Wilson, Sayette, & Fiez, 2004a). The differential engagement of prefrontal regions in response to drug cues likely reflects differences between the groups in motivational/cognitive control processes (Wilson et al., 2004b), and in some cases, expectancy to smoke (McBride, Barrett, Kelly, Aw, & Dagher, 2006). To date, the large majority of neuroimaging studies on nicotine addiction are based on non-treatment seeking smokers. The observation of Wilson et al. (2004) and a recent study by this same group (Wilson, Sayette, & Fiez, 2012) raise the question of whether relapse- or abstinence-related effects observed in non-treatment seeking smokers can be applied to treatment-seeking smokers for whom this research is most pertinent. Moving forward it is therefore important to assess effects in both non-treatment and treatment seeking smoker populations to establish convergent and divergent mechanisms and to provide a greater understanding of how motivation to quit smoking interacts with relapse- and abstinent-related processes.

1.6. Transcranial Direct Current Stimulation

Sections 1.1 to 1.5 of the introduction highlight the challenge of treating nicotine dependence. Cigarette smoking remains the leading cause of preventable death and disease in the United States, accounting for 480,000 deaths each year (CDC 2016). Despite our improved understanding of the neurobiology of nicotine addiction and drug abuse over the past 10 years (Nestler 2005, Everitt and Robbins 2005, Koob and Volkow 2010, Goldstein and Volkow 2011), current pharmacological interventions for smoking cessation remain highly inadequate, with the most efficacious treatment (varenicline) having only about 27% absolute cessation rate according to recent meta-analysis (Cahill 2013, 2014). Additionally, symptoms of nicotine withdrawal remain a major impediment for smokers trying to quit, with most quit attempts failing within the first week of abstinence when withdrawal symptoms are at their height (Hughes 1992, Sutherland et al 2017).

As introduced in *Sections 1.3 (The Brain's Default Mode Network) and 1.4 (Inhibitory Control & Impulsive Decision Making during Nicotine Withdrawal),* recent findings based on resting state functional connectivity (rsFC) revealed that the brain can be divided into multiple large-scale functional networks, many of which have been found to contribute to psychiatric disease (Lu and Stein 2014, Turk-Browne 2013, Yeo et al 2011, Fox et al 2014). Addiction has been hypothesized as an imbalance within and between three of these networks (Lerman et al 2014, Pariyadath et al 2015, Fedota and Stein 2015, Sutherland et al 2012). Specifically, the cognitive and affective disturbances of nicotine withdrawal syndrome have been attributed to a reduced coherence within-network connectivity of the Executive Control Network (ECN) and increased within-network connectivitystrength of the Default Mode Network (Sutherland et al 2017). Additionally, the Salience Network (SN) has been called the "gatekeeper" for these two networks, and its function may be dysregulated in addictive disorders, as well (Sutherland et al 2017).

A treatment that acts by modifying these large-scale brain networks with specificity may help address the underlying pathophysiology of nicotine addiction and improve clinical outcomes (Dunlop et al 2016). Transcranial Direct Current Stimulation (tDCS), a type of Non-invasive Brain Stimulation (NIBS), has the potential to modify neuronal circuits by application of a subthreshold conductive current through the scalp. Two potential targets for tDCS as a smoking cessation aid are the dorsolateral pre-frontal cortex (dlPFC), a node of the ECN, and the ventromedial prefrontal cortex (vmPFC), a node of the DMN (Lerman et al 2014). tDCS can potentially strengthen the control of the ECN through excitatory stimulation of the dlPFC, and weaken the influence of the DMN by inhibitory stimulation of the vmPFC. It is important to study the acute effects of tDCS applied to these networks to better understand its potential to be used as a smoking cessation aid in the future.

1.7. Considerations when conducting nicotine-withdrawal research

Nicotine Abstinence Period. The affective (e.g., anhedonia, anxiety, irritability) and cognitive (e.g., difficulty concentrating) withdrawal symptoms following acute nicotine abstinence show an onset of 2 to 12 hrs post-cessation and peak within 2-3 days (J R Hughes, Higgins, & Bickel, 1994). Most prior imaging and EEG studies have assessed acute withdrawal effects following overnight abstinence (typically 8 to12 hrs of smoking deprivation; e.g., Azizian et al., 2010; Beaver et al., 2011; D. M. Cole et al., 2010; Loughead et al., 2010; Sweet et al., 2010) and a few, 24 hours abstinence (Kozink, Kollins, & McClernon, 2010a; McClernon et al., 2009b).

Two different durations of the abstinence period have been selected for the Main Study Arm (36 hours deprivation) and the tDCS Arm (12 hours deprivation). Each has advantages and disadvantages, and the time periods selected best suit the individual aims within the particular arms.

We have selected 36 hours for the Main Study Arm because it approximates maximal acute withdrawal effects, producing robust effects across MRI, EEG and behavioral measures that can be used as predictors for success on sustained abstinence later in the study design. This time period ensures that the study can capture the neurobiological changes and behavioral phenomena that underlie acute nicotine withdrawal effects. However, this long period of abstinence comes at the expense of subject compliance.

Although a longer abstinence period accentuates the nicotine withdrawal effects, shorter periods of abstinence (such as 12 hours) have been found to result in changes in behavior, resting state functional connectivity, and BOLD patterns. Previous studies using overnight abstinence periods (10-12 hrs) have shown acute nicotine effects when assessing behavioral performance (e.g., Mancuso et al., 1999; Dawkins et al., 2006, 2007b), EEG/ERPs (e.g., Knott et al., 1999; Pritchard et al., 2004; Gilbert et al., 2007), and fMRI signals (e.g., Xu et al., 2005; Brody, 2006; Wang et al., 2007). For example, using a battery of behavioral tests, acute smoking deprivation (12hrs) has been associated with impaired reward motivation (Dawkins et al., 2006) and a reduced capacity to inhibit prepotent motor responses (Dawkins et al., 2007b). With regard to fMRI signals, nicotine-abstinent states (12hr), in comparison to satiated states, have been associated with significant regional cerebral blood flow increases in ACC during “resting” periods (Wang et al., 2007) and alterations in dorsal lateral PFC activity during working memory tasks (Xu et al., 2005).

We have selected 12 hours abstinence for the tDCS Arm because we have shown it sufficient to achieve a “delta” in these outcome measures when compared to the non-deprived condition, allows us to test the hypotheses germane to this arm, and has the added benefit of improved subject compliance. The 12 hours

abstinence will allow an evaluation of how tDCS can *acutely* modify large-scale networks in both the nicotine sated and nicotine deprived conditions.

Individually Tailored Nicotine Dose. The purpose of the current study is to understand the neurobiological mechanisms underlying acute nicotine withdrawal in humans. Chronic nicotine administration in animals (Marks, Stitzel, & Collins, 1986) and humans (Breese et al., 1997) produces a dose-dependent increase in neural adaptations which ultimately underlie acute withdrawal effects (Buisson & Bertrand, 2002). In an effort to minimize nicotine withdrawal effects in the non-deprived condition it is therefore critical that we match each participant's nicotine-patch dose to their typical daily nicotine intake (how? Who does this?). Individually tailored nicotine patch doses have been used in smoking cessation trials (Hurt, 2003) and higher doses have been shown to produce a greater reduction of withdrawal symptoms in heavier users (Ebbert et al., 2007) . In addition, NIDA protocol 398 also employs the use of an individually tailored nicotine dose based on the number of daily cigarettes.

2. Study objectives or hypotheses

Overview

The Main Study will focus on the anhedonia, negative affect, inhibitory control deficits and impulsive decision making which accompany acute nicotine withdrawal in humans. The extent to which these acute withdrawal-related processes predict the success of smoking-cessation treatment will be assessed. The primary objective is to gain a greater understanding of the neurobiological mechanisms underlying acute nicotine withdrawal and contributing to the maintenance of, or return to smoking behavior among nicotine-dependent individuals. Secondary objectives are to understand the neurobiological basis of responsivity to nicotine replacement with respect to acute nicotine withdrawal symptoms, and to examine the neurobiological mechanisms underlying motivation to quit smoking and the interaction between motivation to quit and symptoms, which arise during acute withdrawal from nicotine.

Study population

We aim to recruit 85 nicotine-dependent individuals aged 18 to 55 years who are currently seeking treatment for smoking cessation (treatment-seeking smokers) and 35 nicotine-dependent individuals aged 18-55 years who are *not* currently seeking treatment for smoking cessation (non-treatment seeking smokers). Nicotine dependency will be based on a urine cotinine level corresponding to smoker status for the specific test being used, typically corresponding to a urine cotinine above about 200 ng/ml, and a history of smoking consistently for at least one year.

Design

The study will be a within (nicotine deprivation), between (treatment-seeking status) subjects double blind placebo controlled design. All participants will complete two scanning sessions, each preceded by a 36 hour smoking abstinence period. During one abstinence period and scanning session participants will wear a dose-matched nicotine patch (non-deprived), and during the other abstinence period and scanning session, a placebo-patch (nicotine-deprived). Following participation in both scan sessions treatment-seeking participants will commence 12 weeks of treatment involving daily varenicline administration with weekly counseling. Follow-up assessments will be conducted with treatment-seeking smokers at 1, 6 and 12 months after their last treatment visit.

2.1. Primary goals

To characterize the neurobiological mechanisms underlying acute nicotine withdrawal and the maintenance of smoking behavior in current cigarette smokers. To achieve this, the current protocol will employ multiple task-based and resting state measures to probe the anhedonic and anxiogenic effects of acute nicotine withdrawal as well as reported inhibitory control deficits and increased impulsive decision-making. Changes in RSN dynamics will also be examined in relation to affective and cognitive symptoms associated with acute nicotine withdrawal. Acute nicotine-withdrawal will be manipulated by assessing dependent smokers twice: once in a non-deprived state (following 36 hours of smoking abstinence during which time they will continually wear a dose-matched nicotine patch) and on a second occasion in a nicotine-deprived state (following 36 hours of smoking abstinence whilst wearing a placebo patch). In the nicotine-deprived condition smokers are expected to report anhedonia and negative affective symptoms and associated changes in mesolimbic dopaminergic pathways

as well as amygdala and BNST functioning. As illustrated in Figure 1, we expect these changes to drive inhibitory control deficits and elevated impulsive decision making by altering functioning within and between intrinsic default mode, executive control and salience networks. Finally, the extent to which neurobiological changes resulting from acute nicotine withdrawal predict success of treatment for smoking cessation will be assessed.

2.2. Secondary goals

An additional aim of the current protocol is to identify a neurobiological basis for variance in responsivity to nicotine replacement. Individual differences in response to nicotine replacement exist both in the degree of alleviation of acute withdrawal symptoms (Cole et al., 2010) as well as in terms of longer-term relapse outcomes (Uhl et al., 2008). In the current protocol responsivity will be operationalized as change in craving, affective and cognitive symptoms following nicotine-deprivation relative to nicotine-satiety with a dose-matched nicotine patch. By examining the source of individual differences in responsivity to nicotine replacement, we may be able to identify neurobiological targets for adjunct or alternative treatments in poor responders. We will also examine the extent to which pre-existing traits predict neurobiological correlates of responsivity to nicotine replacement.

This protocol also aims to examine the common short/long allele polymorphism in the 5-HTTLPR gene as a mediator of acute nicotine withdrawal effects on amygdala reactivity and rACC-amygdala connectivity.

A final goal of the present research is to examine the neurobiological mechanisms underlying motivation to quit smoking and the interaction between motivation to quit and symptoms which arise during acute withdrawal from nicotine. To achieve this aim, non-treatment seeking smokers will be recruited and will complete the same experimental procedures outlined in Section 2.1 under nicotine-deprived and non-deprived state but will not enter treatment for smoking cessation and will not be followed-up to assess for treatment success. We will examine the same processes outlined in Section 2.1 which we are assessing as a function of acute nicotine withdrawal and will look for between group differences (treatment-seeking vs non-treatment seeking smokers) on these measures.

2.3. Aims and hypotheses

Aim 1: To examine the effect of acute nicotine withdrawal on self-reported anhedonia and its relationship to reward responsiveness and striatal reactivity, and in turn the extent to which these withdrawal-related neurobiological processes predict treatment success. Acute nicotine withdrawal produces a state of anhedonia and reduced reward responsiveness. Preclinical work suggests that these effects may result from reduced dopamine release at the NAcc (manifesting in reduced striatal reactivity), mediated by increased central CRF and potentially by reduced glutamate signaling in a circuit from the PFC to the NAcc, and potentially the hippocampus.

Measures: Self-reported anhedonia; behavioral and EEG indices of reward responsiveness; BOLD response and functional connectivity during striatal reactivity task; task measure of striatal reactivity, EEG and behavioral correlates of reward responsiveness.

Mediators: Plasma ACTH; Glutamate concentration at the PFC/ACC and/or hippocampus measured with MRS.

Hypothesis 1.1: Compared with nicotine satiety, acute nicotine withdrawal will result in increased self-reported anhedonia, accompanied behavioral correlates of reward responsiveness and reduced ventral striatal reactivity. The degree of reduced striatal reactivity and reward responsiveness will in turn predict treatment success.

Hypothesis 1.2: The effect of acute nicotine withdrawal on striatal reactivity will be partially mediated by an increase in plasma adrenocorticotropic hormone (ACTH; an index of central CRF).

Hypothesis 1.3: Acute nicotine withdrawal will result in reduced glutamate measured with MRS at the hippocampus and PFC, with the latter predicting reduced PFC-NAcc connectivity. Reduced PFC glutamate, as well as reduced PFC-NAcc signaling will partially mediate the predicted reduction in ventral striatal reactivity.

Aim 2: To examine the effect of acute nicotine withdrawal on self-reported negative affect (anxiety, irritability) and stress reactivity, and in turn on reactivity in the amygdala and BNST. Acute nicotine withdrawal produces marked elevations in negative affect and enhanced stress reactivity. Although preclinical literature is limited, there is some evidence that these effects may be mediated by enhanced central CRF acting at the amygdala and BNST. Neuroimaging studies have linked individual differences in negative affect and stress reactivity to amygdala reactivity as well as top down regulation from the rostral ACC. Both amygdala reactivity and rACC-amygdala connectivity are in turn mediated by the short/long allele polymorphism in the 5-HTTLPR gene.

Measures: Self-reported negative affect (e.g., anxiety, irritability) and craving; BOLD response and functional connectivity during amygdala reactivity task and USCRT.
Mediators: Plasma ACTH; 5-HTTLPR short/long allelic polymorphism

Hypothesis 2.1: Compared with nicotine satiety, acute nicotine withdrawal will result in enhanced negative affect (e.g., anxiety, irritability), accompanied by enhanced amygdala and BNST reactivity. These effects will be partially mediated by an increase in plasma ACTH (as a marker of central CRF).

Hypothesis 2.2: The effect of nicotine withdrawal on amygdala reactivity will be partially mediated by reduced regulatory input from the rACC. During nicotine withdrawal we expect the rACC (and other DMN regions) to be engaged in rumination on craving and affective symptoms. Consequently, we expect reduced task-related regulatory input from the rACC to the amygdala, manifesting in reduced rACC-amygdala effective connectivity during an amygdala reactivity task.

Hypothesis 2.3: The effect of acute nicotine withdrawal on amygdala reactivity and rACC-amygdala connectivity will be most pronounced among individuals expressing the short allele of the 5-HTTLPR gene.

Hypothesis 2.4: Larger changes in amygdala and BNST reactivity will be associated with poorer treatment outcomes.

Aim 3: To examine the neurobiological basis of inhibitory control deficits and impulsive decision making observed during acute nicotine withdrawal and the extent to which these processes predict treatment success. Inhibitory control deficits may arise from poor monitoring for salient events (errors, conflict) which signal the need to adjust behavior (insula-dorsal ACC network) or problems adjusting behavior following errors or conflict (ECN or rACC). During acute nicotine-withdrawal we expect reduced task-related DMN deactivation, driven by enhanced craving and cognitive symptoms. This should in turn result in reduced engagement of the dACC in extrinsic performance monitoring and reduced ECN and rACC engagement in post-conflict/error adjustment processes and decision-making.

Measures: BOLD, ERP and behavior during go-nogo, affective flanker, and striatal reactivity tasks; behavior during the delay discounting task, Self-reported craving and affective symptoms, BOLD rsFC and CRF at rest.

Mediators: Task-related BOLD

Hypothesis 3.1: Relative to nicotine satiety, acute nicotine withdrawal will be associated with reduced dACC engagement in performance monitoring. Due to lower dACC engagement during on-going performance monitoring (e.g., congruent or go trials which typically function as a baseline), detection of errors or conflict will be associated with an enhanced dACC signal and enhanced amplitude in ERP correlates of conflict detection. Behaviorally, reduced dACC engagement during performance monitoring will be mediated by reduced DMN deactivation, each of which will be associated with greater variance response times and more performance errors.

Hypothesis 3.2: Nicotine deprivation (relative to nicotine satiety) will be associated reduced DMN deactivation during task, will be greatest in individuals reporting more severe craving and affective symptoms. Reduced task-related deactivation in the DMN will in turn compromise the ability of the rACC (being part of the DMN) and the ECN (which is negatively coupled to the DMN) to engage in task-related conflict-resolution and post-error adjustment processes.

Hypothesis 3.3: Acute nicotine withdrawal is expected to lead to a steeper discounting of rewards as a function of time (more impulsive decision making). More impulsive decision making will be associated with enhanced rsFC connectivity in the DMN and between the DMN and insula at rest, and greater CBF to DMN regions. We expect the relationship between impulsive decision making and rsFC and resting CBF changes following acute nicotine withdrawal to be strongest in medial PFC components of the DMN

Aim 4: To examine the impact of 36 hrs of acute nicotine-withdrawal, relative to nicotine satiety, on rsFC and CBF within and between the DMN, ECN and salience network. We also wish to examine the relationship between these changes and affective and cognitive withdrawal symptoms, urge to smoke and treatment success.

Measures: self-reported affective and cognitive withdrawal symptoms; self-reported urge to smoke; resting connectivity within and between the DMN, ECN and salience networks; CBF to DMN, ECN and salience network regions at rest.

Hypothesis 4.1: Compared to nicotine satiety, acute nicotine withdrawal will be associated with enhanced resting state connectivity within the DMN and between the DMN and insula node of the salience network. The degree of elevation in resting connectivity within the DMN and between the DMN and insula will predict the change in self-reported urge to smoking, severity of affective withdrawal symptoms and treatment success.

Hypothesis 4.2: Acute nicotine withdrawal will be associated with reduced connectivity within the ECN and between the ECN and insula, as well as reduced negative coupling between the ECN and DMN. The degree of reduced connectivity and negative coupling will predict with the severity of cognitive withdrawal symptoms as well as treatment success.

Aim 5: To examine characteristics of rsFC associated with long-term abstinence following treatment for smoking cessation. To address this aim we will examine the following exploratory questions:

Q1. What characteristics of rsFC are associated with treatment success (vs failure) at 1, 6 and 12 months post-treatment?

Q2. Are there characteristics of rsFC that vary as a function of time post-treatment in successfully abstinent individuals?

Aim 6: To examine the neurobiological basis of motivation to quit smoking and the interaction between motivation to quit and mechanisms underlie acute nicotine withdrawal.

Q1. Do the self-reported symptoms of acute nicotine withdrawal (e.g., anhedonia, anxiety/irritability, difficulty concentrating, impulsive decision making) and the putative neurobiological processes which underlie these (e.g., enhanced amygdala reactivity, reduced striatal reactivity, hypothesized shift in resting state network dynamics in Fig 1) vary as a function of motivation to quit (treatment-seeking vs non-treatment seeking smokers).

Q2. Is there a main effect of motivation to smoke (treatment-seeking vs non-treatment seeking smokers) on measures of striatal and amygdala reactivity, cognitive control, drug cue reactivity and resting state network dynamics.

3. Subjects:

3.1. Study population

Treatment-seeking smokers. This study will enroll healthy male and female adults who have a history of 1 or more of consistently years smoking cigarettes with a urine cotinine level corresponding to smoker status for the specific test being used, typically corresponding to a urine cotinine above about 200 ng/ml, and are actively seeking treatment for smoking cessation. We are requesting to enroll a total of 120 treatment-seeking smokers, with 85 participants expected to a) complete the two pre-treatment scan visits; 2) set a quit date; 3) attempt to quit for at least 24 hours; 4) have their quit status established (i.e., relapsed vs abstinent) at 1, 6 and 12 months following their quit date.

Non-treatment seeking smokers. This study will also recruit 42 healthy male and female adults who have a history of 1 or more years of consistently smoking cigarettes with a urine cotinine level corresponding to smoker status for the specific test being used, typically corresponding to a urine cotinine above about 200 ng/ml, but who are *not* currently seeking treatment for smoking cessation. 35 participants are expected to complete the two pre-treatment scan visits. Non-treatment seeking smokers will *not* set a quit date or make a quit attempt, will *not* complete a treatment program as part of this study and will *not* be followed up after they complete the two pre-treatment visits.

3.2. Inclusion criteria

All participants must:

(1) Be between the ages of 18-55. Be right-handed. *Assessment tool(s):* Edinburgh *Handedness Inventor*y.

(2) Be in good health. *Justification:* Many illnesses may alter fMRI signals as well as cognitive processes and neural functioning. *Assessment tool(s):* Participants will provide a brief health history during phone screening, and undergo a medical history and physical examination with a qualified IRP clinician.

(3) Be free of active DSM-IV dependence, or dependence in partial remission, on alcohol or any drug except nicotine. Past active dependence is acceptable provided it is at least five years in the past and total time of active dependence did not exceed 4 years. Those with past dependence on any substance other than alcohol or marijuana may not have any current use (past 6 months) of the substance on which they were dependent. For individuals with past alcohol or marijuana dependence, current use of the previously

dependent substance will be allowed providing they do not meet any current DSM-IV criteria for substance dependence, with the exception of tolerance. *Justification:* Dependence on other substances (drugs or alcohol) may result in unique CNS deficits that could confound results and introduce excessive variance. *Assessment tool(s)*: The computerized SCID and clinical substance abuse/dependence assessment. While recreational/intermittent use of alcohol and/or marijuana will be tolerated in all participant groups, individuals will be excluded if they meet current or recent (within 5 years) DSM-IV diagnostic criteria for dependence on any substances. A positive drug test for marijuana will not be exclusionary as long as participants have not used in the 24hrs preceding the imaging visits. In the event of a positive drug test for marijuana, self-reports of current marijuana use will be used to differentiate intermittent/infrequent from chronic/frequent users. Given a possible link between marijuana use and psychosis (Ben Amar and Potvin, 2007) frequent users, defined as those using twice or more per week within the last month, will be excluded from participation.

(4) Be able to abstain from alcohol 24hrs before each of the imaging sessions and able to moderate their caffeine intake 12hrs before each session. *Justification:* Alcohol and caffeine modulate neural functioning in a way that would complicate data interpretation. *Assessment tool(s):* Self-report and breathalyzer.

(5) Must have a urine cotinine level corresponding to smoker status for the specific test being used, typically corresponding to a urine cotinine above about 200 ng/ml, and have been smoking consistently for at least one year. For lighter smokers (less than 10 cpd), this is defined as smoking at their current level or more for at least the past year (excluding any quit attempts in the last year). For heavier smokers (more than 10 cpd), they must have been smoking at least an average of 10 cpd for at least the past year (excluding quit attempts). Quit attempts will be assessed via clinical interview and judgment. Smokers vary in their smoking topography (puff volume, time held, number of puffs etc.,) (Patterson et al., 2003) a factor which can significantly alter the amount of nicotine consumed from a single cigarette (Patterson et al., 2003). Cotinine levels should therefore provide a more accurate assessment of actual nicotine exposure than self-reported cigarette use (Perezstable et al., 1995).. *Justification:* The present protocol is interested in neurobiological mechanisms that underlie nicotine withdrawal, and is thus contingent on the presence of nicotine dependence. Assessment tool(s): Self-report, commercial urine cotinine test corresponding to smoker status for the specific test being used, typically corresponding to a urine cotinine above about 200 ng/ml .

(6) Be able to abstain from smoking for 36hrs on two occasions during the study. *Justification:* The present protocol will investigate the effect of acute nicotine withdrawal on affective, inhibitory control and decision making processes and their neurobiological correlates. Previous research suggests that a 36hrs deprivation period is appropriate to produce robust effects across these domains (Kozink et al., 2010a; McClernon et al., 2009b; VanderVeen, Cohen, Cukrowicz, & Trotter, 2008). To isolate effects to nicotine-withdrawal (as opposed to tobacco withdrawal), participants will be required to abstain from smoking prior to both scanning session. They will be delivered nicotine during one abstinence period (non-deprived) via a dose-matched transdermal patch, and will wear a placebo patch during the other abstinence period (nicotine-deprived). *Assessment tool(s)*: video recorded measurement of expired CO levels plasma nicotine levels.

(7) Agree to also participate in NIDA-IRP protocol 10-DA-N457.

<u>Treatment-seeking</u> smokers will also have to meet the following inclusion criteria:

(8) Be actively seeking treatment for smoking cessation and willing to engage in 12-weeks of treatment involving daily administration of Varenicline and weekly counseling sessions, as well as follow-up assessments at 1, 6 and 12 months following treatment onset.

3.3. Exclusion criteria

All participants will be excluded if they:

(1) are not suitable to undergo an fMRI experiment due to certain implanted devices (cardiac pacemaker or neurostimulator, some artificial joints, metal pins, surgical clips or other implanted metal parts), body morphology, or claustrophobia. *Justification:* MR scanning is one of the primary measurement tools used in the protocol. *Assessment tool(s):* Prospective participants will fill out an MRI screening questionnaire and undergo an interview with an MR technologist. Questions concerning suitability for scanning will be referred to the MR Medical Safety Officer. Prospective participants will be questioned about symptoms of claustrophobia and placed in the mock scanner during their first visit to assess for possible difficulty tolerating the confinement of the scanner and for ability to fit into the scanner.

(2) have coagulopathies, history of, current superficial, or deep vein thrombosis, musculoskeletal abnormalities restricting an individual's ability to lie flat for extended periods of time. *Justification:* MR scanning sessions require participants to lie flat on their backs and remain perfectly still for approximately two hours. Therefore, conditions that would make that difficult (e.g. chronic back pain, significant scoliosis) or dangerous (e.g. familial hypercoagulability syndrome, history of thrombosis) will be exclusionary. *Assessment tool(s):* History and physical examination by a qualified IRP clinician, supplemented with a trial of lying in the mock scanner to assess comfort issues.

(3) have HIV or Syphilis. *Justification:* HIV and Syphilis both can have central nervous system (CNS) sequelae, thus introducing unnecessary variability into the data. *Assessment tool(s):* Oral HIV followed by blood test if oral test is + and STS+ without adequate prior treatment

(4) regularly use any prescription (e.g., antidepressants, benzodiazepines, antipsychotics, anticonvulsants, barbiturates), over-the-counter (e.g., cold medicine) or herbal medication (e.g., Kava, Gingko biloba, St. John's wort) that may alter CNS function, cardiovascular function, or neuronal-vascular coupling. *Justification:* The use of these substances may alter the fMRI signal and/or neural functions of interest in the current study. *Assessment tool(s):* History and comprehensive urine drug screening to detect antidepressants, benzodiazepines, antipsychotics, anticonvulsants, and barbiturates.

(5) have any current neurological illnesses including, but not limited to, seizure disorders, frequent migraines or on prophylaxis, multiple sclerosis, movement disorders, history of significant head trauma, or CNS tumor. *Justification:* Neurological diseases alter CNS function and, possibly, the neuronal-vascular coupling that forms the basis of the fMRI signal. *Assessment tool(s):* History and physical examination by a qualified IRP clinician, urine drug screening for anticonvulsants not disclosed by history. History of head trauma with loss of consciousness of more than 30 minutes or with post-concussive sequelae lasting

more than two days, regardless of loss of consciousness, will be exclusionary. The MAI who will also retain discretion to exclude based on a history of neurological illness that may compromise data integrity.

(6) Have any current major psychiatric disorders to include, but not limited to, mood, anxiety, psychotic disorders, or substance-induced psychiatric disorders, or any current suicidal ideations or history of suicide attempts or currently under antidepressant or antipsychotic medication treatment. The MAI will reserve the right to exclude on the basis of psychiatric history not explicitly described in this criterion *Justification:* Psychiatric disorders involve the central neural system (CNS) and, therefore, can be expected to alter the fMRI measures being used in this study. A recent FDA communication (2/1/2008) indicates that there may be an association between varenicline and the worsening of current psychiatric illness even if the illness is currently under control. *Assessment tool(s):* Computerized SCID, Beck Depression Inventory, Beck Anxiety Inventory, Adult ADHD Self-Report Scales and clinical interview confirmation by clinician.

(7) Are cognitively impaired or learning disabled. *Justification:* Cognitive impairment and learning disabilities may be associated with altered brain functioning in regions recruited during laboratory task performance. Cognitive impairment may affect one's ability to give informed consent. *Assessment tool(s):* History of placement in special-education classes as a consequence of serious learning problems and not solely as a consequence of behavioral problems, assessed during the History and Physical screening assessment.

(8) have significant cardiovascular or cerebrovascular conditions. *Justification*: Such conditions may alter blood flow, the fMRI signal and other autonomic signals, and increase risks associated with nicotine patch use. *Assessment tool(s):* History and physical exam, including 12-lead EKG.

(9) have any other major medical condition that in the view of the investigators would compromise the safety of an individual during participation. *Justification:* Many illness not explicitly covered here may increase risk or alter important outcome measures. *Assessment tool(s):* History and physical examination by a qualified IRP clinician and CBC, urinalysis, NIDA chemistry panel (liver function tests, electrolytes, kidney function). The following lab values will result in exclusion from the study:

i. Hemoglobin < 10 g/dl
ii. White Blood Cell Count < 2400/µl
iii. Liver Function Tests > 3X normal
iv. Serum glucose > 200 mg/dl
v. Urine protein > 2+
vi. Serum creatinine > 2 mg/dl
vii. Estimated glomerular filtration rate < 60 ml/min

a. The MAI will retain discretion to exclude based on less extreme lab results. After the screening process has been completed, the MAI will take into account all data collected in order to decide if there is an existing medical illness that would compromise participation in this research.

(10) are pregnant, planning to become pregnant, or breastfeeding. Females are instructed in the consent to use effective forms of birth control during the study period. Justification: study procedures and drugs

used in the current protocol may complicate pregnancy or be transferred to nursing children. Assessment tool(s): Urine and/or serum pregnancy tests, and clinical interview. Urine pregnancy tests will be conducted at the beginning of each imaging visit.

(11) are non-English speaking. Justification: To include non-English speakers, we would have to translate the consent and other study documents and hire and train bilingual staff, which would require resources that we do not have and could not justify given the small sample size for each experiment. Additionally, the data integrity of some of the cognitive tasks and standardized questionnaires used in this study would be compromised as they have only been validated in English. Most importantly, ongoing communication regarding safety procedures is necessary when participants are undergoing MRI procedures. The inability to effectively communicate MRI safety procedures in a language other than English could compromise the safety of non-English speaking participants. Assessment tool(s): self-report.

Treatment-seeking smokers will also be excluded if they:

(12) have moderate to severe renal impairment. *Justification. Given that renal* secretion is varenicline's major route of clearance, kidney impairment may result in higher systemic levels of the drug than intended. Per Pfizer's chantix insert, varenicline pharmacokinetics were *unchanged in s*ubjects with mild renal impairment in comparison to those with normal renal function, whereas individuals with moderate and severe impairment presented with varenicline levels 1.5 and 2.1-fold higher, respectively. *Assessment tool(s): Estimated* glomerular filtration rate. Renal insufficiency with estimated creatinine clearance < 60 ml/min calculated by the Cockcroft-Gault equation will be excluded.

(13) are diabetic. *Justification.* A recent case report describes multiple episodes of severe hypoglycemia experienced by a 51 year old Type-I diabetic after beginning varenicline treatment (Kristensen et al., 2008). The discontinuation of varenicline resolved any further hypoglycemic episodes. The safety of varenicline has not been investigated in patients with diabetes. *Assessment tool(s):* Casual plasma glucose testing. Individuals with glucose levels above 200 mg/dl may be further evaluated for diabetes using a fasting glucose test or be excluded.

4. Study Design and Methods:

4.1. Study overview

Treatment seeking smokers will be asked to complete three study phases: pre-treatment study visits, treatment visits and post-treatment follow-up visits.

Non-treatment seeking smokers will *only* be asked to complete Phase 1: Pre-treatment study visits.

PHASE 1 - Pre-treatment study visits

Phase 1 will typically require three outpatient visits to NIDA-IRP (one orientation visit, two fMRI imaging visits). Visits will take place over approximately a 3-5 week period. If an individual participant's schedule makes it difficult for them to complete the visits as they are described here, we will try to accommodate

their needs when this is possible and does not interfere with the study design or study aims. For example, orientation training day procedures could be completed over 2 or more days.

The orientation visit will take approximately 6 hours and each scanning visit, approximately 9 hours. After screening and admission into the study, participants will be scheduled for an orientation visit. During this visit they will complete the informed consent process as well as task training for the scanning sessions. All participants will complete two scanning sessions: one whilst nicotine-deprived, and another non-deprived session. Nicotine deprivation will be manipulated by asking participants to either wear a dose-matched nicotine (non-deprived) or placebo (nicotine-deprived) patch during a 36 hour smoking abstinence period completed prior to both scans. The order of nicotine-deprived and non-deprived sessions will be counterbalanced across participants. Participants will be blinded as to which sessions/abstinence periods involve nicotine- or placebo-patches. The double-blind with respect to the nicotine/placebo patch condition will be maintained by Dr. Betty Jo Salmeron or the protocol MAI. The investigator/s interacting with the participants will be blind to the nicotine/placebo patch condition the participant is undergoing, at the time of their interactions. "Investigator/s interacting with participants" includes the post-doc or research assistant who runs the study session and gathers research data. It does not include medical personnel such as nurses who may interact with the participant during their study visit but do not gather research data.

Participants will generally receive their scan 1 nicotine and placebo patches during the orientation session, as well as a CO monitor, video camera and questionnaires. The patches and equipment for scan 2 will generally be given to participants during their scan 1 session. During both 36hr abstinence periods participants will use a video camera to record themselves taking their CO levels and will report their craving and affective symptoms on the paper copies of take-home questionnaires. Training to measure and video record the measurement of CO levels will take place during the orientation session. Follow-up calls will be made to each participant as a reminder to commence their abstinence period.

During each scanning visit participants will complete a series of imaging measures and several tasks to be complete outside of the scanner. In addition to imaging measures and bench tasks, participants will also be asked to complete several psychological questionnaires (see detailed list below) and will also provide blood samples for cortisol and ACTH.

Nicotine administration during abstinence and scanning sessions. Two essential pharmacological issues that are of importance in the current design relate to participant's recent exposure to nicotine and to the selected dose administered to each participant. Doses will be individually tailored based on the average number of cigarettes smoked per day to better match daily nicotine intake (please see "*Individually Tailored Nicotine Dose*" in Section 1.7 above for justification). The dose for smokers consuming less than 10 cigarettes per day will be 14mg to start, If they are still experiencing cravings their dose will be increased to 21mg. The dose for smokers consuming 10-15 cigarettes per day will be a 21mg patch and this will be increased by 7mg for each additional 5 cigarettes/day (i.e., 21mg for individuals who smoked 10-15 cigarettes/day; 28mg for 15-20 cigarettes; 35mg for 20-25 cigarettes, and 42mg for more than 25 cigarettes). Nicotine patch doses up to 63mg/d have been shown to be safe and generally well-tolerated by smokers (Ebbert et al., 2007; Zevin, Jacob, & Benowitz, 1998).

An IND has not been obtained for the use of the nicotine patch in this study. This study meets the criteria for exemption for an IND as this investigation is not intended to support a new indication for use or any other significant change to the labeling; the nicotine patch is already approved and marketed and the investigation is not intended to support a significant change in advertising; and the investigation does not involve a route of administration or dosage level in use in a patient population or other factor that significantly increases the risks associated with the use of the drug product. Individually tailored nicotine patch doses have been used in smoking cessation trials (Hurt, 2003) and higher

doses have been shown to produce a greater reduction of withdrawal symptoms in heavier users (Ebbert et al., 2007) . As stated above, nicotine patch doses up to 63mg/d have been shown to be safe and generally well-tolerated by smokers (Ebbert et al., 2007; Zevin, Jacob, & Benowitz, 1998).

PHASE 2 - Treatment visits

Phase 2 will involve 15-18 visits over a period of about 16-19 weeks. Within approximately 2 weeks of their second scan visit, participants will be scheduled for their first of up to 3 pre-treatment preparation visits. The number of preparation visits (1 to 3) will be determined by the study therapist on the basis of the participants assessed level of preparation and motivation to quit smoking. During their final pretreatment preparation visit, participants will work with the therapist to develop a treatment plan and set a quit date. They will also receive their first 2-weeks supply of varenicline during this visit (or their first treatment visit) and will meet with the MAI or the designated NIDA-IRP physician in the MAIs absence who will describe the medication, prescribed dosing schedule and potential side-effects. Participants will be instructed to take their first dose of varenicline 1 week prior to their quit date and will receive a phone call reminder on this day from a study staff member if they are not coming in for a treatment session on this day. From this date participants will complete 12-weeks of daily varenicline administration as well as weekly and biweekly 30min counseling sessions. Participants will also complete a 15 min nurse assessment during each weekly visit.

PHASE 3 – Follow-up visits

Participants will be asked to complete follow up visits at 1, 6, and 12 months after their last treatment visit. During these visits participants will complete a nurse assessment, meet with an investigator to assess quit status and smoking abstinence since last visit, complete a series of state measures and complete a 15-20 min scan session.

4.2. Recruitment

Participants will be recruited from the general population through advertising in city, regional or campus media, such as internet, newspapers, flyers, via referrals, or radio and television advertisements (see Appendix 14). Participants will also be recruited from among past candidates for NIDA-IRP studies if written consent to be re-contacted has been obtained. We anticipate an average accrual rate of 1-2 participants per week.

Recruitment Plan

Individuals will be recruited for this protocol through IRB-approved advertisements and outreach initiatives, by referral from healthcare workers or facilities, or by referral from other study participants. In order to more efficiently identify and screen volunteers for research participation, NIDA-IRP, through their recruitment contractors, regularly sponsor media advertisements. Radio, television, internet and print media, as well as circulars, brochures, and flyers, are routinely employed in accordance with NIDA IRP clinical policy. In addition to media advertising the recruitment contractor may also directly contact community providers and businesses to increase awareness of research study participation opportunities. The Recruitment contractor may alter the size and/or color of these ads. Color changes will not change the emphasis of the ad in a way that might be impermissible.

Community providers of care and services in Baltimore, Washington DC and the surrounding areas may be contacted for recruitment. IRB approved materials will be offered to providers such as medical care providers (e.g. health clinics, urgent care clinics, individual practitioners, hospital ED's, etc.), county or city service agencies (e.g. social services, health departments, human resources, adult education centers, etc.), and community service providers (e.g. shelters, Goodwill Industries, employment centers, crisis centers, etc.). A list of providers contacted will be submitted to the IRB at the time of each Continuing Review.

NIDA Staff or contractors may also go to community businesses such as libraries, community centers, convenience stores, grocery stores, food venders, and other local businesses, etc. to request permission to post IRB approved recruitment materials. Posting approval will be consistent with the venue such that the customary approval process for posting will be followed and documented.

In addition, staff from the NIDA IRP and NIDA IRP recruitment contractor may go on site to community locations to recruit participants. When NIDA outreach staff is on site, they may post signs in the lobby or waiting area advertising that NIDA will be recruiting for one of its studies. Also, an announcement may be made that NIDA is on-site that day to recruit for one of its studies and that anyone interested in more information can go to the designated area to learn more. Interested people will meet one-on-one at the site with an outreach staff person who will answer specific questions about the protocol. All advertisements will be submitted for approval by the IRB before being used. Written permission by the director from each site will be obtained prior to recruiting. This permission will include a statement about their approval of our presence in their site to recruit for studies, plus, where applicable, an assurance that a person's choice to be involved in this screening or study will not in any way affect their participation there.

Outreach Events:

B'More Healthy Expo
Saturday, February 28, 2015 (10A-5P)
Baltimore Convention Center
30,000 + attendees
20x20 booth
Email advertisement - WBFF will send out our currently approved email, twice, to up to 50,000 people who agreed to be emailed sponsor information. The email used will the exact same one approved by the IRB under protocol 474 amendment 12(L), which is reattached with this PTMS submission or reference.

NIDA-IRP personnel would be available to meet potential candidates interested in NRB smoking cessation studies to tell them about our active studies and to answer any questions they may have. We would hand out IRB approved materials such as fliers, business cards and study fact sheets. Our booth would only be decorated with IRB approved materials.

We will take NIH-issued, password-protected laptops so that interested people can leave their information with us. We have worked with Massoud Vahabzadeh, Chief of the Biomedical Informatics Section (BIS), to create a system to protect the privacy of individuals interested in participating in studies. BIS will create a database to record candidate information directly so that they cannot see other people's information. He also suggested that as a back-up, (e.g. power failure) we should take index cards so that we can have people write down their

information for us. Again, this method would eliminate the possibility that they would see anyone else's information as we would give each person their own card.

These are the fields BIS will use to create the database, some with dropdown choices:

First Name
Last Name
Primary Phone#
Alternative Phone#
Email address
Best contact method: (email, phone)
Preferred contact time: (anytime, morning, afternoon, evenings)
Smoking Status: (non-smoker, smoker interested in treatment, smoker not looking for treatment, ex-smoker)

Participant Testimonials:
We will develop ads using real participant testimonials with a photo and a quote from participants who are willing to have their photos and quotes used as ads. Participant testimonials are currently used at NIH (https://www.drugabuse.gov/related-topics/treatment/one-patients-story-nida-clinical-trials-bring-new-life-to-woman-struggling-opioid-addiction and https://www.nih.gov/health-information/nih-clinical-research-trials-you/personal-stories). We are interested in using high-impact quotes from similar interviews with our current and former participants (both treatment seeking and non-treatment) to improve the authenticity and relatability of our advertising campaigns.

We would ask participants if 1) they would like to tell us about their experience in this study and have us use their picture and quote used for advertising purposes. If they are interested, we may ask them questions such as those listed below, then develop the ads, submit to PILB and then to IRB. The ads would look very similar to current ads with a quote from the participant. These ads would be posted as currently approved ads do, in print, online, social media, and on the NIDA website. Past subjects may also be contacted via phone so that we may ask them if they are interested in doing a testimonial ad with NIDA. Participants will be notified that not all quotes will be used and that the final ad may not be published. They will also be asked to sign a media release form if they would like to have their photo and/or quote used in an ad.

"What would you say to someone who is thinking about being in this study?"
"How has being in this study affected your daily life?"
"When considering personal interactions throughout the duration of the trial, how would you describe your experience?"

4.3. Screening methods

Candidate participants will be screened for participation based on the Inclusion criteria/Exclusion criteria assessment tools listed above, under the aegis of the IRB approved NIDA-IRP screening protocol, 06-DA-N415. Informed consent will be obtained from prospective participants prior to the commencement of any screening procedures in a private area where participants are free to ask questions. During screening participants will be given some initial information regarding the study (see Appendix 2 "Fact Sheet") to determine if they are interested in possible participation.

The assessments to be done during screening include the following:

1. *Beck Anxiety Inventory* (BAI; Beck, 1993) - A 21-item self-report inventory, which assesses the severity of anxiety. The BAI has demonstrated high internal consistency with an alpha reliability of .92, and high test-retest reliability demonstrated by a correlation of .75 between tests taken one week apart. In addition, the BAI has shown strong convergent validity with BAI scores correlating significantly with other clinical measures of anxiety (correlations range from .25 to .51), and strong discriminant validity with patients diagnosed with an anxiety disorder scoring significantly higher on the BAI than depressed patients and controls (Beck, 1993). This questionnaire is estimated to take less than 5 minutes to complete.
2. *Beck Depression Inventory* (BDI; Beck, 1996) - The BDI-II is 21-item self-report inventory that assesses symptoms of depression currently and in the preceding seven days. Each item is rated on a 0-3 scale with summary scores ranging between 0 and 63. Studies have shown the BDI-II to have high test-retest reliability (r=.93, p<.001), high convergent validity in that it correlates more positively with other measures of depression (r=.71) than measures of anxiety (r=.47), and no association with demographic variables such as gender, ethnicity, and age (Beck, Steer, Ball, & Ranieri, 1996). These findings suggest that the BDI-II is a valid and reliable assessment tool regardless of population. This questionnaire is estimated to take less than 5 minutes to complete.
3. *Adult ADHD Self-Report Scale* (ASRS v1.1; Harvard School of Medicine, 2005) – This questionnaire is an 18-item checklist designed by the World Health Organization (WHO) and Workgroup on Adult ADHD to determine whether a participant is suffering from symptoms related to attention-deficit/hyperactivity disorder. It requires about 5 minutes to complete.
4. *Edinburgh Handedness Inventory* (Oldfield, 1971) – This 12-item questionnaire assists in determining the dominant hand (or handedness) of a particular participant by inquiring which hand the participant uses for various tasks. This task requires an estimated 2-5 minutes to complete
5. *Fagerstrom Test for Nicotine Dependence* (FTND; Heatherton et al., 2001) A 6- item test that measures the severity of nicotine addiction. Estimated time to complete is 1-5 minutes.
6. *Brain Imaging Information Handout (see attached material)* – A general information sheet on brain imaging research with common questions and answers is given to subjects as an informational item only. Subjects are given time to read the handout and to ask questions about it while in clinic (time to complete, 20 minutes).
7. *Medical Assessments* – Vital Signs, height/weight measurements, urinalysis, EKG, oral HIV test, blood draw, urine qualitative drug screen for methadone, benzodiazepines, cocaine, amphetamine/methamphetamine, opiates, barbiturates, tetrahydrocannabinol, and tricyclic antidepressants, urine toxicology which analyzes for presence of a broad range of prescription and nonprescription drugs, as well as urine pregnancy testing for menstruant females, and urine cotinine level testing to verify smoking or nonsmoking status. Breathalyzer for alcohol and exhaled breath CO measurement is taken at this time as well. Urine specimens may be collected under observed conditions if required by the specific protocol being screened for. Staff member observing urine collection will be same sex as subject and will observe through one way mirror. Estimated time to complete is 30-40 minutes.

8. *Contact Information Form* - This form requests multiple methods of contact for a participant, as well as the preferred contact method, preferred contact time, and emergency contact information. Estimated time to complete is 2-5 minutes.
9. *Participant Safety – MRI Screening Form* - A questionnaire that aids in determining whether a participant can safely have an MRI scan. Estimated time to complete is 3-5 minutes.
10. *Medical History and Physical Examination* –This is a standard physical and detailed medical history and physical examination designed to capture the inclusion and criteria for NIDA-IRP protocols. It is administered by a Physician's Assistant or other credentialed medical staff. The estimated time to complete the H&P exam is variable; it is dependent on both the individual and the protocol(s) for which s/he is being considered. The examination typically lasts between 45 – 90 minutes.
11. *Mock Scanner:* Subjects who qualify for MRI scanning as determined by the interview and the MRI Safety form are taken to the Mock scanner during their screening visit. If the mock scanner is occupied during their screening visit, subjects are brought back to try the mock scanner before any actual MRI scanning session. The scanner environment is explained to the participant and they are asked to lie in the scanner to make sure there are no problems with body fit or comfort. This give subjects the opportunity to opt out of scanning experiments if they are not comfortable with the scanner set up. Estimated time to complete is 5-10 minutes.
12. *Blood Draw* – Blood is taken from a vein in the hand or arm. Tests on the sample include CBC, complete metabolic profile, TSH, ESR, STS, Hepatitis B surface antigen, Hepatitis C antibody and HIV (if needed to confirm a positive salivary test for HIV), Prothrombin time (PT), Parial Thromboplastin Time (PTT), and Creatine Kinase (CPK). Estimated time to complete is 20-30 minutes.
13. *SCID Screen Patient Questionnaire – Extended (SSPQ-X)* (1) This test is the computer-administered screening version of the Structured Clinical Interview for DSM-IV™ (SCID). While there are a total of 589 questions in this program, participants are not presented with all questions. Questions that are presented are dependent upon the participant's answer to the previous question. The disorders covered include Mood Disorders, Anxiety Disorders, Substance Use Disorders, Psychotic Symptoms, Somatoform Disorders, and Eating Disorders. SSPQ-X also differentiates between Substance Dependence and Substance Abuse and includes questions for each type of drug endorsed. Estimated time to complete is 25 to 45 minutes.
14. *Drug Use Survey (DUS)* An interviewer-administered questionnaire designed to collect information regarding lifetime history of substance use. It requires 10-40 minutes to complete, depending on history of use.
15. *Toronto Alexithymia Scale (TAS-20)* (6) A twenty item scale that measures difficulty with awareness of feelings. Estimated time to complete is 2-5 minutes.

4.4. **Study procedures**

(i) Orientation and task training

Before commencing the scanning sessions, participants will complete a training session where they will be instructed on and practice the experimental tasks in the mock scanner and be trained for abstinence period procedures. Participants will not be able to smoke during the orientation visit.

1) Nurse Assessment. After completing the consent interview, participants will undergo a medical update, which will include vital sign assessment, ECG (during orientation), MRI compatible ECG electrode placement (scan sessions), a breathalyzer test for expired ethanol, an expired CO test for smoking-abstinence compliance, a urine test for common abused drugs, a metal/MRI safety screening on scan days, and if female, a urine pregnancy test. In the event of a positive drug test for marijuana, an interview to assess last drug use and a Neuromotor Drug Influence Evaluation (Dr. Steve Heishman, personal communication) to test for acute intoxication will be performed. The purpose of the nurse assessment is to provide additional screening for characteristics (e.g., pregnancy, positive drug tests, metal in body) which would exclude an individual from participation in the study on that day. If participants test positive for drugs other than marijuana they will be rescheduled, however, if they test positive on a second occasion they will be excluded from the study. This will be made clear to the participant in the consent process. In addition, participants will be asked to report time of last meal, hours slept the previous night, recent medication use, changes is medical history since last visit and, if female, last menstrual period and whether or not they are currently taking a contraceptive pill.

Information obtained during the nurse assessment is stored confidentially and securely in the individual's medical file (See section 5.0 – Storage of Data and Samples). ~30min

2) Baseline and characterization questionnaires. A series of questionnaires (see Appendix 3) will also be administered during the orientation visit. ~1hr

4) Nicotine patch toleration test. Participants will complete a nicotine patch toleration test during the orientation visit. Nursing staff will apply the dose-matched nicotine patch at the end of the nurse assessment and will describe potential side effects to the participant. Participants will wear the patch for approximately 3 hours and will be asked to notify the LI or nursing staff immediately if they experience any worrisome side-effects. A nurse will remove the patch and conduct a post patch assessment including vital signs, neck/back pain assessment, mental status exam, and skin assessment.

3) Mock scanner training session. The mock scanner training session will familiarize participants with study procedures and the scanner environment. Given that training and scanning will take place on separate days, individuals will receive training refreshers during subsequent visits. ~ 1.5hrs

5) Abstinence period procedure training. Participants will also be trained to measure their CO levels and record this using a small video camera. (see *Abstinence Periods* section for details). At the end of this visit they will generally also receive their first set of nicotine or placebo patches and will be instructed on the application of these during the abstinence periods as well as the time points and procedure for recording their CO and mood/withdrawal symptoms during their upcoming abstinence period. Subjects will also be provided with written instructions on how to apply each patch (see Appendix 5). CO monitors and video cameras and paper copies of self-report measures to complete during the abstinence period will also be distributed to participants at the end of their orientation session. Participants will also be provided instruction in cognitive behavioral techniques (e.g., progressive relaxation) designed to reduce withdrawal discomfort and improve compliance to the 36 hour abstinence procedure ~40min

6) Assessment of motivation and preparation to quit. Several measures assessing participant motivation and preparation to quit smoking (see Appendix 9) will be administered to the treatment-seeking smokers and those who enroll in the motivational arm. ~20min

(ii) Abstinence periods

During both the nicotine-deprived and non-deprived abstinence period participants will use a small video camera to video record themselves measuring their CO levels. They will also report mood and withdrawal symptoms at 6 time points throughout each abstinence period: 10pm on day 1 (2hrs post-cessation); 8am on day 2 (12 hrs post-cessation); 12noon on day 2 (16 hrs post-cessation); 4pm on day 2 (20hrs post-cessation); 8pm on day 2 (24 hours post-cessation); 10pm on day 2 (26 hrs post-cessation). All times are approximate. Their CO levels will be measured again at 8am on day 3 (36 hrs post-abstinence) when they arrive at NIDA-IRP for their scanning session. Research staff will generally phone participants on the afternoon they are to commence their abstinence period. Participants may also receive text message reminders from research staff at each of the six measurement time-points depending on participant needs. This procedure will allow the research team to monitor participant CO levels throughout the 36 hr abstinence periods, validating smoking abstinence. It will also provide a method of measuring changes in mood and withdrawal symptoms throughout the 36 hr abstinence periods across both deprived and non-deprived conditions. This information will provide a measurement of responsivity to nicotine replacement.

(iii) Scanning Visits.

There will be two scanning visits, both preceded by a 36 hr abstinence period. During one abstinence period and scanning session participants will wear a dose-matched nicotine patch. A placebo patch will be applied throughout the nicotine-deprived abstinence period and scanning session. The order of deprived and non-deprived sessions will be counterbalanced across subjects. Scanning sessions will be separated by a minimum of 5 days. The two scanning visits will be essentially identical. Each visit will consist of two 1.5 to 2 hr scanning sessions, one taking place in the morning (AM) and one in the afternoon (PM). Participants will also complete several pre- and post-scan tasks and a series of questionnaires throughout the day. All imaging visits will take approximately 9hrs (8am to 5pm) to complete. The order of procedures completed during each scan visit is outlined schematically in Figure 2, and described in detail below.

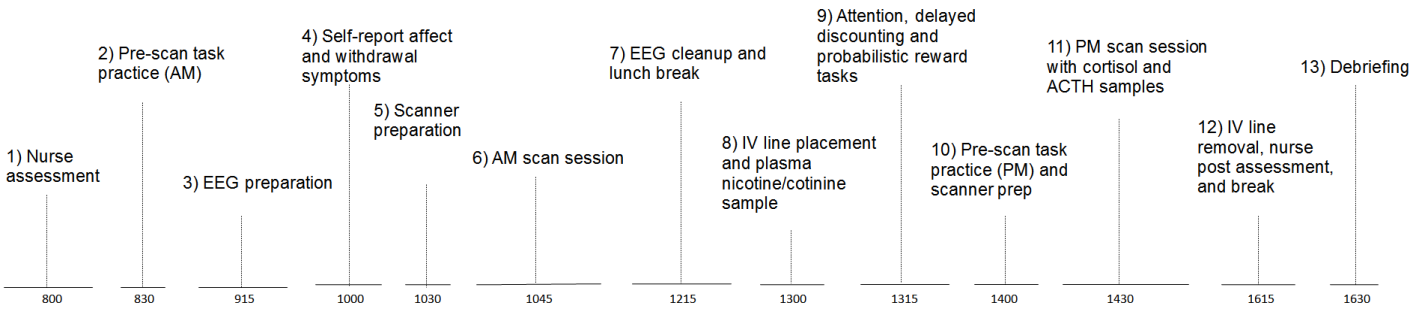


Figure 2. A schematic outline of the procedures completed during both deprived and non-deprived scanning sessions (note: all times approximate and order of tasks and scans generally fixed but may be switched in the event of time constraints, instrument problems or subject compliance).

1) Nurse Assessment. Upon arrival at NIDA-IRP participants will return their video camera and CO monitor to research staff. They will then undergo a scan day nurse assessment. See orientation day Nurse Assessment for procedure details.

2) Pre-scan task practice (AM). Prior to scanner prep, participants will be reacquainted with the task instructions and will complete brief training runs. The task instruction/practice period will last ~30 min.

3) EEG Preparation. Simultaneous EEG and fMRI data will be collected during all scanning sessions. Therefore, an EEG preparation period lasting approximately 45min will always precede the morning scanning session.

Participants will be asked to wash their hair the night before/morning of the scan, or alternatively, upon arrival at NIDA-IRP. They will also be asked not to wear any hair product on the day of the scan. Prior to cap placement they will be given a comb and asked to brush their hair thoroughly. The investigator will then place the fMRI compatible EEG cap onto the participant's head and then adjust the cap position so that electrode locations correspond to conventional recording locations (10/20 system). In addition, an electrode will be placed on the upper back to monitor cardiac activity. The experimenter will then insert a conductive gel into each of the electrodes so that good electrical contact can be made between the scalp and the electrodes. Electrode impedances will be reduced below 10kΩ during preparation to ensure quality EEG recording. The location of each of the electrodes will be digitally recorded and used to model the electrical generators of EEG activity in subsequent analyses.

4) Self-report measures. Participants will be administered several brief measures to assess current anxiety, arousal, mood, and other withdrawal symptoms. These measures will be administered on a computer in a private area and include those indicated in Appendix 4.

5) Scanner Preparation. Approximately 15min is needed to position the participant in the scanner bed, setup subject response pads, and prepare other equipment for the recording session.

All scanning experiments will be performed on a research-dedicated 3T MRI scanner, located at the NIDA-IRP. The 3T MRI machine used in this protocol is FDA approved and is used as a data collection tool. This study may use the MRI in research mode, including custom pulse sequences. All sequences, including those in research mode, are within FDA approved specific absorption radiation (SAR) limits. No additional risk is incurred in research mode, and we are not testing sequences to evaluate safety or risk.

Participants' heads will be placed inside the RF coil and held in place by a head restraint that may consist of soft foam, a vacuum bag, a bite bar and/or hardened polyurethane foam (expanding packing foam). For most scanning experiments, a blipped, gradient-echo, echo-planar image (EPI) pulse sequence (e.g. TE = 27ms) is used with a TR of about 2 seconds. Images will be acquired with an in-plane resolution of 64 x 64 and with a 22cm field of view. Specific imaging parameters (e.g. number of slices, slice angle, TR, TE, flip angle etc.) may be changed in order to maximize signal integrity. Please note, that we use a "watchdog" program that runs continuously on the MRI computer during the execution of all pulse sequences and ensures that no scanning parameter ever exceeds FDA guidelines. The MRI is used as a data collection tool. All sequences and scans conducted are within FDA approved parameters, and the scans are not conducted to assess safety or efficacy of the scans themselves.

6) AM Scan Session (note: all times approximate and order of scans generally fixed but may be switched in the event of instrument problems or subject compliance).

Structural Images. A set of high resolution structural MR images will be collected. The images will be acquired using a T1-weighed three-dimensional MPRAGE sequence and will be used for the display of functional data. Completion time: ~5 min.

Affective Multi-source Interference Task (affective MSIT). Participants will complete a modified Multi-source Interference Task designed to study the ERP and fMRI correlates of performance monitoring, conflict resolution and post-error adjustment processes within an affective context. The MSIT combines several sources of combines aspects of the simon, stroop and flanker tasks to produce a strong and reliable conflict manipulation (Bush, Shin, Holmes, Rosen, & Vogt, 2003; Bush & Shin, 2006). Each trial involves the presentation of three digits which will include 0s, 1s, 2s or 3s. One of the three digits will be different from the other two (the oddball). Participants indicate the identity of the oddball (which will always be a 1, 2 or 3) by pressing one of the three buttons. On control trials (no conflict) the oddball digit is in the same spatial location as the response button and the other two digits are zeros (e.g., 100 or 020). On conflict trials the oddball is in a different location to the response button and the other two digits are competing response choices (e.g., 211, or 232). We have included an additional affective component to the standard MSIT, by manipulating the consequences of correct (win 0, 5 or 50 points) and slow/incorrect responses (lose 0, 10 or 100 points). To increase the likelihood of errors, participants are only awarded points for correct responses if they exceed an individually determined time threshold. Participants will be able to "cash in" points at the end of task and earn up to $50 depending on the number of points accumulated. Completion time ~40min

Diffusion tensor imaging (DTI) scan. A DTI scan will be performed for the assessment of white matter microstructure. Previous research has suggested that some addiction-related deficits in cognitive task performance can be related to impaired anatomical connectivity between brain regions (Pfefferbaum et al., 2000; Lim et al., 2002; Madden et al., 2004; Moeller et al., 2005). There is also evidence that the integrity of connectivity between medial PFC and amygdala regions may mediate trait reactivity to stress (Kim & Whalen, 2009), a central construct of interest in the current protocol. Thus DTI may reveal important pre-existing structural characteristics of relevance to individual differences in acute nicotine withdrawal. Completion time: ~5min.

Go/No-go Task: Inhibitory control processes - cognitive context. Participants will perform multiple blocks of a Go-NoGo task designed to study the ERP and fMRI correlates of response inhibition, error detection and post-error behavioral adjustments. The goal of the go-nogo task is to press a button following each target stimulus (go-trial) and to withhold a response following each lure stimulus (NoGo-trial). In each block of the task participants will view a serial stream of alternating Xs and Ys. Participants will be instructed to make a right-handed button press response to each target stimulus (an X or Y that is different from the last X or Y stimuli presented) while the stimulus is still on the screen. In contrast, responses will need to be inhibited following lure stimuli, defined as a non-alternating target stimulus (i.e., the second of two consecutively presented identical stimuli such as XX). Within each block, most of the stimuli will be targets, while only a small portion will be lures. Task blocks will be interspersed with resting fixation blocks which will be employed as a baseline for some analyses. Performance on this task is quantified as the number of successful inhibitions that participants are able to achieve. Stimulus duration on this task will be individually tailored to produce an equivalent number of successful inhibitions and commission errors across participants. Specifically, during training in the mock scanner environment an appropriate difficulty-level will be selected. The combination of shorter stimulus durations and the instruction to respond while the stimulus is on-screen, yields increased numbers of commission errors (Garavan et al., 2002). Robust differences in brain activity and behavioral performance have been observed using this paradigm to study cocaine addicts (Kaufman et al., 2003). Completion time: ~20 min.

7) Break. Following completion of the AM-session, participants will take a break. Prior to the break laboratory technicians will remove the EEG cap from their head and allow the participants to wash and dry their

hair. During the break time participants will be able to have lunch, relax, and spend time in the day room at their leisure. The break will last ~60 min.

8) Plasma nicotine. Immediately after lunch a nurse will fit an indwelling catheter into a vein on the participants arm, wrist, hand or foot. The selection of which vein to fit the indwelling catheter may vary between participants. Immediately after the indwelling catheter has been successfully inserted, and before the PM scan session, a nurse will take a blood sample from participants that will later be used to assess for plasma nicotine and cotinine levels.

9) Bench tasks:

Rapid Visual Information Processing Task. Attention will be assessed with the *rapid visual information processing* task (RVIP; Wesnes & Warburton, 1983). Individual differences in attention can significantly alter performance across a range of tasks. It is thus important to control for treatment and withdrawal-related changes in attention. The RVIP provides a quick and reliable measure of sustained attention. The task has participants monitor a continuous stream of single digits presented on a screen in front of them and respond with a button press when they notice three odd or three even numbers presented consecutively (e.g., 5,9,7 or 4,2,8). Completion time ~10 min

Delayed Discounting. Following the break participants will complete a delay discounting task (Hariri et al., 2006), which assesses the discounting of incentive value with delay to receipt of reward. On each trial participants will be asked to select between a hypothetical amount of money received that day (e.g., $0.15 to $150) and a large amount (e.g., $150) received after a delay ranging from 0 days to 5 years. For each delay interval (e.g., 0 days, 7 days, 2 years) a 'switch point' is calculated as the average (in dollars) of the last immediate value that the participant selected and the next lower immediate value (i.e., the value at which participants opted for the delayed reward). Switch point values are plotted against delay intervals to calculate a delayed discounting curve – with steeper curves indicating greater discounting of incentive value with delay to receipt of reward. Completion time: ~10-20min.

Probabilistic reward task. The probabilistic reward task was designed to produce a behavioral index of reward responsiveness and shows sensitivity to anhedonic symptoms. The task involves three blocks of 100 trials which each commence with the presentation of a simple line-drawing of mouthless face. The mouthless face is first presented for 500ms, followed by the presentation of the same face with either a short or long mouth superimposed for 100ms. Participants are required to indicate whether the mouth presented was long or short. For each 100 trials, only 40 correct responses were awarded with positive feedback and points ("Correct!! You won 20pts"). For half of the participants correct responses to the short mouth will be awarded three times more than correct responses to the long mouth, and vice versa for the other half of the participants. The task yields two indices of reward responsiveness: reward bias, which is the systematic preference for the more heavily rewarded stimulus, and reward learning, which is the difference in reward bias from block 1 to block 3. Participants will be able to "cash in" points at the end of task and earn up to $10 depending on the number of points accumulated. Completion time ~20min.

10) Pre-scan task practice (PM). As above for AM session.

11) PM scan session (note: all times approximate and order of scans generally fixed but may be switched in the event of instrument problems or subject compliance).

Structural scans. As above. ~5min

Unpredictable Shock and Cue Reactivity Task (USCRT): to assess activity in the BNST and CeA. The USCRT was developed to assess the effect of state activation in the BNST and CeA upon reactivity to drug-related and neutral cues. Unpredictable shock produces a state of sustained anxiety mediated by the BNST in rodents (Davis et al., 2010) and shown to activate the BNST and CeA in humans (Alvarez et al., 2011).

Smoking-related (e.g., person inhaling on cigarette) and neutral (e.g., person holding a pencil in their mouth) pictures will be presented under either an unpredictable shock condition (during which a shock could occur at any time) or a safe condition (during which participants are explicitly informed that no shock will occur). The shocks will be delivered through two disk electrodes placed on one of the feet or ankles, using a constant current AC stimulator. The shock will have intensity up to 4.0 mA and duration up to 500ms. The shock is generally described by participants as rather anxiogenic and unpleasant, but not painful. The mean rating of aversiveness on a scale of 1 (not at all painful) to 10 (extremely painful) is about 5. The procedure for administering the unpredictable shock is based on that described by our colleagues at NIMH (led by Christian Grillon) who developed the unpredictable shock paradigm. Their experience is that over 95% of participants who received the shock chose to participate in the experiment. Participants will not receive the shock until the first scan visit for two reasons. Firstly, additional exposure to the shock is likely to produce habituation to the unpleasant quality of the shock and reduce the degree of anticipatory anxiety that presentation of the unpredictable shock stimulus elicits. Secondly, the level of shock presented to participants in the current study is determined via a shock work up procedure, which commences with a very low level of shock delivery which most individuals cannot detect. The level is then increased to a level which produces discomfort but is also tolerable for participants. We therefore do not anticipate any withdrawal from the study due to inability to tolerate shock.

Once the electrodes are placed, and prior to commencing the experiment, we will perform a shock work-up procedure during which participants receive between 1and 5 shocks of increasing intensity and select and are asked to rate each shock, indicate when the level of the shock is perceived as unpleasant and tolerable but not painful. As a consequence, no participant is presented with a shock that exceeds this perceived level of discomfort. Participants will have the opportunity to withdraw from the study if they wish at any moment, and are informed explicitly about this option in the informed consent. Self-report measures of craving, mood, arousal and distress will be taken throughout the procedure. Completion time ~ 25min

Spectroscopy (MRS) scan: Measure of intra and extracellular glutamate. As described above, acute nicotine withdrawal is associated reduced glutamatergic transmission at the VTA and PFC/ACC. A proton magnetic resonance spectroscopy (MRS) scan of pre-defined ROIs within these regions will be conducted to examine the effect of acute nicotine withdrawal on extra- and intra-cellular glutamate detected by MRS. Completion time: ~20 min

Amygdala Reactivity Task. Participants will complete a simple perceptual task previously shown to produce robust bilateral amygdala activity (Hariri et al., 2002; Pezawas et al., 2005; Foland-Ross et al., 2010), as well as top-down prefrontal regulation of amygdala reactivity (Hariri et al., 2002; Foland-Ross et al., 2010). This blocked fMRI paradigm consists of two experimental conditions: a face matching condition, and a sensorimotor control condition. During the emotion matching condition, participants view an array of 3 faces and their task is to select (via button press) one of the two faces matches the identity of the target face. During the sensorimotor control task, participants view an array of 3 geometric shapes (circles, vertical and horizontal ellipses) and select one of the two shapes (bottom) that matches the target shape (top). Each block begins with a brief instruction screen ("match face" or "match shape"). During each emotion block different images selected from a standard set of pictures of facial affect (Ekman and Friesen, 1976), are presented. During the control blocks, different geometric shapes are presented in a pseudo-random order. Completion time: ~7min.

Striatal Reactivity Paradigm (SRP). The SRP provides a robust probe of ventral striatal (VS) activity associated with positive and negative feedback (Hariri et al., 2006). At the start of each trial participants will be presented with a card displaying a question mark on the center of the screen. They will be instructed to guess whether the value of the card was greater than or less than 5 by pressing one of two buttons. Participants are then presented with the value of the card and a green up arrow (positive feedback) or red down arrow (negative feedback) indicating the accuracy of their guess. They are also informed that their performance will determine the size of a monetary reward to be received at the end of the scanning session. Unknown to the participants, the outcome of each trial (i.e., positive or negative feedback) is predetermined. The task will be presented in a blocked design with positive feedback (correct on 75% of trials) and negative feedback blocks (correct on 25% of trials) interspersed with control blocks during which participants are instructed to make alternating button press responses when they see an 'x' on the center of the screen. Participants can earn up to $10 on the task. Completion time: ~5min

Resting Scan. An EPI scan to assess resting-state fluctuations (RSF) will be conducted. These scans will provide us with information relating to trait and perhaps state connectivity between different prefrontal and limbic brain regions. Completion time: ~10 min.

Heart rate, heart-rate variability and respiration. Heart rate (HR) and heart-rate variability (HRV) will be measured throughout the two scanning sessions via MRI compatible ECG electrodes placed on the participant's chest or back and a Pulse-Ox monitor attached to the individuals forefinger HR provides a sensitive measure of tonic and phasic changes in physiological arousal in response to errors (Hajcak et al., 2004), drug cues and (for review see Carter & Tiffany, 1999) and threat of shock (Rhudy and Meagher, 2000; Bradley et al., 2008). Resting HRV provides a trait measure related to enhanced resilience to stress and affect regulation (Friedman and Thayer, 1998; Hansen et al., 2009). Respiration will be measured with a thoracic strain-gauge belt that will be fitted around the individual's waist to control for respiration-related changes in HR and HRV. HR, HRV and respiration will be measured during all task and resting functional scans.

11a) <u>Plasma cortisol and ACTH: collection and analysis.</u> Blood samples will be taken before and during the PM scan session in order to measure levels of plasma cortisol and ACTH. The nurse will be present throughout the PM scan session to take one 7 ml sample of blood at the three different time-points illustrated in Figure 3.

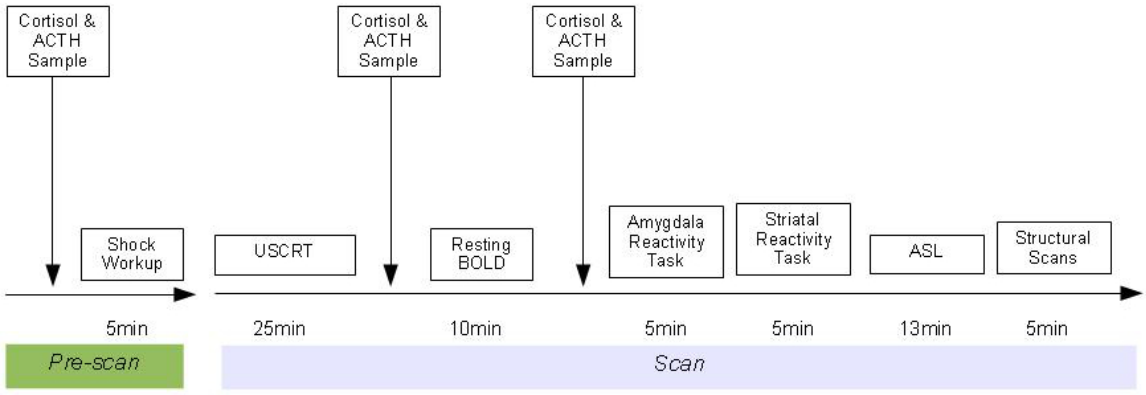


Figure 3. A schematic outline of the scanning procedures and cortisol/ACTH plasma sampling time points during the PM scan session.

12) Post Scan Nurse Assessment. A nurse will remove the IV line and patch, obtain vital signs, and complete a neck/back pain assessment, mental status exam, and skin assessment.

13) Debriefing (end of day). At the end of the PM scanning session on scan day 1 there will be a debriefing period for discussion of questions/issues related to the day's tasks, scheduling/reminder of subsequent visit. They will also schedule a time close to their next abstinence period during which they will briefly visit one of the research staff to pick up their second set of placebo or nicotine patches, CO monitor, flip camera and forms to record mood/withdrawal symptoms. They may take these items home if their scan 2 is within a week or two and the equipment is not needed for another participant.

After Scan day 2 debriefing, treatment-seeking participant's motivation and preparation to quit will be reassessed and they will be scheduled for their first treatment preparation visit with the study therapist.

(iv) Research and Clinical Care Procedures.

Clinical care for the treatment-seeking smokers will be provided as soon as participation in the first two scan sessions is complete. For details of clinical care procedures please refer to *Section 4.4 Study Procedures (v) Treatment* below. All participants entering into the study will be advised during the consent process that nicotine patches used during the first two abstinence periods and scan visits are strictly for research purposes.

(v)Treatment

1) Treatment Overview:

Within approximately 2 weeks of their second scan visit, participants will be scheduled for their first of up to 3 pre-treatment preparation visits. The number of preparation visits (1 to 3) will be determined by the study therapist on the basis of the participants assessed level of preparation and motivation to quit smoking. During their final pretreatment preparation visit, participants will work with the therapist to develop a treatment plan and set a quit date. They will also receive their first 2-weeks supply of varenicline during this visit or their first treatment visit. During either visit they will meet with the MAI or the designated NIDA-IRP physician in the MAIs absence who will describe the medication, prescribed dosing schedule and potential side-effects. Participants will be instructed to take their first dose of Varenicline 1 week prior to their quit date and will receive a phone call reminder on this day from a study staff member if they are not already here for a treatment session that day. From this date participants will complete 12-weeks of treatment including daily varenicline administration (for 12 weeks) as well as weekly and biweekly 30min counseling sessions. Participants will also complete a 15 min nurse assessment during each weekly visit.

Week 1	begin taking varenicline (0.5 – 1mg/day); counseling; nurse assessment; questionnaires
Week 2	Quit Date; varenicline (2mg/day); counseling; nurse assessment; questionnaires.
Week 3	varenicline (2mg/day); counseling; nurse assessment; questionnaires
Week 4	varenicline (2mg/day); counseling; nurse assessment; questionnaires
Week 5	varenicline (2mg/day); counseling; nurse assessment; questionnaires

Week 6	varenicline (2mg/day); counseling; nurse assessment; questionnaires
Week 7	varenicline (2mg/day); counseling; nurse assessment; questionnaires
Week 8	varenicline (2mg/day); counseling; nurse assessment; questionnaires
Week 9	varenicline (2mg/day); phone assessment
Week 10	varenicline (2mg/day); counseling; nurse assessment; questionnaires
Week 11	varenicline (2mg/day); phone assessment
Week 12	varenicline (2mg/day); counseling; nurse assessment; questionnaires
Week 13	phone assessment
Week 14	counseling; nurse assessment; questionnaires.
Week 16	1 month follow-up counseling; nurse assessment; questionnaires

Please note that all times described in the treatment outline are approximate and if a person cannot attend a scheduled visit we will reschedule it for the next available date.

2) Varenicline Administration. All participants will follow the dose schedule outlined below:

Week 1:
Days 1 to 3: Take 1 x 0.5mg pill each day in the morning.
Days 4 to 7: Take 2 x 0.5mg pills each day. Take one in the morning and one in the evening.

Week 2 to 12:
Take 2 x 1mg pills each day. Take one in the morning and one in the evening.

Participants will be instructed to eat prior to dosing, to take their dose with a full glass of water, and to take doses at least 8hrs apart (please see Appendix 8 for take-home study pill instructions). As noted above, they will receive a 2 week supply of varenicline during their initial interview. They will then receive an additional 1-2 week supply of varenicline on a weekly or bi-weekly basis from weeks 2 to 10.

3) Pretreatment preparation visits. Participants will be scheduled for 1 to 3 pretreatment preparation visits with a study therapist. The number of preparation visits will be determined by the study therapist on the basis of the participants assessed level of preparation and motivation to quit smoking. Pretreatment preparation visits will involve motivational interviewing and cognitive behavioral techniques to increase motivation to quit and prepare participants for their quit attempt and treatment.

4) Counseling. Throughout the treatment phase, all participants will complete weekly or biweekly counseling sessions with a therapist trained and supervised in smoking-cessation treatment who will review the participant's quit plan (including target quit date and tobacco use reduction plans) and quit status. As part of this assessment participants will complete a Time-Line Follow-back form to assess self-reported smoking behavior since their last treatment visit (see Appendix 4). The study therapist will also administer the Varenicline Toleration Assessment (Appendix 7) during the counseling session, including sessions that occur after the participant has taken their final dose of Varenicline. Counseling will include cognitive-behavioral approaches emphasizing the participant's understanding of the antecedents and consequences of his or her smoking, and

skills training to help the participant cope with situations associated with smoking. Materials that counselors may use during sessions or give to participants as take home materials are listed in Appendix 10. One counseling session, lasting approximately 30 min, will be scheduled weekly or biweekly. Study therapists will receive extensive training and supervision by the study MAI (who may also serve as a therapist as needed).

Therapists will either be Masters level counselors or hold a PhD in a relevant field (e.g., psychology) with prior clinical experience. Those with a Master's degree or higher in a relevant field but without clinical experience may also be trained as counselors, but will complete more extensive training that will consist of didactic instruction in principles of cognitive behavioral therapy and cognitive behavioral techniques specific to smoking cessation, observation of counseling sessions with a trained counselor, and observation by a trained counselor of treatment delivery. The MAI and the lead study therapist will meet with all new counselors for an overview of the NRB nicotine cessation counseling program, as well as an in-depth discussion of the theories and techniques behind cognitive-behavioral therapy (CBT) and motivational interviewing (MI). Trainees will also be provided with a manual which outlines the nicotine treatment program, and discusses the MI and CB strategies to be employed at each level of treatment. The individual worksheets and exercises included in this manual will be reviewed with the trainee. New counselors will observe a minimum of 3 treatment sessions, and will be observed a minimum of 3 times by the lead study therapist or Dr. Salmeron. They will also attend the weekly supervision meetings with Dr. Salmeron and the lead study therapist. During the supervision meetings, counselors discuss current cases and bring up any concerns or challenges in order to gain direction and insight in future treatment applications. Bachelor's level staff with a background in psychology or related field may also be trained to fill in for counseling sessions when the primary therapist is not available. They will complete the aforementioned training.

5) Nurse assessment. On the day of their scheduled treatment session participants will meet with a study nurse who will assess their vital signs, and administer an expired CO measurement. CO levels of ≥ 4ppm will be considered positive. The study nurse will also administer a urine cotinine test on their first visit after self-reported abstinence and then every 2 to 3 weeks after this, depending on how late in their course of treatment they achieve abstinence. Urine cotinine assessments will only be administered if the participant reports abstinence for the past 7 days ~15min.

6) Phone Assessment. During weeks where participants are not scheduled for in-person counseling the study therapist, LI or study clinician will conduct telephone assessments to monitor for changes in physical and psychiatric symptoms (administering the Varenicline Toleration Assessment, Appendix 7) and assess smoking status.

7) Questionnaires. During each weekly visit participants will complete the state measures indicated in Appendix 4. ~15min

(vi) Follow-up.

Participants will be asked to complete follow up visits at 1, 6, and 12 months after their last varenicline dose. During these visits participants will complete a nurse assessment (same as nurse assessment outlined above for scan visits with a definite urine cotinine test), meet with an investigator to assess quit status and

smoking abstinence since last visit, completing state measures indicated in Appendix 4, and complete a 15-20 min scan session involving the following procedures:

Structural Images. A set of high resolution structural MR images will be collected. The images will be acquired using a T1-weighed three-dimensional MPRAGE sequence and will be used for the display of functional data. Completion time: ~5 min.

Resting Scan. An EPI scan to assess resting-state fluctuations (RSF) will be conducted. These scans will provide us with information relating to trait and perhaps state connectivity between different prefrontal and limbic brain regions. Completion time: ~10 min.

Heart-rate variability and respiration. Heart-rate variability (HRV) will be measured throughout the two scanning sessions via MRI compatible ECG electrodes placed on the participant's chest or back. Resting HRV provides a trait measure related to enhanced resilience to stress and affect regulation (Friedman and Thayer, 1998; Hansen et al., 2009). Respiration will be measured with a thoracic strain-gauge belt that will be fitted around the individual's waist to control for respiration-related changes HRV. HRV and respiration will be measured during all task and resting functional scans.

(vi)Potential problems and alternatives

1) Missing a treatment visit. If a participant cannot attend their scheduled weekly visit, they will be offered the next available time within that week. If they cannot attend an appointment that week they will be asked to complete their medical monitoring assessment (see *Section 8 Subject Monitoring*) and counseling over the phone at a convenient time that week.

2) Inability to tolerate varenicline. If participants are unable to tolerate varenicline, they can continue to attend the scheduled counseling visits and assessments and will be offered nicotine replacement therapy (NRT) as an alternative to varenicline treatment. NRT will involve daily application of a dose-matched nicotine patch/patches as well as optional 2 or 4 mg nicotine lozenges. Dose matching will be conducted according to the following schedule of daily patch administration:

10-15 cigarettes per day: 21mg patch
16-20 cigarettes per day: 21mg + 7mg patch
21-25 cigarettes per day: 21 mg + 14 mg patch
26 or more cigarettes per day: 21mg +21mg patch

3) Other issues or concerns expressed by participants regarding the treatment schedule. If any participant expresses concerns or experiences issues regarding attendance at counseling sessions or varenicline administration (e.g., no longer wishes to receive varenicline treatment) we will work with the individual participant to derive a treatment plan that is suitable for them. This will help participants remain in treatment for the full 12-weeks. For example, if any participant wishes to discontinue varenicline treatment but still wishes to attend the counseling sessions, they will be offered NRT as an alternative treatment, but may still continue the counseling sessions in the event that they refuse NRT. Alternatively, if a participant wishes to continue varenicline treatment but has issues/concerns with attendance at counseling sessions, they can continue to receive varenicline treatment without counseling. However, we will require that at a minimum they attend NIDA-IRP at least biweekly to pick up their weeks supply of varenicline, complete a nurse assessment, complete their questionnaires

and receive a brief medical monitoring and abstinence assessment from a study therapist. During the weeks that participants do not come in, study therapists will call participants to conduct telephone assessments to monitor for changes in physical and psychiatric symptoms (administering the varenicline toleration assessment) and assess smoking status.

Participants do not wish to attend follow-up visits. If a participant is contacted at the 1, 6 or 12 month follow-up time point and at this point states they do not wish to attend a follow-up visit, they will be asked to complete a part of their follow-up assessment over the phone. The assessment will be conducted by one of the study therapists who will assess the participants quit status and smoking history since their last contact with the study team.

4.5. End of participation

Participation in the study will be terminated once all study procedures including treatment and follow-up sessions are completed or under circumstances warranting participant withdrawal from the study (see section "7. Subject Monitoring"). Participants can also withdraw from the study at any time should they no longer wish to participate. Participants who withdraw from the study will be provided with information regarding alternative treatment options within the community. If participants decide during the pre-treatment study phase that they wish to quit smoking immediately (that is, prior to completing their pre-treatment scan sessions), they will be withdrawn from the study and provided information regarding alternative treatment options within the community. If participants withdraw during the treatment phase of the study, they will be asked if they wish to consent to be contacted at the three follow-up points. They will also be provided with information regarding alternative treatment options within the community should they wish to seek treatment elsewhere. Typically, no data obtained during the course of this study will be shared with the individual participant or their health care providers.

4.6. Relationship to other protocols.

All participants will be asked to participate in protocol 10-DA-N457. Participation in protocol 10-DA-N457 is required for inclusion in the current study. Genotyping and blood draw for genotyping described in the current protocol will carried out under protocol 10-DA-N457. Participants who complete the Motivational Interviewing Arm of the study (see Section 22) may be offered admission to protocol 13-DA-N485.

5. Storage of data and samples

Clinical data will be stored in the Clinical Data Warehouse (CDW) and in clinical research charts or research binders. Research charts or binders will be stored in locked filing cabinet, in a locked room at the NIDA-IRP. Imaging data are stored on a password protected drive on a UNIX server, which is maintained by the NRB. One copy of the consent form is kept in the participant's NIDA-IRP medical chart, the other is kept in a limited access, locked cabinet with other consent forms from the same protocol. Summaries of data analyses (e.g. demographics, clinical laboratory results, consent audit) are stored on a password protected shared NRB data drive. Participant data contained within files on the NRB data drive are identified by ARC number, and not by other personal identifiers such as name.

Plasma samples acquired for measurement of cortisol and ACTH, will be destroyed once valid assays have been obtained.

6. Data Sharing Plan

Unless specified otherwise, data collected in this study may also be combined with data collected across IRB approved protocols, including the NRB Repository Protocol, 06-DA-N802 and the NRB protocol, 10-DAN-457, "Characterization of Phenotypic and Genotypic Regressors to allow for greater power to detect otherwise small effect size analyses untestable with a single data set. Additionally, de-identified data may be shared with properly administered databases and/or with collaborators with whom proper data sharing agreements are in place.

7. Additional Considerations

N/A

8. Risks/ discomforts

8.1. Questionnaire and Characterization Measures.

The questions asked may be perceived as embarrassing or may cause anxiety or distress. Nevertheless, such reactions are expected to be temporary and to rapidly dissipate spontaneously.

Risk Minimization. Participants will be advised prior to completing these measures that they may refuse to answer any questions that cause them discomfort. Furthermore, any participant who experiences anxiety or distress as a result of these assessments will be able to speak to a NIDA-IRP mental health professional to discuss this.

8.2. Nicotine Patch Administration.

Adverse events (AEs) related to the nicotine patch include:

- Abdominal Pain
- Chest pain
- Coughing
- Diaphoresis
- Dizziness
- Dry mouth
- Headache
- Palpitations
- Rash
- Restlessness
- Sleepiness
- Temporary mood change
- Minor skin irritation
- Nausea/Vomiting

The most common possible side effects of nicotine patch administration are skin irritation, sleep disturbances, and nausea and vomiting. All effects are temporary and will dissipate spontaneously and quickly (1-2 hrs) after patch removal.

More severe toxic effects of nicotine may include cardiac tachyarrythmias, hypertensive crisis, dizziness, weakness, confusion, and convulsions. The lethal dose of nicotine in adults is from 0.5-1.0 mg/kg or a total single bolus dose of 40-60mg (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5218a3.htm) (US-EPA. Chemical

profile: Nicotine. US Environmental protection Agency 1987). Nicotine death may likely result from paralysis of respiratory muscles or central respiratory failure. Individuals likely have widely different levels of tolerance to the toxic effects of nicotine based on previous exposure. Nicotine patch doses up to 63mg/d have been shown to be safe and generally well-tolerated by smokers (Zevin et al., 1998; Ebbert et al., 2007). Most reports of nicotine poisonings and deaths are typically the result of contact with nicotine-containing pesticides (Benowitz et al., 1987). Although ingestion of nicotine is common, deaths due to poisoning are extremely rare, due to early vomiting and the short half-life of the drug. In the current protocol, the protracted release of nicotine via the transdermal (24hr) patch should keep nicotine plasma concentrations well within a safe range. If participants show initial signs of nicotine poisoning during the patch-toleration test (i.e., burning throat, nausea, vomiting) they may be excluded from further participation depending on the severity of their symptoms

Risk Minimization. In order to minimize risks associated with nicotine, only individuals who meet certain medical history criteria will be recruited into the study. Participants will be clearly instructed on potential side-effects during their orientation visit and will be provided with a phone number to speak with a research team member should they experience any side-effects following patch application. These instructions will be re-iterated to all participants over the phone when they are contacted to commence their abstinence periods. They will be given identical instructions prior to both the placebo and nicotine-patch abstinence periods. During imaging visits, all subjects receiving a nicotine or placebo patch will be monitored for blood pressure, respiratory rate, and pulse rate changes prior to scanning sessions and compared to their admission baseline values. Nicotine or placebo patch administration will be terminated immediately upon participant's request.

8.3. Varenicline administration. (Applicable to treatment-seeking smokers only)

Varenicline is being used in accordance with its FDA-approved labeling in this study. The most frequent AE reported in varenicline treatment groups during Phase II and III trials were nausea, insomnia, abnormal dreams, and headaches (Gonzales et al., 2006; Jorenby et al., 2006; Nides et al., 2006; Oncken et al., 2006). Nausea, the most common AE, was reported by ~30-50% of participants receiving varenicline in comparison to ~10% of those receiving placebo (Glover and Rath, 2007). Reports of nausea are typically described as transient and mild to moderate in severity. Discontinuation rates for varenicline are generally similar to those seen for placebo- and buproprion-groups in these trials (e.g., Nides et al., 2006). Titration of doses has been shown to reduce the overall incidence of nausea to ~16-34% (Oncken et al., 2006). Generally, very few participants (i.e., < 5%) discontinue study medication because of nausea (Oncken et al., 2006). A long-term study assessing the safety and efficacy of varenicline administration concluded that varenicline can be safely administered for up to 52 weeks, the duration of that study (Williams et al., 2007). Based on extant clinical data there appear to be no clinically meaningful pharmacokinetic drug-drug interactions and no evidence for human abuse liability (Glover and Rath, 2007).

Other discomforts that have been reported include vomiting, abdominal pain, gas, indigestion, constipation, reflux, dry mouth, diarrhea, and mouth ulceration. Additional frequent side effects include difficulty sleeping (18%), abnormal dreams and nightmare (13%), headache (15%), feeling fatigued or sleepy (7%), and change in appetite (3%). Additional listed side effects include tinnitus (ringing ear), increased urination, blurred vision, sweating, hot flush, increase or decrease of blood pressure, increased heart rate or palpitation, flu-like symptoms, drug allergy, abnormal liver function test, increase in weight, abnormal electrocardiogram, back and muscle pain, disturbance in attention, dizziness or fainting, feeling restless, anxiety, depression or euphoria, irritability, and agitation. A complete list of the expected AEs and side effects can be found in the drug insert (see attached chantix_pfizer_insert.pdf). In addition, based on Pfizer and FDA's ongoing safety review of post-marketing reports, Pfizer recently updated the varenicline label in the U.S. to include a warning that patients who are attempting to quit smoking with varenicline should be observed for serious neuropsychiatric symptoms, including changes in behavior, agitation, depressed mood, suicidal/violent ideation, and suicidal/violent behavior. Pfizer

also stated that a causal relationship between varenicline and these reported symptoms has not been established. In some reports, however, an association could not be excluded. More specifically, some reports may have been complicated by the symptoms of nicotine withdrawal in patients who stopped smoking, but not all patients with these symptoms had quit smoking. Some patients with pre-existing psychiatric illness experienced a worsening of their conditions. A recent report compared patients with mental illness and patients with no mental illness who were on varenicline treatment for smoking cessation reported that "there was no evidence that varenicline exacerbated mental illness" (Stapleton et al., 2008).

A recent case report describes multiple episodes of severe hypoglycemia experienced by a 51 year old Type-I diabetic after the onset of varenicline treatment (Kristensen et al., 2008). The discontinuation of varenicline resolved any further hypoglycemic episodes. Thus, it is possible that diagnosed/undiagnosed diabetics may experience severe hypoglycemic episodes while taking varenicline. In the consent, severe hypoglycemic events will be noted as a potential side effect.

A list of treatment-emergent adverse events reported by patients during all clinical trials involving varenicline indicates that cardiac issues (e.g., arrhythmia, myocardial infarct) occurred infrequently (Pfizer insert). Given that cigarette smoking has a negative impact on the heart and the potential for cardiac side effects, in the consent cardiac disorders are noted as a potential side effect.

Risk Minimization. Only individuals who meet certain medical history criteria will be recruited into the study. Participants with a history of heart problems, diabetes, or abnormal renal functions will not be included in the present study. Doses will be titrated to reduce the severity/incidence of side effects. The daily dose of varenicline may be reduced (on a case-by-case basis) to alleviate side effects such as nausea. We have excluded individuals with current or past major psychiatric illness, and will closely monitor potential psychiatric symptoms, depression, and suicidal ideation as part of the clinical monitoring plan. The monitoring plan (*see Section 8 Subject Monitoring*) consists of weekly in-person assessments at NIDA-IRP during 12 week treatment visits. Drug administration procedures will be terminated immediately upon participant request. In the event of a severe side effect such as a hypoglycemic event, myocardial infarction/arrhythmia, or increase in suicidal ideation, participants will be advised to immediately discontinue study pill administration. If seeking medical care, participants will be instructed to notify their physician or medical care providers of the fact that they are taking varenicline.

8.4. Smoking abstinence/nicotine deprivation

The 36 hr nicotine deprivation is expected to produce acute nicotine withdrawal symptoms, and hence some degree of discomfort. According to the DSM-IV (APA, 1994) acute nicotine withdrawal is associated with the following symptoms:

- dysphoric or depressed mood
- insomnia
- irritability, frustration, or anger
- anxiety
- difficulty concentrating
- restlessness
- decreased heart rate
- increased appetite

As our primary interest in the current protocol is the neurobiological mechanisms underlying acute nicotine withdrawal symptoms, no specific efforts will be made to minimize the discomfort associated with these

symptoms during the participant's abstinence period or scanning session. However, participants will be fully aware that their participation is voluntary and that they can withdraw from the study at any time, including during the abstinence period or scanning session.

8.5. Blood draw

AEs related to blood draw may include:

- Minor Skin irritation
- Pain/Discomfort
- Scarring
- Syncope
- Weakness
- light-headedness
- Bleeding
- Swelling at draw site
- Infection

Risk Minimization. Blood draws will only be performed by experienced medical or nursing staff. Risk of Infection is minimized by the use of sterile blood drawing procedures.

8.6. Intravenous Catheter

Possible side effects of intravenous catheter insertion might include acute pain, infection, phlebitis, lightheadedness/fainting or bruising.

Risk Minimization. Intravenous catheter insertion and blood draw will only be performed by experienced medical or nursing staff.

8.7. MRI Scanning.

When used on appropriately qualified individuals, MRI presents minimal risk as long as technical scan parameters remain within FDA guidelines. There is no exposure to x-rays or radioactivity. The radio waves used have produced burns (most of these minor) in about one in a million exams. The magnet may move metal implants in the body, the motion of which could be painful and harmful. Metal implants may also cause burns from the radio frequency energy used. While inside the magnet, subjects may experience an acute panic attack due to claustrophobia. Subjects may also experience mild, remittable discomfort from lying in the scanner.

Risk Minimization. To ensure adherence to FDA guidelines for MRI, a trained MRI operator who has been instructed in these guidelines performs all machine manipulations. Other preventative measures include assuring that all equipment to be used during imaging sessions is MR compatible, that participants are familiar with the MRI environment (using the mock scanner), and that participants are aware of how to signal the MR operator if they need to do so during the session. Furthermore, participants are screened prior to each MRI session for any MR contraindications, including metal implants, pregnancy, fear of small, enclosed spaces, and inability to lie still for prolonged periods of time.

8.8. EEG Recording.

When recording EEG an elastic cap containing electrodes is placed on the participants head. During preparation conductive gel is inserted into each electrode cup and worked into place using the blunt end of a cotton swab. Subjects may experience mild scalp irritation during the preparation process. Extended periods of time (typically > 6hrs) wearing the EEG cap may produce tension headaches. Some people may experience tension headaches after shorter periods of time, particularly if the cap fits tightly around their heads. Participants will need to wash the conductive gel from their hair at the end of the recording session.

Risk Minimization. All EEG preparation was conducted by Meredith McHugh, who has extensive experience conducting EEG research. Participants will not wear the EEG cap for more than 6 hrs (typically no more than 3 hours) and can ask to have it removed at any time if too uncomfortable. Participants will be informed of the EEG clean-up process before enrolling.

8.9. Physiological monitoring.

The tape (or electrodes) used to attach the wires may cause minor skin rash.

Risk minimization. If this happens, a decision will be made by the MAI about whether the participant should complete this part of the experiment.

8.10. Tasks completed inside and outside of the scanner.

Participants may find the tasks that they are asked to perform during, before and after scanning sessions boring, difficult, and/or frustrating.

Risk Minimization. In order to reduce discomforts associated with performing the emotional and cognitive tasks, scanning sessions and task sessions outside of the scanner are organized in such a way that participants will not perform tasks for extended periods of time and will be given a chance to rest between tasks. In addition, the tasks are designed to make performance neither too difficult nor too easy. Ideally, individual adjustments of task difficulty enable participants to remain actively engaged in the task. Participants receive extensive training before the actual experimental task begins.

8.11. Presentation of smoking-related pictures.

This protocol includes several procedures which involve the presentation of smoking-related pictures which are likely to induce or increase craving/urge to smoke and may also cause some mild distress.

Risk minimization. The craving/mild distress elicited by these procedures is a primary outcome measure of interest and central to the manipulation. There will thus be no effort to minimize the craving/mild distress experienced by participants. However, participants will be made fully aware during the consent process that they have the right to discontinue this procedure without consequence at any time.

8.12. Self-report characterization and state measures

Some questions asked may be perceived as embarrassing or may cause anxiety or distress. Nevertheless, such reactions are expected to be temporary and to rapidly dissipate spontaneously.

Risk Minimization. Participants will be advised prior to completing these measures that they may refuse to answer any questions that cause them discomfort. Furthermore, any participant who experiences anxiety or distress as a result of these assessments will be able to discuss it with a NIDA IRP mental-health professional.

8.13. Electric shock.

Electric shock as administered in the proposed study poses minimal risk to participants and some discomfort which is similar to that of having a tight rubber band snapped against the skin.

Risk Minimization. Risk of discomfort is minimized by employing the shock workup procedure described in section 4.4 Study Procedures. During the consent process and again after the shock workup procedure, participants are explicitly informed of their right to discontinue the procedure without consequence at any point.

8.14. Electric shock within the MRI scanner.

Introduction of electrical wires that are connected to the participant in the strong magnetic field of the scanner may constitute an additional risk. The main risk associated with administering shocks is the introduction of electrode wires directly in the RF (radiofrequency) field of the magnet. To minimize this risk, wires will not be exposed to the radiofrequencies induced in the RF head coil. The shock stimulator equipment will be outside the magnetic area, and a plastic and copper cable is taken through the wall for connection to the participant electrodes. To eliminate the possibility that electrode wires will enter the RF-field from participant movements, shock electrodes will be attached to either the foot or ankle region. Area between the wires will be minimized by twisting the wires together, and coiling of the wires will be prevented by inspection prior to each session (i.e., wires run without loops). MRI compatibility of all devices/wires entering the magnetic field of the scanner is determined by Thomas Ross, PhD, a staff scientist here at NIDA-IRP. Dr. Ross has a doctorate in Experimental Plasma Physics and has extensive experience in the acquisition and analysis MRI data and in the development of MRI compatible procedures.

8.15. Acoustic Noise.

Some subjects may experience temporary, reversible shifts in hearing threshold after MRI.

Risk Minimization. The sound generated by an MR system usually consists of a series of repetitive pulses. Acoustic noise is a result of the mechanical vibration produced by the gradient coils when the large currents are applied to them to create time varying imaging gradient fields. The relevant safety parameters required to characterize such a noise are the peak impulse sound pressure level (L-peak) and the time integral of the A-weighted sound pressure level (Leq). In MR applications, the peak impulse sound pressure level is dependent upon the peak amplitude of the individual pulses, while the time integral of the A-weighted sound pressure level is dependent upon the continuous exposure to a series of such pulses. Based on Occupational Safety and Health Administration (OSHA, Occupational Noise Exposure – 1910.95, www.osha.gov) regulations: 1) exposure to impulsive or impact noise should not exceed 140 dB peak sound pressure level, and 2) exposure to continuous noise for 2 hours per day should not exceed 100 dB. Note that these regulations are based on employees' daily exposure to a noisy environment over their entire working career, whereas we use them here as a comparison for participants' occasional exposure to MRI scanner noise, thus making these values extremely conservative measures. Measurements within our MRI scanner (provided by Siemens, the manufacturer) indicate that the maximum noise level produced by our standard fMRI pulse sequence is 116.5 dB. Based upon our typical echo spacing, this sound is in the 3-4 kHz range.

According to the Occupational Safety and Health Administration regulations (OSHA, occupational noise exposure 1910.95, www.osha.gov):

1) Exposure to impulsive or impact noise should not exceed 140 dB peak sound pressure level, and

2) Exposure to continuous noise for 2 hours per day should not exceed 100 dB.

These guidelines are based on employees' daily exposure to a noisy environment over their entire working career, whereas we use them here as a comparison for participants' occasional exposure to MRI scanner noise.

This study consists of a series of scans ranging in length from a few seconds to about ten minutes. Rest periods of up to a few (1-5) minutes occur regularly between scans while the next scan is being set up.

During fMRI experiments, MRI compatible headphones with tubes for audio input and/or earplugs with or without tubes for audio input are routinely used for hearing protection. In addition, a vacuum pillow or other padding used for head stabilization may also attenuate noise. Based on the manufactures specifications, the earplugs with and without audio tubes have noise reduction ratings of approximately 25dB and 33 dB. The headphones have a noise reduction rating of 29 dB. The vacuum pillow / padding foam provides additional noise reduction (values not available, but estimated at 5-15 dB). The noise reduction rating is a conservative measure that averages over frequencies and attempts to account for improper protection use. The mean noise attenuations at 3150 Hz, a frequency near that of our scanner, are actually 35dB, 47dB for the 2 types of earplugs and 40dB for the headphones used. Thus, the use of any two of these devices in combination reduces the noise level to levels much lower than that required by OSHA for lifetime exposure, and provides effective hearing protection for the participants. Nevertheless, some subjects may experience temporary, reversible shifts in hearing threshold after MRI.

8.16. Mock Scanner.

Potential side effects associated with the mock scanner include mild backache from having to lie still for a prolonged period of time, temporary difficulty hearing soft sounds after the exam, and being uncomfortable being in a small enclosed space.

Risk Minimization. Pre-study screening for history of back problems or claustrophobia will minimize the likelihood of side effect from mock scanning. In addition, participants will be given adequate hearing protection to minimize hearing difficulties resulting from mock scanning procedures (see Acoustic Noise section above for details).

9. Subject monitoring

9.1. Parameters to be monitored

Participants will complete a nicotine patch toleration test during the orientation visit. Nursing staff will apply the dose-matched nicotine patch at the end of the nurse assessment and will describe potential side effects to the participant. Participants will wear the patch for approximately 3 hours and will be asked to notify the LI or nursing staff immediately if they experience any worrisome side-effects.

Participants will be instructed during their orientation visit, and on the phone immediately prior to the commencement of each abstinence period, on the potential side effects of nicotine administration via transdermal patch. The details of what side effects are possible, when to remove the patch, and details of who to contact should participants experience side effects are clearly outlined in the Nicotine Patch Instructions (Appendix 5) which participants will take home with them from the orientation session. During telephone and in-person assessments participants will be directly asked open-ended questions will be used to assess for the presence of adverse effects. During placebo-patch and nicotine scans heart rates will be monitored via pulse oximeter. After completion of the scan, nursing will remove the patch and measure blood pressure and heart rate.

Based on Pfizer and FDA's ongoing safety review of post-marketing reports, Pfizer have modified the varenicline label in the U.S. to include a warning that patients who are attempting to quit smoking with varenicline should be observed for serious neuropsychiatric symptoms, including changes in behavior, agitation, depressed mood, suicidal ideation, and suicidal behavior. However, a recent cohort study based on the UK General Practice

Research Database (N = 80, 660) found no significant difference between varenicline and nicotine replacement therapy in reported incidents of fatal and non-fatal self-harm (0.16% vs 0.22%), suicidal ideation (0.05% vs 0.05%) or initiation of anti-depressant use (3.18% vs 3.62%) (Gunnell, Irvine, Wise, Davies, & Martin, 2009). Nevertheless, to help mitigate potential risks related to changes in mood, behavior, or suicidal ideation and to assess for all side effects, a clinical monitoring plan involving weekly in-person assessments during the 12-week treatment period will be implemented. During their initial treatment interview (the day they set their quit date) or during their final pretreatment visit they will briefly meet with the study MAI or the designated NIDA-IRP physician in the MAI's absence who will distribute a 2 weeks supply of varenicline and carefully describe the medication, prescribed dosing schedule and possible side-effects. Participants will also receive a set of take-home instructions and information sheet (see Appendix 8). During each weekly visit across the entire 12-week treatment participants will receive a nurse assessment to assess vital signs and complete a series of questionnaires (see Appendix 4). During their counseling session the therapist will administer a semi-structured interview (see Appendix 7) to assess for psychiatric and physical symptoms. If participants are unable to attend their weekly counseling session in person, study therapists, LI or study clinician will conduct telephone assessments to monitor for changes in physical and psychiatric symptoms (administering the varenicline toleration assessment, Appendix 7). The same assessment will also be conducted each week for the first two weeks after their last Varenicline dose. At any time during the study, endorsement of one or more items listed in Appendix 7 will be referred to the study MAI or a NIDA-IRP clinician in the MAIs absence to who will determine if the endorsement requires further action. Participants with suicidal ideations, atypical changes in behavior, or experiencing clinically significant or difficult to tolerate side effects may be provided with a referral, instructed to stop taking the study pills, and/or be withdrawn from the study.

Study therapists will be extensively trained and supervised in administration of the semi-structured interview by the study MAI. The participant will be referred to the MAI if any questions or issues arise.

At each visit to NIDA, participants are queried about their general medical and emotional health (including current medications and drug use history) since their last visit to NIDA and are observed throughout each visit at NIDA by study investigators. Any change in health status is noted by protocol staff and evaluated for relevance to the participant's participation. Investigators will refer the participant to the MAI for any medical adverse events or questions about recent medical conditions. The MAI will be informed of and respond to any adverse events.

(1) All female participants will have a urine pregnancy test performed before each visit that requires an MRI.
(2) All participants participating in an MRI study will receive a comprehensive screening for metal and other possible devices within the body that are contraindicated in the MRI environment.
(3) The MRI is for research purposes only and not the type used to look for neurophathological changes; they will not be routinely reviewed for such problems. However, sometimes the MRI will show what could be an abnormal finding. In this case, the participant will be referred to the proper expert(s) for further diagnostic procedures.

Medical Emergencies. During fMRI sessions, which will take place at NIDA-IRP arrangements are in place for the emergency transport of subjects to the Johns Hopkins Bayview Emergency Department, if required.

9.2. Criteria to be used

Participants who experience a serious expected or unexpected adverse event (i.e., require hospitalization, experience significant disability/incapacitation, death, or have a life-threatening reaction) with likely or greater relationship to the experimental procedures will be excluded from any further participation. Participants may also be withdrawn from the study should they experience a serious allergic reaction to the nicotine or placebo patch adhesive. Should participants fail to comply with the 36 hr smoking abstinence, they will be asked to reschedule their scanning visit and recomplete the 36 hr abstinence. If they do not wish to reschedule, they will be withdrawn from the study.

No specific data on the toxic dose of varenicline appear to be available. The toxic dose of varenicline may be dependent on previous experience with nicotine. Specifically, smokers are able to tolerate higher single-doses of varenicline than non-smokers (Faessel et al., 2006a). Varenicline is well tolerated in single doses up to 3mg (Faessel et al., 2006a) and in multiple doses up to and including 2mg daily (Faessel et al., 2006b) with nausea and vomiting being limiting factors. Participants may be discontinued from varenicline treatment and offered alternative treatment (i.e., nicotine replacement therapy) depending on the severity of varenicline related side effects. The daily dose of varenicline may be reduced (on a case-by-case basis) to alleviate side effects and increase retention rates if necessary.

9.3. Criteria for individual subject withdrawal

Individual participation will be terminated if the individual no longer wishes to participate, is continually non-compliant (repeatedly fails to show for appointments without calling to reschedule, does not complete procedures as instructed, does not comply with restrictions such as limited caffeine use, drug use), if they no longer meet study inclusion criteria, or if a circumstance arises that makes continued participation unsafe or ill-advised (for example, an inability to tolerate nicotine patch; participants will be allowed to continue in the study without varenicline if they cannot tolerate it). Subjects will also be withdrawn if they do not complete the following required procedures:

1) the two pre-treatment scan visits;

2) set a quit date;

3) attempted to quit for at least 24 hours;

4) we have been able to establish their quit status (i.e., relapsed vs non-relapse) at 1, 6 OR 12 months following their last treatment visit.

10. Outcome measures

10.1. Primary outcomes measures

1) Change in BOLD signal and FC related to task parameters, between drug conditions: will provide important insight into the neurobiological mechanisms underlying acute nicotine withdrawal, in particular those related to anhedonia, negative affect, inhibitory control and impulsive decision making.

2) Behavioral performance on each of the tasks assessing inhibitory control processes, reward responsiveness, amygdala, striatal, BNST reactivity and impulsive decision making (e.g., reaction time, error rate, hit rate, reward bias): will provide task-related parameters necessary for analysis of BOLD data. Performance data will function as a secondary outcome by providing behavioral validation of acute nicotine withdrawal effects.

3) *Self-reported craving, withdrawal symptoms and mood/affect*: will be employed as regressors in the analysis of task and resting BOLD data. They will also provide the primary means of validating the acute nicotine withdrawal manipulation.

4) *Smoking status at 1, 6 and 12 months*: Smoking status (relapse vs abstinent) at each of the follow-up time points will be based on 7-day point prevalence defined as no smoking (not even a puff) or use of any tobacco products for the past 7 days. This is a standard method of assessing abstinence and dichotomizing relapse status at follow-up (Keating & Siddiqui, 2006; Leventhal et al., 2008; Swan et al., 2010b). Whenever abstinence at follow-up is assessed in-person, self-reported abstinence will be corroborated with breath CO and urine cotinine levels.

10.2. Secondary outcome measures include:

4) MRS for glutamate concentration: Glutamate concentration at the PFC and hippocampus is expected to change as a function of acute nicotine withdrawal and may also play a role in the expected reduction in VS reactivity and anhedonia.

5) Plasma ACTH and cortisol: are expected to increase as a function of acute nicotine withdrawal and is expected to play role in both anhedonia and negative affective symptoms, potentially by acting at the amygdala, BNST and VS.

6) Resting state CBF from ASL: To test for changes in neural activity (i.e., not connectivity) at rest it is necessary to employ a measure which does not require a baseline. CBF provides such a measure as changes in blood flow are referenced to a common arterial location outside of the brain.

7) ERP and EEG measures: Secondly, ERP and EEG indices will provide information about neural processing that cannot be derived from the BOLD signal and will be important to dissociating temporally tightly coupled processes underlying inhibitory control.

8) *Ratings and scores on self-report characterization measures:* will be employed to examine potential mediation of primary hypothesized effects. E.g., does degree of nicotine dependence mediate craving related changes in rsFC in the DMN?

9) *Structural MRI and DTI data:* will be employed to examine potential mediation of primary hypothesized effects. E.g., does increased WM integrity between the rACC and amygdala effect rsFC between these two structures and in turn amygdala reactivity?

Resting state MRI at follow-up. Resting state MRI will be assessed between- and within-groups as a function of relapse status and time since last treatment visit at each of the three follow-up time-points (1, 6 and 12 months). This will allow us to address the following exploratory questions:

Q1. What characteristics of rsFC are associated with treatment success (vs failure) at 1, 6 and 12 months post-treatment?

Q2. Are there characteristics of rsFC that vary as a function of time post-treatment in successfully abstinent individuals?

11. Statistical Analysis

11.1. Pre-processing

The imaging data will be pre-processed with AFNI (Analysis of Functional NeuroImages, http://afni.nimh.nih.gov/afni/ (Cox, 1996)) and Brainvoyager 1.7 (BrainInnovation, Masstricht, The Netherlands). All functional images will be directly registered upon high resolution MPRAGE anatomical scans obtained during the same imaging session. Location and intensity of activations from individual and/or grouped data will be translated into 3D stereotaxic coordinates (Talairach and Tournoux, 1988). Functional images of activation-induced BOLD signal changes will be determined using cross correlation or multiple regression analyses (Worsley and Friston, 1995; Bandettini and Wong, 1997).

Glutamate concentrations derived from MRS will be quantified for the PFC/ACC and or hippocampus with LCModel software. The LCModel software utilizes a library of reference spectra and a curve fitting algorithm to estimate the tissue concentration of neural metabolites. Values are given in institutional units approximating millimolar (ppm) concentration.

EEG/ERP data will be pre-processed and analyzed using commercially (BrainVision Analyzer) and freely available (Delorme and Makeig, 2004) software packages. Individual and group ERPs will be calculated and the amplitude of selected components (e.g., ERN, P300, evoked theta). BESA (Brain Electrical Source Analysis, MEGIS, Germany) will also be used to identify the putative neural generators of ERP components using dipole source models.

11.2. Data-Analysis

Unless otherwise specified, all BOLD, genetic, behavioral, self-report and physiological data will be analyzed with AFNI or *R* (http://www.R-project.org; R Development Core Team, 2008). The types of statistical analysis to be utilized in the current protocol are summarized below.

Nicotine deprived vs non-deprived. Between condition (nicotine-deprived vs non-deprived) comparisons will be conducted on self-report, behavioral, BOLD ROI, resting CBF and ERP responses with a series of one-way repeated measures ANCOVAs, with nicotine-deprivation (nicotine-deprived vs non-deprived) as the within-subjects factor and nicotine dose as the covariate. By controlling for nicotine dose we can partially dissociate the

effects from acute nicotine-withdrawal effects. Treatment seeking status (Treatment seeking vs non-treatment seeking) will be included as a between-subjects factor in these models to assess the main effects and of motivation to quit as well as any interaction between motivation to quit and acute nicotine withdrawal.

Mediation. Where mediation has been hypothesized (e.g., central CRF as a mediator of VS reactivity following acute nicotine withdrawal), regression coefficients for the paths of each mediation model will be derived with multiple regression and the mediation effect will be tested with ProdClin (Mackinnon, Fritz, Williams & Lockwood, 2007). ProdClin is a program developed by MacKinnon, Fritz, Williams, and Lockwood (2007) that uses the distribution of the product of paths a and b to compute asymmetric confidence intervals for the mediated effect. ProdClin is an alternative to the Sobel (1982) method that provides a more powerful and accurate test of the mediation effect because it is based on an asymmetric distribution which more closely resembles the true distribution of the product terms. Mediation models will be conducted separately for treatment-seeking and non-treatment seeking smokers.

Functional Connectivity. Seed regions for FC will be based on peak BOLD responses in ROIs for the parameter of interest. Strength of functional connectivity will be measured by correlating the BOLD time-series between seed and target regions. Fisher *r* to *z* transformations will be conducted to test for significant differences in functional connectivity strength between the non-deprived and nicotine-deprived conditions. Treatment seeking status (Treatment seeking vs non-treatment seeking) will be included as a between-subjects factor in these models to assess the main effects and of motivation to quit as well as any interaction between motivation to quit and acute nicotine withdrawal.

Correlational analyses. Bivariate partial correlations controlling for nicotine dose will also be conducted between self-report and behavioral indices of acute nicotine withdrawal and BOLD indices. Correlational analyses will serve to identify neural markers of responsivity to nicotine replacement. A series of bivariate partial correlations (controlling for nicotine dose) will also be conducted between BOLD, EEG, psychophysiological and neuroendocrine markers between nicotine-deprived and non-deprived conditions. Correlations will be conducted separately for non-treatment and treatment seeking groups.

Genotype by nicotine-deprivation interaction. 2 x 2 mixed-design ANOVAs will be conducted with genotype (short vs long allele of the 5-HTTLPR gene) varied between-subjects, and nicotine-deprivation (nicotine deprived vs non-deprived) varied within-subjects. The DVs will be BOLD signal change and functional connectivity. Treatment seeking status (Treatment seeking vs non-treatment seeking) will be included as a between-subjects factor in these models to assess the main effects and of motivation to quit as well as any interaction between motivation to quit and acute nicotine withdrawal.

RSN dynamics under acute nicotine withdrawal. The presence of three independent RSNs resembling the ECN, DMN and SN will be confirmed by applying an Independent Component Analysis (ICA) to the band pass filtered (0.01 to 0.08 Hz) resting BOLD time-series. ICA analysis will be conducted using the FSL Melodic ICA software (http://www.fmrib.ox.ac.uk/analysis/research/melodic/). The time-series of six seed regions reflecting core-nodes within each of these networks (ECN: dlPFC, PPC; DMN: vmPFC, PCC; SN: dACC, anterior insula) will then be regressed against each other to assess within and between network rsFC. Fisher *r* to *z* transformations will be applied to assess changes in rsFC between nicotine-deprived and non-deprived conditions. Bivariate partial correlations (controlling for nicotine dose) will also be conducted between the degree of change in rsFC within/between the three networks and changes in self-reported affective/cognitive withdrawal symptoms/urge to smoke as well as changes in working memory and attention indices. Treatment seeking status (Treatment seeking vs non-treatment seeking) will be included as a between-subjects factor in these models to assess the main effects and of motivation to quit as well as any interaction between motivation to quit and acute nicotine withdrawal. Correlations will be conducted separately for non-treatment and treatment seeking groups.

Relapse vs non-relapse group comparisons. Neurobiological processes underlying acute nicotine withdrawal will be assessed as a function of relapse status at each of the follow-up time-points by conducting between group comparisons.

11.3. Criteria for significance

For behavioral, EEG/ERP and self-report measures alone, a statistical effect exceeding an α-level of 0.05 will be considered statistically significant. The appropriate adjustments for multiple comparison follow-up tests (e.g., Bonferroni, Tukey, Scheffe) will be employed where necessary. For the fMRI analysis, where ROIs are stipulated a priori, the threshold for statistical significance will also be set at an α-level of 0.05. For whole-brain analyses, probability thresholding of individual voxels is used in combination with minimum cluster size thresholding to increase statistical power whilst minimizing the chance of type 1 errors. The underlying principle is that true regions of activation will tend to occur over contiguous voxels whereas noise has much less of a tendency to form clusters of activated voxels. Although tables of false detection probability vs. cluster size have been published, they are limited to 2D images, and are not directly applicable; even if tables were published, they could not cover every possible combination of experimental parameters. Therefore, we empirically investigate the tradeoff between probability and cluster threshold to achieve the desired significance level for a particular experimental condition.

The AlphaSim program (within AFNI) is a Monte Carlo simulation technique that provides a method for multiple comparison correction while persevering statistical power. Using random image generation, Gaussian filtering (to simulate voxel correlations), thresholding, and tabulation of cluster size frequencies, the program generates an estimate of the overall significance level achieved for various combinations of probability and cluster size thresholds assuming spatially uncorrelated voxels. Overall, our simulations indicate that by accepting a minimum cluster size it is possible to obtain an order-of-magnitude improvement in probability threshold over the value of probability threshold required if cluster size thresholding is not used. An approximately 200-400 µl minimum cluster size will generally be used in these studies to maintain our alpha level of 0.05.The criteria for significance (alpha) will be 0.05.

11.4. Power analysis

General fMRI power. Since fMRI is the main outcome measurement utilized in the present protocol, the key power analysis pertains to the fMRI data. However, prospective power analyses for fMRI data are complicated for several reasons. First, fMRI data are analyzed in a hierarchal manner such that both the *intra*-participant variance from the time-course data and the *inter*-participant variance across individuals could affect statistical power. A large number of time points tend to mitigate effects of intra-participant variance, but temporal autocorrelation and scanner limitations limit the number of independent measurements per unit time and thus the number of independent time points that are collected. In addition, effect sizes and both types of variance will vary spatially. Because of this, a given study may have sufficient power to detect differences in some brain regions, but lack sufficient power in other regions where the null hypothesis is false. Finally, fMRI analysis consists of a very large number of non-independent multiple comparisons, greater than 1.5 million at the group level, necessitating correction methods less severe than a Bonferroni correction, as discussed above. Thus, a proper power analysis on fMRI data requires simulating all of these effects. Desmond and Glover (2002) have performed such a simulation. They show for a relatively modest signal change of 0.5% during a cognitive task and with an intra-participant standard deviation of 0.75% and an inter-participant standard deviation of 0.5%, that 11 participants are required for a power of 0.8 using $p < 0.05$. Using a false positive rate of $p < 0.002$, a level more consistent with a cluster size threshold to correct for multiple comparisons (Forman et al., 1995), and with the variances kept the same, approximately 21 participants are needed for an expected signal change of 0.5% and

11 participants for a signal change of 0.75%. For more than ~100 independent time points, power (and hence the intra-participant variance) is relatively independent of the number of time points (Desmond and Glover, 2002). All current analyses should fall within this range.

Relapse vs non-relapse group comparisons. Based on previous studies comparing individuals who relapse following smoking cessation treatment to those who remain abstinent (Janes et al., 2010; Versace et al., 2011), we anticipate a medium-large effect size (Cohen's $d = 0.65$) when comparing relapsers vs non-relapsers across each of our neurobiological outcome measures. As we have proposed clear directional hypotheses regarding the relationship between treatment outcomes and neurobiological changes resulting from acute nicotine withdrawal, a one-tailed *t*-test model was used to calculate power. A power level (1-β) of 0.8 was selected to achieve an appropriate balance between the ability to detect significant differences and the number of participants needed. Approximately 30 participants per group will be required to detect significant differences between these two independent groups at a nominal α level of 0.05 in a one-tailed test assuming a medium-large effect size (calculated by G*Power, http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/).

Nicotine-deprived vs non-deprived condition comparisons. Estimating a dose-matched nicotine vs placebo effect size in 36 hr deprived smokers is complicated by several factors. Firstly, previous within-subject nicotine vs placebo studies have employed significantly shorter abstinence periods (e.g., 3 - 4 Hahn, Hong, Cole, or 8 hrs, Cole and Beaver). Secondly, studies addressing acute nicotine withdrawal effects using longer abstinence periods (e.g., 24 hrs) typically compare smoking deprivation to a smoke adlib condition (Azizian et al., 2010b; Field, Santarcangelo, Sumnall, Goudie, & Cole, 2006b; Kozink, Kollins, & McClernon, 2010b; McClernon et al., 2008b; McClernon, Kozink, Lutz, & Rose, 2009a; Mitchell, 2004b). Following 8 hrs of smoking deprivation, nicotine (relative to placebo) produces small to medium effects on self-report withdrawal symptoms, mood and craving measures (Cohen's $d = 0.15$ to 0.52), medium effects on behavioral measures ($d = 0.48$ to 0.618) (Beaver et al., 2011; Cole et al., 2010). Effects of 12 to 24hrs of smoking deprivation (relative to adlib smoking) on self-reported craving and affect/mood vary from medium to large ($d = 0.51$ to 4.91). The same deprivation periods produce small to medium effect sizes ($d = 0.23$ to 1.435) for go-nogo, attention and delay discounting tasks with larger effects on RT and discounting rate and smaller effects on error rates.

In the current study we aim to increase the severity of nicotine-withdrawal effects by employing a 36 hour deprivation period. At the same time, individual differences in responsivity to nicotine replacement will likely reduce between-condition effects relative to smoking deprivation vs smoke adlib studies cited above. Together the above studies suggest that the weakest effects of a dose-matched nicotine vs placebo manipulation in 36 hr deprived smokers smoking deprivation manipulation will arise for behavioral measures and will be medium in size ($d = 0.5$). We selected a power level (1-β) of 0.80 to achieve an appropriate balance between the ability to detect significant differences and the number of participants needed. Assuming an alpha-level of 0.05, a correlation among repeated measures of r = 0.5 and a medium effect size, approximately 35 participants will be required to achieve a power of 0.80 (calculated by G*Power http://www.psycho.uniduesseldorf.de/abteilungen/aap/gpower3/).

Motivation to quit by nicotine-deprivation. To generate an estimated effect size for a 2 (treatment seeking vs non-treatment eeking) x 2 (deprived vs non-deprived) we referred to the single study conducted to date comparing treatment seeking smokers to non-treatment seeking smokers (Wilson et al., 2012). In this study, which employed self-report physiological and neuroimaging outcome measures, the smallest effect size reported for a 2 x 2 between-within interaction, where the between subjects factor was treatment seeking status, was $\eta^2 = .08$. Based on an effect size of $\eta^2 = .08$, and a power of 0.80, and an estimated correlation between repeated measures of 0.5, the required sample size to detect a true 2 x 2 between-within interaction effect is 26 individuals.

Genotype by nicotine-deprivation. To derive an estimated effect size for the genotype by nicotine-deprivation interaction we examined effects of the 5-HTTLPR polymorphism on pharmacological manipulations where conditions are varied within-subjects. Genotype by treatment effects on behavioral and self-report outcome

measures in these studies display a small to large effect size (d = 0.30 to 0.90) (Kellner et al., 2009; Marsh et al., 2006; Thakur, Grizenko, Sengupta, Schmitz, & Joober, 2010). Recently, (Minnix et al., 2011) reported a large effect (d = 1.45) of the 5-HTTLPR polymorphism on startle reactivity in overnight deprived smokers following nicotine vs placebo nasal spray. Although the effect of the serotonin transporter polymorphism on brain (BOLD or EEG/ERP) activity changes following nicotine or smoking deprivation has yet to be investigated, large effect sizes (d > 0.80) have been reported for a smoking deprivation by COMT genotype interaction (Wang et al., 2008).

Due to the large variance in reported effects we conducted two power analyses assuming a medium (d =0.50) and large effect size (d = 0.80) effect size. Based on these analyses and assuming a correlation among repeated measures of r = 0.5, we would require approximately 16 to 35 individuals to achieve an 80% chance of detecting a genotype by nicotine-deprivation effect in the current study across our BOLD and behavioral indices (calculated by G*Power, http://www.psycho.uniduesseldorf.de/abteilungen/aap/gpower3/).

Correlational. Bivariate partial correlations (controlling for nicotine dose) will be conducted between the change in BOLD, ERP/EEG, psychophysiological and neuroendocrine markers, behavioral and self-report under nicotine-deprivation (relative to nicotine satiety). Large effects (d > 0.80) are typically observed between changes in self-report (e.g., craving, withdrawal symptoms) and BOLD measures (e.g., insula or amygdala activity or functional connectivity) during smoking abstinence relative to smoke adlib (Cole et al., 2010; Kozink et al., 2010a; McClernon et al., 2009b). Similarly large effect sizes are reported between changes in behavioral indices (e.g., RT variability) and BOLD responses (e.g., PCC task response) under nicotine (relative to placebo) in minimally deprived smokers (Hahn et al., 2007). Large effect sizes also arise between BOLD responses and neuroendocrine and psychophysiological markers (Ahs, Sollers, Furmark, Fredrikson, & Thayer, 2009; Drevets et al., 2002) as well as between behavioral indices and time-to-smoke in the laboratory relapse model (Dallery & Raiff, 2007). Based on a slope of 0.59 between each predictor and outcome measure (a large effect size according to J. Cohen, 1988) a sample size of at least 20 would be required to achieve a power of 0.80. (Calculated by G*Power, http://www.psycho.uniduesseldorf.de/abteilungen/aap/gpower3/).

Mediation Analyses. Based on the literature reviewed above, we expect a large effect size between each predictor, outcome and mediation variable in the mediation models described above. Based on a large effect size for paths *a* and *b*, Fritz and McKinnon (2007) suggest a sample of 35 to achieve a 0.80 power to detect each mediation effect with Prodclin.

11.5. Accrual number request, taking into account screening/ dropouts

Treatment-seeking smokers. In the current study, an individual will be considered a completer if they meet the following criteria: 1) have completed the two pre-treatment scan visits; 2) have set a quit date; 3) attempted to quit for at least 24 hours; 4) we have been able to establish their quit status (i.e., relapsed vs non-relapse) at 1, 6 and 12 months following their last treatment visit. Based on the power analyses above we anticipate that a minimum group size of 30 in each of our two relapse groups (relapse vs abstinent) at each follow-up time point (1, 6 and 12 months) would provide a reasonable level of statistical power allowing for the detection of effects of interest in the present study. Based clinical trials of varenicline with similar follow-up time points (Aubin et al., 2008; Keating & Siddiqui, 2006; Krebs & Sherman, 2012; Swan et al., 2010a; Tsai et al., 2007), we estimate that 35% of completers at 12 months will meet criteria for 7-day point prevalent abstinence. The 12 month follow-up is the point at which we would expect our abstinent group to be smallest. Achieving a minimum of 30 abstinent smokers at 12 months would require 85 completers (i.e., 35% abstinence rate). Based on an expected 30% attrition rate at 12 months (Keating & Siddiqui, 2006) a total of 120 treatment-seeking smokers (85 completers) is therefore requested.

Non-treatment seeking smokers. An individual will be considered a completer if they complete procedures outlined for Phase 1 of the protocol (see *Section 4 Study Design and Methods)*. Based on power analyses described above and in *Section 10.4* we anticipate that 35 non-treatment seeking smokers with complete

and useable data would provide a reasonable level of statistical power allowing for the detection of effects of interest in the present study. However, since acute nicotine-withdrawal effects may increase head movement and given the potential for unforeseeable errors during data collection, an estimated 20% of the participants who complete all phases of the study will likely be removed from the final analyses. Thus, a total of 42 non-treatment seeking smokers is requested.

12. Human Subjects Protection

12.1 Subject selection

Participant recruitment will be based strictly on the inclusion/exclusion criteria. No preferences in participant recruitment will be made on the bases of gender, race, or ethnic background. Efforts will be made to avoid participant distribution bias such that if skewing is noted, subjects in the over-represented group may temporarily be excluded from the study until additional participates from under-represented groups can be established.

Efforts will be made to include ethnic minorities in proportion to their presence in the major metropolitan Baltimore area (Baltimore City, Anne Arundel, and Baltimore and Howard counties; http://quickfacts.census.gov/qfd/states/24/24510.html).

Treatment seeking smokers

APPROVED STUDY POPULATION						
	FEMALE ENROLLMENT	MALE ENROLLMENT	**TOTAL** ENROLLMENT	FEMALE COMPLETERS	MALE COMPLETERS	**TOTAL** COMPLETERS
APPROVED CEILING	61	59	120	43	42	85

NIH TARGETED/PLANNED ENROLLMENT			
ETHNIC CATEGORY	Sex/Gender		
	Females	Males	Total
Hispanic or Latino	3	2	5**
Not Hispanic or Latino	58	57	115
Ethnic Category: Total of All Subjects*	61	59	120*
RACIAL CATEGORIES			
American Indian/Alaska Native	0	0	0
Asian	2	2	4
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	39	38	77
White	20	19	39
Racial Categories: Total of All Subjects*	61	59	120*

*The "Ethnic Category: Total of All Subjects" must be equal to the "Racial Categories: total of All Subjects."

Non-treatment seeking smokers

APPROVED STUDY POPULATION						
	FEMALE ENROLLMENT	MALE ENROLLMENT	**TOTAL** ENROLLMENT	FEMALE COMPLETERS	MALE COMPLETERS	**TOTAL** COMPLETERS
APPROVED CEILING	21	21	42	18	17	35

NIH TARGETED/PLANNED ENROLLMENT			
ETHNIC CATEGORY	Sex/Gender		
	Females	Males	Total
Hispanic or Latino	1	1	2**
Not Hispanic or Latino	20	20	40
Ethnic Category: Total of All Subjects*			42*
RACIAL CATEGORIES			
American Indian/Alaska Native	0	0	0
Asian	1	0	1
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	13	13	26
White	7	8	15
Racial Categories: Total of All Subjects*	21	21	42*

*The "Ethnic Category: Total of All Subjects" must be equal to the "Racial Categories: total of All Subjects."

Older individuals, defined as those over 55, will be excluded from the present study. *Justification:* Many cognitive processes change with age. In addition, the risk of difficult-to-detect medical abnormalities such as silent cerebral infarcts increases with age.

Left-handed individuals will also be excluded. *Justification:* Some of the neural processes assessed in this protocol may be lateralized in the brain. In order to reduce potential variance, participants will be required to be right-handed.

12.2 Justification for exclusion of children

Children will not be included in the present study. This study focuses on the effects of acute nicotine withdrawal processes. Given that nicotine is a controlled substance (must be 18 or older to purchase nicotine containing products) children under the age of 18 will not be recruited.

12.3 Justification for exclusion of vulnerable populations

There will be no inclusion of vulnerable populations in the current Study. The study focuses on the effects of acute nicotine withdrawal on a diverse range of affective, cognitive, behavioral and neurobiological indices. To control for variance in these domains we need to exclude individuals suffering from mental illness, intellectual impairment or any other condition that may affect neurological functioning (e.g. HIV). Pregnant women are also explicitly excluded due to the unknown risks that MRI poses to the unborn fetus.

12.4 Safeguards for vulnerable populations

Measures of intellectual functioning, mental health and HIV screening will be conducted under protocol 06-DA-N415 and used in the current protocol to identify and exclude individuals in vulnerable populations. Urine and/or blood serum pregnancy testing will also be employed in the current protocol to screen for pregnancy.

12.5 Justification of sensitive procedures.

The use of a placebo condition in the current protocol may be considered a sensitive procedure. A placebo condition has been included in the current design to avoid the potential for demand characteristics (i.e., expectancy bias) to impact on participant behavior/self-report.

In addition, participants will be required to abstain from smoking for 36 hours prior to each scanning session. As outlined previously, the primary purpose of the current protocol is to understand the neurobiological mechanisms that underlie acute nicotine withdrawal and drive early relapse in smokers. The affective (e.g., anhedonia, anxiety, irritability) and cognitive (e.g., difficulty concentrating) withdrawal symptoms following acute nicotine abstinence show an onset of 2 to 12 hrs post-cessation and peak within 2-3 days (J R Hughes et al., 1994). Most prior imaging and EEG studies have assessed acute withdrawal effects following overnight abstinence (typically 8 to12 hrs of smoking deprivation; e.g., Azizian et al., 2010; Beaver et al., 2011; D. M. Cole et al., 2010; Loughead et al., 2010; Sweet et al., 2010) and a few, 24 hours abstinence (Kozink et al., 2010a; McClernon et al., 2009b). Although abstinence effects on task and resting state BOLD or EEG activity have been observed across most studies, behavioral effects appear to be less robust with shorter abstinence periods. By limiting their abstinence period to 12 or 24 hours studies may be failing to capture or detect neurobiological changes and behavioral phenomena which underlie the later peak in acute nicotine withdrawal effects. In the current protocol, 36 hrs was selected as an abstinence period that would approximate peak acute withdrawal effects, producing robust effects across MRI, EEG and behavioral measures, whilst also maximizing non-smoking compliance.

The administration of varenicline may be considered a sensitive procedure. Varenicline is an FDA approved first-line treatment for smoking cessation which is more effective than current alternative smoking cessation aids (Aubin et al., 2008). In the current study, varenicline will be administered to treatment seeking smokers as part of a 12-week treatment for smoking cessation and will therefore be of direct benefit to participants. Employing Varenicline with counseling will also reduce relapse rates at 1, 6 and 12 months relative to alternative treatments and thus increase our statistical power to be able to examine differences between those individuals who relapse and those who remain abstinent at each of these time points. Moreover, varenicline is reasonably well tolerated, has an acceptable safety profile, and is associated with low abuse liability (Glover and Rath, 2007). Notably, varenicline was associated with significantly greater abstinence rates than NRT at 4 weeks (55.9% vs 43.2%) and 52 weeks (26.1% vs 20.3%). Varenicline was also associated with significantly less craving, withdrawal symptoms and smoking satisfaction compared with NRT (Aubin et al., 2001).

12.6 Staff Participation

NIDA-IRP staff will be allowed to participate in this protocol. Recruitment of staff will be via IRB-approved announcements and advertisements to the IRP as a whole. No staff member will be directly approached to participate in an experiment. To ensure against issues of breach of confidentiality or coercion, only staff from outside the Neuroimaging Research Branch and who are not under the supervision of any study investigator listed on this protocol will be allowed to participate. Additionally, employee participation will be limited to those doing so on their own time so that the incentive tasks will not be undermined by the lack of a payment incentive. No personal identifiers will be tagged to any of the data - behavioral, physiological or neurobiological. During the screening and recruitment process, non-NIDA participants will be explicitly asked for permission to re-contact them in the future. Appropriate methods for such contact (e.g. phone, email, letter) will be determined at the time of consent.

13. Anticipated Benefit

Treatment-seeking smokers

Direct benefits: Varenicline administration and smoking-cessation counseling (or alternative treatment plans described above) should help decrease participants' smoking and withdrawal discomfort. They may also benefit more generally by receiving psychological assistance in developing skills to enable them to cope with stress and other situations which precipitate cigarette smoking.

Non-treatment-seeking smokers

There are no direct benefits to participants in the non-treatment seeking arm of this protocol. The findings may benefit future populations by extending understanding of the neurobiological mechanisms underlying motivation to quit and the interaction with mechanisms underlying symptoms experienced during acute withdrawal from nicotine.

14. Consent documents/Process

Written informed consent will be obtained from each subject at entry into the study. The consent document will include details regarding all study procedures (e.g. fMRI, EEG, and genotyping). Informed consent is obtained by the following process: Subject reviews the study consent form; PI or co-investigator then meets with the subject in a private area to review the consent, confirm subject understanding, and to answer any questions. This process will include reading through the document with the participant in order to further ensure participant understanding. Once the subject verbally demonstrates understanding to the investigator and agrees to the process, a consent quiz is administered. Provided the participant answers at least 80% of the questions correctly (which must include correct answers to questions #5 and #9), the participant is invited to sign the consent form. If the score on the consent quiz is less than 80% correct, the investigator reviews the incorrect answers and re-administers the consent quiz. Failure to obtain 80% correct (including correct answers to questions #5 and #9) on the second administration of the quiz excludes the subject from participating in the study. If the participant does score ≥ 80% on the consent quiz, they are asked to sign and date the consent form. The investigator co-signs the consent form and, if necessary, the consent is also signed by a third-party witness to the subject's signature. Two copies are made of the consent. The original copy of the consent and quiz are attached to the participant's medical record, the participant is given a copy of the consent form for his/her own records and the second copy of the consent and a copy of the quiz are stored with other protocol consents in a binder which is located in a locked filing cabinet. Once the signed consent has been obtained, the investigator will note the participant's enrollment in the study in the CDW database. Only after this last step has been completed will study procedures begin.

15. Data and Safety monitoring

a. **Selection of a Data and Safety Monitoring Mechanism** - Data and safety will be monitored by the NIDA Principal Investigator and MAI. Subjects will be monitored during the study for any adverse reactions and the overall protocol will be monitored by reviewing the AE's on a yearly basis. The NIDA PI will report all adverse events according to established NIH IRB guidelines. This protocol involves no more than minimal risk to participants.

b. **Frequency of the Monitoring -** Subjects will be monitored during the study for any adverse reactions and the overall protocol will be monitored by reviewing the AE's on a yearly basis.
c. **Stop or Change Rules -** An unexpected serious adverse event related to study procedures or the revelation of information or data not available at the time of study preparation which renders the risk/benefit ratio for this study unfavorable will result in study cessation.
d. **Advanced Plans for Any Interim Analyses and/or Futility Analyses -** There are no current plans for interim analyses and/or futility analyses.
e. **Information To Be Monitored**
    a. Progress of the study, including assessment of participant recruitment and accrual and adverse events will be reviewed to determine whether there is any change to the risk/benefit ratio of the study. This information will be reported to the IRB at the time of continuing review.
    b. Subjects will be monitored for adverse events by study staff. Adverse events will be recorded by staff in the subjects' medical record and reported to the Clinical Director and NIH IRB in accordance with all NIH and NIDA requirements for adverse event reporting.
f. There are no external factors (e.g., developments in the literature, results of related studies, etc.) that may have an impact on the safety of participants or on the ethics of the research study.
g. **Communication** - Adverse events will be recorded by staff in the subjects' medical record and reported to the Clinical Director and NIH IRB in accordance with all NIH and NIDA requirements for adverse event reporting. Additionally, a summary of study progress, including assessment of participant recruitment and accrual and adverse events will be reported to the IRB at the time of continuing review.

15.1. **Criteria for Study Cessation**

For the Study as a Whole: A particular arm of the study will be suspended by the PI, pending CD review, if any subject has a serious adverse event that is probably or definitely related to study procedures.

16. Quality Assurance

Quality-assurance monitor: The Principal Investigator and the MAI will be monitoring data collection and the study on an ongoing basis. Quality assurance will monitored by the NIDA Quality-Assurance Team on a schedule determined by the Clinical Director.

Quality-assurance plan: We will use the quality-assurance plan that is being developed by the NIDA IRP Clinical Director.

17. Event Characterization and reporting to the IRB, Clinical Director (CD) and Sponsor

NIH OHSRP Policies 801 and 802 (effective July 1, 2019; supersedes SOP 16) will be followed for reportable events and non-compliance.

18. Alternatives to participation or alternative therapies

Treatment-seeking smokers who are not eligible to participate in this protocol or who no longer want to participate in this protocol may be able to receive smoking cessation treatment outside of the study, under the

care of their own physician. They will be given referrals, when appropriate, for smoking-cessation help in the community.

Non-treatment seeking smokers who are not eligible to participate in this protocol or who longer wish to participate in this protocol and who express a desire at this point to seek treatment for smoking cessation will be given referrals to smoking cessation treatment outside of the study, as described above for treatment-seeking smokers.

Nonsmokers (enrolled in tDCS arm) who are not eligible to participate in this protocol, or who no longer wish to participate in this protocol, may choose not to join the study or to withdraw at any time.

19. Confidentiality

Strict subject confidentiality will be maintained throughout. Participants will be assigned a code number without personally-identifying information following their first contact in the protocol. This number will be used throughout the experiment and will be the only identifier on specimen samples, behavioral and physiological archival data, fMRI/MRI and EEG/ERP data. The identity of participants will not be revealed at scientific meetings, in publications or other vehicles of public communication. The PI and co-investigators will have access to the ID code, which will be maintained in a separate electronic file from the study data. Medical and questionnaire data will be gathered in the CDW, a secure database on a closed network. Access to records in the CDW is protected by a system of password-protected accounts and monitored by the Clinical Director (CD). Data downloaded from the CDW for analysis will be identified only by participant number.

19.1. **Medical Records.**

All medical history information is stored in the CDW database, which is password protected and has limited access. In addition, each participant is assigned a medical records folder during the screening process, which is used to store paper copies of medically relevant documents. This record is kept in locked cabinet and access to these files is limited to study personnel, including study investigators, nursing staff and clinicians.

19.2. **Research Records/Data.**

Paper copies of research records such as PI copies of consents and 'Run Sheets' (procedural checklists) used by investigators will be kept in binders. These binders will be kept in a locked cabinet, in a locked room, which has limited access. Binders remain in this room at all times, apart from when required for study sessions. At the completion of each paper copies of research records or binders will be returned to the locked cabinet by the investigator or research associate responsible for the experimental session. Data (physiological, imaging, behavioral) obtained during experimental sessions is stored on password protected, network drives, which have limited access. Data stored on these drives is identified by study number, participant ARC and/or task. No personal identifiers are stored with the data.

19.3. **Stored Samples.**

No biological samples will be stored for future use. Plasma samples acquired for measurement of cortisol and ACTH will be destroyed once valid assays have been obtained.

19.4. **Special Precautions.**

A Certificate of Confidentiality will be obtained for this protocol and strict subject confidentiality will be maintained throughout.

20. Conflict of Interest/Technology Transfer

NIH guidelines on conflict of interest have been distributed to all investigators. There are no conflicts-of-interest to report.

A human Material Transfer Agreement (MTA) between NIDA-IRP and the Board of Supervisors of Louisiana State University and Agricultural and Mechanical College acting on behalf of LSU Health Sciences Center at Shreveport for the analysis of plasma samples has been obtained. The Recipient Scientist at LSU, Hyung Nam, Ph.D., will receive coded samples to conduct analysis of plasma neuro-steroids and amino acids to compare with nicotine satiety, the effect of acute nicotine withdrawal on plasma neuro-steroids and amino acid profiles and their relationship with measured glutamate concentration (MRS) in the cingulate cortex.

21. Research and Travel Compensation

Treatment seeking smokers

All participants will be compensated for their participation in the pre-treatment scan and orientation visits and the three follow-up visits and related procedures according to NIDA-IRP policy summarized in *Table 1*. Compensation will be prorated for parts completed if subjects do not complete the study. Participants will also earn points on several tasks/procedures which will be exchanged at the end of each scanning session for additional monetary payment (as outlined). They will also receive incentives for successful abstinence during the pre-treatment study phase. At the end of the second pre-treatment scan day they will also receive a 10% completion bonus based on payment for study related procedures and MRI scanning. This completion bonus is only based on the hours participated during Phase 1, not on any other compensation received during Phase 1. For example, 28 hours of Phase 1 procedures @ $20 per hour = $560, so 10% of this is $56. Participants will have the option of cash or check payment. If participation runs beyond 6:30 pm or occurs on weekends, payment by check may be the only option. Participants will receive payment earned for each day at the end of each day of participation. In the rare instances where a check is pre-issued because procedures are scheduled after 6:30 or on weekends, the amount due the participant will be estimated on the low end, and the participant will receive any balance due either at their next session, or via a check in the mail (whichever they prefer). The minimum amount of time required for study procedures per scan visit is 8 hours (with 1 hour lunch break). We have based maximum payment on 10 hours of study related procedures per visit to allow for additional time when procedures take longer than expected. Participants will receive travel compensation for all Phase 1, 2 and 3 visits (up to 21 visits). Payment for at home procedures will be contingent upon maintaining abstinence for the full 36 hour period.

	Rate	**Time/frequency**	**Total**
Study related procedures incl. MRI scanning	$20/hr	32hrs	$640
At home procedures	$20 per period	2 periods	$40
Performance incentives	$25 day	2 days	$50
Task payment	Up to $70 per day	2 days	$140
Indwelling catheter	$10/day	2 days	$20
Travel	$15 per round trip	21 trips	$315
Completion bonus	10% total	1	$56.00
Max Total			**$1261.00**

Non-treatment seeking smokers

All participants will be compensated for their participation in the pre-treatment scan and orientation visits related procedures according to NIDA-IRP policy summarized in *Table 1*. Compensation will be prorated for parts completed if subjects do not complete the study. Participants will also earn points on several tasks/procedures which will be exchanged at the end of each scanning session for additional monetary payment (as outlined). They will also receive incentives for successful abstinence during the pre-treatment study phase. At the end of the second pre-treatment scan day they will also receive a 10% completion bonus based on payment for study related procedures and MRI scanning. This completion bonus is only based on the hours participated during Phase 1, not on any other compensation received during Phase 1. For example, 28 hours of Phase 1 procedures @ $20 per hour = $560, so 10% of this is $56. Participants will have the option of cash or check payment. If participation runs beyond 6:30 pm or occurs on weekends, payment by check may be the only option. Participants will receive payment earned for each day at the end of each day of participation. In the rare instances where a check is pre-issued because procedures are scheduled after 6:30 or on weekends, the amount due the participant will be estimated on the low end, and the participant will receive any balance due either at their next session, or via a check in the mail (whichever they prefer). The minimum amount of time required for study procedures per scan visit is 8 hours (with 1 hour lunch break). We have based maximum payment on 10 hours of study related procedures per visit to allow for additional time when procedures take longer than expected. Participants will receive travel compensation for all Phase 1 visits. Payment for at home procedures will be contingent upon maintaining abstinence for the full 36 hour period.

	Rate	**Time/frequency**	**Total**
Study related procedures incl. MRI scanning	$20/hr	28hrs	$560
At home procedures	$20 per period	2 periods	$40
Performance incentives	$25 day	2 days	$50
Task payment	Up to $70 per day	2 days	$140
Indwelling catheter	$10/day	2 days	$20
Travel	$15 per round trip	3	$45
Completion bonus	10% total	1	$56.00
Max Total			**$911.00**

22. Motivational Interviewing Arm

22.1 Arm Title

474 – Motivational interviewing to increase engagement in smoking cessation treatment.

22.2 Arm Background

The 2010 National Health Interview survey (*n* = 27, 157) reported that 69% of current smokers indicate a desire to stop smoking completely (Malarcher, Dube, Shaw, Babb, & Kaufmann, 2011). In contrast, the prevalence of successful smoking cessation (> 6 months without smoking) within the past 12 months fell at 6% (Malarcher et al., 2011). One of the primary factors driving the discrepancy between self-reported interest in quitting and successful cessation is the degree of motivation and preparation to quit smoking and maintain long-term abstinence (Center for Substance Abuse Treatment, 1999). Thus while smokers may express a desire to quit, this is often coupled with a reluctance to set a quit date and commit to long-term smoking abstinence. Motivational interviewing is a therapeutic technique designed to directly address this ambivalence and increase engagement in substance abuse treatment (Center for Substance Abuse Treatment, 1999).

22.3. Arm Objective

The purpose of the Motivational Interviewing Arm is to increase motivation and preparation to for smoking cessation treatment among individuals who express an interest in quitting smoking but are not currently ready to enter smoking cessation treatment, and are otherwise eligible for participation in the current protocol 12-DA-N474 or protocol 13-DA-N485. If, after a prescribed period of motivational interviewing, the participant and the study therapist feel that the participant is ready to enter treatment for smoking cessation, they will be offered admission into either protocol 12-DA-N474 or protocol 13-DA-N485.

22.4 Number and type of subjects

This study arm will recruit 300 current smokers who meet criteria for participation in protocol 12-DA-N474 (see section 3 Subjects) or protocol 13-DA-N485) but who are not currently ready to enter treatment for smoking cessation.

APPROVED STUDY POPULATION						
	FEMALE ENROLLMENT	MALE ENROLLMENT	**TOTAL** ENROLLMENT	FEMALE COMPLETERS	MALE COMPLETERS	**TOTAL** COMPLETERS
APPROVED CEILING	150	150	300	150	150	300

NIH TARGETED/PLANNED ENROLLMENT			
ETHNIC CATEGORY	Sex/Gender		
	Females	Males	Total
Hispanic or Latino	6	6	12**
Not Hispanic or Latino	144	144	288
Ethnic Category: Total of All Subjects*			300*
RACIAL CATEGORIES			
American Indian/Alaska Native	1	1	2
Asian	3	3	6
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	99	99	198
White	47	47	94
Racial Categories: Total of All Subjects*	150	150	300*

*The "Ethnic Category: Total of All Subjects" must be equal to the "Racial Categories: total of All Subjects."

23.5. Arm design and procedures

An initial session will be conducted to assess individual motivation/preparation to quit. During this session participants will complete the treatment preparation and motivation to quit measures outlined in Appendix 9 and meet briefly with a study therapist (~60min). This initial session will be followed by 1 to 10 sessions of motivational interviewing with a study therapist (each session ~20-60min). Motivational interviewing techniques are based on the SAMHSA Treatment Improvement Protocol (TIP) for enhancing motivation for substance abuse treatment (Center for Substance Abuse Treatment, 1999). Individuals recruited for this study will vary in their motivation and preparation to quit. Consequently, the number of motivational interviewing sessions will vary according to individual need as determined by the study therapist, with a maximum of 10 sessions per individual.

23.6 Storage of data

Clinical data will be stored in the Clinical Data Warehouse (CDW) and in clinical research charts. Research charts will be stored in locked filing cabinet, in a locked room at the NIDA-IRP. One copy of the consent form is kept in the participant's NIDA-IRP medical chart, the other is kept in a limited access, locked cabinet with other consent forms from the same protocol. Participant data contained within files on the NRB data drive are identified by ARC number, and not by other personal identifiers such as name.

23.7 Risks/discomforts

Questionnaire and Characterization Measures

The questions asked may be perceived as embarrassing or may cause anxiety or distress. Nevertheless, such reactions are expected to be temporary and to rapidly dissipate spontaneously.

Risk Minimization. Participants will be advised prior to completing these measures that they may refuse to answer any questions that cause them discomfort. Furthermore, any participant who experiences anxiety or distress as a result of these assessments will be able to speak to a NIDA-IRP mental health professional to discuss this.

23.8 Outcome measures/Statistical Analysis

There will be no outcome measures or collation and analysis of data at a group-level in this arm of the study.

23.9. Human subjects protection

The same as outlined for above in *Section 12. Human Subjects Protection*

23.10. Anticipated benefit

Direct benefits: motivational interviewing should help increase participant's motivation to participate in smoking cessation treatment, which should in turn decrease their smoking behavior and withdrawal discomfort.

23.11 Consent documents/process, Data and safety Monitoring, Quality Assurance, Adverse event and unanticipated problem reporting, Alternatives to participation, Confidentiality, Conflict of Interest

Details for the above sections are identical to those outlined within Sections 14-19 of the main protocol document.

23.12 Research and Travel Compensation

Participants will be reimbursed $20 per hour for the initial assessment visit but will not receive payment for the motivational interviewing visits. They will also receive $15 travel compensation for each visit. Based on motivational counseling visits and a single pre-assessment visit, anticipated maximum compensation for participation in the Motivational Interviewing Arm of this study is $185.

23. Transcranial Direct Current Stimulation (tDCS) Arm

24.1. Arm Title

474 – Transcranial Direct Current Stimulation (tDCS) to reduce effects of the Nicotine Withdrawal Syndrome

24.2. Arm Background

Cigarette smoking remains the leading cause of preventable death and disease in the United States, accounting for 480,000 deaths each year (CDC 2016). Despite our deeper understanding of the neurobiology of nicotine addiction and drug abuse over the past 10 years (Nestler 2005, Everitt and Robbins 2005, Koob and Volkow 2010, Goldstein and Volkow 2011), current pharmacological interventions for smoking cessation are highly inadequate, with the most efficacious treatment (varenicline) having only about 27% absolute cessation rate according to recent meta-analysis (Cahill 2013, 2014). Additionally, symptoms of nicotine withdrawal remain a major impediment for smokers trying to quit, with most quit attempts failing within the first week of abstinence when withdrawal symptoms are at their height (Hughes 1992, Sutherland et al 2017).

Recent findings based on resting state functional connectivity (rsFC) revealed that the brain can be divided into multiple large-scale functional networks, many of which have been found to contribute to psychiatric disease (Lu and Stein 2014, Turk-Browne 2013, Yeo et al 2011, Fox et al 2014). Addiction has been hypothesized as an imbalance within and between three of these networks (Lerman et al 2014, Pariyadath et al 2015, Fedota and Stein 2015, Sutherland et al 2012). Specifically, the cognitive and affective disturbances of nicotine withdrawal syndrome have been attributed to a reduced within-network connectivity of the Executive Control Network (ECN) and increased within-network connectivity of the Default Mode Network (Sutherland et al 2017). Additionally, the Salience Network (SN) has been called the "gatekeeper" for these two networks, and its function may be dysregulated in addictive disorders, as well (Sutherland et al 2017).

A treatment that acts by modifying these large-scale brain networks with specificity may help address the underlying pathophysiology of nicotine addiction and improve clinical outcomes (Dunlop et al 2016). Transcranial Direct Current Stimulation (tDCS), a type of Non-invasive Brain Stimulation (NIBS), has the potential to modify neuronal circuits by application of a subthreshold conductive current through the scalp. Two potential targets for tDCS as a smoking cessation aid are the dorsolateral pre-frontal cortex (dlPFC), a node of the ECN, and the ventromedial prefrontal cortex (vmPFC), a node of the DMN (Lerman et al 2014). tDCS can potentially strengthen the control of the ECN through excitatory stimulation of the dlPFC, and weaken the influence of the DMN by inhibitory stimulation of the vmPFC.

24.3. Arm Objective

The goal of the Transcranial Direct Current Stimulation (tDCS) Arm is to investigate the effects of tDCS on modulating large-scale brain networks dysregulated in nicotine addiction and withdrawal. Non-smokers and matched smokers (in both nicotine deprived and sated conditions) will be recruited for a randomized, sham-controlled, double-blind, repeated measures tDCS crossover study. tDCS will be used to probe the ECN and DMN by targeting the dlPFC with anodal (excitatory) stimulation, and the vmPFC with cathodal (inhibitory) stimulation (Pariyadath et al 2015). The effects of tDCS on brain activation patterns and cognitive control will be measured "online" while the subject is being scanned via MRI, allowing greater insight into the neurophysiological effects of tDCS.

Arm Hypothesis: The nicotine withdrawal syndrome is a **3-network dysregulation** characterized by reduced network connectivity (1) within the ECN and (2) between the ECN and the SN, as well as increased network connectivity (1) within the DMN, and (2) between the DMN and the SN. These deficits can be remediated by application of tDCS to cortical nodes of the ECN and DMN networks. (**Figure 1a**).

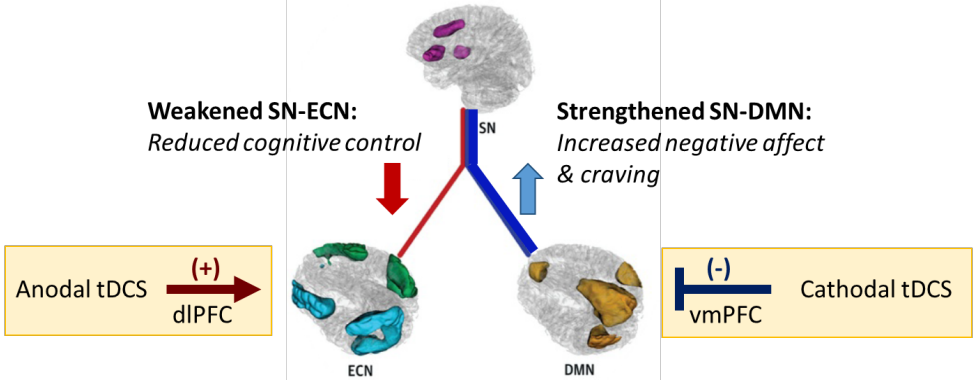


Figure 1a: Hypothesis. Three network dysregulation in nicotine addiction and hypothesized effects of tDCS. The ECN and DMN are anticorrelated. In nicotine withdrawal syndrome, the within-network connectivity of the DMN is stronger than normal, and the within-network connectivity of the ECN is weaker than normal. This imbalance is mediated by dysregulated connectivity between the SN - DMN and the SN - ECN. *SN = Salience Network. ECN = Executive Control Network. DMN = Default Mode Network. dlPFC = dorsolateral prefrontral cortex. vmPFC = ventromedial prefrontal cortex. Image adapted from Lerman et al 2014.*

Alternative Hypothesis (1): Nicotine withdrawal syndrome is a **2-network dysregulation** characterized by altered homeostatic regulation between the ECN and DMN. It is possible that the ECN normally acts to weaken the DMN, while the DMN normally acts to weaken the ECN. In the nicotine withdrawal syndrome, the ECN may have weakened control over the DMN, leading to a stronger within-network connectivity of the DMN and weaker within-network connectivity of the ECN. Anodal tDCS applied to the dlPFC can return the 2 networks to homeostatic regulation by strengthening the within-network connectivity of the ECN, while cathodal tDCS applied to the vmPFC can return the 2 networks to homeostatic regulation by weakening the within-network connectivity of the DMN. (**Figure 1b).**

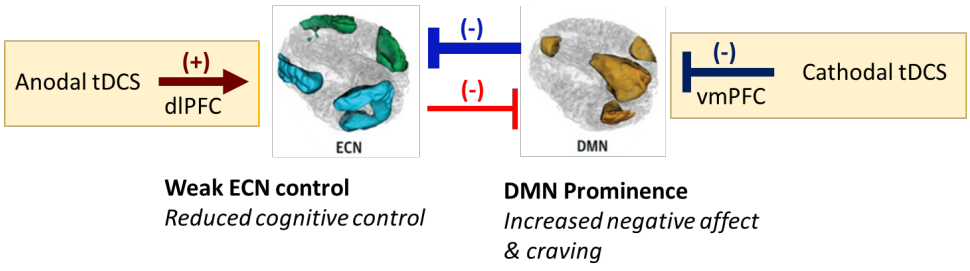


Figure 1b: Alternative hypothesis. Two network dysregulation in nicotine addiction and hypothesized effects of tDCS. *SN = Salience Network. ECN = Executive Control Network. DMN = Default Mode Network. dlPFC = dorsolateral prefrontral cortex. vmPFC = ventromedial prefrontal cortex. Image adapted from Lerman et al 2014.*

Alternative Hypothesis (2): It is also possible that the imbalances seen in nicotine addiction and withdrawal are due to an abnormal connectivity of only one of the two networks (DMN or ECN), either internally within the network or in connection with the Salience Network. The within-network connectivity of the DMN, or its between-network connectivity with the SN, may be stronger independently of changes to the ECN. Alternatively, the within-network connectivity of the ECN, or its between-network connectivity with the SN, may be weaker independently of changes to the DMN. The exact relationship may differ across individuals. In either case, anodal-dlPFC + cathodal-vmPFC tDCS is expected to bring the within and between network connectivities of the DMN and the ECN closer to the healthy control state.

Specific Aims

Aim 1: To determine the effect of excitatory tDCS stimulation of the dlPFC and inhibitory tDCS stimulation of the vmPFC on the within-network and between-network connectivity of the Executive Control Network (ECN), Default Mode Network (DMN), and Salience Network (SN).

To understand the effect of anodal stimulation of the dlPFC, and cathodal stimulation of the vmPFC, we will compare the following tDCS montages: **(1) anodal left-dlPFC + cathodal right-vmPFC** stimulation, with anode over the left dlPFC and cathode over the right-vmPFC; **(2) cathodal left-dlPFC + anodal right-vmPFC** stimulation, in which polarity is reversed between the two electrodes, and **(3) sham** stimulation..

Sub-Aim 1.1: To determine the effect of tDCS stimulation on the Executive Control Network (ECN) and cognitive control during nicotine withdrawal. Resting state connectivity, behavioral tasks and functional imaging will be used to measure the strength of the ECN and cognitive control mechanisms in response to excitatory tDCS stimulation of the left dlPFC, a cortical node of the ECN.

Hypothesis 1.1.1. Anodal-dlPFC tDCS will strengthen the within-network connectivity of the Executive Control Network compared to sham as measured by resting state functional connectivity in *abstinent smokers > sated smokers > healthy controls*. Cathodal-dlPFC tDCS will have the inverse effect on ECN rsFC.

Hypothesis 1.1.2. Anodal-dlPFC tDCS will improve reaction time and response accuracy on cognitive control tasks (Parametric Flanker, Amygdala Reactivity Task, and N- back) in *abstinent smokers > sated smokers > healthy controls*. The improved behavioral performance will be correlated with increased BOLD signal in nodes of the ECN. Cathodal-dlPFC tDCS will have the inverse effect on BOLD signal and behavior.

Sub-Aim 1.2: To determine the effect of tDCS stimulation on the Default Mode Network (DMN) during nicotine withdrawal. Resting state connectivity, behavioral tasks and functional imaging, magnetic resonance spectroscopy, and white matter integrity will be used to measure the strength of the DMN and cognitive control mechanisms in response to excitatory tDCS stimulation of the left dlPFC, a cortical node of the ECN, and inhibitory tDCS stimulation of the left vmPFC, a cortical node of the DMN.

Hypothesis 1.2.1. Cathodal-vmPFC tDCS will weaken the within-network connectivity of the Default Mode Network as measured by resting state functional connectivity in *abstinent smokers > sated smokers > healthy controls.* Anodal-vmPFC tDCS will have the inverse effect on DMN rsFC.

Hypothesis 1.2.2. Cathodal-vmPFC tDCS will improve reaction time and response accuracy on cognitive control tasks (Parametric Flanker, Amygdala Reactivity Task, and N-back) in *abstinent smokers > sated*

smokers > healthy controls. The improved behavioral performance will be correlated with greater BOLD deactivation in nodes of the DMN during these tasks. Anodal-vmPFC tDCS will have the inverse effect on BOLD signal.

Sub-Aim 1.3: To determine the effect of tDCS stimulation on the Salience Network (SN) and its connectivity with the ECN and the DMN.

Hypothesis 1.3.1. Anodal-dlPFC tDCS will strengthen the between-network connectivity of the Executive Control Network to the Salience Network compared to sham as measured by resting state functional connectivity in *abstinent smokers > sated smokers > healthy controls*. Cathodal-dlPFC tDCS will have the inverse effect on ECN-SN rsFC.

Hypothesis 1.3.2. Cathodal-vmPFC tDCS will weaken the between-network connectivity of the Default Mode Network to the Salience Network as measured by resting state functional connectivity in *abstinent smokers > sated smokers > healthy controls.* Anodal-vmPFC tDCS will have the inverse effect on DMN-SN rsFC.

Hypothesis 1.3.3. Anodal-dlPFC + Cathodal-vmPFC tDCS will improve BOLD reactivity in nodes of the Salience Network in tasks that are known to those nodes (e.g. the insula in the Parametric Flanker Task). Cathodal-dlPFC + Anodal-vmPFC will have the inverse effect on BOLD reactivity in the SN.

Aim 2: To compare the effects of tDCS stimulation parameters on behavior, resting state functional connectivity, and BOLD fMRI signal.

Sub-Aim 2.1: To compare the effect of tDCS stimulation on a working memory task given during stimulation (online) and after stimulation (offline).

Hypothesis 2.1.1: Active tDCS stimulation will provide greater benefit on accuracy and response time, and increase BOLD activation in the ECN to a greater degree, during the "online" working memory task than during the "offline" working memory task.

Alternative Hypothesis 2.1.1(a): Active tDCS stimulation will provide greater the same degree of benefit on accuracy and response time, and increase BOLD activation in the ECN to the same degree, during the "online" working memory task as during the "offline" working memory task.

Alternative Hypothesis 2.1.1(b): Active tDCS stimulation will provide less benefit on accuracy and response time, and increase BOLD activation in the ECN to a smaller degree, during the "online" working memory task than during the "offline" working memory task.

Aim 3: To compare the effects of tDCS stimulation on nonsmokers vs. smokers, in both the nicotine sated and nicotine deprived states.

Hypothesis 3.1. Active tDCS will have the greatest effect on the group farthest from normal resting functional connectivity at pre-stimulation baseline, i.e. smokers in withdrawal. The non-smoker group will respond the least to active tDCS. Smokers using a nicotine patch will respond to tDCS more than non-smokers, but not as much as smokers in withdrawal. Thus*, tDCS will have the greatest impact on network connectivity and behavior in the smoker-nicotine withdrawal group,* as measured by outcomes listed in Aim 1.

Aim 4: To evaluate the effect of individual variability on current localization, behavioral outcomes, and brain activation patterns in response to active tDCS stimulation.

Hypothesis 4.1. Anatomical Variability. As each individual has variation in head and brain size, skull thickness, and brain morphometry, it is expected that a standard tDCS application across all individuals will produce varied effects in each individual participant. Computer modeling of each participant's brain can approximate how standard tDCS stimulation parameters will affect each individual based on their own anatomy. We will use a post-hoc regression analysis to determine how outcome measures correlate with inter-individual anatomical variability.

Individuals who are predicted to have greater current flow through the dlPFC and vmPFC by computer modeling will also have greater responsivity to tDCS stimulation, as measured by outcomes listed in Aim 1.

Hypothesis 4.2. Psychological Variability. Cigarette smoking behavior is usually accompanied by a variety of co-morbid conditions and traits, such as depressed mood, increased anxiety, and use of substances such as marijuana and alcohol. Although participants will be excluded for serious co-morbidities, including moderate to severe mood or anxiety disorders, or substance use disorders (other than nicotine), the traits of these disorders fall on continuous scales that show variance among individuals before the diagnostic threshold. These individual differences can affect performance on the fMRI tasks, resting state functional connectivity, and response to tDCS.

We will use a post-hoc regression analysis to determine how these outcome measures correlate with inter-individual differences in personality, mood, anxiety, and substance use. It is predicted that individuals prone to depressed mood, increased anxiety, and more frequent use of marijuana or alcohol will have more abnormal resting connectivity at baseline, and therefore will be more responsive to tDCS stimulation, as measured by outcomes listed in Aim 1.

24.4. Number and type of subjects

There will be two study populations: 1) healthy nicotine-dependent adult participants who are current non-treatment seeking smokers; and 2) healthy non-smoking, non-drug dependent controls.

This study arm will recruit 60 current **non-treatment seeking smokers** (enrollees) with the expectation of 35 completers. Additionally, we will recruit 55 **non-smokers** (enrollees) attempted to match based on recruitment availability, by education, age, race, and gender, with the expectation of 45 completers (of either the Behavioral Pilot or tDCS Imaging study).

24.5. Modified Inclusion/Exclusion Criteria for tDCS Study Arm

The inclusion and exclusion criteria have been modified from the main protocol to best address the Aims of the Arm. In general, the spirit of the criteria in the main protocol remain the same. For the reader's convenience, the complete Inclusion/Exclusion criteria for this Arm are found below. *Checklist can be found in Appendix 1.*

Modified Inclusion Criteria. All Subjects must:

(1) Be between the ages of 18-65.

(2) Be right-handed. *Assessment tool:* Edinburgh Handedness Inventory.

(3) Be in good health. *Justification:* Many illnesses may alter fMRI signals as well as cognitive processes and neural functioning. *Assessment tool(s):* Participants will provide a brief health history during phone screening, and undergo a medical history and physical (H&P) examination with a qualified IRP clinician.

(4) Be free of current moderate to severe DSM-V Substance Use Disorder on any drug, except nicotine in smokers. Those with past moderate to severe use disorder on substances may be included, provided they are in sustained remission (and not on maintenance therapy for opioid use disorder) and are not intoxicated on the day of the imaging session. *Justification:* Moderate to severe use disorder on other substances may result in unique CNS deficits that could confound results and introduce excessive variance, while mild substance use disorder and substance use disorder in remission are common in community samples of smokers. *Assessment tool(s):* Computerized SCID or comparable assessment and DSM-5 substance use disorder assessment.

(5) Be able to abstain from alcohol and other recreational drugs for 24 hours before each imaging session, and able to moderate caffeine intake 12 hours before each imaging session. *Justification:* Recent substance use, including alcohol, and caffeine modulate neural functioning in a way that would complicate data interpretation. *Assessment tool(s):* Self-report, breathalyzer, and urine toxicology screen with follow up neuromotor assessment to ensure absence of acute impairment with positive urine test.

<u>Smokers</u> must meet the additional criteria (6) and (7):

(6) Have a urine cotinine level corresponding to smoker status for the specific test being used, typically corresponding to a urine cotinine above about 200 ng/ml, and have been smoking for at least 1 year. *Justification:* The present protocol is interested in neurobiological mechanisms that underlie nicotine withdrawal, and is thus contingent on the presence of nicotine dependence. *Assessment tool(s):* Self-report, commercial urine cotinine test corresponding to smoker status for the specific test being used, typically corresponding to a urine cotinine above about 200 ng/ml.

(7) Be able to abstain from smoking for 12 hours prior to MRI-tDCS study sessions. *Justification:* The present protocol will investigate the effect of acute nicotine withdrawal on cognitive processes and response to tDCS. *Assessment:* Self-report and expired CO levels.

In addition, <u>non-smokers</u> must meet criteria (8):

(8) Not have a history of daily cigarette smoking or have used any nicotine products continuously lasting more than a month, and no smoking or continuous use of any nicotine products within the past year. *Assessment Tools:* Self-report, urine cotinine test, and expired CO levels.

Modified Exclusion Criteria. All participants will be excluded if they:

(1) Are not suitable to undergo an fMRI experiment due to certain implanted devices (cardiac pacemaker or neurostimulator, some artificial joints, metal pins, surgical clips or other implanted metal parts), body morphology, or claustrophobia. *Justification:* MR scanning is one of the primary measurement tools used in the protocol. *Assessment tool(s):* Prospective participants will fill out an MRI screening questionnaire and undergo an interview with an MR technologist. Questions concerning suitability for scanning will be referred to the MR Medical Safety Officer. Prospective participants will be questioned about symptoms of claustrophobia and placed in the mock scanner during their first visit to assess for possible difficulty tolerating the confinement of the scanner and for ability to fit into the scanner.

(2) Have musculoskeletal abnormalities restricting an individual's ability to lie flat for extended periods of time. *Justification:* MR scanning sessions require participants to lie flat on their backs and remain perfectly still for approximately one hour. Therefore, conditions that would make that difficult (e.g. chronic back pain, significant scoliosis) will be exclusionary. *Assessment tool(s):* History and physical examination by a qualified IRP clinician, supplemented with a trial of lying in the mock scanner to assess comfort issues.

(3) Have HIV or Syphilis. *Justification:* HIV and Syphilis both can have central nervous system (CNS) sequelae, thus introducing unnecessary variability into the data. *Assessment tool(s):* Oral HIV followed by blood test if oral test is + and STS+ without adequate prior treatment

(4) Regularly or intermittently use any prescription (e.g., benzodiazepines, barbiturates), over-the-counter (e.g., cold medicine) medications that are likely to alter BOLD signal (neuronal-vascular coupling). Justification: The use of these substances may alter the fMRI signal and/or neural functions of interest in the current study. Assessment tool(s): History and comprehensive urine drug screening to detect benzodiazepines, antipsychotics, anticonvulsants, and barbiturates. Note: If a participant is intermittently taking a medication likely to affect BOLD signal, the participant may be excluded or if scanned, will be scanned in the same medication state for data continuity purposes (i.e. either all scan days are scheduled after 5 half-lives since last medication use; or all scan days are scheduled on medication).

(5) Have any current neurological illnesses including, but not limited to, seizure disorders, frequent migraines or on prophylaxis, multiple sclerosis, cerebrovascular accident, movement disorders (except essential tremor, so long as it would not interfere with study tasks such as button pressing), history of significant head trauma, or CNS tumor. *Justification:* Neurological diseases alter CNS function and, possibly, the neuronal-vascular coupling that forms the basis of the fMRI signal. *Assessment tool(s):* History and physical examination by a qualified IRP clinician, urine drug screening for anticonvulsants not disclosed

by history. History of head trauma with loss of consciousness of more than 30 minutes or with post-concussive sequelae lasting more than two days, regardless of loss of consciousness, will be exclusionary. The MAI who will also retain discretion to exclude based on a history of neurological illness that may compromise data integrity.

(6) Have current major psychotic disorders, mania, substance-induced psychiatric disorders, or any current suicidal ideations or history of suicide attempts. Moderate to severe current symptoms of mood or anxiety disorders will be exclusionary as well. However, mild mood or anxiety disorder symptoms will *not* be exclusionary, whether medicated or unmedicated. The MAI will reserve the right to exclude on the basis of psychiatric history not explicitly described in this criterion. *Justification:* Psychiatric disorders involve the central neural system (CNS) and, therefore, can be expected to alter the fMRI measures being used in this study, however, some degree of mood and anxiety symptoms are common in community samples of smokers. *Assessment tool(s):* Computerized SCID or comparable assessment, and clinical interview confirmation by clinician.

(7) Are cognitively impaired or learning disabled. *Justification:* Cognitive impairment and learning disabilities may be associated with altered brain functioning in regions recruited during laboratory task performance. Cognitive impairment may affect one's ability to give informed consent. *Assessment tool(s):* History of placement in special-education classes as a consequence of serious learning problems and not solely as a consequence of behavioral problems, assessed during the History and Physical screening assessment.

(8) Have significant cardiovascular conditions that would make use of nicotine patch unsafe. *Justification*: Nicotine patch may cause significant arrhythmias in susceptible individuals. *Assessment tool(s):* History and physical exam, including 12-lead EKG.

(9) Have any other major medical condition, such as diabetes mellitus, that in the view of the investigators would compromise the safety of an individual during participation, or the quality of data obtainable. *Justification:* Many illnesses not explicitly covered here may increase risk or alter important outcome measures. *Assessment tool(s):* History and physical examination by a qualified IRP clinician and CBC, urinalysis, NIDA chemistry panel (liver function tests, electrolytes, kidney function). The following lab values will result in exclusion from the study:

i. Hemoglobin < 10 g/dl
ii. White Blood Cell Count < 2400/µl
iii. Liver Function Tests > 3X upper limit of normal
iv. Serum glucose > 200 mg/dl
v. Urine protein > 2+
vi. Serum creatinine > 2 mg/dl

The MAI will retain discretion to exclude based on less extreme lab results. After the screening process has been completed, the MAI will take into account all data collected in order to decide if there is an existing medical illness that would compromise participation in this research.

(10) Are pregnant, planning to become pregnant, or breastfeeding. Females are instructed in the consent to use effective forms of birth control during the study period. *Justification:* study procedures and drugs used in the current protocol may complicate pregnancy or be transferred to nursing children. *Assessment*

tool(s): Urine and/or serum pregnancy tests, and clinical interview. Urine pregnancy tests will be conducted at the beginning of each imaging visit.

(11) Are non-English speaking. *Justification:* There is no direct benefit to participants in this study. To include non-English speakers, we would have to translate the consent and other study documents and hire and train bilingual staff, which would require resources that we do not have and could not justify given the small sample size for each experiment. Additionally, the data integrity of some of the cognitive tasks and standardized questionnaires used in this study would be compromised as they have only been validated in English. Most importantly, ongoing communication regarding safety procedures is necessary when participants are undergoing MRI and tDCS procedures. The inability to effectively communicate MRI and tDCS safety procedures in a language other than English could compromise the safety of non-English speaking participants. *Assessment tool(s):* self-report.

The following exclusion criteria are new for the tDCS study:

(12) Participated in any brain stimulation session less than two weeks ago, or underwent brain stimulation exposure for treatment purposes in the last 6 months, including tDCS or transcranial magnetic stimulation (TMS). *Justification:* Prior exposure to brain stimulation may result in carry-over effects that could confound the results of this study. Participants may enroll in the study once the listed time periods have passed. *Assessment tool(s):* Participant medical history and physical (H&P).

24.6. Arm design and procedures

We will recruit smokers and matched non-smokers for a randomized, sham-controlled crossover study of 3 conditions of tDCS: **(1) anodal left-dlPFC + cathodal right-vmPFC** stimulation, with anode over the left dlPFC and cathode over the right-vmPFC; **(2) cathodal left-dlPFC + anodal right-vmPFC** stimulation, in which polarity is reversed between the two electrodes, and **(3) sham** stimulation. These are summarized in **Table 1.** Because tDCS is MRI scanner-compatible (Wörsching et al. 2016; Johnstone, Hinson, & Stagg 2016; Downar & Davis 2015; Antal et al. 2014), all tDCS sessions will be performed "online" while the participant is in the MRI scanner. The tDCS model we will use is the *neuroConn DC-Stimulator MR* (neuroCare Group GmbH, Munchen, Germany).

Smoking subjects will attend a total of 3 visits (Orientation, MRI/tDCS +Nicotine patch, and MRI/tDCS + Placebo patch), and non-smoking subjects will attend a total of 2 visits (Orientation and MRI/tDCS without patch). Each visit day will consist of the 3 independent MRI/tDCS sessions (**Figure 1),** and the nature of the tDCS condition will be double blinded and randomized. The 3 MRI/tDCS sessions will correspond with the 3 tDCS conditions: (1) anodal left-dlPFC + cathodal right-vmPFC; (2) cathodal left-dlPFC + anodal right-vmPFC; and (3) sham.

Each tDCS stimulation will last approximately 25 minutes, and will occur concurrently while the participant is inside the MRI scanner. MRI/tDCS sessions will last about 60-100 minutes, with scans taken before, during, and after the tDCS stimulation (**Figure 2**). Each of the 3 MRI/tDCS sessions has a "core block" of scans that is the same across sessions. These include the Resting scan during tDCS stimulation, and task scans during and after stimulation. The "core block" takes about 1 hour to complete.

In addition to the "core block", the first MRI/tDCS session of the day contains a Baseline Resting Scan, and at least one of the MRI/tDCS sessions will contain a DTI scan (unless tDCS is found to induce artifact in DTI, *see note on next page*). The resting and DTI scans each add ~10-15 minutes to the total session time. The addition of these scans will not affect participant safety or study design. Only 1 DTI scan is needed per person, and would only be repeated if the first scan was of poor quality.

The MRI/tDCS visit day will last approximately 8 hours, with the three MRI/tDCS sessions separated by an interscan break of about 40-60 minutes, accounting for an approximate 1.5 hour break between tDCS sessions ("Inter-tDCS interval", **Figure 3**).

Before beginning the MRI/tDCS visit days, all subjects will attend a half day orientation visit. This time will consist of an informed consent session, nurse assessment, task training, mock scanner session, and tDCS toleration test *(described below)*. Questionnaires will also be administered during this time. If subjects cannot complete questionnaires on this day, they can finish them at a later visit. The visit will last about 4.5-5.5 hours. Some participants may take longer to answer questionnaires, complete training tasks, or other procedures, and in this case their visit would be extended. If structural scans are added to orientation day (*see note on next page*), the day will be extended by about 30-45 minutes. Additional time or scans on orientation day will be included in compensation according to the remuneration table.

tDCS toleration test: Participants will experience tDCS during the orientation session using the ramp up that they will experience during tDCS sessions to make sure they tolerate the sensation. This will last long enough for them to get the full strength (2mA intensity) and to decide if they want to proceed with the experiment, i.e. generally less than 2 minutes but not more than 5 minutes. During the toleration test, the participant will be

seated comfortably in a chair outside the scanner (mock or real) environment. This brief tDCS application allows the participant to experience tDCS in a comfortable environment prior to receiving tDCS in the scanner, ask further questions, and decide whether they wish to participate further in the study.

The MRI/tDCS visit days will consist of a brief nurse visit, refresher training on fMRI tasks, and the 3 MRI/tDCS scanning sessions. Questionnaires will also be administered on this day, prior to scanning, during the inter-scan breaks, and/or after the scans. The day will last about 8 hours.

Smoker participants will be given nicotine patches during the study. One of these patches will be a Nicotine Tolerance Test Patch (see Appendix 12), and will be applied by the nurse at the orientation session. The other two will be blinded Study Patches containing either nicotine or placebo. The Study Patches should be applied 2 hours prior to the first MRI/tDCS session, due to the pharmacokinetic profile of nicotine. Smoking participants will be given blinded study patches (Study Patch 1, Study Patch 2) to apply the morning of the MRI-tDCS sessions, along with an instruction sheet for how to use the patches (see Appendix 12).

Smokers will attend 1 Orientation visit (half-day) and 2 MRI/tDCS visits (full days), for a total of 3 visit days (**Figure 1A**). Smokers will need to abstain for 12 hours prior to the MRI/tDCS visit days. All 3 tDCS conditions will be applied during each MRI/tDCS visit day. One MRI/tDCS visit day will be completed with a patch containing nicotine, and the second MRI/tDCS visit day will be completed with a patch containing placebo. On the morning of their first imaging day, smokers will apply Study Patch 1. The content of the patch (nicotine or placebo) will be randomized and double-blinded. The subject will apply Study Patch 2 on the morning of their second MRI/tDCS visit day.

Non-smokers will attend 1 Orientation visit (half-day) and 1 MRI/tDCS visit day (full day) (**Figure 1B)**, *without nicotine or placebo patch conditions*. All 3 tDCS conditions will be applied during the MRI/tDCS visit day. Non-smokers will be matched by age, gender, race, and education, as recruitment allows.

To assess the online and after-effects of tDCS on the ECN, DMN and SN, the MRI/tDCS session will measure resting state functional connectivity, and BOLD signal and behavioral performance during cognitive fMRI tasks. Three classical behavioral tasks will be presented during the scanning session: (**1**) Parametric Flanker Task known to elicit activity from the ECN and SN, (**2**) N-back task known to target the ECN, and (**3**) Amygdala Reactivity Task, known to be modulated by the SN and DMN. *(See Section 24.8 for task details)*. An example of an MRI/tDCS session timeline is shown in **Figure 2**, the exact order of tasks may differ across participants.

High-resolution structural images will be acquired on each scan day. These structural scans may include T1 weighted, T2 weighted, or Diffusion Tensor Imaging (DTI) scans. Either a T2 weighted or a DTI scan will be needed for post-hoc analysis on the effect of inter-individual anatomical variability on the effects of tDCS. Pilot studies and discussions with modeling experts on the study team will be used to determine whether a T2 or DTI scan will be best suited for this analysis.

Note about structural scans: Preliminary studies using a phantom in the scanner have found that some structural scans may be affected by the presence of tDCS electrodes. The significance of this artifact in human subjects will not be known until the study begins. Structural scans are important for conducting post-hoc modeling studies of predicted current flow through body and brain tissues such as the skull. Artifact may reduce the accuracy of these models. It may be necessary to collect structural scans during a separate session when the participant is not

wearing the tDCS device. If this is the case, the structural scans will be collected either during orientation day, or through Protocol 457. Additionally, if we find that the DTI scan collected with tDCS equipment is not useable, it will be removed from the imaging day procedures for future participants.

Regulatory status for drugs/devices in the tDCS arm:

Nicotine Patch: An IND has not been obtained for the use of the nicotine patch in this study. This study meets the criteria for exemption for an IND as this investigation is not intended to support a new indication for use or any other significant change to the labeling; the nicotine patch is already approved and marketed and the investigation is not intended to support a significant change in advertising; and the investigation does not involve a route of administration or dosage level in use in a patient population or other factor that significantly increases the risks associated with the use of the drug product. Individually tailored nicotine patch doses have been used in smoking cessation trials (Hurt, 2003) and higher doses have been shown to produce a greater reduction of withdrawal symptoms in heavier users (Ebbert et al., 2007) . As stated above, nicotine patch doses up to 63mg/d have been shown to be safe and generally well-tolerated by smokers (Ebbert et al., 2007; Zevin, Jacob, & Benowitz, 1998).

MRI: The 3T MRI machine used in this protocol is FDA approved and is used as a data collection tool. This study may use the MRI in research mode, including custom pulse sequences. All sequences, including those in research mode, are within FDA approved specific absorption radiation (SAR) limits. No additional risk is incurred in research mode, and we are not testing sequences to evaluate safety or risk.

tDCS: The tDCS device weuse is the neuroConn DC-Stimulator MR (neuroCare Group GmbH, Munchen, Germany). This tDCS device has not been cleared by the FDA for the treatment of any medical indication, and it's use in this study is investigational. tDCS does NOT meet the criteria for a significant risk device, per FDA 21 CFR 812. The tDCS stimulation parameters used in this study follow standard safety guidelines and minimize risk of adverse events (Fregni et al 2015, Bikson et al 2016). The tDCS parameter boundaries are listed below in section *2. tDCS Parameter boundaries.*
The safety and efficacy of tDCS is well established and is not being studied in this protocol. The procedure is considered minimal risk based on systematic reviews of the published literature (Brunoni et al 2011; Fregni et al., 2015). No serious adverse events have been reported in the modern tDCS literature (since 1998). A recent review by an expert panel has summarized and evaluated risks and the current regulatory status for tDCS based on data from over 400 studies (Fregni et al, 2015). As the authors state, "To our knowledge, in the US, IRBs ubiquitously (at a minimum overwhelmingly) designate tDCS trials NSR […], thus not requiring a formal IDE application to the FDA." (Fregni et al, 2015, p10). In cases where investigators have asked the FDA for a risk-designation for a tDCS study, the FDA has considered tDCS trials as NSR (Fregni et al, 2015).

Repeat visits & alternative scheduling: Sometimes, due to equipment or scheduling issues, or poor data quality, subjects may need schedule an additional visit(s) to finish or repeat study procedures. Smokers who repeat scan days will also repeat the Patch procedures, and will be provided the appropriate patch for the repeat visit by the pharmacy. Additionally, some subjects may not be able to attend an 8-hour visit day. If this is not possible, we will allow participants to break up MRI/tDCS sessions across days in a manner conducive to both the participant's and the NRB's scheduling needs.

tDCS Assessment at the end of Imaging Day (Nonsmokers): RA, LAI, AI or study team member will remove the tDCS electrodes at the end of the last tDCS session. The team member will verbally assess the participant

for adverse reaction to tDCS, and specifically inquire about headache or skin discomfort. The team member will assess the site of skin application for erythema or other adverse reaction. The team member will determine whether a nurse, PA, or MAI needs to be alerted for medical care or further assessment.

Figure 1: tDCS Stimulation Condition Timeline and Randomization

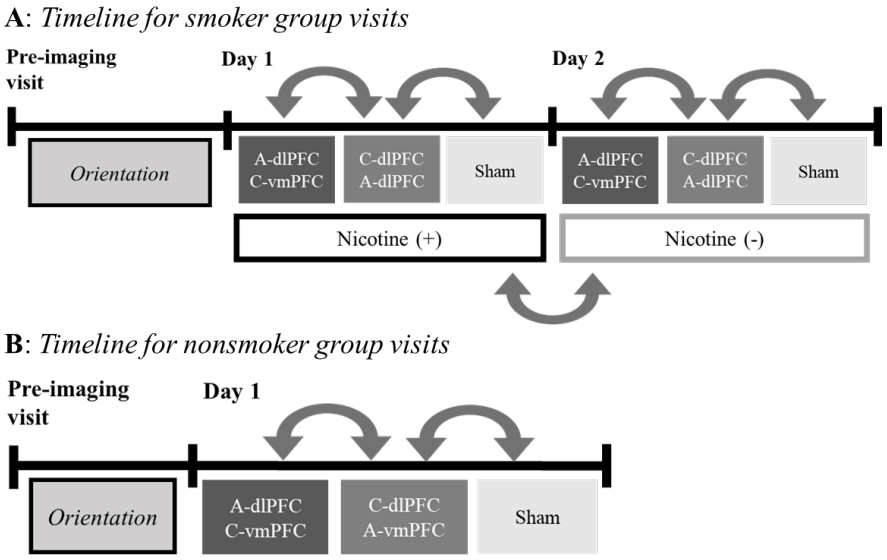


Figure 1: tDCS stimulation condition timeline and randomization method. The three tDCS conditions are (**1**) anodal left dlPFC and cathodal right vmPFC (supra-orbital area), (**2**) cathodal left-dlPFC and anodal right-vmPFC (**3**) sham tDCS. **The orientation visit** consists of a informed consent session, nurse visit, task training, mock scanner session, and questionnaires. **A**) Visit Day timelines for smoker group. **B**) Visit Day timelines for non-smoker group. *dlPFC = dorsolateral prefrontal cortex, vmPFC = ventromedial prefrontal cortex*, *A = anodal, C = cathodal.*

Table 1: Within-Subject Study Conditions

Abstinent Smokers	Sham	Anodal dlPFC + Cathodal vmPFC	Cathodal dlPFC + Anodal vmPFC
Placebo Patch	1. Sham / Placebo Patch	2. A-dlPFC + C-vmPFC / Placebo Patch	3. C-dlPFC + A-vmPFC / Placebo Patch
Nicotine Patch	4. Sham / Nicotine Patch	5. A-dlPFC + C-vmPFC / Nicotine Patch	6. C-dlPFC + A-vmPFC / Nicotine Patch
Non-smokers			
No patch	1. Sham	2. A-dlPFC + C-vmPFC	3. C-dlPFC + A-vmPFC

***** *dlPFC = dorsolateral prefrontal cortex, vmPFC = ventromedial prefrontal cortex*, *A = anodal, C = cathodal.*

Figure 2: Sample of single MRI/tDCS session. Estimated* timeline.
**All times are approximate and show idealized conditions. On occasion, due to equipment, compliance, or unforeseen disruptions, timing may change. Some scans may be repeated due to excessive motion and other technical problems, if it does not interfere with study design. Exact order of scanner tasks may differ from the order shown below. Time in magnet will never exceed 2.5 hours for one MRI/tDCS session.*

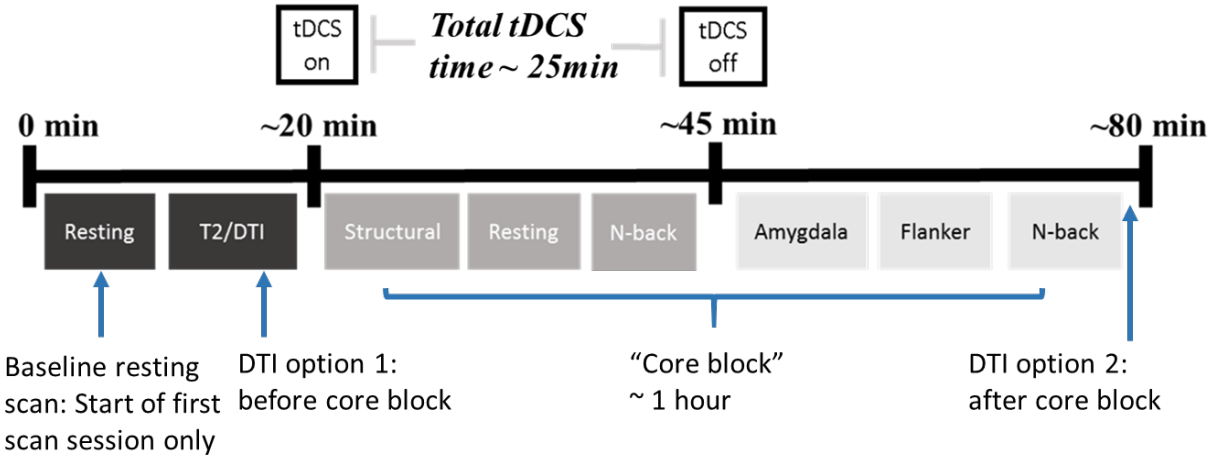
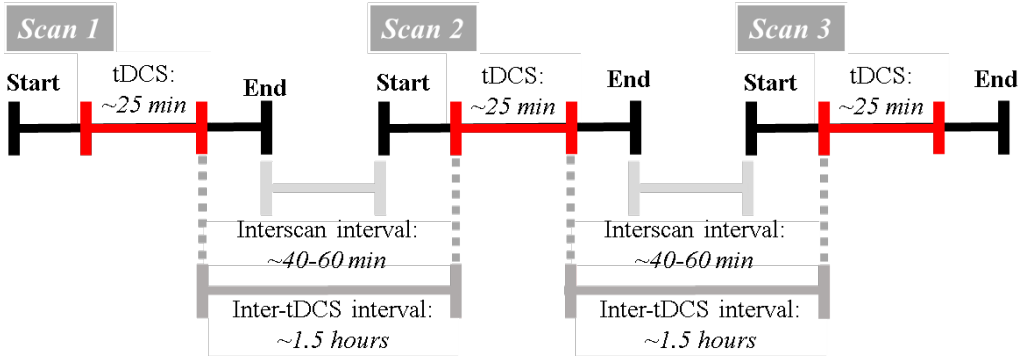


Figure 3: Inter-scan interval and Inter-tDCS interval.Estimated* timeline.
**All times are approximate and show idealized conditions. On occasion, due to equipment, compliance, or unforeseen disruptions, timing may change. Some scans may be repeated due to excessive motion and other technical problems, if it does not interfere with study design. Time in magnet will never exceed 2.5 hours for one MRI/tDCS session.*



1. Nurse Assessments for tDCS Arm:

The nursing assessments for the tDCS Arm have been adjusted for the specific needs of the tDCS experiment. The nurse assessments are broken down into: Behavioral Pilot Assessments, Imaging Phase Assessments (Smokers), and Imaging Phase Assessments (Nonsmokers). Each of these is further broken down into tDCS/scanner and non-tDCS/scanner study visit assessments.

For all cases, the nurse assessment will take place after the informed consent interview, prior to beginning the orientation or tDCS sessions. If the participant returns for another visit to complete remaining tDCS sessions or orientation procedures, the nurse assessment will take place prior to beginning the other study procedures that are scheduled for that day. Information obtained during the nurse assessment is stored confidentially and securely in the individual's medical file (See section 5.0 – Storage of Data and Samples).

Behavioral Pilot Nurse Assessments: The assessments will include:

Non-tDCS/Mock Scanner Visit (Orientation)
- Breathalyzer for expired ethanol
- Urine toxicology screen for commonly abused drugs*
- Urine pregnancy (females)

tDCS/Mock Scanner Visit

Pre-assessment
- Breathalyzer for expired ethanol
- Urine toxicology screen for commonly abused drugs*
- Urine pregnancy (females)
- Weight & Vital Signs
- Assess for recent caffeine intake and approximate last use of all substances, by participant self-report
- May assist with set up of some physiological monitoring equipment (e.g. ECG electrodes, Pulse-Ox monitor, Respiratory belt)

Imaging Phase Nurse Assessments

Non-smokers: The assessment will include:

Non-tDCS/MRI Visit (Orientation).
- Breathalyzer for expired ethanol
- Expired CO reading
- Urine toxicology screen for commonly abused drugs*
- Urine pregnancy (females)

tDCS/MRI Visit.

Pre-assessment
- Breathalyzer for expired ethanol
- Expired CO reading
- Urine toxicology screen for commonly abused drugs*
- Urine pregnancy (females)
- Weight & Vital Signs

- Assess for recent caffeine intake and approximate last use of all substances, by participant self-report
- Menstrual History Questionnaire (females)
- metal/MRI Safety Screen
-

Smokers. The assessment will include**:**

Non-tDCS/MRI Visit (Orientation).

Pre-assessment

- Breathalyzer for expired ethanol
- Expired CO reading
- Urine toxicology screen for commonly abused drugs*
- Urine pregnancy (females)
- Weight & Vital Signs
- Assess for recent caffeine intake and approximate last use of all substances, by participant self-report
- ECG, due to use of the nicotine patch
- Placement of a nicotine test patch (Nicotine Tolerance Test, see above *Section 4.4(i)4.*)

Post-assessment (only on days when the Nicotine Tolerance Patch is worn)

- Removal of nicotine test patch and assessment of skin at site of patch application
- If participant experiences any adverse reactions from the patch, the nurse will notify the MAI (or covering clinician) and obtain vital signs.

tDCS/MRI Visit.

Pre-assessment

- Checking that participant applied the patch properly
- Breathalyzer for expired ethanol
- Expired CO reading
- Urine toxicology screen for commonly abused drugs*
- Urine pregnancy (females)
- Weight & Vital Signs
- Assess for recent caffeine intake and approximate last use of all substances, by participant self-report
- Menstrual History Questionnaire (females)
- metal/MRI Safety Screen

Post-assessment

- Removal of nicotine patch and assessment of skin at site of patch application

*In the event of a positive drug test for marijuana, an interview to assess last drug use and a Neuromotor Drug Influence Evaluation (Dr. Steve Heishman, personal communication) to test for acute intoxication will be performed. The purpose of the nurse assessment is to provide additional screening for characteristics (e.g., pregnancy, positive drug tests) which would exclude an individual from participation in the study on that day. If participants test positive for drugs other than marijuana they will be rescheduled, however, if they test positive on a second occasion they will be excluded from the study. This will be made clear to the participant in the consent process.

2. tDCS Parameter boundaries:

The tDCS stimulation parameters used in this current study follow standard safety guidelines and minimize risk of adverse events (Fregni et al 2015, Bikson et al 2016). Parameters are summarized in **Figure 3** and **Table 2.**

- **Number of tDCS sessions:** There is no upper limit set in the literature for maximum number of tDCS sessions. Smoking subjects are asked to complete 6 total tDCS sessions (4 active, 2 sham), and non-smoking subjects are asked to complete 3 total tDCS sessions (2 active, 1 sham). As there is no increased risk associated with participating in more total tDCS sessions (Fregni et al 2015, Bikson et al 2016, Russo 2013), subjects may be asked to attend an MRI/tDCS re-visit if imaging data from an original visit day does not pass quality control standards set by the lab. For example, one study reported subjects receiving >100 tDCS sessions of 30 min duration at 2 mA without adverse effects arising from cumulative exposure (Russo et al 2013).

- **Number of tDCS sessions per day:** Subjects will be asked to complete 3 tDCS sessions per day, one of which is a sham. Current recommendations for tDCS are to keep active sessions to no more than 2 per day (Fregni et al 2015). With this in mind, participants may be asked to reschedule an additional active or sham MRI/tDCS session if the original scan does not pass quality control standards set by the lab.

- **Consecutive days of tDCS sessions and separation of visit days:** There is no restriction on consecutive days of tDCS session or minimum separation of visit days, as there are no known adverse effects from consecutive tDCS days. MRI/tDCS visit days will be scheduled according to participant and NIDA resource availability.

- **Duration of tDCS stimulation:** The duration of tDCS stimulation will be approximately 25 minutes. This tDCS stimulation duration falls under conventional safety parameters, which extend up to 60 minutes (Fregni et al 2015, Bikson et al 2016). Stimulation will not exceed 60 minutes at any one session.

- **Interval between tDCS sessions ("inter-tDCS interval):** There will be an inter-tDCS interval of approximately 1 hour and 30 minutes between each tDCS session. Duration of inter-tDCS interval ranging from 1h to 2 weeks was not found to be a confounding factor in recent meta-analysis of single session, sham controlled anodal dlPFC tDCS (Dedoncker et al 2016).

- **Stimulation intensity:** Stimulation intensity will be 2mA for the duration of the tDCS session. This falls within established safety parameters and standard guidelines, which extend between 1- 2mA (Fregni et al 2015, Bikson et al 2016). Stimulation will not exceed 2mA.

- **Stimulation polarity:** Anodal tDCS is considered excitatory, while cathodal tDCS is considered inhibitory. Anodal stimulation will be applied to the left-dlPFC, a cortical node of the ECN; cathodal stimulation will be applied to the right-vmPFC (located at the supra-orbital area), a cortical node of the DMN. Polarity will also be reversed between the left-dlPFC and right-vmPFC.

- **tDCS electrodes:** We will use standard MR-compatible tDCS electrodes. The electrodes will be covered with a layer of conductive paste/gel, and then applied to the participant's skin. Non-adhesive

bandages or netting may be used to hold the electrodes in place. Prior to applying the electrodes, a standard skin preparation may be used, such as cleaning with an alcohol swab, and/or using a mildly abrasive gel.

- **Placement of tDCS electrodes:** Placement of the dlPFC electrode will be accomplished by the 10-20 EEG system and/or the Beam method (Beam et al 2009), involving a set of scalp measurements to localize the F3 site. The vmPFC location will be estimated by the supraorbital edge.

- **Sham tDCS:** One of the benefits of tDCS for experimental research is its effective sham condition. Participants become desensitized to the mild tingling sensation of tDCS after the initial ramping of current (Woods et al 2016). To simulate the experience of tDCS stimulation, current is ramped on and turned off at the beginning and end of the tDCS session. An additional sham option is to have the current ramp up and down only at the beginning of the sham session, and not at the end. This second sham is supported in the literature as an effective blinding technique, which subjects cannot distinguish from active stimulation (Gandiga et al 2006, Brunoni et al 2012). One of these sham options will be used for data that will be analyzed together, to be determined based on equipment capabilities and preliminary analysis of blinding efficacy in our cross over design. We will assess the efficacy of sham condition by providing participants and the investigator with a questionnaire on the MRI/tDCS session, wherein they will report whether they thought the tDCS session was active or sham (see Appendix 13). The MRI operator (or other non protocol personnel) will control active/sham conditions.

Table 2: tDCS Parameter Summary

Parameters	
Number of MRI/tDCS Visit Days	Smokers: 2 Non-smokers: 1
Number of tDCS sessions	Smokers: 6 (4 active, 2 sham) Nonsmokers: 3 (2 active, 1 sham)
Number of tDCS sessions/day	3 tDCS sessions/day
Duration of tDCS	~ 25 minutes/tDCS session, not to exceed 60 min
Inter-tDCS interval	~ 90 min interval between tDCS sessions
Stimulation intensity	2 mA
Stimulation polarity	Anodal dlPFC + Cathodal vmPFC (supra-orbital area) Cathodal dlPFC + Anodal vmPFC (supra-orbital area)
Placement of electrodes	dlPFC: 10-20 EEG system, Beam method vmPFC: Supra orbital edge
Sham procedure	On/Off ramp at tDCS session start (+/- end, see above)

3. Characterization Measures

The following questionnaires will be administered to participants either during the screening process, orientation visit, or during the imaging visit days as time permits. State measures will be collected at each visit. Trait measures will be collected during orientation, but may also be completed as time allows during subsequent study visits. Content for each questionnaire can be found in Appendix 3 (Characterization Measures) or Appendix 4 (State Measures).

Some of these questionnaires are also found in NIDA-IRP protocol 10-DA-N457. If the participant has enrolled in 10-DA-N457, these questionnaires may be completed within that protocol instead. These include: Fagerstrom Test for Nicotine Dependence, Toronto Alexithymia Scale (TAS-20), Adult ADHD Self-Report Scale, Positive and Negative Affect Schedule, and the State-Trait Anxiety Inventory Form Y (STAI). Questionnaires answered during screening, under Protocol 415, may also be included for analysis. Questionnaires do not need to be repeated on visit days if they were conducted during either the screening Protocol 415 or Protocol 457.

Nicotine Dependence and Withdrawal Scales (Smoking Group Only).

1) **The Fagerström Test for Nicotine Dependence** (Heatherton et al., 1991) is a six item test that measures the severity of nicotine addiction on a 0-10 scale. To be completed once (Trait). Completion time: ~2-3min.

2) **The Tobacco Craving Questionnaire** (Singleton et al., 2003) is a brief instrument used to assess current feelings related to smoking and craving using 12 Likert-type items. Each item is rated on a 7-point scale from strongly disagree to strongly agree. To be completed each visit (State). Completion time: ~2min.

3) **Tobacco Craving Scale** consists of 5 self-report items pertaining to desire for a cigarette that are rated on a 10-point scales. To be completed each visit (State). Completion time: ~2 min.

4) **The Minnesota Nicotine Withdrawal scale** (Hughes and Hatsukami, 1998) yields a measure of total withdrawal related discomfort based on ratings from 15 self-report items. To be completed each visit (State). Completion time: ~2-3 min.

5) **The Cigarette Dependence Scale** (Etter et al., 2003) is a brief 12-item self report that assesses the main components of DSM-IV and ICD-10 definitions of dependence, which include compulsion, withdrawal symptoms, loss of control, time allocation, neglect of other activities, and persistence despite harm. To be completed once (Trait). Completion time: ~2-3min.

6) A **general smoking history questionnaire** will also be administered. To be completed once (Trait). Completion time: ~5 min.

7) **Visual analog scales** (100mm) will also be used to assess the degree to which various nicotine withdrawal symptoms may be experienced: depressed, clumsy, tired, anxious, happy, drowsy, sad, dizzy, alert, energetic, light-headed, irritable, frustrated, nervous, sad, desire to smoke, difficulty concentrating, increased appetite, hunger, weight-gain, difficultly sleeping, awakening at night, insomnia, restless, impatient, panicked, missing cigarettes, urge to smoke, disoriented. To be completed each visit (State). Completion time ~10 min.

Personality Scales

8) **The Brief Externalizing Inventory** (Hall et al., 2007), adapted from the full Externalizing inventory (Krueger et al., 2007), is a subset of 159 self-report items used to assess a range of behavioral and personality characteristics that have been attributed to a broad psychological construct termed externalizing. These characteristics include: physical/relational destructive-aggression, boredom proneness, irresponsibility, problematic impulsivity, drug and alcohol use or problems, theft, fraud, rebelliousness, alienation, and blame externalizing. A recent report demonstrated that participants rated as high externalizing displayed reduced amplitude ERNs in comparison to those with low externalizing scores (Hall et al., 2007). Thus, this measure may be a useful tool to account for variability in ERN amplitudes. To be completed once (Trait). Completion time: ~15min.

9) **The Temperament and Character Inventory** (Cloninger et al., 1994) is a widely used test that assesses dimensions of personality (e.g., harm avoidance, novelty seeking, reward dependence, and persistence) that are considered to be related to monoaminergic function. To be completed once (Trait). Completion time: ~25min.

10) **Sensation Seeking Scale V** (SSS-V, Zuckerman et al., 1978) is a 40-item self-report questionnaire that assesses individual differences in sensation seeking. The scale consists of four subscales (Boredom Susceptibility [BS], Thrill and adventure seeking [TAS], Experience seeking [ES], and Disinhibition [Dis]) composed of 10-items each. To be completed once (Trait). Completion time: ~5-10min.

11) **Attitudes Towards Risk Questionnaire** consists of 34-self report items rated on a 5-point Likert scale that assess attitudes towards physical and psychological risk. To be completed once (Trait). Completion time: ~5-10min.

12) **Toronto Alexithymia Scale** (TAS-20): a 20-item self report questionnaire to assess emotional awareness To be completed once (Trait). Completion time: ~5min.

13) **Adult ADHD Self-Report Scale** (ASRS v1.1): This questionnaire is an 18-item checklist designed by the World Health Organization (WHO) and Workgroup on Adult ADHD to determine whether a participant is suffering from symptoms related to attention-deficit/hyperactivity disorder. To be completed once (Trait). Completion time: ~ 5 minutes.

Mood and Anxiety Scales

14) **The Positive and Negative Affect Schedule** (or similar self-rated questionnaire) is a 20-item scale composed of 10 items describing negative affect and 10 items describing positive affect. To be completed each visit (State). Completion time: ~5 min.

15) **Profile of Mood States (POMS)** measures present mood state by a list of adjectives on a 5-point Likert scale and measures six dimensions of affect, including tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, and confusion-bewilderment. The measure has been shown to produce reliable and valid profiles of mood state (McNair et al., ; Cella et al., 1989). To be completed each visit (State). Completion time ~5min.

16) **The State-Trait Anxiety Inventory Form Y (STAI)** is an instrument for measuring anxiety in adults. The STAI differentiates between the temporary condition of "state anxiety" and the more general and long-standing quality of "trait anxiety." The essential qualities evaluated by the STAI-anxiety scale are feelings of apprehension, tension, nervousness, and worry. Form Y1 (State): To be completed each visit. Form Y2 (Trait): To be completed once. Completion time: ~5min each.

17) **Beck Depression Inventory-II** (BDI-II: Beck et al., 1996): a brief self-administered inventory that assesses depressive symptoms both at the time of completion and in the preceding 7 days. The NIDA-IRP Critical Alert System will be activated in CDW to address critical responses to this questionnaire. To be completed once (Trait). Completion time: ~5 min.

18) **Beck Anxiety Inventory** (Beck et al., 1988): a brief inventory similar to the BDI-II that assesses symptoms of anxiety. To be completed once (Trait). Completion time: ~5 min.

Other Drug Use

19) **Drug Use Survey (DUS):** An interviewer-administered questionnaire designed to collect information regarding lifetime history of substance use. To be completed once (Trait). Completion time: < 40 minutes.

Other Questionnaires

20) **Menstrual Cycle History Questionnaire:** To be administered at the start of each visit. Menstrual cycle information can be used to better understand gender differences should they be apparent in this study. To be completed each imaging visit (State). Completion time ~2 min.

24.7 tDCS Behavioral Pilot

Up to the first **25 non-smoking participants** will participate in the tDCS Behavioral Pilot. This pilot study will examine the effect of tDCS stimulation on behavioral outcomes on the Parametric Flanker, N-back, and Amygdala Reactivity tasks in the *mock scanner*. **All procedures will be done as currently approved in the main tDCS study, except without MRI** (i.e. in the mock scanner only) **or characterization questionnaires** (only the tDCS Blinding Questionnaire will be administered to this group).

The tDCS Pilot will consist of an Orientation Session (consent, nurse visit, and task training, questionnaires and tDCS toleration test; approximately 3.5 hours), and three Mock Scanner/tDCS sessions (same as below, *24.6:Arm Design and Procedures;* approximately 5 - 6 hours for three 1-hour mock scanner sessions separated by two 1-hour breaks). The orientation and each of the 3 mock scanner/tDCS sessions can be completed on the same or separate days, as NRB resources and participant scheduling allow.

Because the Pilot will not include real MRI, the mock/scanner tDCS session will not include time for resting scans or structural image scans. The order of tasks will be re-arranged to reflect this difference. Order of tasks does not affect participant safety or study design. tDCS stimulation parameters will be the same as currently approved in the main tDCS protocol.

During orientation, participants will complete the following questionnaires: (1) Brief Externalizing Inventory, (2) Temperament and Character Inventory, (3) Sensation Seeking Scale V, (4) State-Trait Anxiety Inventory Form Y (STAI), (5) Attitudes Towards Risk Questionnaire. These questionnaires collectively take ~60 min to complete. We may also analyze questionnaires completed by the participant, when the data was collected from Protocols 457 and/or 415. These data will be used to explore how individual differences affect response to tDCS.

Participants in the pilot will be compensated at the same rate as described in the main tDCS protocol, minus the MRI compensation. They will still receive the completion bonus. Pilot participants can make up to about $258.50 if all sessions are completed on the same day. If sessions are completed on separate days, compensation will vary according to the remuneration table (see below, *24.18: Research and Travel Compensation*).

24.8. fMRI tasks

1. Parametric Flanker Task to Engage the Executive Control Network and the Salience Network: Participants will complete a modified Flanker task designed to yield sufficient commission errors to study EEG and fMRI signatures of error processing (e.g., de Bruijn et al., 2004). In this task, participants respond according to the central item/letter (e.g., H or S) of a congruent (HHHHHHH or SSSSSSS) or incongruent (HHHSHHH or SSSHSSS) stimulus array. Parametric manipulations of the agreement between flankers and the central target (e.g. SSSHSSS > SSHHHSS > SHHHHHS > HHHHHHH) elict varying degrees of response conflict (e.g. SSSHSSS > SSHHHSS > SHHHHHS > HHHHHHH) as measured by speed and accuracy of response. Participants are presented with a fixation mark on the screen, after which the stimulus array appears. Participants are instructed to respond as fast as possible to avoid feedback indicating that their response was too slow according to a preset reaction time (RT) deadline. Whenever participants respond too slowly, feedback (e.g., "!") is presented indicating that they need to respond faster on future trials. This task measures areas (e.g. the anterior cingulate) thought to be involved in error monitoring or conflict and has been shown to be dysfunctional in drug abusers.

2. N-back Task to Engage the Executive Control Network: The n-back task is designed to parametrically manipulate working memory (WM) by asking participants to determine whether a given stimulus matches a stimulus presented in either the previous trial (1-back), 2 trials previously (2-back), or 3 trials previously (3-back). To control for attentional mechanisms, participants may also complete either 0-back trials, in which they simply respond to a predefined stimulus item; 1-back trials can also be chosen as a control condition. Variants of this task have been previously published (Cohen et al, 1994). The N-back task is known to engage the Executive Control Network (ECN) (Lerman et al 2014).

   In the version of the task used for this study, we will choose only 2 of the parametric conditions, e.g. 1-back and 3-back. This will reduce the time required to complete the task so that it can fit within the relatively short 1-hour MRI scan session. The task will be given both during and after tDCS stimulation, to compare the "online" (during) and "offline" (after-effects) of stimulation on working memory.

3. Amygdala Reactivity Task to measure top-down control: Participants will complete a simple perceptual task previously shown to produce robust bilateral amygdala activity (Hariri et al., 2002; Pezawas et al., 2005; Foland-Ross et al., 2010), as well as top-down prefrontal regulation of amygdala reactivity (Hariri et al., 2002; Foland-Ross et al., 2010). This blocked fMRI paradigm consists of two experimental conditions: a face matching condition, and a sensorimotor control condition. During the emotion matching condition, participants view an array of 3 faces and their task is to select (via button press) one of the two faces that matches the identity of the target face. During the sensorimotor control task, participants view an array of 3 geometric shapes (circles, vertical and horizontal ellipses) and select one of the two shapes (bottom) that matches the target shape (top). Each block begins with a brief instruction screen ("match face" or "match shape"). During each emotion block different images selected from a standard set of pictures of facial affect (Ekman and Friesen, 1976), are presented. During the control blocks, different geometric shapes are presented in a pseudo-random order.

24.9. Storage of data and Data Sharing Plan

The same as outlined for above in *Section 5., Storage of data and samples* and *Section 6., Data Sharing Plan.*

24.10. Risks/discomforts

tDCS: tDCS is a minimal risk procedure and side effects are mild, benign and transient. These may include itching, tingling, a reddening or a burning sensation of the skin underneath the stimulating electrode. These symptoms are deemed a discomfort in 10-14% of tDCS participants (Brunoni et al 2011). In order to further reduce these minimal risks, the impedance of the tDCS system will be continuously monitored throughout all sessions. Instead of the more popular sponge pads soaked in saline, bicarbon electrodes with a thick layer of conductive paste will be used to prevent drying of the electrodes during the scanning session. The stimulator will automatically turn off when the impedance value exceeds the critical range.

If discomforts occur, a decision will be made by the MAI or designate about whether the participant should complete the experiment. Headaches can also be a side effect of tDCS, and these subside with removal of the strap or with non-prescription medication. Non-prescription pain killers will be provided as decided on a case-by-case basis by the MAI.

Nicotine patch: the risks/discomforts of the nicotine patch are the same as listed in *Section 8.2. Nicotine Patch Administration.* Only the smoker group will receive nicotine patches. Nicotine dose will be individually tailored based on the average number of cigarettes smoked per day to better match daily nicotine intake. The following graded dosing scale will be used to individually tailor nicotine dose, it is identical to the scale found in *Section 4.1, Study Overview*:

> *"Nicotine administration during abstinence and scanning sessions.* Two essential pharmacological issues that are of importance in the current design relate to participant's recent exposure to nicotine and to the selected dose administered to each participant. Doses will be individually tailored based on the average number of cigarettes smoked per day to better match daily nicotine intake (please see "*Individually Tailored Nicotine Dose*" in Section 1.5 above for justification). The dose for smokers consuming less than 10 cigarettes per day will be 14mg to start. If they are still experiencing cravings their dose will be increased to 21mg . The dose for smokers consuming 10-15 cigarettes per day will be a 21mg patch and this will be increased by 7mg for each additional 5 cigarettes/day (i.e., 21mg for individuals who smoked 10-15 cigarettes/day; 28mg for 15-20 cigarettes; 35mg for 20-25 cigarettes, and 42mg for more than 25 cigarettes). Nicotine patch doses up to 63mg/d have been shown to be safe and generally well-tolerated by smokers (Ebbert et al., 2007; Zevin, Jacob, & Benowitz, 1998)."

24.11. Outcome measures

There are no additional outcome measures beyond those described in the main protocol document (*Sections 10.1 and 10.2).*

24.12. Statistical Analysis

The same as outlined above in *Section 11,* with the addition of the following power analyses.

Power Analysis.

General fMRI power. The same as outlined above in *Section 11.4:* "Since fMRI is the main outcome measurement utilized in the present protocol, the key power analysis pertains to the fMRI data. However, prospective power analyses for fMRI data are complicated for several reasons. First, fMRI data are analyzed in a hierarchal manner such that both the *intra*-participant variance from the time-course data and the *inter*-participant variance across individuals could affect statistical power. A large number of time points tend to mitigate effects of intra-participant variance, but temporal autocorrelation and scanner limitations limit the number of independent measurements per unit time and thus the number of independent time points that are collected. In addition, effect sizes and both types of variance will vary spatially. Because of this, a given study may have sufficient power to detect differences in some brain regions, but lack sufficient power in other regions where the null hypothesis is false. Finally, fMRI analysis consists of a very large number of non-independent multiple comparisons, greater than 1.5 million at the group level, necessitating correction methods less severe than a Bonferroni correction, as discussed above. Thus, a proper power analysis on fMRI data requires simulating all of these effects. Desmond and Glover (2002) have performed such a simulation. They show for a relatively modest signal change of 0.5% during a cognitive task and with an intra-participant standard deviation of 0.75% and an inter-participant standard deviation of 0.5%, that 11 participants are required for a power of 0.8 using $p < 0.05$. Using a false positive rate of $p < 0.002$, a level more consistent with a cluster size threshold to correct for multiple comparisons (Forman et al., 1995), and with the variances kept the same, **approximately 21 participants are needed for an expected signal change of 0.5% and 11 participants for a signal change of 0.75%.** For more than ~100 independent time points, power (and hence the intra-participant variance) is relatively independent of the number of time points (Desmond and Glover, 2002). All current analyses should fall within this range."

tDCS power. Based on laboratory experience and literature review, the effect size for tDCS is the smallest of the study manipulations. Therefore, sample sizes falling in the appropriate power range for tDCS will also fall within the appropriate range for other manipulations in this study. In a meta-analytic review of the effect of tDCS on behavioral performance in cognitive tasks, it was found that effect size (Cohen's *d*) for anodal stimulation was about 0.49 (CI: 0.26, 0.72) for tDCS vs sham stimulation (Jacobson, Koslowsky, & Lavidor, 2011). Cathodal tDCS has been found to produce less reliable effect sizes than anodal tDCS. There is no independent cathodal tDCS condition in this study, and we have calculated power for anodal tDCS stimulation. The direction of tDCS effect is not classically known across each of the task paradigms, thus a two-tailed dependent samples t-test will be used. **Approximately 34 participants in each group (smoker and non-smoker) will be required to achieve a power of 0.8 when assessing the effect of anodal tDCS vs sham (α-level of 0.05, two-tailed, matched pairs t-test, assuming a medium effect size for anodal tDCS stimulation**).

Accounting for non-completion and non-compliance, we are requesting a maximum accrual of 60 smokers (enrollees) and 55 non-smokers (enrollees), with expectation of 35 completers in the smoker group, and 45 completers in the nonsmoker group.

24.13. Human subjects protection

The same as outlined for above in *Section 12. Human Subjects Protection.* Enrollment for the tDCS Arm is shown below:

APPROVED STUDY POPULATION						
	FEMALE ENROLLMENT	MALE ENROLLMENT	**TOTAL** ENROLLMENT	FEMALE COMPLETERS	MALE COMPLETERS	**TOTAL** COMPLETERS
APPROVED CEILING	57	58	115	40	40	80

NIH TARGETED/PLANNED ENROLLMENT			
ETHNIC CATEGORY	Sex/Gender		
	Females	Males	Total
Hispanic or Latino	2	3	5**
Not Hispanic or Latino	55	55	110
Ethnic Category: Total of All Subjects*			115*
RACIAL CATEGORIES			
American Indian/Alaska Native	1	1	2
Asian	1	2	3
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	37	37	74
White	18	18	36
Racial Categories: Total of All Subjects*	57	58	115*

*The "Ethnic Category: Total of All Subjects" must be equal to the "Racial Categories: total of All Subjects."

24.14. Anticipated benefit

There are no direct health benefits to subjects in this arm of the study. The results of this work may benefit the health of society if the findings contribute to a better understanding of brain function as related to drug abuse.

24.15. Consent documents/process

The consent process is the same as for the main protocol *Section 15 Consent documents/Procedure*, however a different quiz will be used that contains information relevant to the tDCS Arm. Please see attached Consent Forms D1 (Smokers) and D2 (Nonsmokers), and Quizzes D1 (Smokers) and D2 (Nonsmokers). As in the main protocol, participants must answer at least 80% of questions correctly with correct answers to #5 and #9.

24.16. Data and Safety Monitoring. Quality Assurance. Adverse event and unanticipated problem reporting. Alternatives to participation. Confidentiality. Conflict of Interest.

The same as those outlined within *Sections 16-21* of the main protocol document.

24.17. Research and Travel Compensation.

Subjects will be compensated according to NIDA-IRP guidelines below:

Item	Compensation Rate
IRP Study Visit	$20/hour

fMRI	$15 per scan session
tDCS	$10 per tDCS session
Travel to NIDA	$15 round-trip per visit day, or Full reimbursement based on receipts & prior permission
Total Completion	10% completion bonus, paid on completion of final visit

Subjects who voluntarily withdraw before completing the study will only be paid for that part of the study they complete. However, if medical reasons preclude completion of the study, subjects will be paid the full stipend for that day.

Based on MRI/tDCS scanning visits (each lasting approximately 8 hours) and a single half-day pre-assessment session (4 hours), anticipated approximate maximum compensation for participation in the Transcranial Direct Current Stimulation (tDCS) Arm of this study is about $654.50 for smokers (3 total visit days) and about $379.50 for non-smokers (2 total visit days).

Remuneration will generally be in cash payments. If participation runs into the evening or occurs on weekends, payment by check may be the only option. In the rare instances where a check is pre-issued because procedures are scheduled in the evenings or on weekends, the amount due to the participant will be estimated on the low end, and the participant will receive any balance due either at their next session, or via a check in the mail (whichever they prefer). Participants who complete and MRI session will also be offered a T-shirt with an ironed-on picture of their brain as part of their compensation.

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24. Appendix 1: Participant Eligibility Checklists

Eligibility Checklist for Protocol 474-Nontreatment

Screening

Inclusion Criteria

1. Be between the ages of 18-55. Be right-handed. *Assessment tool(s):* Edinburgh *Handedness Inventor*y.

   (YES/NO)

2. Be free of active DSM-IV dependence, or dependence in partial remission, on alcohol or any drug except nicotine. Past active dependence is acceptable provided it is at least five years in the past and total time of active dependence did not exceed 4 years. Those with past dependence on any substance other than alcohol or marijuana may not have any current use (past 6 months) of the substance on which they were dependent. For individuals with past alcohol or marijuana dependence, current use of the previously dependent substance will be allowed providing they do not meet any current DSM-IV criteria for substance dependence, with the exception of tolerance. *Assessment tool(s)*: The computerized SCID and clinical substance abuse/dependence assessment. While recreational/intermittent use of alcohol and/or marijuana will be tolerated in all participant groups, individuals will be excluded if they meet current or recent (within 5 years) DSM-IV diagnostic criteria for dependence on any substances.

   (YES/NO)

3. Be able to abstain from alcohol 24hrs before each of the imaging sessions and able to moderate their caffeine intake 12hrs before each session *Assessment tool:* Direct question and documented response

   (YES/NO)

4. Must have a urine cotinine level corresponding to smoker status for the specific test being used, typically corresponding to a urine cotinine above about 200 ng/ml, and have been smoking consistently for at least one year. For lighter smokers (less than 10 cpd), this is defined as smoking at their current level or more for at least the past year (excluding any quit attempts in the last year). For heavier smokers (more than 10 cpd), they must have been smoking at least an average of 10 cpd for at least the past year (excluding quit attempts). *Assessment tool(s):* Self-report, commercial urine cotinine test corresponding to smoker status for the specific test being used, typically corresponding to a urine cotinine above about 200 ng/ml.

   (YES/NO)

5. Be able to abstain from smoking for 36hrs on two occasions during the study. *Assessment tool:* Direct question and documented response

(YES/NO)

6. Be comfortable with the scanning requirements of the study

   Prospective participants will be questioned about symptoms of claustrophobia and placed in the mock scanner during their first visit to assess for possible difficulty tolerating the confinement of the scanner and for ability to fit into the scanner. *Assessment tool:* mock Scanner Trial

   (YES/NO)

7. Be English-speaking. *Assessment tool*: self-report.

   (YES/NO)

Medical

Inclusion criteria

1. Be in good health. *Assessment tool(s):* Participants will provide a brief health history during phone screening, and undergo a medical history and physical examination with a qualified IRP clinician.

   (YES/NO)

Exclusion criteria

1. Are cognitively impaired or learning disabled. *Assessment tool(s):* History of placement in special-education classes as a consequence of serious learning problems and not solely as a consequence of behavioral problems, assessed during the History and Physical screening assessment.

   (YES/NO)

2. are not suitable to undergo an fMRI experiment due to certain implanted devices (cardiac pacemaker or neurostimulator, some artificial joints, metal pins, surgical clips or other implanted metal parts), body morphology, or claustrophobia. *Assessment tool(s):* Prospective participants will fill out an MRI screening questionnaire and undergo an interview with an MR technologist. Questions concerning suitability for scanning will be referred to the MR Medical Safety Officer.

   (YES/NO)

3. have coagulopathies, history of, current superficial, or deep vein thrombosis, musculoskeletal abnormalities restricting an individual's ability to lie flat for extended periods of time. *Assessment tool(s):* History and physical examination by a qualified IRP clinician, supplemented with a trial of lying in the mock scanner to assess comfort issues.

   (YES/NO)

4. have HIV or Syphilis. *Assessment tool(s):* Oral HIV followed by blood test if oral test is + and STS+ without adequate prior treatment.

   (YES/NO)

5. regularly use any prescription (e.g., antidepressants, benzodiazepines, antipsychotics, anticonvulsants, barbiturates), over-the-counter (e.g., cold medicine) or herbal medication (e.g., Kava, Gingko biloba, St. John's wort) that may alter CNS function, cardiovascular function, or neuronal-vascular coupling. *Assessment tool(s):* History and comprehensive urine drug screening to detect antidepressants, benzodiazepines, antipsychotics, anticonvulsants, and barbiturates.

   (YES/NO)

6. have any current neurological illnesses including, but not limited to, seizure disorders, frequent migraines or on prophylaxis, multiple sclerosis, movement disorders, history of significant head trauma, or CNS tumor. *Assessment tool(s):* History and physical examination by a qualified IRP clinician, urine drug screening for anticonvulsants not disclosed by history. History of head trauma with loss of consciousness of more than 30 minutes or with post-concussive sequelae lasting more than two days, regardless of loss of consciousness, will be exclusionary. The MAI will also retain discretion to exclude based on a history of neurological illness that may compromise data integrity.

   (YES/NO)

7. Have any current major psychiatric disorders to include, but not limited to, mood, anxiety, psychotic disorders, or substance-induced psychiatric disorders, or any current suicidal ideations or history of suicide attempts or currently under antidepressant or antipsychotic medication treatment. The MAI will reserve the right to exclude on the basis of psychiatric history not explicitly described in this criterion *Assessment tool(s):* Computerized SCID-NP, ASRS (adult ADHD self-report scale), Beck Depression Inventory, Beck Anxiety Inventory, and DSM-IV (DSM-IV, APA, 1994) confirmed by clinician interview.

   (YES/NO)

8. have significant cardiovascular or cerebrovascular conditions. *Assessment tool(s):* History and physical exam, including 12-lead EKG.

   (YES/NO)

9. have any other major medical condition that in the view of the investigators would compromise the safety of an individual during participation**. *Assessment tool(s):* History and physical examination by a qualified IRP clinician and CBC, urinalysis, NIDA chemistry panel (liver function tests, electrolytes, kidney function). The following lab values will result in exclusion from the study:

   (a) Hemoglobin < 10 g/dl (YES/NO)
   (b) White Blood Cell Count < 2400/µl (YES/NO)

(c) Liver Function Tests > 3X normal (YES/NO)
(d) Serum glucose > 200 mg/dl (YES/NO)
(e) Urine protein > 2+ (YES/NO)
(f) Serum creatinine > 2 mg/dl (YES/NO)
(g) Estimated creatinine clearance <60ml/min (YES/NO)

(YES/NO)

**The MAI will retain discretion to exclude based on less extreme lab results. After the screening process has been completed, the MAI will take into account all data collected in order to decide if there is an existing medical illness that would compromise participation in this research.

10. pregnant, planning to become pregnant, or breastfeeding. Females are instructed in the consent to use effective forms of birth control during the study period. *Assessment tool(s):* Urine and/or serum pregnancy tests, and clinical interview.

(YES/NO)

Eligibility Checklist for Protocol 474-treatment

Screening

Inclusion Criteria

1. Be between the ages of 18-55. Be right-handed. *Assessment tool(s):* Edinburgh *Handedness Inventor*y.

(YES/NO)

2. Be free of active DSM-IV dependence, or dependence in partial remission, on alcohol or any drug except nicotine. Past active dependence is acceptable provided it is at least five years in the past and total time of active dependence did not exceed 4 years. Those with past dependence on any substance other than alcohol or marijuana may not have any current use (past 6 months) of the substance on which they were dependent. For individuals with past alcohol or marijuana dependence, current use of the previously dependent substance will be allowed providing they do not meet any current DSM-IV criteria for substance dependence, with the exception of tolerance. *Assessment tool(s)*: The computerized SCID and clinical substance abuse/dependence assessment. While recreational/intermittent use of alcohol and/or marijuana will be tolerated in all participant groups, individuals will be excluded if they meet current or recent (within 5 years) DSM-IV diagnostic criteria for dependence on any substances.

(YES/NO)

3. Be able to abstain from alcohol 24hrs before each of the imaging sessions and able to moderate their caffeine intake 12hrs before each session *Assessment tool:* Direct question and documented response

(YES/NO)

4. Must have a urine cotinine level corresponding to smoker status for the specific test being used, typically corresponding to a urine cotinine above about 200 ng/ml, and have been smoking consistently for at least one year. For lighter smokers (less than 10 cpd), this is defined as smoking at their current level or more for at least the past year (excluding any quit attempts in the last year). For heavier smokers (more than 10 cpd), they must have been smoking at least an average of 10 cpd for at least the past year (excluding quit attempts). *Assessment tool(s):* Self-report, commercial urine cotinine test corresponding to smoker status for the specific test being used, typically corresponding to a urine cotinine above about 200 ng/ml.

   (YES/NO)

5. Be able to abstain from smoking for 36hrs on two occasions during the study. *Assessment tool:* Direct question and documented response

   (YES/NO)

6. Be comfortable with the scanning requirements of the study
   Prospective participants will be questioned about symptoms of claustrophobia and placed in the mock scanner during their first visit to assess for possible difficulty tolerating the confinement of the scanner and for ability to fit into the scanner. *Assessment tool:* mock Scanner Trial

   (YES/NO)

7. Be actively seeking treatment for smoking cessation and willing to engage in 12-weeks of treatment involving daily administration of Varenicline and weekly counseling sessions, as well as follow-up assessments at 1, 6 and 12 months following treatment onset. *Assessment tool:* Treatment motivation screening tool as well as direct question and documented response

   (YES/NO)

8. Be English-speaking. *Assessment tool*: self-report.

   (YES/NO)

Medical

Inclusion criteria

1. Be in good health. *Assessment tool(s):* Participants will provide a brief health history during phone screening, and undergo a medical history and physical examination with a qualified IRP clinician.

   (YES/NO)

Exclusion criteria

1. Are cognitively impaired or learning disabled. *Assessment tool(s):* History of placement in special-education classes as a consequence of serious learning problems and not solely as a consequence of behavioral problems, assessed during the History and Physical screening assessment.

   (YES/NO)

2. are not suitable to undergo an fMRI experiment due to certain implanted devices (cardiac pacemaker or neurostimulator, some artificial joints, metal pins, surgical clips or other implanted metal parts), body morphology, or claustrophobia. *Assessment tool(s):* Prospective participants will fill out an MRI screening questionnaire and undergo an interview with an MR technologist. Questions concerning suitability for scanning will be referred to the MR Medical Safety Officer.

   (YES/NO)

3. have coagulopathies, history of, current superficial, or deep vein thrombosis, musculoskeletal abnormalities restricting an individual's ability to lie flat for extended periods of time. *Assessment tool(s):* History and physical examination by a qualified IRP clinician, supplemented with a trial of lying in the mock scanner to assess comfort issues.

   (YES/NO)

4. have HIV or Syphilis. *Assessment tool(s):* Oral HIV followed by blood test if oral test is + and STS+ without adequate prior treatment.

   (YES/NO)

5. regularly use any prescription (e.g., antidepressants, benzodiazepines, antipsychotics, anticonvulsants, barbiturates), over-the-counter (e.g., cold medicine) or herbal medication (e.g., Kava, Gingko biloba, St. John's wort) that may alter CNS function, cardiovascular function, or neuronal-vascular coupling. *Assessment tool(s):* History and comprehensive urine drug screening to detect antidepressants, benzodiazepines, antipsychotics, anticonvulsants, and barbiturates.

   (YES/NO)

6. have any current neurological illnesses including, but not limited to, seizure disorders, frequent migraines or on prophylaxis, multiple sclerosis, movement disorders, history of significant head trauma, or CNS tumor. *Assessment tool(s):* History and physical examination by a qualified IRP clinician, urine drug screening for anticonvulsants not disclosed by history. History of head trauma with loss of consciousness of more than 30 minutes or with post-concussive sequelae lasting more than two days, regardless of loss of consciousness, will be exclusionary. The MAI will also retain discretion to exclude based on a history of neurological illness that may compromise data integrity.

   (YES/NO)

7. Have any current major psychiatric disorders to include, but not limited to, mood, anxiety, psychotic disorders, or substance-induced psychiatric disorders, or any current suicidal ideations or history of suicide attempts or currently under antidepressant or antipsychotic medication treatment. The MAI will reserve the right to exclude on the basis of psychiatric history not explicitly described in this criterion *Assessment tool(s):* Computerized SCID-NP, ASRS (adult ADHD self-report scale), Beck Depression Inventory, Beck Anxiety Inventory, and DSM-IV (DSM-IV, APA, 1994) confirmed by clinician interview.

   (YES/NO)

8. have significant cardiovascular or cerebrovascular conditions. *Assessment tool(s):* History and physical exam, including 12-lead EKG.

   (YES/NO)

9. have any other major medical condition that in the view of the investigators would compromise the safety of an individual during participation**. *Assessment tool(s):* History and physical examination by a qualified IRP clinician and CBC, urinalysis, NIDA chemistry panel (liver function tests, electrolytes, kidney function). The following lab values will result in exclusion from the study:

   (h) Hemoglobin < 10 g/dl (YES/NO)
   (i) White Blood Cell Count < 2400/µl (YES/NO)
   (j) Liver Function Tests > 3X normal (YES/NO)
   (k) Serum glucose > 200 mg/dl (YES/NO)
   (l) Urine protein > 2+ (YES/NO)
   (m) Serum creatinine > 2 mg/dl (YES/NO)
   (n) Estimated glomerular filtration rate <60ml/min (YES/NO)

   (YES/NO)

   **The MAI will retain discretion to exclude based on less extreme lab results. After the screening process has been completed, the MAI will take into account all data collected in order to decide if there is an existing medical illness that would compromise participation in this research.

10. pregnant, planning to become pregnant, or breastfeeding. Females are instructed in the consent to use effective forms of birth control during the study period. *Assessment tool(s):* Urine and/or serum pregnancy tests, and clinical interview.

    (YES/NO)

11. have moderate to severe renal impairment. *Assessment tool(s): Estimated* glomerular filtration rate. Renal insufficiency with estimated creatinine clearance < 60 ml/min calculated by the Cockcroft-Gault equation will be excluded.

(YES/NO)

12. Are diabetic. *Assessment tool(s):* Casual plasma glucose testing. Individuals with glucose levels above 200 mg/dl may be further evaluated for diabetes using a fasting glucose test or be excluded.

    (YES/NO)

Eligibility Checklist for Protocol 474-tDCS – Main Arm

Screening

All participants

		Yes	No	MAI	*Assessment Tool(s)*
1.	Age 18-65 years.	☒	☐	☐	
2.	Right handed.	☒	☐	☐	*Edinburg Handedness Inventory*
3.	Free of current moderate-severe DSM-V Substance Use Disorder on any drug, except nicotine in smokers. If there is past moderate to severe substance dependence, must currently be in sustained remission (and not on maintenance therapy for opioid use disorder) and not intoxicated on the day of the imaging session.	☒	☐	☐	*Computerized SCID* *DSM-5 SUD Assessment* *Clinical Assessment* *Urine Toxicology Screen* *Breathalyzer*
4.	Able to abstain from alcohol and other recreational drugs for 24 hours before each imaging session, and able to moderate caffeine intake 12 hours before each imaging session.	☒	☐	☐	*Self report*
5.	English-speaking	☒	☐	☐	*Self report*

Smoker Group

		Yes	No	MAI	*Assessment Tool(s)*
6.	Have a urine cotinine equivalent of about 200ng/ml or higher and have been smoking for at least 1 year.	☒	☐	☐	*Self report* *Urine cotinine test*
7.	Able to abstain from smoking for 12 hours prior to MRI-tDCS study sessions.	☒	☐	☐	*Self-report and expired CO levels*

Non-smoker Group

		Yes	No	MAI	*Assessment Tool(s)*
6.	Not have a history of daily cigarette smoking or have used any nicotine products continuously lasting more than a month, and no smoking or continuous use of any nicotine products within the past year.	☒	☐	☐	*Self report*

Medical

All participants

		Yes	No	MAI	*Assessment Tool(s)*
1.	Be in good health.	☒	☐	☐	*Health history, H&P*
2.	Are suitable for MRI scanning (i.e. Free of certain implanted devices (cardiac pacemaker or neurostimulator, some artificial joints, metal pins, surgical clips or other implanted metal parts), have appropriate body morphology, free of claustrophobia.)	☒	☐	☐	*MRI screening questionnaire and interview by MR technologist.* *Questions concerning suitability referred to MR Medical Safety Officer.*
3.	Free of musculoskeletal abnormalities restricting an individual's ability to lie flat for extended periods of time.	☒	☐	☐	*H&P* *Mock scanner trial*
4.	Free of HIV or Syphilis	☒	☐	☐	*Oral HIV, Blood test if (+) Oral (+) STS (w/o Prior Tx)*
5.	Does NOT regularly use any prescription (e.g., benzodiazepines, antipsychotics, anticonvulsants, barbiturates), over-the-counter (e.g., cold medicine) or herbal medication (e.g., Kava, Gingko biloba, St. John's wort) that may alter neuronal-vascular coupling, with some exceptions below: May use anti-depressant medications. May use certain medications intermittently, provided the participant can be scanned in the same medication state across multiple study days for data consistency (i.e. either all scan days are scheduled after 5 half-lives since last medication use; or all scan days are scheduled on medication).	☒	☐	☐	*History* *Comprehensive Urine Drug Screen*
6.	Free of current neurological illnesses including, but not limited to, seizure disorders, frequent migraines or on prophylaxis, multiple sclerosis, movement disorders (except essential tremor, so long as	☒	☐	☐	*H & P* *Comprehensive Urine Drug Screen*

	it would not interfere with study tasks such as button pressing), cerebrovascular accident, history of significant head trauma, or CNS tumor.				*History of head trauma (loss of consciousness > 30 minutes OR sequelae lasting >2 days)*
7.	Free of current major psychotic disorders, mania, substance-induced psychiatric disorders, or any current suicidal ideations or history of suicide attempts. Free of moderate to severe *current symptoms* of mood or anxiety disorders. (Mild mood or anxiety disorder *symptoms* are *not* exclusionary, whether medicated or unmedicated.)	☒	☐	☐	*Computerized SCID,* *Clinician interview*
8.	NOT cognitively impaired or learning disabled.	☒	☐	☐	*H&P Screening (history of placement in special education classes as a consequence of serious learning problems, and not solely as a consequence of behavioral problems)*
9.	Free of significant cardiovascular conditions that would make use of nicotine patch unsafe.	☒	☐	☐	*H&P, 12-lead EKG*
10.	Free of any other major medical condition that in the view of the investigators would compromise the safety of an individual during participation, or the quality of the data obtainable, including but not limited to lab values outside the following parameters: Hemoglobin < 10 g/dl White Blood Cell Count < 2400/µl Liver Function Tests > 3X upper limit of normal Serum glucose > 200 mg/dl Urine protein > 2+ Serum creatinine > 2 mg/dl Must be free of Diabetes Mellitus	☒	☐	☐	*H&P, CBC, urinalysis, NIDA chemistry panel (liver function tests, electrolytes, kidney function).* *The MAI will retain discretion to exclude based on less extreme lab results, or other major medical condition not otherwise specified*
11.	Is NOT pregnant, planning to become pregnant, or breastfeeding (females).	☒	☐	☐	*Urine and/or serum pregnancy tests,*

					Clinical interview. *Females are instructed in the consent to use effective forms of birth control during the study period.*
12.	Has NOT participated in any brain stimulation session less than two weeks ago, or undergone brain stimulation exposure for treatment purposes in the last 6 months, including tDCS or transcranial magnetic stimulation (TMS).	☒	☐	☐	*H&P, Clinical interview*

Eligibility Checklist for Protocol 474-tDCS – Pilot

Screening

		Yes	No	MAI	*Assessment Tool(s)*
1.	Age 18-65 years.	☒	☐	☐	
2.	Right handed.	☒	☐	☐	*Edinburg Handedness Inventory*
3.	Free of current moderate-severe DSM-V Substance Use Disorder on any drug, except nicotine in smokers. If there is past moderate to severe substance dependence, must currently be in sustained remission (and not on maintenance therapy for opioid use disorder) and not intoxicated on the day of the imaging session.	☒	☐	☐	*Computerized SCID* *DSM-5 SUD Assessment* *Clinical Assessment* *Urine Toxicology Screen* *Breathalyzer*
4.	Able to abstain from alcohol and other recreational drugs for 24 hours before each imaging session, and able to moderate caffeine intake 12 hours before each imaging session.	☒	☐	☐	*Self report*
5.	Not have a history of daily cigarette smoking or have used any nicotine products continuously lasting more than a month, and no smoking or continuous use of any nicotine products within the past year.	☒	☐	☐	*Self report*
6.	English-speaking	☒	☐	☐	*Self report*

Medical

		Yes	No	MAI	*Assessment Tool(s)*
1.	Be in good health.	☒	☐	☐	*Health history, H&P*
2.	Are suitable for MRI scanning (i.e. Free of certain implanted devices (cardiac pacemaker or neurostimulator, some artificial joints, metal pins, surgical clips or other implanted metal parts), have appropriate body morphology, free of claustrophobia.)	☒	☐	☐	*MRI screening questionnaire and interview by MR technologist.* *Questions concerning suitability referred to MR Medical Safety Officer.*
3.	Free of musculoskeletal abnormalities restricting an individual's ability to lie flat for extended periods of time.	☒	☐	☐	*H&P* *Mock scanner trial*
4.	Free of HIV or Syphilis	☒	☐	☐	*Oral HIV, Blood test if (+) Oral (+) STS (w/o Prior Tx)*
5.	Does NOT regularly use any prescription (e.g., benzodiazepines, antipsychotics, anticonvulsants, barbiturates), over-the-counter (e.g., cold medicine) or herbal medication (e.g., Kava, Gingko biloba, St. John's wort) that may alter neuronal-vascular coupling, with some exceptions below: May use anti-depressant medications. May use certain medications intermittently, provided the participant can be scanned in the same medication state across multiple study days for data consistency	☒	☐	☐	*History* *Comprehensive Urine Drug Screen*
6.	Free of current neurological illnesses including, but not limited to, seizure disorders, frequent migraines or on prophylaxis, multiple sclerosis, movement disorders (except essential tremor, so long as it would not interfere with study tasks such as button pressing), cerebrovascular accident, history of significant head trauma, or CNS tumor.	☒	☐	☐	*H & P* *Comprehensive Urine Drug Screen* *History of head trauma (loss of consciousness > 30 minutes OR sequelae lasting >2 days)*

7.	Free of current major psychotic disorders, mania, substance-induced psychiatric disorders, or any current suicidal ideations or history of suicide attempts. Free of moderate to severe current symptoms of mood or anxiety disorders. (Mild mood or anxiety disorder symptoms are not exclusionary, whether medicated or unmedicated.)	☒	☐	☐	*Computerized SCID, Clinician interview*
8.	NOT cognitively impaired or learning disabled.	☒	☐	☐	*H&P Screening (history of placement in special education classes as a consequence of serious learning problems, and not solely as a consequence of behavioral problems)*
9.	Free of significant cardiovascular conditions that would make use of nicotine patch unsafe.	☒	☐	☐	*H&P, 12-lead EKG*
10.	Free of any other major medical condition that in the view of the investigators would compromise the safety of an individual during participation, or the quality of the data obtainable, including but not limited to lab values outside the following parameters: Hemoglobin < 10 g/dl White Blood Cell Count < 2400/µl Liver Function Tests > 3X upper limit of normal Serum glucose > 200 mg/dl Urine protein > 2+ Serum creatinine > 2 mg/dl Must be free of Diabetes Mellitus	☒	☐	☐	*H&P, CBC, urinalysis, NIDA chemistry panel (liver function tests, electrolytes, kidney function).* *The MAI will retain discretion to exclude based on less extreme lab results, or other major medical condition not otherwise specified*
11.	Is NOT pregnant, planning to become pregnant, or breastfeeding (females).	☒	☐	☐	*Urine and/or serum pregnancy tests,* *Clinical interview.* *Females are instructed in the consent to use effective forms of birth control during the study period.*

12.	Has NOT participated in any brain stimulation session less than two weeks ago, or undergone brain stimulation exposure for treatment purposes in the last 6 months, including tDCS or transcranial magnetic stimulation (TMS).	☒	☐	☐	*H&P, Clinical interview*

Appendix 2: Participant Fact Sheets

STUDY 474 Treatment Seekers FACT SHEET

474: "Identifying neurobiological mechanisms that underlie acute nicotine withdrawal syndrome and drive early relapse in smokers"

All visits will take place at the NIH/NIDA research facility on the Johns Hopkins Bayview Medical Campus.

Participation in this study will follow 3 phases:

PHASE 1: Pre-treatment study visits: involves 3 visits over a period of about 3-5 weeks. Visit 1 will be an orientation visit (about 6 hours) and visits 2 and 3 will use MRI to measure brain activity (about 9 hours per visit). During PHASE 1 you will:

- NOT be able to smoke for 36 hours before the two imaging visits.
- Wear a nicotine skin patch or a placebo (fake) patch during your 36 hour smoking abstinence period and study visits.
- Have your blood drawn to test for levels of stress-related hormones.
- Complete multiple MRI scanning sessions that last about 1.5 to 2 hours each.
- Undergo EEG (brain waves) recording.
- Answer questionnaires about how you think and feel.
- Complete various tasks and procedures inside and outside of the MRI scanner.

Compensation: You will receive up to $911 compensation for participation in PHASE 1.

PHASE 2: Treatment visits: involves 13 visits over a period of about 12-15 weeks. Each visit will last about 1 hour. During PHASE 2 you will:

- Set a quit date and develop a treatment plan with a study therapist.
- Take Chantix® (varenicline) every day for a period of 12 weeks. See page 2 for more information on Chantix®.
- Meet for weekly and biweekly counseling sessions with a therapist.
- Answer questionnaires about how you think and feel.

Compensation: You will receive free treatment, including medication (Chantix®) and weekly counseling sessions and $15 travel reimbursement for each visit to the NIH/NIDA Research facility

PHASE 3: Post-treatment follow-up visits: Approximately 1, 6 and 12 months after your last treatment visit you will be contacted by study staff who will schedule a follow-up visit with you. Each of the three follow-up visits will last approximately 1.5 hours. During PHASE 3 you will:

Complete an MRI scanning session that will last about 20min each visit.

Meet with a study staff member on each visit who will ask you questions about your smoking behavior and how you think and feel.

Compensation: You will receive up to $165 compensation for participation in PHASE 3.

Each phase also includes 15-30 min medical assessments with a study nurse
Weekend and weeknight appointments scans and counseling available where necessary

Varenicline (Chantix®)

- Varenicline (Chantix®) is approved by the FDA to help people quit smoking.
- Varenicline is currently the most effective smoking medication available.
- Like all medications, taking varenicline may result in side effects. The most common side effects include: nausea (16-34% of the people who took varenicline in clinical trials experienced this side effect), difficulty sleeping (18%), abnormal dreams (13%), headaches (15%), feeling tired or sleepy (7%) and change in appetite (3%). In rare cases heart problems have been reported. Low blood sugar (hypoglycemia) may also be a possibility, especially for diabetics.

Other side effects that have been reported include:

-vomiting	-back and muscle pain	-mouth sores	-hot flush
-stomach pain	-disturbances in attention	-nightmares	-blood pressure changes
-gas	-dizziness	-ringing in the ears	-increased heart rate
-indigestion	-fainting	-increased urination	-flu-like symptoms
-constipation	-restlessness	-blurred vision	-drug allergy
-reflux	-anxiety	-sweating	-increases in weight
-dry mouth	-depression	-mouth sores	-hot flush
-diarrhea	-back and muscle pain	-nightmares	-blood pressure changes
-irritability	-violent/aggressive thoughts or actions	-low blood sugar	-heart problems
-agitation	-strange behavior	-suicidal thoughts	

- The maker of varenicline and the FDA have issued a 'black box' warning indicating that people taking this medication should be observed for rare but serious changes in behavior such as agitation, depressed mood, and suicidal/violent ideation or suicidal/violent behavior.
- A recent study of 80 660 men and women using smoking cessation products in the United Kingdom found that compared with other smoking cessation products (e.g., nicotine replacement skin patches) varenicline was not associated with an increased risk of self-harm, suicidal thoughts or suicidal behavior.

474: "Identifying neurobiological mechanisms that underlie acute nicotine withdrawal syndrome and drive early relapse in smokers

All visits will take place at the NIH/NIDA research facility on the Johns Hopkins Bayview Medical Campus.

Participation in this study will involve 3 visits over a period of about 3-5 weeks. Visit 1 will be an orientation visit (about 6 hours) and visits 2 and 3 will use MRI to measure brain activity (about 9 hours per visit). During the study you will:

- NOT be able to smoke for 36 hours before the two imaging visits.
- Wear a nicotine skin patch or a placebo (fake) patch during your 36 hour smoking abstinence period and study visits.
- Have your blood drawn to test for levels of stress-related hormones.
- Complete multiple MRI scanning sessions that last about 1.5 to 2 hours each.
- Undergo EEG (brain waves) recording.
- Answer questionnaires about how you think and feel.
- Complete various tasks and procedures inside and outside of the MRI scanner.

- Compensation: You may receive up to about $911 compensation for participation in this study.

Each phase also includes 15-30 min medical assessments with a study nurse
Weekend and weeknight appointments scans and counseling available where necessary

STUDY 474 tDCS FACT SHEET for PARTICIPANTS WHO SMOKE CIGARETTES

474: "Transcranial Direct Current Stimulation (tDCS) to reduce effects of the Nicotine Withdrawal Syndrome"

All visits will take place at the NIH/NIDA research facility on the Johns Hopkins Bayview Medical Campus.

Brain research with functional magnetic resonance imaging (fMRI) has shown that nicotine can change some patterns of brain activity, and that smokers have different patterns of brain activity when compared to non-smokers. fMRI can be used to see changes in brain patterns when a person performs various cognitive thinking tasks – such as a memory game – or even when lying still inside the MRI scanner.

tDCS is a new technology that may help improve brain activity in both smokers and non-smokers. tDCS works by passing a small current through electrodes placed on your head. When tDCS is turned on, you may feel a tingling or "pins and needles" sensation where the electrodes are placed. This small stimulation appears to make some parts of the brain work a little more or less effectively. This effect goes away in less than 1 hour after the stimulation.

This study will use fMRI to measure the effect of tDCS on brain function in both smokers and non-smokers. The study will also measure the effect of tDCS in smokers experiencing withdrawal. This study may help us understand the potential for tDCS as a future treatment for smoking.
Participation in this study will involve 3 visits. Visit 1 will be an orientation visit (about 4.5-5.5 hours) and Visits 2 and 3 will use MRI and tDCS to measure and stimulate brain activity (about 8 hours per visit). During the study you will:

- NOT be able to smoke for 12 hours before the two imaging visits.
- Wear a nicotine skin patch or a placebo (fake) patch during the day of your study visits.
- Complete multiple tDCS sessions that last about 25 minutes each. There will be 3 stimulation sessions per day for the two imaging visits, 2 of these will be active (real) and 1 will be sham (fake). The tDCS brain stimulation will be given inside the MRI scanner.
- tDCS requires contact with the skin on your scalp, and you may be asked to move hair out of the way to allow placement of tDCS electrodes.
- Complete multiple MRI scanning sessions that last about 1 hour each. There will be 3 MRI scanning sessions per day for the two imaging visits.
- There may be an additional, shorter, MRI scan on orientation day. If so, orientation will be ~30-45 minutes longer. This scan would not include tDCS.
- Answer questionnaires about how you think and feel.
- Complete various tasks and procedures inside and outside of the MRI scanner.

  Compensation: You may receive up to about $654.50 compensation for participation in this study.

Each visit to NIDA also includes a medical assessment with a study nurse

STUDY 474 tDCS FACT SHEET for NONSMOKING PARTICIPANTS

474: "Transcranial Direct Current Stimulation (tDCS) to reduce effects of the Nicotine Withdrawal Syndrome"

All visits will take place at the NIH/NIDA research facility on the Johns Hopkins Bayview Medical Campus.

Brain research with functional magnetic resonance imaging (fMRI) has shown that nicotine can change some patterns of brain activity, and that smokers have different patterns of brain activity when compared to non-smokers. fMRI can be used to see changes in brain patterns when a person performs various cognitive thinking tasks – such as a memory game – or even when lying still inside the MRI scanner.

tDCS is a new technology that may help improve brain activity in both smokers and non-smokers. tDCS works by passing a small current through electrodes placed on your head. When tDCS is turned on, you may feel a tingling or "pins and needles" sensation where the electrodes are placed. This small stimulation appears to make some parts of the brain work a little more or less effectively. This effect goes away in less than 1 hour after the stimulation.

This study will use fMRI to measure the effect of tDCS on brain function in both smokers and non-smokers. The study will also measure the effect of tDCS in smokers experiencing withdrawal. This study may help us understand the potential for tDCS as a future treatment for smoking.

Participation in this study will involve 2 visits. Visit 1 will be an orientation visit (about 4-5 hours) and Visit 2 will use MRI and tDCS to measure and stimulate brain activity (about 8 hours). During the study you will:

- Complete multiple tDCS sessions that last about 25 minutes each. There will be 3 stimulation sessions on the imaging visit day, 2 of these will be active (real) and 1 will be sham (fake). The tDCS brain stimulation will be given inside the MRI scanner.
- tDCS requires contact with the skin on your scalp, and you may be asked to move hair out of the way to allow placement of tDCS electrodes.
- Complete multiple MRI scanning sessions that last about 1 hour each. There will be 3 MRI scanning sessions on the imaging visit day.
- There may be an additional, shorter, MRI scan on orientation day. If so, orientation will be ~30-45 minutes longer. This scan would not include tDCS.
- Answer questionnaires about how you think and feel.
- Complete various tasks and procedures inside and outside of the MRI scanner.

- Compensation: You may receive up to about $379.50 compensation for participation in this study.

Each visit to NIDA also includes a medical assessment with a study nurse

STUDY 474 tDCS BEHAVIORAL PILOT FACT SHEET

474: "Transcranial Direct Current Stimulation (tDCS) to reduce effects of the Nicotine Withdrawal Syndrome"

All visits will take place at the NIH/NIDA research facility on the Johns Hopkins Bayview Medical Campus.

tDCS is a new technology that may help improve brain activity. tDCS works by passing a small current through electrodes placed on your head. This small stimulation appears to make some parts of the brain work a little more or less effectively. This effect goes away in less than 1 hour after the stimulation. This study is a pilot study (a smaller study) to determine the effect of tDCS on behavior in healthy people. This study may help us understand the potential for tDCS as a future treatment for smoking. When tDCS is turned on, you may feel a tingling or "pins and needles" sensation where the electrodes are placed.

Participation in this study will involve an Orientation (about 3.5 hours) and a Study Phase, where tDCS is used to stimulate brain activity (about 5-6 hours). The Orientation and Study Phase can be completed on the same or separate days. During the study you will:

- Complete multiple tDCS sessions that last about 25 minutes each. There will be 3 stimulation sessions during the Study Phase. 2 of these will be active (real) and 1 will be sham (fake). The tDCS brain stimulation will be given inside a mock (fake) scanner.
- tDCS requires contact with the skin on your scalp, and you may be asked to move hair out of the way to allow placement of tDCS electrodes.
- Complete multiple mock scanner sessions that last about 1 hour each. There will be 3 mock scanner sessions during the Study Phase.
- Answer questionnaires about how you normally think and feel, and how tDCS made you feel.
- Complete various tasks and procedures inside and outside of the mock scanner.

- Compensation: You may receive up to approximately $250.00 compensation for participation in this study if all procedures are completed on the same day.

Each visit to NIDA also includes a medical assessment with a study nurse

Appendix 3: Characterization Measures

1) Fagerström Test for Nicotine Dependence: (Heatherton, Kozlowski, Frecker, & Fagerström, 1991) is a six item test that measures the severity of nicotine addiction on a 0-10 scale. Completion time: ~>5min.

Directions: Please circle the answer that best describes your typically smoking behaviors.

How soon after you wake up do you smoke your first cigarette?
After 60 minutes (0)
31-60 minutes (1)
6-30 minutes (2)
Within 5 minutes (3)

Do you find it difficult to refrain from smoking in places where it is forbidden?
No (0)
Yes (1)

Which cigarette would you hate most to give up?
The first in the morning (1)
Any other (0)

How many cigarettes per day do you smoke?
10 or less (0)
11-20 (1)
21-30 (2)
31 or more (3)

Do you smoke more frequently during the first hours after awakening than during the rest of the day?
No (0)
Yes (1)

Do you smoke even if you are so ill that you are in bed most of the day?
No (0)
Yes (1)

2) The Cigarette Dependence Scale (Etter, Le Houezec, & Perneger, 2003)is a brief 12-item self report that assesses the main components of DSM-IV and ICD-10 definitions of dependence, which include compulsion, withdrawal symptoms, loss of control, time allocation, neglect of other activities, and persistence despite harm. Completion time: ~5min.

Directions: Please answer each of the following questions

Please rate your addiction to cigarettes on a scale of 0-100 (0= I am NOT addicted to cigarettes at all, 100 = I am extremely addicted to cigarettes) _____.

On average, how many cigarettes do you smoke per day? _____

2b) Since your last visit how many cigarettes per day have you smoked? _____.

Usually, how soon after waking up do you smoke you first cigarette? _____minutes.

For you quitting smoking for good would be:
Impossible
Very difficult
Fairly difficult
Fairly easy
Very easy

Please indicate whether you agree with each of the following statements:

After a few hours without smoking, I feel an irresistible urge to smoke
Totally agree
Somewhat agree
Neither agree nor disagree
Somewhat disagree
Totally disagree

The idea of not having any cigarettes causes me stress (response options same as item 5)
Before going out, I always make sure that I have cigarettes with me.
I am a prisoner of cigarettes
I smoke too much
Sometimes I drop everything to go out and buy cigarettes
I smoke all the time
I smoke despite the risks to my health

3) A general smoking history questionnaire will also be administered (Completion time: ~5 min):

Directions: Please answer each of the questions regarding your smoking behavior.

Number of years smoking_____
age when first cigarette smoked_____
age when started daily smoking_____
average number of cigarettes/day_____
number of quit attempts_____
strategies used during quit attempts (nicotine replacement, buproprion, counseling, cold turkey),
longest period of time not smoking since smoking everyday,
Please rate your desire to quit smoking on a 0-100 scale (0= no desire to quit, 1= extreme desire to quit) _____.
Please rate your confidence in your ability to quit (0= quitting would be impossible for me, 100= It would be easy for me to quit)_____.
Please rate your level of addiction to cigarettes (0=not addicted at all, 100= Extremely addicted)

4) The Revised Social Anhedonia Scale (Eckblad, Chapman, Chapman, & Mishlove, 1982) is a 40-item true-false self-report questionnaire intended to measure decreased pleasure derived from interpersonal sources. This scale demonstrates good psychometric properties and has been extensively used in schizophrenia research (Edell, 1995).

Directions: Please answer each item True or False. Please do not skip any items. It is important that you answer every item, even if you are not quite certain which is the best answer.

1. Having close friends is not as important as many people say. T F (for each item)
2. I attach very little importance to having close friends.
3. I prefer watching television to going out with other people.
4. A car ride is much more enjoyable if someone is with me.
5. I like to make long distance phone calls to friends and relatives.
6. Playing with children is a real chore.
7. I have always enjoyed looking at photographs of friends.
8. Although there are things that I enjoy doing by myself, I usually seem to have more fun when I do things with other people.
9. I sometimes become deeply attached to people I spend a lot of time with.
10. People sometimes think that I am shy when I really just want to be left alone.
11. When things are going really good for my close friends, it makes me feel good too.
12. When someone close to me is depressed, it brings me down also.
13. My emotional responses seem very different from those of other people.
14. When I am alone, I often resent people telephoning me or knocking on my door.
15. Just being with friends can make me feel really good.
16. When things are bothering me, I like to talk to other people about it.
17. I prefer hobbies and leisure activities that do not involve other people.
18. It's fun to sing with other people.
19. Knowing that I have friends who care about me gives me a sense of security.
20. When I move to a new city, I feel a strong need to make new friends.
21. People are usually better off if they stay aloof from emotional involvements with most others.
22. Although I know I should have affection for certain people, I don't really feel it.
23. People often expect me to spend more time talking with them than I would like.
24. I feel pleased and gratified as I learn more and more about the emotional life of my friends.
25. When others try to tell me about their problems and hang-ups, I usually listen with interest and attention.
26. I never had really close friends in high school.
27. I am usually content to just sit alone, thinking and daydreaming.
28. I'm much too independent to really get involved with other people.
29. There are few things more tiring than to have a long, personal discussion with someone.
30. It made me sad to see all my high school friends go their separate ways when high school was over.
31. I have often found it hard to resist talking to a good friend, even when I have other things to do.
32. Making new friends isn't worth the energy it takes.
33. There are things that are more important to me than privacy.
34. People who try to get to know me better usually give up after awhile.
35. I could be happy living all alone in a cabin in the woods or mountains.
36. If given the choice, I would much rather be with others than be alone.
37. I find that people too often assume that their daily activities and opinions will be interesting to me.

38. I don't really feel very close to my friends.
39. My relationships with other people never get very intense.
40. In many ways, I prefer the company of pets to the company of people.

5) The Physical Anhedonia Scale (Chapman & Chapman, 1978) is a 61-item true-false self-report that taps a range of presumably pleasurable experiences involving eating, touching, feeling, sex, movement, smell, and sound. This scale demonstrates good psychometric properties and has been extensively used in schizophrenia research (Edell, 1995).

Directions: Please answer each item True or False. Please do not skip any items. It is important that you answer every item, even if you are not quite certain which is the best answer.

1. I have usually found lovemaking to be intensely pleasurable. T F (for each item)
2. When eating a favorite food, I have often tried to eat slowly to make it last longer.
3. I have often enjoyed the feel of silk, velvet, or fur.
4. I have sometimes enjoyed feeling the strength in my muscles.
5. Dancing, or the idea of it, has always seemed dull to me.
6. I have always found organ music dull and unexciting.
7. The taste of food has always been important to me.
8. I have had very little fun from physical activities like walking, swimming, or sports.
9. I have seldom enjoyed any kind of sexual experience.
10. On hearing a good song, I have seldom wanted to sing along with it.
11. I have always hated the feeling of exhaustion that comes from vigorous activity.
12. The color that things are painted has seldom mattered to me.
13. The sound of rustling leaves has never much pleased me.
14. Sunbathing isn't really more fun than lying down indoors.
15. There just are not many things that I have ever really enjoyed doing.
16. I don't know why some people are so interested in music.
17. Flowers aren't as beautiful as many people claim.
18. I have always loved having my back massaged.
19. I never wanted to go on any of the rides at an amusement park.
20. Trying new foods is something I have always enjoyed.
21. The warmth of an open fireplace hasn't especially soothed and calmed me.
22. Poets always exaggerate the beauty and joys of nature.
23. When I have seen a statue, I have had the urge to feel it.
24. I have always had a number of favorite foods.
25. I don't understand why people enjoy looking at the stars at night.
26. I have had very little desire to try new kinds of foods.
27. I never have the desire to take off my shoes and walk through a puddle barefoot.
28. I've never cared much about the texture of food.
29. When I have walked by a bakery, the smell of fresh bread has often made me hungry.
30. I have often enjoyed receiving a strong, warm handshake.
31. I have often felt uncomfortable when my friends touch me.
32. I have never found a thunderstorm exhilarating.
33. Standing on a high place and looking out over the view is very exciting.
34. I have often found walks to be relaxing and enjoyable.
35. The sound of the rain falling on the roof has made me feel snug and secure.
36. I like playing with and petting soft little kittens or puppies.
37. The sound of organ music has often thrilled me.
38. Beautiful scenery has been a great delight to me.
39. The first winter snowfall has often looked pretty to me.
40. Sex is okay, but not as much fun as most people claim it is.

41. I have sometimes danced by myself just to feel my body move with the music.
42. I have seldom cared to sing in the shower.
43. One food tastes as good as another to me.
44. On seeing a soft, thick carpet, I have sometimes had the impulse to take off my shoes and walk barefoot on it.
45. After a busy day, a slow walk has often felt relaxing.
46. The bright lights of a city are exciting to look at.
47. The beauty of sunsets is greatly overrated.
48. It has always made me feel good when someone I care about reaches out to touch me.
49. I have usually found soft music boring rather than relaxing.
50. I have usually finished my bath or shower as quickly as possible just to get it over with.
51. The smell of dinner cooking has hardly ever aroused my appetite.
52. When I pass by flowers, I have often stopped to smell them.
53. Sex is the most intensely enjoyable thing in life.
54. I think that flying a kite is silly.
55. I've never cared to sunbathe; it just makes me hot.
56. The sounds of a parade have never excited me.
57. It has often felt good to massage my muscles when they are tired or sore.
58. When I'm feeling a little sad, singing has often made me feel happier.
59. A good soap lather when I'm bathing has sometimes soothed and refreshed me.
60. A brisk walk has sometimes made me feel good all over.
61. I have been fascinated with the dancing of flames in a fireplace.

6) Barratt Impulsiveness Scale (BIS-11, Patton et al., 1995) was designed to measure three aspects of behavioral and cognitive impulsiveness: attentional, motor and nonplanning. The scale has 30 self-descriptive items to on a 4 point likert scale ranging from 1- Rarely/Never to 4 Almost Always/Always.

DIRECTIONS: People differ in the ways they act and think in different situations. This is a test to measure some of the ways in which you act and think. Read each statement and put an X on the appropriate circle on the right side of this page. Do not spend too much time on any statement. Answer quickly and honestly.

	① Rarely/Never	② Occasionally	③ Often	④ Almost Always/Always
1	I plan tasks carefully.			① ② ③ ④
2	I do things without thinking.			① ② ③ ④
3	I make-up my mind quickly.			① ② ③ ④
4	I am happy-go-lucky.			① ② ③ ④
5	I don't "pay attention."			① ② ③ ④
6	I have "racing" thoughts.			① ② ③ ④
7	I plan trips well ahead of time.			① ② ③ ④
8	I am self controlled.			① ② ③ ④
9	I concentrate easily.			① ② ③ ④
10	I save regularly.			① ② ③ ④
11	I "squirm" at plays or lectures.			① ② ③ ④
12	I am a careful thinker.			① ② ③ ④
13	I plan for job security.			① ② ③ ④
14	I say things without thinking.			① ② ③ ④
15	I like to think about complex problems.			① ② ③ ④
16	I change jobs.			① ② ③ ④
17	I act "on impulse."			① ② ③ ④
18	I get easily bored when solving thought problems.			① ② ③ ④
19	I act on the spur of the moment.			① ② ③ ④
20	I am a steady thinker.			① ② ③ ④
21	I change residences.			① ② ③ ④
22	I buy things on impulse.			① ② ③ ④
23	I can only think about one thing at a time.			① ② ③ ④
24	I change hobbies.			① ② ③ ④
25	I spend or charge more than I earn.			① ② ③ ④
26	I often have extraneous thoughts when thinking.			① ② ③ ④
27	I am more interested in the present than the future.			① ② ③ ④
28	I am restless at the theater or lectures.			① ② ③ ④
29	I like puzzles.			① ② ③ ④
30	I am future oriented.			① ② ③ ④

Patton, Stanford, Barratt (1995). *J Clin Psy*, vol. 51, pp. 768-774

7) Rehearsal Scale of the Emotion Control Questionnaire (RS-ECQ) Roger & Najarian, 1989). The RS-ECQ was designed assess the trait tendency to ruminate or rehearse

Instructions: Please indicate how you feel about each item by circling either 'True' or 'False'. If you feel that an item is neither entirely true nor false, please choose the alternative that is most like you. If you haven't been in the situation described, please say how you feel you would behave in that situation.

1. I remember things that upset me or make me angry for a long time afterwards.
2. I generally don't bear a grudge-when something is over, it's over, and I don't think about it again.
3. I get 'worked up' just thinking about things that have upset me in the past.
4. I often find myself thinking over and over about things that have made me angry.
5. I can usually settle things quickly and be friendly again after an argument.
6. If I see or hear about an accident, I find myself thinking about something similar happening to me or to people close to me.
7. I think about ways of getting back at people who have made me angry long after the event has happened.
8. I never forget people making me angry or upset, even about small things.
9. I find it hard to get thoughts about things that have upset me out of my mind.
10. I often daydream about situations where I'm getting my own back at people.
11. If I see something that frightens or upsets me, the image of it stays in my mind for a long time afterwards.
12. Thinking about upsetting things just seems to keep them going, so I try to put them out of my mind.
13. If I lose out on something, I get over it quickly.
14. If I have to confront someone, I try not to think too much about it beforehand.

tDCS Arm Questionnaires

The Brief Externalizing Inventory (Hall et al., 2007), adapted from the full Externalizing inventory (Krueger et al., 2007), is a subset of 159 self-report items used to assess a range of behavioral and personality characteristics that have been attributed to a broad psychological construct termed externalizing. Completion time: ~30min.

Directions: This questionnaire contains statements that different people might use to describe themselves. Most of these statements are followed by four choices T, t, f, F. The meaning of these four different choices is given below: T = True t = mostly true, f = mostly false, F = false.

For each statement, circle the choice that describes you best.

1. I have broken someone's things to prevent them from being used. T t f F (for each item)
2. I've smoked marijuana before going to work or school.
3. I have had problems at work because I was irresponsible.
4. If I could control my impulses, my life would be much better.
5. I enjoy pushing people around sometimes.
6. I have lied to avoid paying back loans.
7. I have done things that put others in danger.
8. My drug use led to problems at work or school.
9. I have damaged someone's things to get something I wanted.
10. I tried an illegal drug at a party.
11. I've told lies about someone just to see how it would affect them.
12. I've never used marijuana in my life.
13. I have stolen something out of a vehicle.
14. I get in trouble for not considering the consequences of my actions.
15. I've smoked marijuana at parties.
16. I have been unfairly blamed when I was just taking advantage of others' mistakes.
17. I have run up big debts that I had trouble paying.
18. I don't see any point in worrying if what I do hurts someone else.
19. I've stood friends up.
20. I have brought a weapon into a fight.
21. I have borrowed money with no thought of paying it back.
22. I have rolled a marijuana joint.
23. I have missed work without bothering to call in.
24. I seek out thrills almost everywhere I go.
25. I do lots of things just to get a thrill.
26. I have not lived up to my end of a contract.
27. I've told lies about someone who upset me.
28. I've made big decisions without thinking them over.
29. At some point in my life, I needed more drugs to get the same effect.
30. I've asked someone to help bail me out of debt.
31. I've vandalized public property just for kicks.

32. I keep appointments I make.
33. I have thrown something at a person who angered me.
34. Many problems in my life are caused by doing things without thinking.
35. I sometimes insult people on purpose to get a reaction from them.
36. People often abuse my trust.
37. I've quit a job without giving two weeks notice.
38. I get bored easily.
39. I have gotten things from people by making them feel sorry for me.
40. I have taken a drug like LSD or magic mushrooms.
41. I don't lie very much.
42. Others have told me they are concerned about my lack of self-control.
43. I've used downers like Valium or Xanax for non-medical reasons.
44. I have used a weapon against someone who insulted me.
45. I often get bored quickly and lose interest.
46. I've kept using marijuana even though it caused problems with my memory or health.
47. At times I kept drinking alcohol even though it caused problems with family or friends.
48. I have talked a stranger into giving me money.
49. I have taken items from a store without paying for them.
50. When I want something, I want it right now.
51. I have robbed someone.
52. I taunt people just to stir things up.
53. I've gotten in trouble because I missed too much school.
54. I have tried smoking marijuana.
55. I've gone on drinking binges.
56. I have missed a final exam.
57. I have taken money from someone's purse or wallet without asking.
58. I've spent more money on marijuana than I should have.
59. I have started a fight because it was exciting.
60. I've lost control of my alcohol use.
61. I have hit someone in the face or head in anger.
62. I have never bought drugs.
63. I've made fun of someone to impress other people.
64. Sometimes I threaten people.
65. I gave up things I used to enjoy because of drugs.
66. I have lied to get someone to sleep with me.
67. My drinking led to problems at home.
68. I lose control of myself and do things I probably shouldn't.
69. I've held someone down to get what I wanted from them.
70. I have written a check knowing it would not cash.
71. I have broken into a house, school, or other building.
72. I enjoy a good physical fight.
73. Sometimes I use my wits to take advantage of people.
74. At times, marijuana has been more important to me than work, friends, or school.
75. I've used drugs when it might be hazardous, like while driving a car.
76. People think of me as dependable.
77. I've used marijuana when it might be hazardous, like while driving a car.
78. I have used physical force to take something from someone.

79. I hate waiting to get things that I want.
80. I have spread rumors about people who were competing with me.
81. I've taken an illegal drug that gave me a rush and made me more awake.
82. I have snuck marijuana or hash into a public event.
83. I have used a weapon to get something I wanted.
84. I have lost valuable goods or money because I decided things too quickly.
85. One or more times in my life, I have beaten someone up for bothering me.
86. I rarely lie.
87. I've told lies about someone else to make myself look better.
88. I lie sometimes without even thinking about it.
89. I've bought items used for smoking marijuana.
90. I've had legal problems because of my drug use.
91. I've had legal problems because I couldn't resist my impulses.
92. Many people consider me a rule breaker.
93. I've gotten high using marijuana.
94. I've ruined the friendships of people who made me angry.
95. I've driven while drunk.
96. I have lied to get ahead at work.
97. I've spent big parts of my day using marijuana.
98. I've never taken illegal drugs.
99. I have a hard time waiting patiently for things I want.
100. My impulsive decisions have caused problems with loved ones.
101. I have gotten money from people by threatening to tell their secrets.
102. I've never used street drugs.
103. I think about things before I do them.
104. How other people feel is important to me
105. I've taken prescription medicine to get high.
106. I have a habit of breaking rules.
107. I've missed a rent or mortgage payment.
108. I don't think about the outcomes of my decisions enough.
109. I don't drink.
110. I have failed to pay my taxes on time.
111. I get blamed for things that I don't do.
112. I often disobey rules.
113. I don't care much if what I do hurts others.
114. I've thought about doing physical harm to someone who hurt me.
115. I have damaged someone's things because it was exciting.
116. I have lied to the police.
117. I have quit a job without having another source of support lined up.
118. I often get in trouble for breaking rules.
119. I gave up things I used to enjoy because of my drinking.
120. My marijuana use has led to problems at home, work, or school.
121. I have failed to show up to court when I was supposed to.
122. I have lied on a job application.
123. I have damaged someone's property because I was angry with them.
124. My drinking led to problems at work or school.
125. I've broken something belonging to someone else to get back at them.

126. I've hurt someone's feelings on purpose to get back at them.
127. I've failed to make payments on a loan.
128. I don't hesitate to complicate the lives of people who upset me.
129. I vandalized someone's house or things because they were rude to me.
130. My drug use has caused problems with my family.
131. I plan before I act.
132. I've made a fool of someone because it made me feel good.
133. I have used more drugs for longer than I meant to.
134. I have been in trouble with the law for something I did on impulse.
135. I have smacked someone who upset me.
136. People use me.
137. I have failed to pay a traffic fine.
138. My lack of self-control gets me in trouble.
139. I've never had any desire to try an illegal drug.
140. I have been called a bully.
141. I get unfairly blamed for things.
142. I have destroyed property just for kicks.
143. I have conned people to get money from them.
144. I have broken into someone's home and taken things.
145. I am sensitive to the feelings of others.
146. I've broken the law to get money for drugs.
147. I've skipped work or meetings to satisfy sudden urges.
148. I have been caught shoplifting.
149. I'm not a drinker.
150. I have left a restaurant or gas station without paying my bill.
151. I have bought marijuana.
152. I like risky activities.
153. I've gotten into trouble after blindly going after what I wanted.
154. I have stolen something worth more than $10.
155. I talk badly about people who cause me trouble.
156. When I want something, nothing else seems important.
157. I've been fired from more than one job.
158. After trying to cut down on drinking alcohol, I've felt sad or irritable.
159. I've hit someone because they made fun of me.

The Temperament and Character Inventory (Cloninger et al., 1994) is a widely used test that assesses dimensions of personality (e.g., harm avoidance, novelty seeking, reward dependence, and persistence) that are considered to be related to monoaminergic function. Completion time: ~25min.

Directions: In this booklet you will find statements people might use to describe their attitudes, opinions, interests, and other personal feelings.

Each statement can be answered TRUE or FALSE. Read the statement and describe which choice best describes you. Try to describe the way you USUALLY or generally act and feel, not just how you are feeling noe

We would like you to fill out this questionnaire on your own using a pencil. When you are finished, please return the questionnaire.

To answer you only need to circle either "T" or "F" after each question.

1) I often try new things just for fun or thrills, even if most people think it is a waste of time. T F (for each)
2) I usually am confident that everything will go well, even in situation that worry most people.
3) I am often moved deeply by a fine speech or poetry.
4) I often feel that I am the victim of circumstances.
5) I can usually accept other people as they are, even when they are very different from me.
6) I believe that miracles happen
7) I enjoy getting revenge on people who hurt me.
8) Often when I am concentrating on something, I lose awareness of the passage of time.
9) Often I feel that my life has little purpose or meaning.
10) I like to help find a solution to problems so that everyone comes out ahead
11) I could probably accomplish more that I do, but I don't see the point in pushing myself harder than is necessary to get by.
12) I often feel tense and worried in unfamiliar situations, even when others feel there is little to worry about.
13) I often do things based on how I feel at the moment without thinking about how they were done in the past.
14) I usually do things my own way—rather than giving in to the wishes of other people.
15) I often feel so connected to the people around me that it is like there is no separation between us.
16) I generally don't like people who have different ideas from me.
17) In most situations my natural responses are based on good habits that I have developed.
18) I would do almost anything legal in order to become rich and famous, even if I would lose the trust of many old friends.
19) I am much more reserved and controlled than most people.
20) I often have to stop what I am doing because I start worrying about what might go wrong.
21) I like to discuss my experiences and feelings openly with friends instead of keeping them to myself.
22) I have less energy and get tired more quickly than most people.
23) I am often called "absent-minded" because I get so wrapped up in what I am doing that I lose track of everything else.
24) I seldom feel free to choose what I want to do.
25) I often consider another person's feelings as much as my own.
26) Most of the time I would prefer to do something a little risky (like riding in an automobile over steep hills and sharp turns) rather than having to stay quiet and inactive for a few hours.

27) I often avoid meeting strangers because I lack confidence with people I do not know.
28) I like to please other people as much as I can.
29) I like old "tried and true" ways of doing things much better than trying "new and improved" ways.
30) Usually I am not able to do things according to their priority of importance to me because of lack of time.
31) I often do things to help protect animals and plants from extinction.
32) I often wish that I was smarter than everyone else.
33) It gives me pleasure to see my enemies suffer.
34) I like to be very organized and set up rules for people whenever I can.
35) It is difficult for me to keep the same interests for a long time because my attention often shifts to something else.
36) Repeated practice has given me good habits that are stronger than most momentary impulses or persuasions.
37) I am usually so determined that I continued to work long after other people have given up.
38) I am fascinated by the many things in life that cannot be scientifically explained.
39) I have many bad habits that I wish I could break.
40) I often wait for someone else to provide a solution to my problems.
41) I often spend money until I run out of cash or get into debt from using too much credit.
42) I think I will have very good luck in the future.
43) I recover more slowly than most people from minor illnesses or stress.
44) It wouldn't bother me to be alone all the time.
45) Often I have unexpected flashed of insight or understanding while relaxing.
46) I don't care very much whether other people like me or the way I do things.
47) I usually try to get just what I want for myself because it is not possible to satisfy everyone anyway.
48) I have no patience with people who don't accept my views.
49) I don's seem to understand most people very well.
50) You don't have to be dishonest to succeed in business.
51) I sometimes feel so connected to nature that everything seems to be part of one living organism.
52) In conversations I am much better as a listener than as a talker.
53) I lose my temper more quickly than most people.
54) When I have to meet a group of strangers, I am more shy than most people.
55) I am more sentimental than most people.
56) I seem to have a "sixth sense" that sometimes allows me to know what is going to happen.
57) When someone hurts me in any way, I usually try to get even.
58) My attitudes are determined largely by influences outside my control.
59) Each day I try to take another step toward my goals.
60) I often wish I was stronger than everyone else.
61) I like to think about things for a long time before I make a decision.
62) I am more hard-working than most people.
63) I often need naps or extra rest periods because I get tired easily.
64) I like to be of service to others.
65) Regardless of any temporary problems that I have to overcome, I always think it will turn out well.
66) It is hard for me to enjoy spending money on myself, even when I have saved plenty of money.
67) I usually stay calm and secure in situations that most people would find physically dangerous.
68) I like to keep my problems to myself.
69) I don't mind discussing my personal problems with people whom I have known briefly or slightly.
70) I like to stay home better than to travel or explore new places.
71) I do not think it is smart to help weak people who cannot help themselves.
72) I cannot have any peace of mind if I treat other people unfairly, even if they are unfair to me.

73) People will usually tell me how they feel.
74) I often wish I could stay young forever.
75) I am usually more upset than most people by the loss of a close friend.
76) Sometimes I have felt like I was part of something with no limits of boundaries in time and space.
77) I sometimes feel a spiritual connection to other people that I cannot explain in words.
78) I try to be considerate of other people's feelings, even when they have been unfair to me in the past.
79) I like it when people can do whatever they want without strict rules and regulations.
80) I would probably stay relaxed and outgoing when meeting a group of strangers, even if I were told they are unfriendly.
81) Usually I am more worried than most people that something might go wrong in the future.
82) I usually think about all the facts in detail before I make a decision.
83) I feel it is more important to be sympathetic and understanding of other people than to be practical and though-minded.
84) I often feel a strong sense of unity with all the things around me.
85) I often wish I had special powers like Superman.
86) Other people control me too much.
87) I like to share what I have learned with other people.
88) Religious experiences have helped me understand the real purpose of my life.
89) I often learn a lot from people.
90) Repeated practice has allowed me to become good at many things that help me to be successful.
91) I am usually able to get other people to believe me, even when I know that what I am saying is exaggerated or untrue.
92) I need much extra rest, support, or reassurance to recover from minor illnesses or stress.
93) I know there are principles for living that no one can violate without suffering in the long run.
94) I don't want to be richer than everyone else.
95) I would gladly risk my own life to make the world a better place.
96) Even after thinking about something a long time, I have learned to trust my feelings mare than my logical reasons.
97) Sometimes I have felt my life was being directed by a spiritual force greater than any human being.
98) I usually enjoy being mean to anyone who has been mean to me.
99) I have a reputation as someone who is very practical and does not act on emotion.
100) It is easy for me to organize my thoughts while talking to someone.
101) I often react so strongly to unexpected news that I say or do things that I regret.
102) I am strongly moved by sentimental appeals (like when asked to help crippled children)
103) I usually push myself harder than most people do because I want to do as well as I possibly can.
104) I have so many faults that I don't like myself very much.
105) I have too little time to look for long-term solutions for my problems.
106) I often cannot deal with problems because I just don't know what to do.
107) I often wish I could stop the passage of time.
108) I hate to make decisions based only on my first impression.
109) I prefer spending money rather than saving it.
110) I can usually do a good job of stretching the truth to tell a funnier story or to play a joke on someone.
111) Even after there are problems in a friendship, I nearly always try to keep it going anyway.
112) If I am embarrassed or humiliated, I get over it very quickly.
113) It is extremely difficult for me to adjust to changes in my usual way of doing things because I get so tense, tired, or worried.
114) I usually demand very god practical reasons before I am willing to change my old ways of doing things.

115) I need a lot of help from other people to train me to have good habits.
116) I think that extra-sensory perception (ESP, like telepathy or precognitions) is really possible.
117) I would like to have warm and close friends with me most of the time.
118) I often keep trying the same thing over and over again, even when I have not had much success in a long time.
119) I nearly always stay relaxed and carefree, even when nearly everyone else is fearful.
120) I find sad songs and movies pretty boring.
121) Circumstances often force me to do things against my will.
122) It is hard for me to tolerate people who are different from me.
123) I think that most things that are called miracles are just chance.
124) I would rather be kind than to get revenge when someone hurts me.
125)I often become so fascinated with what I'm doing that I get lost in the moment, like I'm detached from time and place.
126) I do not think I have a real sense of purpose in my like.
127) I try to cooperate with others as much as possible.
128) I am satisfied with my accomplishments, and have little desire to do better.
129) I often feel tense and worried in unfamiliar situations, even when other feel there is no danger at all.
130) I often follow my instincts, hunches, or intuition without thinking through all the details.
131) Other people often think that I am too independent because I won't do what they want.
132) I often feel a strong spiritual or emotional connections with all the people around me.
133) It is usually easy for me to like people who have different values from me.
134) I try to do as little work as possible, even when other people expect more of me.
135) Good habits have become "second nature" to me—they are automatic and spontaneous actions nearly all the time.
136) I don't mind the fact that other people often know more than I do about something.
137) I usually try to imagine myself "in other people's shoes", so I can really understand them.
138) Principles like fairness and honesty have little role in some aspects of my life.
139) I am better at saving money than most people.
140) I seldom let myself get upset or frustrated: when things don't work out, I simply move on to other activities.
141) Even when most people feel it is not important, I often insist on things being done in a strict and orderly way.
142) I feel very confident and sure of myself in almost all social situations.
143) My friends find it hard to know my feelings because I seldom tell them about my private thoughts.
144) I hate to change the way that I do things, even if may people tell me there is a new and better way to do it.
145) I think it is unwise to believe in things that cannot be explained scientifically.
146) I like to imagine my enemies suffering.
147) I am more energetic and tire less quickly than most people.
148) I like to pay close attention to details in everything I do.
149) I often stop what I am doing because I get worried, even when my friends tell me everything will go well.
150) I often with I was more powerful than everyone else.
151) I am usually free to choose what I will do.
152) Often I become so involved in what I am doing that I forget where I am for a while.
153) Members of a team rarely get their fair share.
154) Most of the time I would prefer to do something risky (like hang-gliding or parachute jumping_, rather than having to stay quiet and inactive for a few hours.
155) Because I so often spend too much money on impulse, it is hard for me to save money—even for special plans like a vacation.

156) I don't go out of my way to please other people.
157) I am not shy with strangers at all.
158) I often give in to the wishes of friends.
159) I spend most of my time doing things that seem necessary but not really important to me.
160) I don't think that religious or ethical principles about what is right and wrong should have much influence in business decisions.
161) I often try to put aside my own judgments so that I can better understand what other people are experiencing.
162) Many of my habits make it hard for me to accomplish worthwhile goals.
163) I have made real personal sacrifices in order to make the world a better place—like trying to prevent war, poverty, and injustice.
164) I never worry about terrible things that might happen in the future.
165) I almost never get so excited that I lose control of myself.
166) I often give up a job if it takes much longer that I though it would.
167) I prefer to start conversations, rather than waiting for others to talk to me.
168) Most of the time I quickly forgive anyone who does me wrong.
169) My actions are determined largely by influences outside my control.
170) I often have to change my decisions because I had a wrong hunch or mistaken first impression.
171) I prefer to wait for someone else to tae the lead in getting things done.
172) I usually respect the opinions of others.
173) I have had experiences that made my role in life so clear to me that I felt very excited and happy.
174) It is fun for me to buy things for myself.
175) I believe that I have experienced extra-sensory perception in my like.
176) I believe that my brain is not working properly.
177) My behavior is strongly guided by certain goals that I have set for my life.
178) It is usually foolish to promote the success of other people.
179) I often wish I could live forever.
180) I usually like to stay cool and detached from other people.
181) I am more likely to cry at a sad movie than most people.
182) I recover more quickly than most people from minor illnesses or stress.
183) I often break rules and regulations when I think I can get away with it.
184) I need much more practice in developing good habits before I will be able to trust myself in many tempting situations.
185) I wish other people didn't talk as much as they do.
186) Everyone should be treated with dignity and respect, even if they seem to be unimportant or bad.
187) I like to make quick decisions so I can get on with what has to be done.
188) I usually have good luck in whatever I try to do.
189) I am usually confident that I can easily do things that most people would consider dangerous (such as driving an automobile fast on a wet or icy road).
190) I see no point in continuing to work on something unless there is a good chance of success.
191) I like to explore new ways to do things.
192) I enjoy saving money more than spending it on entertainment or thrills.
193) Individual rights are more important than the needs of any group.
194) I have had personal experiences in which I felt in contact with a divine and wonderful spiritual power.
195) I have had moments of great joy in which I suddenly had a clear, deep feeling of oneness with all that exists.
196) Good habits make it easier for me to do things that way I want.
197) Most people seem more resourceful than I am.
198) Other people and conditions are often to blame for my problems.

199) It gives me pleasure to help others, even if they have treated me badly.
200) I often feel like I am a part of the spiritual force on which all like depends.
201) Even when I am with friends, I prefer not to "open up" very much.
202) I usually can stay " on the go" all day without having to push myself.
203) I nearly always think about all the facts in detail before I make a decision, even when other people demand a quick decision.
204) I am not very good at talking my way out of trouble when I am caught doing something wrong.
205) I am more of a perfectionist than most people.
206) Whether something is right or wrong is just a matter of opinion.
207) I think my natural responses now are usually consistent with my principles and long-term goals.
208) I believe that all life depends on some spiritual order or power that cannot be completely explained.
209) I think I would stay confident and relaxed when meeting strangers, even if I were told they are angry at me.
210) People find it easy to come to me for help, sympathy, and warm understanding.
211) I am slower than most people to get excited about new ideas and activities.
212) I have trouble telling a lie, even when it is meant to spare someone else's feelings.
213) There are some people I don't like.
214) I don't want to be more admired than everyone else.
215) Often when I look at an ordinary thing, something wonderful happens—I get the feeling that I am seeing it fresh for the first time.
216) Many people I know look out only for themselves, no matter who else gets hurt.
217) I usually feel tense and worried when I have to do something new and unfamiliar.
218) I often push myself to the point of exhaustion or try to do more than I really can.
219) Some people think I am too stingy or tight with my money.
220) Reports of mystical experiences are probably just wishful thinking.
221) My will power is too weak to overcome very strong temptations, even if I know I will suffer as a consequence.
222) I hate to see anyone suffer.
223) I know what I want to do in my life.
224) I regularly take time to consider whether what I am doing is right or wrong.
225) Things often go wrong for me unless I am very careful.
226) If I am feeling upset, I usually fell better around friends then when left alone.
227) I don't think it is possible for one person to share feelings with someone else who hasn't had the same experiences.
228) It often seems to other people like I am in another world because I am so completely unaware of things going on around me.
229) I wish I were better looking than everyone else.
230) I have lied a lot on this questionnaire.
231) I usually stay away from social situations where I would have to meet strangers, even if I am assured that they will be friendly.
232) I love the blooming of flowers in the spring as much as seeing an old friend again.
233) I usually look at a difficult situation as a challenge or opportunity.
234) People involved with me have to learn how to do things my way.
235) Dishonesty only causes problems if you get caught.
236) I usually feel much more confident and energetic than most people, even after minor illnesses or stress.
237) I like to read everything when I am asked to sign any papers.
238) When nothing new is happening, I usually start looking for something that is thrilling or exciting.
239) Sometimes I get upset.

240) Occasionally I talk about people behind their backs.

Sensation Seeking Scale V (SSS-V, Zuckerman et al., 1978) is a 40-item self-report questionnaire that assesses individual differences in sensation seeking. The scale consists of four subscales (Boredom Susceptibility [BS], Thrill and adventure seeking [TAS], Experience seeking [ES], and Disinhibition [Dis]) composed of 10-items each. Completion time: ~5min.

Directions: Please rate each item with a True or False indication by circling the T or F next to each item.

-BS Items:
1) I can't stand watching a movie that I've seen before. T F (for each item)
2) I get bored seeing the same old faces.
3) When you can predict almost everything a person will do and say he or she must be a bore.
4) I usually don't enjoy a movie or play where I can predict what will happen in advance
5) Looking at someone's home movies or travel slides bores me tremendously.
6) I prefer friends who are excitingly unpredictable.
7) I get restless if I have to stay around home for any length of time.
8) The worst social sin is to be a bore.
9) I have no patience with dull or boring persons.
10) I like people who are sharp and witty even if they do sometimes insult people.

-TAS Items
1) I often wish I could be a mountain climber
2) I sometimes like to do things that are a little frightening.
3) I would like to take up the sport of water-skiing.
4) I would like to try surfboard riding.
5) I would like to learn to fly an airplane.
6) I would like to go scuba diving.
7) I would like to try parachute jumping.
8) I like to dive off the high board.
9) I would like to sail a long distance in a small but seaworthy sailing craft.
10) I think I would enjoy the sensations of skiing very fast down a high mountain slope.

-ES items:
1) I like some of the earthy body smells.
2) I like to explore a strange city or section of town myself, even if it means getting lost.
3) I have tried marijuana or would like to.
4) I would like to try some of the new drugs that produce hallucinations.
5) I like to try new foods that I have never tasted before.
6) I would like to take off on a trip with no pre-planned or definite routes or timetables.
7) I would like to make friends in some of the "far-out" groups like artists or "hippies".
8) I would like to meet some persons who are homosexual (men or women).
9) I often find beauty in the "clashing" of colors and irregular forms of modern painting.
10) People should dress in individual ways even if the effects are sometimes strange.

-DIS items:
1) I like wild "uninhibited" parties.
2) I enjoy the company of real "swingers".

3) I often like to get high (drinking liquor or smoking marijuana).
4) I like to have new and exciting experiences and sensations even if they are a little unconventional or illegal
5) I like to meet members of the opposite sex who are physically exciting.
6) Keeping the drinks full is the key to a good party.
7) A person should have considerable sexual experience before marriage.
8) I could conceive of myself seeking pleasures around the world with the “jet set”.
9) I enjoy watching many of the “sexy” scenes in movies.
10) I feel best after taking a couple of drinks.

Attitudes Towards Risk Questionnaire consists of 34-self report items rated on a 5-point scale that assess attitudes towards physical and psychological risk. Completion time: ~5min.

Directions: Indicate, using the 5-point scale, the degree to which each of the following statements describes you. Use the number 1 if the statement is a very good description of you (like me) and the number 5 to indicate it does not describe you at all (not like me). Use remaining numbers to indicate the varying degrees that the statement is like you or not like you.

Like me Not like me
1.....2.....3.....4.....5

1. I like the feeling that comes with taking physical risks.
2. I like the feeling that comes with taking psychological or social risks.
3. While I don't deliberately seek out situations or activities that involve physical risk, I often end up doing things that involve physical risk.
4. I often seek out situations or activities that society does not approve of.
5. While I don't deliberately seek out situations or activities that society disapproves of, I find that I often end up doing things that society disapproves of.
6. I often do things that I know my parents would disapprove of.
7. I often do things that I know some of my friends would disapprove of.
8. I often find that I am anxious or even scared of things that I am about to do.
9. I often do things that would hurt my reputation.
10. I often do things that would jeopardize my reputation.
11. I often do things that could jeopardize my friendships.
12. I never let fear get in the way of my doing things.
13. I like the feeling that comes from entering a new situation.
14. I don't let what other people think prevent me from doing new things.
15. I like to risk large sums of money.
16. I would be willing to risk my life in order to receive 10 million dollars.
17. I consider myself a risk-taker.
18. Being afraid of doing something new often makes it more fun in the end.
19. The greater the risk the more fun the activity.
20. I like to do things that almost paralyze me with fear.
21. I really don't care what people think of what I say and do.
22. I do not let the fact that something is illegal stop me from doing it.
23. I do not let the fact that something is considered immoral stop me from doing it.

Some people don't actually take risks but think about them. The following questions pertain to how much you think about risks:
24. I often think about doing activities that involve physical risk.
25. I often think about doing activities that involve social risk.
26. I often think about doing things that might jeopardize my health.
27. I often think about doing things that I know my friends would disapprove of.
28. I often think about doing things that I know my parents would disapprove of.
29. I often think about doing things that would arouse a great deal of fear or anxiety in me.
30. I often think about doing things that I know society would disapprove of.
31. I often think about doing things that are illegal.

32. I often think about doing things that are considered immoral.
33. I often think about doing things that would make me a lot of money.
34. I often think about things that would make me famous or notorious.

Toronto Alexithymia Scale (TAS-20): a 20-item self report questionnaire to assess emotional awareness. Completion time: ~5min.

Using the scale provided as a guide, indicate how much you agree or disagree with each of the following statements by circling the corresponding number. Give only one answer for each statement.

Circle1 if you STRONGLY AGREE
Circle 2 if you MODERATELY AGREE
Circle 3 if you NEITHER AGREE NOR DISAGREE
Circle 4 if you MODERATELY AGREE
Circle 5 if you STRONGLY AGREE

1) I am often confused about what emotion I am feeling.
2) It is difficult for me to find the right words for my feelings.
3) I have physical sensations that even doctors don't understand.
4) I am able to describe my feelings easily.
5) I prefer to analyze problems rather than just describe them.
6) When I am upset, I don't know if I am sad, frightened, or angry.
7) I am often puzzled by sensations in my body.
8) I prefer to just let things happen rather than to understand why they turned out that way.
9) I have feelings that I can't quite identify.
10) Being in touch with emotions is essential.
11) I find it hard to describe how I feel about people.
12) People tell me to describe my feelings more.
13) I don't know what's going on inside me.
14) I often don't know why I am angry.
15) I prefer talking to people about their dily activities rather than their feelings.
16) I prefer to watch "light" entertainment shows rather then psychological dramas.
17) It is difficult for me to reveal my innermost feelings, even to close friends.
18) I can feel close to someone, even in moments of silence.
19) I find examination of my feelings useful in solving personal problems.
20) Looking for hidden meanings in movies or plays distracts from their enjoyment.

Beck Depression Inventory (BDI)

Roche | **Beck Depression Inventory** | **Baseline**

V 0477 | CRTN: _____ CRF number: _____ | Page 14 | patient inits: _____

BDI-II | Date:

Name: _____ Marital Status: _____ Age: _____ Sex: _____

Occupation: _____ Education: _____

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the **one statement** in each group that best describes the way you have been feeling during the **past two weeks, including today**. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

1. Sadness
- 0 I do not feel sad.
- 1 I feel sad much of the time.
- 2 I am sad all the time.
- 3 I am so sad or unhappy that I can't stand it.

2. Pessimism
- 0 I am not discouraged about my future.
- 1 I feel more discouraged about my future than I used to be.
- 2 I do not expect things to work out for me.
- 3 I feel my future is hopeless and will only get worse.

3. Past Failure
- 0 I do not feel like a failure.
- 1 I have failed more than I should have.
- 2 As I look back, I see a lot of failures.
- 3 I feel I am a total failure as a person.

4. Loss of Pleasure
- 0 I get as much pleasure as I ever did from the things I enjoy.
- 1 I don't enjoy things as much as I used to.
- 2 I get very little pleasure from the things I used to enjoy.
- 3 I can't get any pleasure from the things I used to enjoy.

5. Guilty Feelings
- 0 I don't feel particularly guilty.
- 1 I feel guilty over many things I have done or should have done.
- 2 I feel quite guilty most of the time.
- 3 I feel guilty all of the time.

6. Punishment Feelings
- 0 I don't feel I am being punished.
- 1 I feel I may be punished.
- 2 I expect to be punished.
- 3 I feel I am being punished.

7. Self-Dislike
- 0 I feel the same about myself as ever.
- 1 I have lost confidence in myself.
- 2 I am disappointed in myself.
- 3 I dislike myself.

8. Self-Criticalness
- 0 I don't criticize or blame myself more than usual.
- 1 I am more critical of myself than I used to be.
- 2 I criticize myself for all of my faults.
- 3 I blame myself for everything bad that happens.

9. Suicidal Thoughts or Wishes
- 0 I don't have any thoughts of killing myself.
- 1 I have thoughts of killing myself, but I would not carry them out.
- 2 I would like to kill myself.
- 3 I would kill myself if I had the chance.

10. Crying
- 0 I don't cry anymore than I used to.
- 1 I cry more than I used to.
- 2 I cry over every little thing.
- 3 I feel like crying, but I can't.

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_____ Subtotal Page 1

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Beck Anxiety Inventory

Beck Anxiety Inventory

Below is a list of common symptoms of anxiety. Please carefully read each item in the list. Indicate how much you have been bothered by that symptom during the past month, including today, by circling the number in the corresponding space in the column next to each symptom.

	Not At All	Mildly but it didn't bother me much	Moderately - it wasn't pleasant at times	Severely – it bothered me a lot
Numbness or tingling	0	1	2	3
Feeling hot	0	1	2	3
Wobbliness in legs	0	1	2	3
Unable to relax	0	1	2	3
Fear of worst happening	0	1	2	3
Dizzy or lightheaded	0	1	2	3
Heart pounding/racing	0	1	2	3
Unsteady	0	1	2	3
Terrified or afraid	0	1	2	3
Nervous	0	1	2	3
Feeling of choking	0	1	2	3
Hands trembling	0	1	2	3
Shaky / unsteady	0	1	2	3
Fear of losing control	0	1	2	3
Difficulty in breathing	0	1	2	3
Fear of dying	0	1	2	3
Scared	0	1	2	3
Indigestion	0	1	2	3
Faint / lightheaded	0	1	2	3
Face flushed	0	1	2	3
Hot/cold sweats	0	1	2	3
Column Sum				

Scoring - Sum each column. Then sum the column totals to achieve a grand score. Write that score here _____ .

Interpretation

A grand sum between **0 – 21** indicates very low anxiety. That is usually a good thing. However, it is possible that you might be unrealistic in either your assessment which would be denial or that you have learned to "mask" the symptoms commonly associated with anxiety. Too little "anxiety" could indicate that you are detached from yourself, others, or your environment.

A grand sum between *22 – 35* indicates moderate anxiety. Your body is trying to tell you something. Look for patterns as to when and why you experience the symptoms described above. For example, if it occurs prior to public speaking and your job requires a lot of presentations you may want to find ways to calm yourself before speaking or let others do some of the presentations. You may have some conflict issues that need to be resolved. Clearly, it is not "panic" time but you want to find ways to manage the stress you feel.

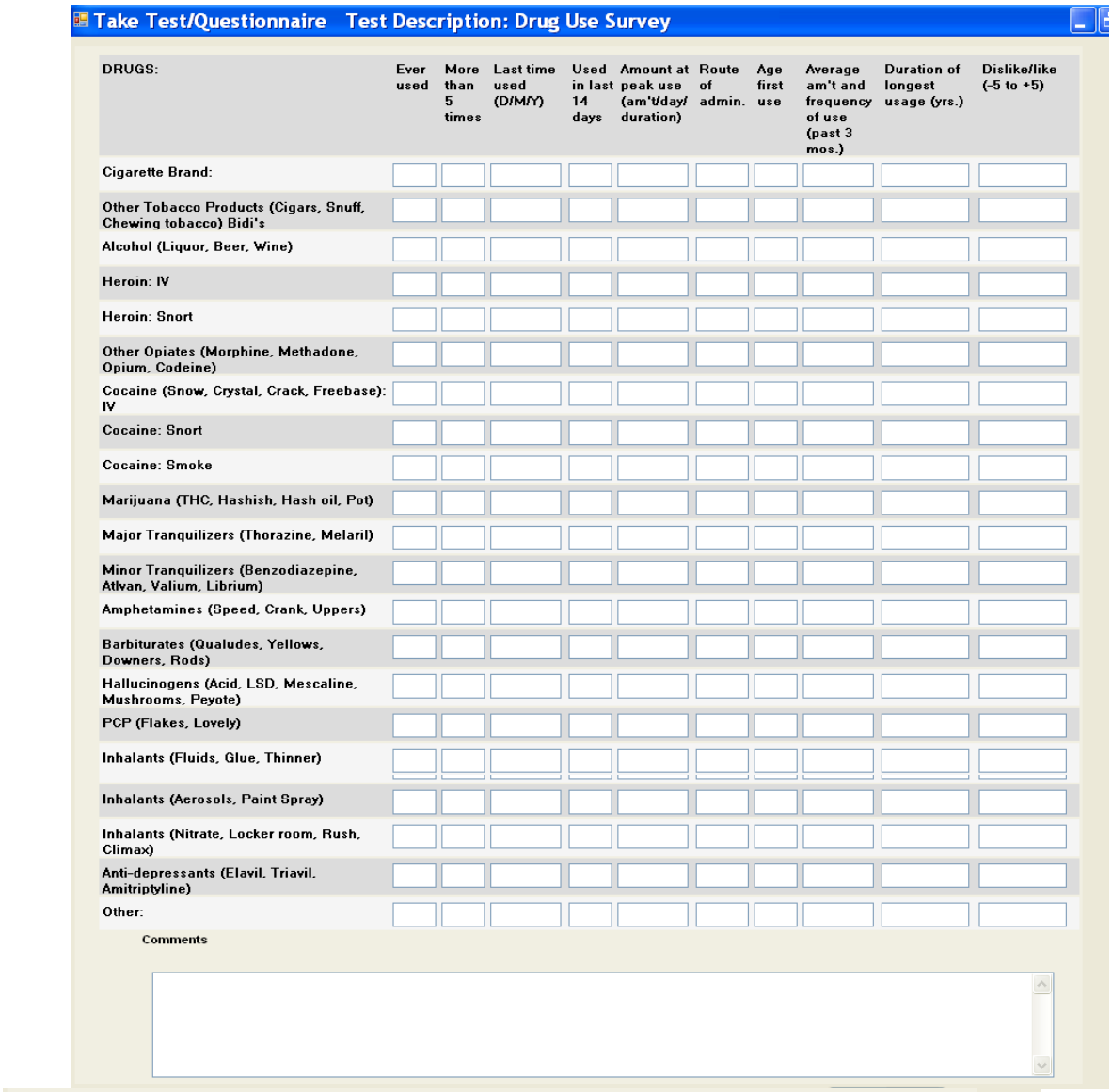
A grand sum that **exceeds 36** is a potential cause for concern. Again, look for patterns or times when you tend to feel the symptoms you have circled. Persistent and high anxiety is not a sign of personal weakness or failure. It is, however, something that needs to be proactively treated or there could be significant impacts to you mentally and physically. You may want to consult a physician or counselor if the feelings persist.

Adult ADHD Self Report Scale (ASRS)

Adult ADHD Self-Report Scale (ASRS-v1.1) Symptom Checklist

Patient Name		Today's Date					
Please answer the questions below, rating yourself on each of the criteria shown using the scale on the right side of the page. As you answer each question, place an X in the box that best describes how you have felt and conducted yourself over the past 6 months. Please give this completed checklist to your healthcare professional to discuss during today's appointment.			Never	Rarely	Sometimes	Often	Very Often
1. How often do you have trouble wrapping up the final details of a project, once the challenging parts have been done?							
2. How often do you have difficulty getting things in order when you have to do a task that requires organization?							
3. How often do you have problems remembering appointments or obligations?							
4. When you have a task that requires a lot of thought, how often do you avoid or delay getting started?							
5. How often do you fidget or squirm with your hands or feet when you have to sit down for a long time?							
6. How often do you feel overly active and compelled to do things, like you were driven by a motor?							
							Part A
7. How often do you make careless mistakes when you have to work on a boring or difficult project?							
8. How often do you have difficulty keeping your attention when you are doing boring or repetitive work?							
9. How often do you have difficulty concentrating on what people say to you, even when they are speaking to you directly?							
10. How often do you misplace or have difficulty finding things at home or at work?							
11. How often are you distracted by activity or noise around you?							
12. How often do you leave your seat in meetings or other situations in which you are expected to remain seated?							
13. How often do you feel restless or fidgety?							
14. How often do you have difficulty unwinding and relaxing when you have time to yourself?							
15. How often do you find yourself talking too much when you are in social situations?							
16. When you're in a conversation, how often do you find yourself finishing the sentences of the people you are talking to, before they can finish them themselves?							
17. How often do you have difficulty waiting your turn in situations when turn taking is required?							
18. How often do you interrupt others when they are busy?							
							Part B

Drug Use Survey



Appendix 4: State Measures (*administered during treatment sessions; #administered during scan sessions; + administered during follow-up scan sessions)

*#+1) The Tobacco Craving Questionnaire (Singleton, Anderson, & Heishman, 2003) is a brief instrument used to assess current feelings related to smoking and craving using 12 Likert-type items. Each item is rated on a 7-point scale from strongly disagree to strongly agree. Completion time: ~10min.

*Directions: Indicate how strongly you agree or disagree with each of the following statements by placing a check mark in one of the spaces between STRONGLY DISAGREE and STRONGLY AGREE. The closer you place your check mark to one end or the other indicates the strength of your agreement or disagreement. If you don't agree or disagree with a statement, place your check mark in the middle space. Please complete every item. We are interested in how you are thinking or feeling **right now** as you are filling out the questionnaire.*

1. I would enjoy a cigarette right now.
STRONGLY DISAGREE ____ : ____ : ____ : ____ : ____ : ____ : ____ STRONGLY AGREE

2. If I smoked right now, I would not be able to stop.
STRONGLY DISAGREE ____ : ____ : ____ : ____ : ____ : ____ : ____ STRONGLY AGREE

3. If I had a lit cigarette in my hand, I probably would smoke it.
STRONGLY DISAGREE ____ : ____ : ____ : ____ : ____ : ____ : ____ STRONGLY AGREE

4. A cigarette would taste good right now.
STRONGLY DISAGREE ____ : ____ : ____ : ____ : ____ : ____ : ____ STRONGLY AGREE

5. I would be less irritable now if I could smoke.
STRONGLY DISAGREE ____ : ____ : ____ : ____ : ____ : ____ : ____ STRONGLY AGREE

6. It would be hard to pass up the chance to smoke.
STRONGLY DISAGREE ____ : ____ : ____ : ____ : ____ : ____ : ____ STRONGLY AGREE

7. I could not stop myself from smoking if I had some cigarettes here.
STRONGLY DISAGREE ____ : ____ : ____ : ____ : ____ : ____ : ____ STRONGLY AGREE

8. Smoking a cigarette would be pleasant.
STRONGLY DISAGREE ____ : ____ : ____ : ____ : ____ : ____ : ____ STRONGLY AGREE

9. If I were smoking now I could think more clearly.
STRONGLY DISAGREE ____ : ____ : ____ : ____ : ____ : ____ : ____ STRONGLY AGREE

10. I would not be able to control how much I smoked if I had some cigarettes here.
STRONGLY DISAGREE ____ : ____ : ____ : ____ : ____ : ____ : ____ STRONGLY AGREE

11. I could not easily limit how much I smoked right now.
STRONGLY DISAGREE ____ : ____ : ____ : ____ : ____ : ____ : ____ STRONGLY AGREE

12. I could control things better right now if I could smoke.
STRONGLY DISAGREE ____ : ____ : ____ : ____ : ____ : ____ : ____ STRONGLY AGREE

*#+2) Tobacco Craving Scale consists of 5 self-report items pertaining to desire for a cigarette that are rated on a 10-point scales. Completion time: > 5 min.

Directions: Please circle the number on the scale that best describes your current feelings.

Please rate how strong your desire for a cigarette is right now.

No desire *Extremely strong*

0 1 2 3 4 5 6 7 8 9 10

Please rate how strong your desire for a cigarette was during the last 24 hours.

No desire *Extremely strong*

0 1 2 3 4 5 6 7 8 9 10

Please rate how often you had the urge to smoke during the past 24 hours.

Not at all *Extremely often*

0 1 2 3 4 5 6 7 8 9 10

In the past 24 hours, please rate how strong your urges have been for a cigarette when something in the environment has reminded you of it.

No urges *Extremely strong*

0 1 2 3 4 5 6 7 8 9 10

Please imagine yourself in the environment in which you previously used drugs and/or alcohol. If you were in this environment right now, what is the likelihood that you would smoke?

Not at all *I'm sure I would use*

0 1 2 3 4 5 6 7 8 9 10

*#+3) Wisconsin Smoking Withdrawal Scale (WSWS) is a 28-item scale that measures the severity of smoking withdrawal symptoms.

Please answer the following questions based on how you have felt or what you have noticed over the last 24 hours. Answer based on how you have felt in general during this time.

0	1	2	3	4
Strongly disagree	Disagree	Feel neutral	Agree	Strongly agree

1. Food is not particularly appealing to me
2. I am getting restful sleep
3. I have been tense or anxious
4. My level of concentration is excellent
5. I awaken from sleep frequently during the night
6. I have felt impatient
7. I have felt upbeat and optimistic
8. I have found myself worrying about my problems
9. I have had frequent urges to smoke
10. I have felt calm lately
11. I have been bothered by the desire to smoke a cigarette
12. I have felt sad or depressed
13. I have been irritable, easily angered
14. I want to nibble on snacks or sweets
15. I have been bothered by negative moods such as anger, frustration, and irritability
16. I have been eating a lot.
17. I am satisfied with my sleep
18. I have felt frustrated
19. I have felt hopeless or discouraged
20. I have thought about smoking a lot.
21. I have felt hungry
22. I feel that I am getting enough sleep.
23. It is hard to pay attention to things
24. I have felt happy and content
25. My sleep has been troubled
26. I have trouble getting cigarettes off my mind
27. It has been difficult to think clearly.
28. I think about food a lot.

*#+4) The Positive and Negative Affect Schedule (Watson, Clark, & Tellegen, 1988) is a 20-item scale composed of 10 items describing negative affect and 10 items describing positive affect. Completion time: ~5 min.

Directions: This scale consists of a number of words that describe different feelings and emotions. Read each item and then select the appropriate answer. Indicate to what extent you feel this way RIGHT NOW, that is, AT THE PRESENT MOMENT.

(1) = Very slightly or not at all (2) = A little (3) = Moderately (4) = Quite a bit (5) = Extremely

	Very slightly or not at all	A little	Moderately	Quite a bit	Extremely
1. Interested	1	2	3	4	5
2. Distressed	1	2	3	4	5
3. Excited	1	2	3	4	5
4. Upset	1	2	3	4	5
5. Strong	1	2	3	4	5
6. Guilty	1	2	3	4	5
7. Scared	1	2	3	4	5
8. Hostile	1	2	3	4	5
9. Enthusiastic	1	2	3	4	5
10. Proud	1	2	3	4	5
11. Irritable	1	2	3	4	5
12. Alert	1	2	3	4	5
13. Ashamed	1	2	3	4	5
14. Inspired	1	2	3	4	5
15. Nervous	1	2	3	4	5
16. Determined	1	2	3	4	5
17. Attentive	1	2	3	4	5
18. Jittery	1	2	3	4	5
19. Active	1	2	3	4	5
20. Afraid	1	2	3	4	5

#5) Visual analog scales will also be used to assess the degree to which various nicotine withdrawal symptoms may be experienced, including stress, anxiety, and desire to smoke (craving). Completion time ~5 min.

Mark: BASELINE POST Scan 1 Scan 2

Please read/answer the following questions. Think about how you are feeling right now.

1. How stressed are you?

Not at all Extremely

0 1 2 3 4 5 6 7 8 9 10

2. How anxious are you?

Not at all Extremely

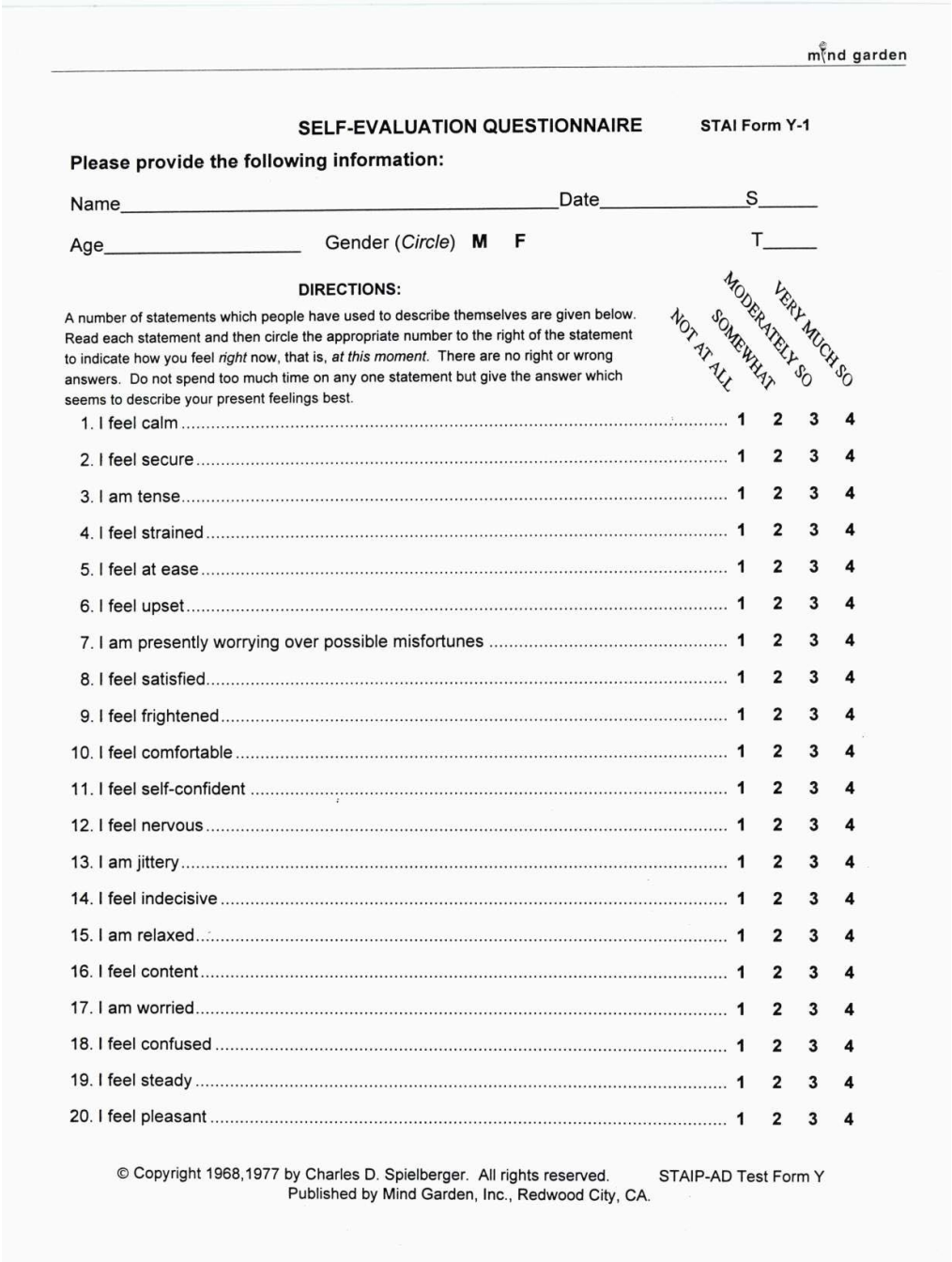
0 1 2 3 4 5 6 7 8 9 10

3. How much are you craving a cigarette?

Not at all Extremely

0 1 2 3 4 5 6 7 8 9 10

#+6) The State-Trait Anxiety Inventory Form Y (STAI) (Spielberger, 1983) is an instrument for measuring anxiety in adults. Participants will complete the state anxiety form which measures the temporary condition of "state anxiety" characterized by feelings of apprehension, tension, nervousness, and worry. Completion time: ~10min.



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SELF-EVALUATION QUESTIONNAIRE **STAI Form Y-1**

Please provide the following information:

Name_____Date_____S_____

Age_____ Gender (*Circle*) **M** **F** T_____

DIRECTIONS:

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel *right* now, that is, *at this moment*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	NOT AT ALL	SOMEWHAT	MODERATELY SO	VERY MUCH SO
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I am tense	1	2	3	4
4. I feel strained	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over possible misfortunes	1	2	3	4
8. I feel satisfied	1	2	3	4
9. I feel frightened	1	2	3	4
10. I feel comfortable	1	2	3	4
11. I feel self-confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I am jittery	1	2	3	4
14. I feel indecisive	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel confused	1	2	3	4
19. I feel steady	1	2	3	4
20. I feel pleasant	1	2	3	4

 STAIP-AD Test Form Y

Published by Mind Garden, Inc., Redwood City, CA.

*#+7) Snaith-Hamilton pleasure scale (Snaith et al., 1995) is designed to measure hedonic tone. Subjects indicate the extent to which they agree or disagree with a series of statements relating to their expected enjoyment of a range of normally pleasurable events.

Directions: This questionnaire is designed to measure your ability to experience pleasure during your current abstinence period. It is important to read each statement very carefully. Check one of the boxes [ ] to indicate how much you agree or disagree with each statement.

For each item: [ ] strongly disagree [ ] disagree [ ] agree [ ] strongly agree

1) I would enjoy my favorite television or radio program:
2) I would enjoy being with my family or close friends:
3) I would find pleasure in my hobbies and pastimes:
4) I would be able to enjoy my favorite meal:
5) I would enjoy a warm bath or refreshing shower:
6) I would find pleasure in the scent of flowers or the smell of a fresh sea breeze or freshly baked bread:
7) I would enjoy seeing other people's smiling faces:
8) I would enjoy looking "smart" when I have made and effort with my appearance:
9) I would enjoy reading a book, magazine, or newspaper:
10) I would enjoy a cup of tea or coffee or my favorite drink:
11) I would find pleasure in small things, e.g., bright sunny day, a telephone call from a friend:
12) I would be able to enjoy a beautiful landscape or view:
13) I would get pleasure from helping others:
14) I would feel pleasure when I receive praise from other people:

*8) Time-line followback. (TLFB). The TLFB will be used to assess smoking behavior since last an individual's last treatment visit (e.g., 7 days). Participants will read the instructions and will be guided by the therapist in filling out the calendar.

Instructions for Filling Out the Timeline Cigarette Use Calendar

To help us evaluate your cigarette use, we need to get an idea of what your smoking was like in the past ____ days. To do this, we would like you to fill out the attached calendar.

- ✓ Filling out the calendar is not hard!
- ✓ Try to be as accurate as possible.
- ✓ We recognize you won't have perfect recall. That's OKAY.

✓ WHAT TO FILL IN

- The idea is to record how many cigarettes you smoked for **each day** on the calendar.
- On days when you **did not smoke cigarettes**, not even one, you should write a "0."

It's important that something is written for every day, even if it is a "0".

✓ YOUR BEST ESTIMATE

- We realize it isn't easy to recall things with 100% accuracy.
- If you are not sure whether you smoked 15 or 16 cigarettes or whether you smoked on a Thursday or a Friday, give it your best guess! What is important is that 15 or 16 cigarettes is very different from 1 cigarette. The goal is to get a sense of how frequently you smoked, how much you smoked, and your patterns of smoking.

✓ HELPFUL HINTS

- If you have an appointment book you can use it to help you recall your use.
- Holidays such as Thanksgiving and Christmas are marked on the calendar to help you recall your smoking. Also, think about how much you smoked on personal holidays & events such as birthdays, vacations, or parties.
- If you have **regular patterns to your smoking**, you can use these to help you recall your use. For example, some people may only smoke during social situations.

✓ COMPLETING THE CALENDAR

- A blank calendar is attached. Write in the number of cigarettes you smoked on **each day**.
- The time period we are talking about on the calendar is

  from _____ to _____.
- In estimating the number of cigarettes you smoked, be as accurate as possible.
- **DOUBLE CHECK THAT <u>ALL</u> DAYS ARE FILLED IN BEFORE RETURNING THE CALENDAR.**
- Before you start look at the **SAMPLE CALENDAR** on the next page.

Instructions for Filling Out the Timeline Cigarette Calendar

✓ **SAMPLE CALENDAR**

2000	SUN	MON	TUES	WED	THURS	FRI	SAT
						1 20	2 0
S	3 20	4 Labor Day 20	5 23	6 28	7 21	8 20	9 23
E	10 20	11 20	12 20	13 28	14 25	15 0	16 24
P	17 20	18 20	19 20	20 20	21 22	22 22	23 24
T	24 21	25 22	26 26	27 24	28 23	29 0	30 22

CDW Entry (for investigator entry):
TLFB – Tobacco Cigarettes

Date of Last Visit:_____
Enter the number of cigarettes smoked per day since the last session, starting with yesterday and working backwards.
Day 1 (yesterday)
Day 2
Day 3
Etc…

*#+9) Perceived Stress Scale (PSS; Cohen, Kamarck, & Mermelstein, 1983). The PSS is a brief instrument used to assess degree of perceived stress during the past month using 10 Likert-type items. Each item is rated on a 5-point scale from never to very often. Completion time: ~5min.

The questions in this scale ask you about your feelings and thoughts **during the last month**. In each case, you will be asked to indicate by circling *how often* you felt or thought a certain way.

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often 4 = Very Often

1. In the last month, how often have you been upset because of something that happened unexpectedly?..... **0 1 2 3 4**

2. In the last month, how often have you felt that you were unable to control the important things in your life?..... **0 1 2 3 4**

3. In the last month, how often have you felt nervous and "stressed"? **0 1 2 3 4**

4. In the last month, how often have you felt confident about your ability to handle your personal problems?..... **0 1 2 3 4**

5. In the last month, how often have you felt that things were going your way?..... **0 1 2 3 4**

6. In the last month, how often have you found that you could not cope with all the things that you had to do? **0 1 2 3 4**

7. In the last month, how often have you been able to control irritations in your life?..... **0 1 2 3 4**

8. In the last month, how often have you felt that you were on top of things?..... **0 1 2 3 4**

9. In the last month, how often have you been angered because of things that were outside of your control?..... **0 1 2 3 4**

10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?..... **0 1 2 3 4**

tDCS Arm Questionnaires

The Minnesota Nicotine Withdrawal scale (Hughes and Hatsukami, 1998) yields a measure of total withdrawal related discomfort based on ratings from 15 self-report items. Completion time: ~5 min.

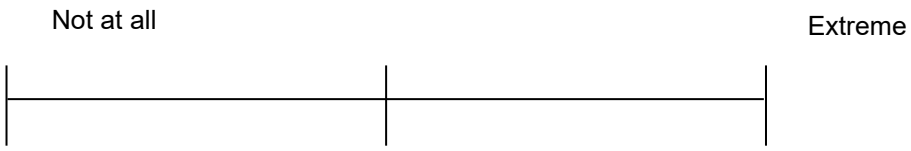
Directions: Please rate the degree to which you have experienced the following, over the past 24 hrs.

0=none, 1=slight, 2=mild, 3=moderate, 4=severe

1)	Angry, Irritable, frustrated	0 1 2 3 4
2)	Anxious, Nervous	0 1 2 3 4
3)	Depressed mood, Sad	0 1 2 3 4
4)	Desire or craving to smoke	0 1 2 3 4
5)	Difficulty concentrating	0 1 2 3 4
6)	Increased appetite	0 1 2 3 4
7)	Insomnia, sleep problems, awakening at night	0 1 2 3 4
8)	Restless	0 1 2 3 4
9)	Impatient	0 1 2 3 4
10)	Constipation	0 1 2 3 4
11)	Dizziness	0 1 2 3 4
12)	Coughing	0 1 2 3 4
13)	Dreaming or Nightmares	0 1 2 3 4
14)	Nausea	0 1 2 3 4
15)	Sore throat	0 1 2 3 4

Visual analog scales (100mm) will also be used to assess the degree to which various nicotine withdrawal symptoms may be experienced: depressed, clumsy, tired, anxious, happy, drowsy, sad, dizzy, alert, energetic, light-headed, irritable, frustrated, nervous, sad, desire to smoke, difficulty concentrating, increased appetite, hunger, weight-gain, difficultly sleeping, awakening at night, insomnia, restless, impatient, panicked, missing cigarettes, urge to smoke, disoriented. Completion time ~10 min.

(This Visual Analog Scale is a different version from the one given as part of the Main Study Arm.)



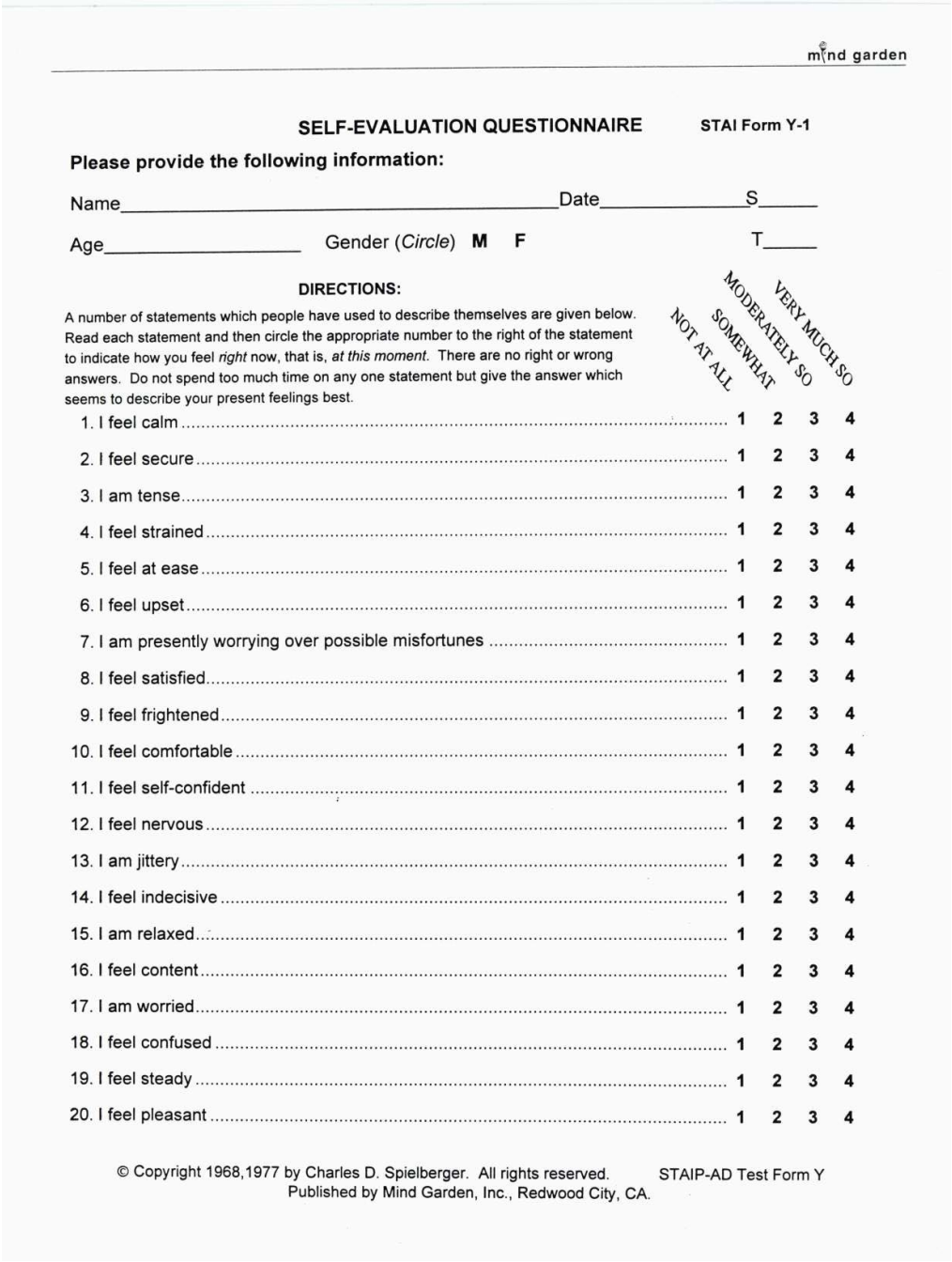
The Profile of Mood States (POMS) measures present mood state (disturbance) by a list of adjectives on a 5-point scale and measures six dimensions of affect or mood, including tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, and confusion-bewilderment. The measure has been shown to produce reliable and valid profiles of mood state (McNair et al., ; Cella et al., 1989). Completion time ~10min.

Directions: Indicate HOW YOU FEEL RIGHT NOW by selecting one of the numbers on the 5-poit scale.

FEELING	Not at all	A little	Moderate	Quite a bit	Extremely
Friendly	1	2	3	4	5

Tense	Lonely
Angry	Miserable
Worn Out	Muddled
Unhappy	Cheerful
Clear-headed	Bitter
Lively	Exhausted
Confused	Anxious
Sorry for things done	Ready to fight
Shaky	Good-natured
Listless	Gloomy
Peeved	Desperate
Considerate	Sluggish
Sad	Rebellious
Active	Helpless
On edge	Weary
Grouchy	Bewildered
Blue	Alert
Energetic	Deceived
Panicky	Furious
Hopeless	Efficacious
Relaxed	Trusting
Unworthy	Full of pep
Spiteful	Bad-tempered
Sympathetic	Worthless
Uneasy	Forgetful
Restless	Carefree
Unable to concentrate	Terrified
Fatigued	Guilty
Helpful	Vigorous
Annoyed	Uncertain about things
Discouraged	Bushed
Resentful	
Nervous	

The State-Trait Anxiety Inventory Form Y (STAI) is an instrument for measuring anxiety in adults. The STAI differentiates between the temporary condition of "state anxiety" and the more general and long-standing quality of "trait anxiety." The essential qualities evaluated by the STAI-anxiety scale are feelings of apprehension, tension, nervousness, and worry. Completion time: ~10min.
The copy below includes the Trait Inventory (in addition to the State Inventory given in the Main Study Arm)



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SELF-EVALUATION QUESTIONNAIRE **STAI Form Y-1**

Please provide the following information:

Name_____Date_____S_____

Age_____ Gender (*Circle*) **M F** T_____

DIRECTIONS:

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel *right* now, that is, *at this moment*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	NOT AT ALL	SOMEWHAT	MODERATELY SO	VERY MUCH SO
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I am tense	1	2	3	4
4. I feel strained	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over possible misfortunes	1	2	3	4
8. I feel satisfied	1	2	3	4
9. I feel frightened	1	2	3	4
10. I feel comfortable	1	2	3	4
11. I feel self-confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I am jittery	1	2	3	4
14. I feel indecisive	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel confused	1	2	3	4
19. I feel steady	1	2	3	4
20. I feel pleasant	1	2	3	4

 STAIP-AD Test Form Y
Published by Mind Garden, Inc., Redwood City, CA.

SELF-EVALUATION QUESTIONNAIRE

STAI Form Y-2

Name_____ Date_____

DIRECTIONS

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you *generally* feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you generally feel.

	ALMOST NEVER	SOMETIMES	OFTEN	ALMOST ALWAYS
21. I feel pleasant	1	2	3	4
22. I feel nervous and restless	1	2	3	4
23. I feel satisfied with myself	1	2	3	4
24. I wish I could be as happy as others seem to be	1	2	3	4
25. I feel like a failure	1	2	3	4
26. I feel rested	1	2	3	4
27. I am "calm, cool, and collected"	1	2	3	4
28. I feel that difficulties are piling up so that I cannot overcome them	1	2	3	4
29. I worry too much over something that really doesn't matter	1	2	3	4
30. I am happy	1	2	3	4
31. I have disturbing thoughts	1	2	3	4
32. I lack self-confidence	1	2	3	4
33. I feel secure	1	2	3	4
34. I make decisions easily	1	2	3	4
35. I feel inadequate	1	2	3	4
36. I am content	1	2	3	4
37. Some unimportant thought runs through my mind and bothers me	1	2	3	4
38. I take disappointments so keenly that I can't put them out of my mind	1	2	3	4
39. I am a steady person	1	2	3	4
40. I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4

Menstrual History Questionnaire

Adapted from the Fernald Community Cohort/Medical Monitoring Program
2007 Questionnaire (Female).
University of Cincinnati College of Medicine, Department of Environmental Health.
URL: https://med.uc.edu/eh/research/projects/fcc/questionnaire, https://med.uc.edu/docs/default-source/Environmental-Health/fcc/y2007-questionnaire_female.pdf?sfvrsn=0

1. How old were you when you started having menstrual periods?
Age: _____
1a. If you cannot remember your exact age, were you:
- □ Younger than 10
- □ 16 or older
- □ 10-12 yrs old
- □ 13-15 yrs old
- □ Don't Know

2. At present which statement best describes your menstrual cycle?
- □ I'm still having regular periods: The date of my last period was: ___/___/_____
- □ My periods are irregular: The date of my last period was: ___/___/_____
- □ I'm pregnant, or my last pregnancy ended within the past 2 months, or I'm breast feeding
- □ My periods have stopped on their own. (I've had menopause.)
- □ I've had menopause, but now have periods because I am taking hormones.
- □ I've had an operation (surgery) which stopped my periods.

If your menstrual periods ceased because of surgery, what did you have removed?
- □ One ovary only
- □ Uterus only
- □ Both ovaries
- □ Uterus and one ovary
- □ Uterus and both ovaries
- □ Don't know
- □ I've taken medication which has stopped my periods.

If your periods stopped because of medication, which medication were you taking? Medication name: _____
□ I"ve had chemotherapy which has stopped my periods.
□ I've had radiation therapy which has stopped my periods.
□ Other: _____

3. If your menstrual periods have stopped, how old were you when your menstrual periods stopped? (Please provide us with the age at which your menstrual periods stopped regardless of why they have stopped – naturally, due to surgery, medication, chemotherapy, or radiation therapy. If your periods have stopped, but you now have periods because of taking hormones, answer with the age at which your periods first stopped.)

Were you:

□ Younger than 20
□ 45-49 yrs old
□ 20-29 yrs old
□ 50-54 yrs old
□ 30-39 yrs old
□ 55 – 59 yrs old
□ 40-44 yrs old
□ 60 or older
OR □ My menstrual periods have not stopped.

4. If your menstrual periods have stopped, how old were you when you first experienced symptoms of menopause such as hot flashes or night sweats?
_____ Years old □ Did not experience symptoms
□ Don't Know
OR □ My menstrual periods have not stopped.

All women should answer the next two questions, whether they currently have menstrual periods or not.

5. When you are (were) having regular menstrual cycles, how many days are (were) there between periods? _____ Days between periods
For how many days do (did) you have your period? _____ Days

6. Between the ages of 18 and 40, excluding times when you may have been on the pill, pregnant, or nursing, which of the following statements BEST describes your menstrual periods? They are (were)…

□ Nearly always regular, that is, you could usually predict when you would start bleeding to within two or three days
□ Fairly Regular
□ Irregular
□ Don't Know

Appendix 5: Nicotine Patch Instructions

Read ALL the instructions before you begin.

Your abstinence period begins at 8pm, two days before your next NIDA visit (e.g., 8pm Saturday night if your visit is at 8am on Monday morning). Smoke a cigarette after dinner, before you commence the abstinence period, if this is usual for you. You will receive a phone call from a study investigator between 5pm and 8pm to ensure that you remember and understand the abstinence procedure. You will place the patch on as soon as you wake up the following day, that is, when you would typically have your first cigarette. To place and change the patch, follow the instructions outlined below. Do not smoke any cigarettes while you are wearing the patch. Since the nicotine patch works by delivering small doses of nicotine into the body, smoking may cause harmful levels of nicotine in your body.

How to apply the nicotine patch:

1. Do not remove the patch from its sealed protective pouch until you are ready to use it.
2. Choose a non-hairy, clean, dry area of skin. The upper arm is OK; we recommend the back where we will place the patch during the study. Do not put the patch on skin that is burned, broken out, cut, or irritated in any way. Make sure your skin is free of lotion and soap before applying the patch.
3. A clear, protective liner covers the sticky backside of the patch – the side that will be put on your skin. The liner has a slit down the middle to help you remove it from the patch. With the sticky backside facing you, pull half the liner away from the patch starting at the middle slit, as shown in the illustration below. Hold the patch at one of the outside edges (touch the sticky side as little as possible), and pull off the other half of the protective liner.

4. Immediately apply the sticky side of the patch to your skin. **Press the patch firmly on your skin with the palm of your hand for at least 10 seconds.** Make sure it sticks well to your skin, especially around the edges. Mild itching, burning or tingling is normal and should go away within an hour.

5. Wash your hands after you apply the patch. Nicotine on your hands could get into your eyes and nose, and cause stinging, redness, or more serious problems.
6. After wearing the patch for 4 hours call the study nurses at **443-740-2294** to tell them how you are responding. If you do not call by 4:00 PM a member of the research team will contact you. Continue wearing the patch for the entire day and evening. Remove the patch just before you go to bed, the night before your NIDA visit.
7. On the morning of your NIDA visit, place a new patch on as soon as you wake up.

If your patch comes off during the day:

The patch generally sticks well to most people's skin. However, a patch may occasionally come off. If your patch falls off during your abstinence period, re-apply as soon as possible and hold in place with medical tape.

To prevent the patch from coming off, do not apply creams or lotions to the area on your skin where you will put the patch. Also, do not bathe, swim or shower during your test.

If you get a minor skin rash:

Use a topical salve (such as a hydrocortisone cream) around the patch.

If you experience any worrisome side effect from the patch (listed below):

Remove it at once and call **Dr. Betty Jo Salmeron at 443-740-2651**. **Also, the study nurses can be reached at 443-740-2294 (available 24 hours every day).**

Side effects of the nicotine patch include: dizziness, headache, upset stomach, vomiting, diarrhea, redness or swelling at the patch site.

Very rare serious side effects of the nicotine patch include: severe rash or swelling, seizures, abnormal heartbeat or rhythm, difficulty breathing.

Appendix 6: Coping strategies

Methods to help you cope with smoking withdrawal symptoms

1. Reminding yourself of the positive consequences of not smoking (such as improved health and appearance, feelings of success and self-control, monetary savings) as well as the negative consequences of smoking (e.g., such as the smell on clothing, hands and breath, negative health consequences).

Exercise

Make a list of reasons you want to quit smoking. Try not to only come up with negative consequences to be avoided, but also benefits to be made. From this list, pick the number 1 reason you want to quit smoking. This can function as a simple internal message of encouragement when you are finding the abstinence period difficult.

spending time with nonsmoking friends, going to the movies, taking long walks, cooking.

3. Keep your hands active. Some smokers miss the tactile sensation of holding a cigarette. Find something you can hold and manipulate to help with this. Manipulating a stress ball or just a pen or pencil can help relieve the desire to hold a cigarette.

4. Have healthy snacks on hand. You may find that your appetite increases when you stop smoking. Having healthy snacks available (e.g., carrot or celery sticks, sugarless candie or chewing gum – not nicotine gum) will help with this. Also, instead of taking a cigarette break, take a break and have a snack instead.

5. Relaxation. Feeling tense or stressed will make it even more difficult for you to stop smoking. We have provided you with a 10min progressive muscle relaxation guide that you can use to help reduce tension, relieve stress and help you relax. Try doing this 10 min relaxation tape when you are feeling particularly tense/stressed or particularly strong desire to smoke.

   Here are some progressive relaxation videos you can follow:
   http://www.youtube.com/watch?v=HFwCKKa--18
   http://www.youtube.com/watch?v=PYsuvRNZfxE
   http://www.youtube.com/watch?v=KmxfjjamcuY

Appendix 7: Varenicline Toleration Assessment

After participants start varenicline, a masters level therapist, LI, or study clinician will conduct a semi-structured interview to assess toleration of varenicline. The assessment will be conducted weekly, either in person or via phone, while participants are taking varenicline, and for two weeks after their last dose. The therapist will be extensively trained by the MAI on question administration. If the interview reveals a possibility of significant side effects, the participant will be put in contact with the MAI for further evaluation. The MAI will decide whether study pill administration needs to be discontinued and other action taken based on the severity of the symptoms.

1) Since we last spoke or met, how have you been feeling? (open-ended question)

2) Have you noticed any physical side effects [or, a change in the severity of any physical side effects], such as nausea, vomiting, dizziness, or headache? Anything else?

3) Have you noticed a change in behavior that would be considered out of the ordinary for you?

4) Do you feel more restless, wound-up, or agitated than usual?

5) Do you feel more irritable or cranky than usual?

6) Have you noticed an increase in feelings of depression, such as sadness, hopelessness, helplessness or worthlessness?

8) Have you had thoughts of violence towards others? …acted aggressively towards someone?

7) Have you had thoughts of death? …wished you were dead? …had thoughts of killing yourself?

Participants will also be reminded to call if any issues should arise between assessments.

Appendix 8: Take-home pill instructions

You have been given a 2 week supply of a medication called Varenicline (Chanitx©). Varenicline is approved by the FDA to help people quit smoking. This package contain 11 x 0.5mg Tablets and 42 x 1mg Tablets.

Week 1:
Days 1 to 3: Take 1 x 0.5mg pill each day in the morning.
Days 4 to 7: Take 2 x 0.5mg pills each day. Take one in the morning and one in the evening.

Week 2 to 12:
Take 2 x 1mg pills each day. Take one in the morning and one in the evening.

How to take the pills:
Pills should be taken at least 8 hours apart. You should take each study pill with food and with a glass of water to minimize possible nausea.

If you forget to take a study pill:
If you forget to take a pill, simply leave the pill in its spot and skip to the next pill.

Side effects:
Common side-effects from varenicline are: ●nausea ●vomiting ●difficulty sleeping ●abnormal dreams ●headaches, and ●feeling tired or sleepy. **Other side effects** could include: ●change in appetite ●stomach pain ●gas ●indigestion ●constipation ●dry mouth ●diarrhea ●blurred vision ●sweating ●hot flush ●increased heart rate ●changes in blood pressure ●back and muscle pain ●disturbances in attention ●dizziness ●fainting ●restlessness ●anxiety ●depression ●irritability and ●agitation. In **rare cases** heart problems, low blood sugar and serious changes in behavior and mood related to anxiety, depression, suicidal thinking, and strange behavior have been reported. A complete list of varenicline related side effects can be found in the consent form.

You will be asked about side-effects and how you are responding to varenicline during your weekly counseling visits.

If you experience any worrisome side effects from the study pills:
Immediately stop taking the study pills and contact the study Nurses or Physician. If you have any questions or concerns, call the Nurses at **443-740-2294 (available 24 hours everyday)**. If you have any issues that you would like to discuss with the study Physician contact **Dr. Betty Jo Salmeron at 443-740-2651 (office).**

If you experience a sudden or drastic change in behavior or mood including severe agitation, depressed mood, suicidal thoughts or behaviors, stop taking the pills and contact Dr. Salmeron immediately.

If you need to seek medical attention for any possible side effects tell your doctor about your participation in this study and that you might be taking varenicline.

Appendix 9: Treatment Preparation and Motivation to Quit Measures

During treatment sessions, participants may be asked to repeat some of the questionnaires assessing motivation to quit in order to assess changes in motivation levels and/or specific issues of concern that should be addressed in session.

1) Processes of Change (Prochaska, Velicer, DiClemente, & Fava, 1988). The Processes of change questionnaire is a self-report measure which assesses readiness to change smoking behavior. The scale includes 40 items which are each assessed on a 5-point likert scale from Never to Repeatedly.

The following experiences can affect the smoking habits of some people. Think of any similar experiences you may be currently having or have had in the last month. Then rate the FREQUENCY of this event on the following five point scale.

1 = Never
2 = Seldom
3 = Occasionally
4 = Often
5 = Repeatedly

1. Special people in my life accept me the same whether I smoke or not. ❑

2. I see "No Smoking" signs in public buildings. ❑

3. I can be open with at least one special person about my experiences with smoking. ❑

4. I tell myself I can choose to smoke or not. ❑

5. Instead of smoking I engage in some physical activity. ❑

6. I recall articles with the problems of quitting smoking. ❑

7. I notice that public places have sections set aside for smokers. ❑

8. I recall information people have personally given me on the benefits of quitting smoking. ❑

9. I am considering the belief that people quitting smoking will help to improve the world. ❑

10. I think about information from articles and advertisements on how to stop smoking. ❑

11. Remembering studies about illnesses caused by smoking upsets me. ❑

12. Other people in my daily life try to make me feel good when I don't smoke 1 2 3 4 5 ❑

13. I tell myself I am able to quit smoking if I want to. ❑

14. I have someone who listens when I need to talk about my smoking. ❑

15. I remove things from my home that remind me of smoking. ❑

16. I tell myself that if I try hard enough I can keep from smoking. ❑

17. I recall information people have personally given me on how to stop smoking. ❑

18. I make commitments not to smoke. ❑

19. I reward myself when I don't smoke. ❑

20. I notice that nonsmokers are asserting their rights. ❑

21. I stop to think that smoking is polluting the environment. ❑

22. I can expect to be rewarded by others if I don't smoke. ❑

23. I keep things around my place of work that remind me not to smoke. ❑

24. I find society changing in ways that make it easier for the nonsmoker. ❑

25. I get upset when I think about my smoking. ❑

26. I find that doing other things with my hands is a good substitute for smoking. ❑

27. When I am tempted to smoke, I think about something else. ❑

28. I do something else instead of smoking when I need to relax or deal with tension. ❑

29. I remove things from my place of work that remind me of smoking. ❑

30. Warnings about the health hazards of smoking move me emotionally. ❑

31. Dramatic portrayals of the evils of smoking affect me emotionally. ❑

32. I react emotionally to warnings about smoking cigarettes. ☐

33. I am rewarded by others if I don't smoke. ☐

34. I consider the view that smoking can be harmful to the environment. ☐

35. I reassess the fact that being content with myself includes changing the smoking habit. ☐

36. I consciously struggle with the issue that smoking contradicts my view of myself as a caring and responsible person. ☐

37. I put things around my home that remind me not to smoke. ☐

38. My dependency on cigarettes makes me feel disappointed in myself. ☐

39. I am considering the idea that the world around me would be a better place without my smoking. ☐

40. I have someone whom I can count on when I'm having problems with smoking. ☐

2) Decisional Balance (Velicer, DiClemente, Prochaska, & Brandenburg, 1985). The decisional balance questionnaire assesses the perceived pros and cons of smoking. The scale is a 20 item self-report measure with each item assessed on a 5-point likert scale from Not Important to Extremely.

The following statements represent different opinions about smoking. Please rate HOW IMPORTANT each statement is to your decision to smoke according to the following five point scale:

1 = Not important
2 = Slightly important
3 = Moderately important
4 = Very important
5 = Extremely important

1. Smoking cigarettes is pleasurable. ☐

2. My smoking affects the health of others. ☐

3. I like the image of a cigarette smoker. ☐

4. Others close to me would suffer if I became ill from smoking. ☐

5.	I am relaxed and therefore more pleasant when smoking.	☐
6.	Because I continue to smoke, some people I know think I lack the character to quit.	☐
7.	If I try to stop smoking I'll be irritable and a pain to be around.	☐
8.	Smoking cigarettes is hazardous to my health.	☐
9.	My family and friends like me better when I am happily smoking than when I am miserably trying to quit.	☐
10.	I'm embarrassed to have to smoke.	☐
11.	I like myself better when I smoke.	☐
12.	My cigarette smoking bothers other people.	☐
13.	Smoking helps me concentrate and do better work.	☐
14.	People think I'm foolish for ignoring the warnings about cigarette smoking.	☐
15.	Smoking cigarettes relieves tension.	☐
16.	People close to me disapprove of my smoking.	☐
17.	By continuing to smoke I feel I am making my own decisions.	☐
18.	I'm foolish to ignore the warnings about cigarettes.	☐
19.	After not smoking for a while a cigarette makes me feel great.	☐
20.	I would be more energetic right now if I didn't smoke.	☐

3) Self-Efficacy/Temptation (Velicer, Diclemente, Rossi, & Prochaska, 1990). The self-efficacy/Temptation model measures perceived self-efficacy to maintain abstinence across a variety of high-risk smoking situations. The measure is a 20 item scale where each item is a assessed on a 5-point likert scale from Not at all tempted to Extremely tempted.

Listed below are situations that lead some people to smoke. We would like to know HOW TEMPTED you may be to smoke in each situation. Please answer the following questions using the following five point scale.

1 = Not at all tempted
2 = Not very tempted
3 = Moderately tempted

4 = Very tempted
5 = Extremely tempted

1.	At a bar or cocktail lounge having a drink.	❑
2.	When I am desiring a cigarette.	❑
3.	When things are not going the way I want and I am frustrated.	❑
4.	With my spouse or close friend who is smoking.	❑
5.	When there are arguments and conflicts with my family.	❑
6.	When I am happy and celebrating.	❑
7.	When I am very angry about something or someone.	❑
8.	When I would experience an emotional crisis, such as an accident or death in the family.	❑
9.	When I see someone smoking and enjoying it.	❑
10.	Over coffee while talking and relaxing.	❑
11.	When I realize that quitting smoking is an extremely difficult task for me.	❑
12.	When I am craving a cigarette.	❑
13.	When I first get up in the morning.	❑
14.	When I feel I need a lift.	❑
15.	When I begin to let down on my concern about my health and am less physically active.	❑
16.	With friends at a party.	❑
17.	When I wake up in the morning and face a tough day.	❑
18.	When I am extremely depressed.	❑
19.	When I am extremely anxious and stressed.	❑
20.	When I realize I haven't smoked for a while.	❑

4) Reasons for Quitting Scale (Curry, Wagner, & Grothaus, 1990). The Reasons for Quitting scale is a self-report measure which assesses endorsement of extrinsic and intrinsic reasons for quitting smoking. The measure contains 20 items which are each assessed on a 5-point likert scale from Not at all true to Extremely true.

What are your reasons for wanting to quit smoking at this time? Below is a list of reasons that smokers may have for quitting. Read each reason and decide how much it applies to you right now. Then circle ONE number for each reason. Remember, there are no "right" or "wrong" reasons for wanting to quit smoking. Any reason is a good one!

0 Not at all
1 A little true
2 Moderately true
3 Quite true
4 Extremely true

I WANT TO QUIT SMOKING:

1. **Because I am concerned that I will suffer from a serious illness if I don't quit smoking.....0.....1.....2.....3.....4**

2. **To show myself that I can quit smoking if I really want to.....0.....1.....2.....3.....4**

3. **So that my hair and clothes won't smell.....0.....1.....2.....3.....4**
4. **Because my spouse, children, or other person I am close to will stop nagging me if I quit smoking.....0.....1.....2.....3.....4**

5. **Because I have noticed physical symptoms that smoking is hurting my health.....0.....1.....2.....3.....4**

6. **Because I will like myself better if I quit smoking.....0.....1.....2.....3.....4**
7. **So that I will save money on smoking related costs such as dry cleaning.....0.....1.....2.....3.....4**

8. **Because someone has given me an ultimatum (made a threat) to quit.....0.....1.....2.....3.....4**

9. **Because I can graphically picture the effects that smoking has on my body.....0.....1.....2.....3.....4**
10. **So that I can feel in control of my life.....0.....1.....2.....3.....4**
11. **Because I won't burn holes in clothing or furniture.....0.....1.....2.....3.....4**

12. Because I will receive a special gift if I quit.....0.....1.....2.....3.....4

13. Because I have known other people who have died from serious illnesses that were caused by smoking.....0.....1.....2.....3.....4

14. Because quitting smoking will prove that I can accomplish other things that are important to me.....0.....1.....2.....3.....4

15. Because I want to save money that I spend on cigarettes.....0.....1.....2.....3.....4

16. Because people I am close to will be upset with me if I don't quit.....0.....1.....2.....3.....4

17. Because I am concerned that smoking will shorten my life.....0.....1.....2.....3.....4

18. To prove to myself that I am not addicted to cigarettes.....0.....1.....2.....3.....4

19. So that I won't have to clean my house or car as often.....0.....1.....2.....3.....4

20. Because I will receive a financial reward for quitting (money from a friend or family member, bonus from work, etc.).....0.....1.....2.....3.....

5) Smoking Cessation Motivation Assessment – a semi-structured interview to assess/ confirm the stage of change and motivation level for participants interested in smoking cessation protocols. Follow up questions and a brief discussion of readiness to quit may ensue. Some items from this questionnaire may be repeated several times throughout the protocol.

1. a) Are you seriously thinking of quitting smoking?
   If yes:
   b) Of the following choices, what would be a more accurate description of your readiness to attempt quitting smoking: **within the next 30 days\ within the next 6 months**?
   Notes:

2. Have you tried to quit before? **Yes/No**

   If yes:

a. How many times and over what period?

_____

b. How long did you remain abstinent and why did you start smoking again?_____

_____

3. On a scale of 0-10 with 0 being not at all and 10 being extremely, how important to you is quitting smoking?

_____

Notes:

4. Do other people in your home smoke? **Yes/No**

Notes:

5. Do you have friends at work who you take smoke breaks with? **Yes/No**

Notes:

6. Do you have friends outside of work you smoke with? **Yes/No**

Notes:

7. In what situations do you enjoy smoking the most?

_____

_____

8. On a scale of 0-10 with 0 being not at all and 10 being extremely, how interested right now are you in quitting?

_____

9. On a scale of 0-10 with 0 being not at all and 10 being extremely, how ready are you to quit smoking **within the next month**?

_____

10. On a scale of 0 to 10 with 0 being not at all and 10 being extremely, how confident are you that you could quit smoking within the next month?

_____

Notes:

GeneralComments:_____

_____

_____

_____

_____

Questions that may be repeated throughout treatment:

1. On a scale of 0-10 with 0 being not at all and 10 being extremely, how important to you is quitting smoking?

   _____

   Notes:

2. On a scale of 0-10 with 0 being not at all and 10 being extremely, how interested right now are you in quitting?

   _____

   Notes:

3. On a scale of 0-10 with 0 being not at all and 10 being extremely, how ready are you to quit smoking?

   _____

   Notes:

4. On a scale of 0 to 10 with 0 being not at all and 10 being extremely, how confident are you that you could quit smoking within the next month?

   _____

   Notes:

Appendix 10: Treatment Materials

Participants may be given these treatment materials during counseling sessions or as take home materials.

Please see attached documents Appendix10.pdf

Appendix 10 (Treatment Session and Take-Home Materials): Table of Contents

1. **Tips for Successful Quitting**
2. **Putting a Stop to Smoky Thinking**
3. **Deep Breathing Relaxation Exercise**
4. **What Are Smoking Triggers**
5. **Triggers for Smoking**
6. **Managing Triggers for Smoking**
7. **Managing Triggers for Smoking Worksheet**
8. **Nonsmoking Game Plan: Coping with High-Risk Situations**
9. **Nonsmoking Game Plan: Coping with High-Risk Situations Worksheet**
10. **Nonsmoking Game Plan: Feeling Badly if You Slip**
11. **Preparing for Quit Day**
12. **Your Quit Day (3 pages)**
13. **Instructions for Mini-Dose Relaxation**
14. **Nonsmoking Game Plan: Lifestyle Change**
15. **Why Quit Smoking – Revisited**
16. **Social Support for Nonsmoking**
17. **Weekly Task Worksheet**
18. **Quitting for Keeps: Remaining a Nonsmoker**
19. **All-Purpose Coping Plan**
20. **Combating Triggers**
21. **Coping with Cravings and Urges**
22. **Decisional Balance Worksheet**
23. **Goals Worksheet**
24. **Reminder Sheet for Problem-Solving**
25. **Seemingly Irrelevant Decisions**
26. **Self-Monitoring Your Smoking Behavior**
27. **Beat Boredom**
28. **Burns You Up**
29. **Coping with Quitting – CB Strategies**
30. **What Happens When You Quit**
31. **NCI – How to Handle Withdrawal and Triggers**
32. **Functional Analysis**
33. **Quit Day Ceremony**
34. **Abstinence Coping Strategies**

Appendix 11: Appointment Cards

Front

Nicotine Treatment Studies

Contact Person: Kim Slater, M.Ed., NCC

Phone: 443-740-2252

Email: QuitSmoking@mail.nih.gov

Back

Appointment Information:

Date:_____ **Time:** _____

Study #:_____ **Session #:**_____

MD Hotline - 24 Hours/7 Days Week:

1-800-Quit-Now ~ www.smokingstopshere.com

NIDA Nursing Phone: 443-740-2294

Nicotine Treatment Studies

Contact Person: _____

Phone: _____

Email: QuitSmoking@mail.nih.gov

Appointment Information:

Date:_____ **Time:** _____

Study #:_____ **Session #:**_____

MD Hotline - 24 Hours/7 Days Week:

1-800-Quit-Now ~ www.smokingstopshere.com

NIDA Nursing Phone: 443-740-2294

Appendix 12: Nicotine Patch Instructions for tDCS Arm

Read ALL the instructions before you begin.

Do not smoke any cigarettes while you are wearing the patches. Since the nicotine patch works by delivering small doses of nicotine into the body, smoking may cause harmful levels of nicotine in your body.

Nicotine Tolerance Test Patch

- The nurse will apply a nicotine patch for you on orientation day to make sure you tolerate it.
- Update the staff if you experience any discomfort with wearing the patch.
- The nursing staff will remove the patch at the end of the orientation session.

Call the study nurses at **443-740-2294** or Dr. Salmeron at **443-740-2651** if you experience any problems after you leave NIDA.

Study Patches (1 and 2)

You will receive 2 take-home patch sets. One of these patches contains nicotine, and the other contains a placebo (no nicotine). The content of the patches is *blinded*, meaning the packaging does not reveal the true content of the patch. These patches will be worn on the *day of your imaging visit.*

Your abstinence period begins **12 hours before your next NIDA visit** (e.g., 8pm Sunday night if your visit is at 8am on Monday morning). Smoke a cigarette after dinner, before you commence the abstinence period, if this is usual for you. You will receive a phone call from a study investigator before your abstinence period begins to ensure that you remember and understand the abstinence procedure.

A team member will tell you what time to place the patch so that you are wearing it for about two hours before your scan. To place the patch, follow the instructions outlined on the next page.

How to apply the nicotine patch:

1. Do not remove the patch from its sealed protective pouch until you are ready to use it.

2. Choose a non-hairy, clean, dry area of skin. We recommend placing it near where the nurse applied your toleration patch. Do not put the patch on skin that is burned, broken out, cut, or irritated in any way. Make sure your skin is free of lotion and soap before applying the patch.

3. A clear, protective liner covers the sticky backside of the patch – the side that will be put on your skin. The liner has a slit down the middle to help you remove it from the patch. With the sticky backside facing you, pull half the liner away from the patch starting at the middle slit, as shown in the illustration below. Hold the patch at one of the outside edges (touch the sticky side as little as possible), and pull off the other half of the protective liner.

4. Immediately apply the sticky side of the patch to your skin. **Press the patch firmly on your skin with the palm of your hand for at least 10 seconds.** Make sure it sticks well to your skin, especially around the edges. Mild itching, burning or tingling is normal and should go away within an hour.

5. Wash your hands after you apply the patch. Nicotine on your hands could get into your eyes and nose, and cause stinging, redness, or more serious problems.

If your patch comes off during the day:

The patch generally sticks well to most people's skin. However, a patch may occasionally come off. If your patch falls off during your abstinence period, re-apply as soon as possible and hold in place with medical tape.

To prevent the patch from coming off, do not apply creams or lotions to the area on your skin where you will put the patch. Also, do not bathe, swim or shower during your test.

If you get a minor skin rash:

Use a topical salve (such as a hydrocortisone cream) around the patch.

If you experience any worrisome side effect from the patch (listed below):

Remove it at once and call **Dr. Betty Jo Salmeron at 443-740-2651**. **Also, the study nurses can be reached at 443-740-2294 (available 24 hours every day).**

Side effects of the nicotine patch include: dizziness, headache, upset stomach, vomiting, diarrhea, redness or swelling at the patch site.

Very rare serious side effects of the nicotine patch include: severe rash or swelling, seizures, abnormal heartbeat or rhythm, difficulty breathing.

Appendix 13: tDCS Stimulation Blinding Questionnaire

(Adapted from Fertonani et al 2015, O'Connell et al 2012, Richardson 2016)

Questions for Participant

Blinding

1. Which treatment do you believe you received? *If you don't know, please provide your best (or random) guess.*

   a) Active
   b) Placebo (sham stimulation)

2. On a scale of 0 to 10 -- 0 being not confident at all and 10 being completely confident -- how confident are you that you received your selection? _____

Side Effects

Did you experience any discomfort or annoyance during the tDCS stimulation? Please answer the following questions regarding the different sensations and indicate the degree of intensity of your discomfort according to the following scale:

None = I did not feel the describe sensation (0)
Mild = I mildly felt the described sensation (1)
Moderate = I felt the described sensation (2)
Considerable = I felt the described sensation to a considerable degree (3)
Strong = I strongly felt the described sensation (4)

1. Itching
2. Pain
3. Burning
4. Warmth/Heat
5. Pinching
6. Metallic/Iron taste
7. Fatigue
8. Other: _____

When did the discomfort begin?
At the beginning of the session, in the middle of the session, towards the end of the session

How long did it last?
It stopped quickly, it stopped in the middle of the session, it stopped at the end of the session

How much did these sensations affect your performance?
Not at all, slightly, considerably, much, very much

Identify whether these sensations were located over the head or in a different location:
On the head ___, Other ____

Questions for Investigator

1. Which treatment do you believe this participant received?

   a) Active (full dose stimulation) with anode over the dlPFC and cathode over the vmPFC
   b) Active (full dose stimulation) with anode over the vmPFC and cathode over the dlPFC
   c) Active (full dose stimulation) but I don't know which polarity
   d) Placebo (sham stimulation)
   e) Don't know

2. If you answered "Don't know" above, can you please provide your best (or random) guess of a treatment the participant received anyway?

   a) Active (full dose stimulation) with anode over the dlPFC and cathode over the vmPFC
   b) Active (full dose stimulation) with anode over the vmPFC and cathode over the dlPFC
   c) Active (full dose stimulation) but I don't know which polarity
   d) Placebo (sham stimulation)

3. On a scale of 0 to 10 -- 0 being not confident at all and 10 being completely confident -- how confident are you that the participant received your selection?

   _____

Side effects
Please report any adverse event/problem (skin irritation, headache, scalp pain, dizziness, or others, please specify) that occurred and rate the event/problem on a scale from 1 to 4 as described above.

Additional comments:

Appendix 14: Recruitment Materials

See attached pdf of advertisements and landing pages. Recruitment through the NIDA Office of the Clinical Director will be done by a general NIDA-IRP recruitment contractor. Under certain circumstances, materials may be developed by other contractors as well. Alternatively, we may develop our own ads or contract these services ourselves. All advertising methods will comply with the most current regulations (NIH and OHSRP SOPs and guidelines, as well as FDA guidelines for FDA-regulated research) and the NIDA policy on recruitment materials. Please see attached pdf of recruitment materials which includes the following: advertisements, landing pages

and alternative phrasing and photos to be substituted for the purpose of A/B testing of best performing ads.

The recruitment contractor may alter the ads in minor ways without prospective IRB review and approval; see below for a list of these types of changes. Other than that, modifications to these materials will be submitted to the IRB for approval as protocol amendments.

The following changes may be made to recruitment materials with no prospective review and approval by the IRB (see next section for further explanation about each bullet point):

- Contact phone numbers
- Color change to graphics
- Overall size, but all content will be increased or decreased proportionately
- Addition of tear-off phone number tags to an approved ad or flyer
- Color or font of the text, but any color change will replace all the text of a certain color. No new emphasis will be created.

All of these changes will be made to the most recently approved recruitment materials and submitted to the IRB annually at the time of continuing review. Materials will only be posted in approved locations.

Contact phone numbers: Phone numbers can vary for several reasons, including A/B efficacy comparison testing on ads and having phone numbers vary by placement, image, style, or wording. This allows us to know which ad or ad placement drew in the phone call for screening since the phone number dialed is tracked. As such, we would like the flexibility to change the phone number(s) listed on any approved ad without prior approval to make this change.

Color change to graphics: Due to requirements from organizations where our recruitment material is placed or A/B testing, we may need to or want to change the colors in the graphics from color to black and white or vice versa, or change a red border to a blue border, etc. Any changes to color in the graphics will not create, amplify, or diminish the emphasis in the original approved materials.

Overall size: We may need to re-size materials to fit the parameters of the hosting organization (e.g, must be 5” x 7” but the originally approved material was 8.5” x 11”, quarter page newspaper ad to a full page newspaper ad, newspaper format size to a bus ad format size) or make an ad from a flyer to a larger format poster for display at an outreach event. We will ensure that all the originally approved content remained in the same proportions when the overall document size was reduced or enlarged.

Addition of tear-off phone number tags: We may add or remove tear-off contact information to approved ads and flyers. This allows us to be flexible in the type of material we provide to organizations to post for us.

Color or font of the text: We may change the color of font in an ad as long as it is all one color to another color (e.g., all blue text changed to green text, all red text to black text). We will not create new emphasis (e.g., we will not change only part of a text field to a color like making the word free blue in what was all black text, we will not make a payment amount a color to highlight it when it was approved as black or the same color as the surrounding text) or remove emphasis. We may change the font of the entire text to meet requirements for placement, for A/B testing, for readability, or other placement needs (e.g., change the font through an entire ad from

Calibri to Times New Roman). We will not add or remove italics, bold, or any other emphasis without prior approval.

Changes outside of the type listed above will be made by making an amendment to the protocol and its recruitment materials. We will not make changes that do not fit within the guidelines above.