Title: A Multi-Modal Investigation of the Smoking Cessation Medication Varenicline: Dopaminergic Modulation of Reward Processing and Cognitive Control
NIDA-IRP Protocol #: 09-DA-N044
Submission Date: February 27, 2012
Version: 10.0

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Total Requested Accrual:
100 Volunteers (50 nicotine-dependent adults; 50 control participants)

Project Uses Ionizing Radiation: ☒ No ☐ Yes
☐ Medically-indicated only
☐ Research-related only
☐ Both

IND/IDE: ☐ No ☒ Yes

Durable Power of Attorney: ☒ No ☐ Yes

Multi-institutional Project ☒ No ☐ Yes

Data Safety Monitoring Board ☐ No ☒ Yes

Transfer Technology Agreement ☒ No ☐ Yes
Agreement type and number: ___

Confidential Disclosure Agreement ☒ No ☐ Yes

Samples are being stored ☐ No ☒ Yes

Consent Reading Level: 8.0 (without privacy boilerplate language)

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A. Précis

Objective. Chronic nicotine exposure is thought to lead to alterations in the dopamine (DA) system that leaves smokers in a hypo-dopaminergic state during periods of abstinence. Varenicline (Chantix), a new efficacious smoking cessation medication, is thought to lead to a modest but sustained increase of DA release thereby reducing nicotine craving and withdrawal. While numerous studies have shown that varenicline is a safe, well-tolerated, and effective pharmacological treatment for nicotine dependence, studies exploring the neurophysiological impact of this drug in the human brain have not been conducted. This protocol will utilize an array of reward processing and cognitive control tasks to explore the effects of subtle DA manipulations (induced by smoking cessation, transdermal nicotine, and varenicline) on brain function and behavioral performance. Brain function will be assessed using functional magnetic resonance imaging (fMRI) and electroencephalography (EEG).

Study Population. There will be two study populations: 1) healthy nicotine-dependent adults who smoke 10 or more cigarettes per day; and 2) healthy non-smoking, non-drug dependent controls. Participants must be generally healthy, right-handed, male or non-pregnant/non-lactating females between the ages of 18-55.

Design. After being medically cleared and giving informed consent, each participant will complete several imaging visits (6 visits, on separate days) before and after taking varenicline. Two of these visits will take place before varenicline administration (baseline), two visits after a two-week varenicline dosing period (post-varenicline), and another two after a two-week placebo-pill period (post-placebo-pill). Each set of two scans will involve the randomized, double blind administration of a nicotine transdermal or placebo patch. fMRI and EEG data will be collected after patch application and will involve several tasks designed to probe brain regions in a corticolimbic circuit that may mediate aspects of reward-processing, learning, attention, goal-directed behaviors, and drug abuse.

Outcome Measures. This study involves assessing neurophysiological and behavior differences between cohorts (smokers vs. non-smokers) and conditions (nicotine vs. placebo-patch; baseline vs. varenicline vs. placebo-pill). The primary outcome measures used to ascertain these differences will be: 1) percentage change in fMRI BOLD signal during performance of cognitive control and reward processing tasks; 2) change in ERP component (e.g., error-related negativity) amplitudes; 3) behavioral measures during task performance including reaction times and error rates; 4) scores on mood, personality, and smoking questionnaires; and 5) variations in genes related to nicotinic receptors and DA functioning.

B. Introduction/Scientific Rationale

Varenicline (Chantix), an α4β2 nicotinic acetylcholine receptor (nAChR) partial agonist, is a novel pharmacotherapy specifically developed for smoking cessation/nicotine dependence. Varenicline was recently approved by the US Food and Drug Administration adding to the list of currently available smoking cessation pharmacotherapies which included nicotine replacement products (patch, gum, lozenge, nasal spray, and inhaler) and the aminoketone antidepressant buproprion. Although previously approved therapies (i.e., nicotine replacement and buproprion) can help some smokers quit, their efficacy is considered to be modest (Doggrell, 2007). Recent clinical trials have shown that varenicline is significantly more efficacious than placebo or buproprion for smoking cessation (Gonzales et al., 2006; Jorenby et al., 2006). Varenicline's efficacy is thought to reflect an α4β2 subunit modulated, modest but sustained increase of dopamine (DA) release in the mesolimbic and prefrontal dopaminergic pathways, thereby reducing craving and withdrawal. While varenicline administration has previously been shown to be an efficacious, safe, and well-tolerated smoking cessation pharmacotherapy (Williams et al., 2007), studies exploring the neurophysiological and cognitive effects of varenicline have yet to be conducted. Elucidating the neurobiological and behavioral consequences of varenicline administration will not only aid in the development of future smoking cessation regimes but may also demonstrate the utility of this compound as a tool for probing the function and dysfunction of the mesocorticolimbic (MCL) DA system in control and drug addicted populations.

The current protocol will focus on relating modulations of the DA systems with reward processing and higher-level cognitive control processes. Recent perspectives suggest a fundamental link between: 1) striatal reward pathways; and 2) the recruitment of a frontal network associated with cognitive control (Holroyd and Coles, 2002; Montague et al., 2004; Holroyd et al., 2005; Satterthwaite et al., 2007). Holroyd and Coles (2002) have postulated an association between these two systems suggesting that the recruitment of frontal resources may in part be mediated by phasic DA activity. Specifically, unexpected negative outcomes (particularly commission errors) produce phasic reductions in DA neuronal firing, which in turn lead to the recruitment of a frontal cognitive control network. The present protocol will investigate the dopaminergic link between a striatal reward system that responds to outcomes and a frontal network (particularly anterior cingulate cortex; ACC) that is sensitive to needs for cognitive control. Multiple imaging modalities, including electroencephalography (EEG), event-related brain potentials (ERPs), magnetic resonance spectroscopy (MRS), and functional magnetic resonance imaging (fMRI), will be employed to assess the effects of mild pharmacological manipulations (nicotine and varenicline) on the DA systems. Assessment of genetic polymorphisms related to DA functioning and nicotinic receptors will also be conducted.

Study Rationale Summary and Specific Aim Overview.

Chronic nicotine exposure results in an alteration in the MCL DA system (Epping-Jordan et al., 1998; Salokangas et al., 2000; Dagher et al., 2001; Liu and Jin, 2004; Rahman et al., 2004; Smolka et al., 2004) such that consistent nicotine administration may become necessary for "normalized" function (Dawkins et al., 2006, 2007b). Here, we hypothesize that a hypo-functioning DA state during periods of abstinence results in decreased reward responsivity and impaired cognitive control functions dependent on DA. Decreased DA-tone may also be responsible for dysphoria and anhedonia often reported by abstinent smokers. Smoker's may continue to use nicotine in a self-medicating fashion to alleviate reduced DA functioning. Given varenicline's mechanism of action, which leads to a sustained increase in DA-tone, this efficacious smoking cessation pharmacotherapy is anticipated to reduce or ameliorate abstinence-induced deficits in reward processing and cognitive control.

Specific Aim 1. To compare brain activation patterns and behavioral performance associated with *reward processing* (MID, Reversal-Learning, and Amygdala Reactivity Tasks) in deprived-smokers to that observed in non-smoking controls both in the presence and absence of *nicotine.*

Specific Aim 2. To compare brain activation patterns and behavioral performance in task paradigms associated with *cognitive control/performance monitoring* (Flanker, Motion Prediction, and Go-NoGo Tasks) in non-smoking controls with that seen in abstinent-smokers both in the presence and absence of *nicotine.*

Specific Aim 3. To determine if the administration of the α4β2 partial agonist *varenicline,* which is thought to produce sustained DA release throughout the MCL system, increases activity in regions associated with *reward processing* and *cognitive control*. A direct comparison of the effects of nicotine, nicotine abstinence, and varenicline on brain activity and behavioral performance will be used to probe for changes in the DA system.

Specific Aim 4. To relate performance monitoring activity in the medial prefrontal cortex with striatal reward-processing activity since both are considered to be mediated by DA activity.

Specific Aim 5. To relate genetic polymorphisms that may mediate nAChR binding and/or monoaminergic-functioning with individual differences in nicotine and varenicline effects.

Mesoorticolimbic Circuit Regulating Drug Seeking.

Drugs of abuse precipitate the release of DA from cells in the ventral tegmental area (VTA) into the nucleus accumbens (NAcc), prefrontal cortex (PFC), and amygdala. Similar to motivationally relevant biological stimuli (e.g., food, water, sex), drugs of abuse increase DA release within this MCL circuit, albeit by different mechanisms (Jay, 2003; Kelley, 2004; Nestler, 2005). The release of DA is considered important for the facilitation of learning and elicitation of motivational elements relating to the acquisition of goals or rewards (Everitt and Robbins, 2005; Kalivas and O'Brien, 2008). Berridge and Robinson (1998) have suggested that DA release plays a role in incentive salience by indicating that an event and associated cues are relatively important, thus requiring attention and mobilization of goal-directed behavior. The intake of addictive drugs precipitates a

larger amplitude and longer duration DA response than that achieved through normal physiological mechanisms. Thus, drugs of abuse usurp the DA-mediated MCL circuit thought to mediate aspects of learning, attention, and goal-directed behavior which can lead to repeated and potentially chronic drug use. In the current protocol we are interested in assessing the effects of mild DA manipulations (induced by acute smoking-abstinence in smokers, as well as nicotine and varenicline administration in smokers and non-smokers) on the functioning of brain regions within the MCL circuit. The current protocol offers a unique opportunity to examine the (dys)function of the MCL DA system using multiple imaging modalities as well as multiple task paradigms selected to interrogate specific nodes in this MCL network.

Figure 1 presents a schematic of the brain regions of interest, specifically: 1) the habenula, 2) ventral striatum (NAcc), 3) amygdala, and 4) prefrontal cortices (orbitofrontal cortex [OFC] and ACC). In the present discussion, the habenula, an epithalamic structure on the dorsomedial surface of the caudal thalamus (Lecourtier and Kelly, 2007), is considered to be the input into the circuit. The habenula is likely involved in reward-related modulation of DA activity, acting as a GABAergic "brake" on VTA cells (Matsumoto and Hikosaka, 2007). Habenula activity will be assessed using a *Motion Prediction Task* previously shown to produce differential activations in this region (Ullsperger and von Cramon, 2003). Continuing on in the network, the VTA sends dopaminergic projections to the NAcc, amygdala, OFC and ACC, areas that have been implicated in reward-based learning, cognitive control, and drug abuse. The ventral striatum/NAcc will be assessed using the *Monetary Incentive Delay* (MID) Task, which has previously been used (Knutson et al., 2000; Knutson et al., 2001; Knutson et al., 2003; Bjork et al., 2004) to assess reward anticipation and delivery. Amygdala functioning will be evaluated using a *Reward Reversal-Learning Task* (Cools et al., 2002; Clark et al., 2004; Cohen et al., 2008) and an *Amygdala Reactivity Task* (Hariri et al., 2002b; Tessitore et al., 2002; Pezawas et al., 2005). OFC activity will also be assessed in the reversal-learning paradigm. Finally, ACC activity related to error monitoring and response inhibition will be assessed using a *Flanker Task* and a *Go-NoGo Task*, respectively. As a general organizational theme the MID Task, Reward-Reversal Learning Task, and Amygdala Reactivity Task are conceptually grouped under the rubric of R*eward Processing* and the Flanker Task, Motion Prediction Task, and Go-NoGo Task are further discussed under the heading of *Cognitive Control/Performance Monitoring*.

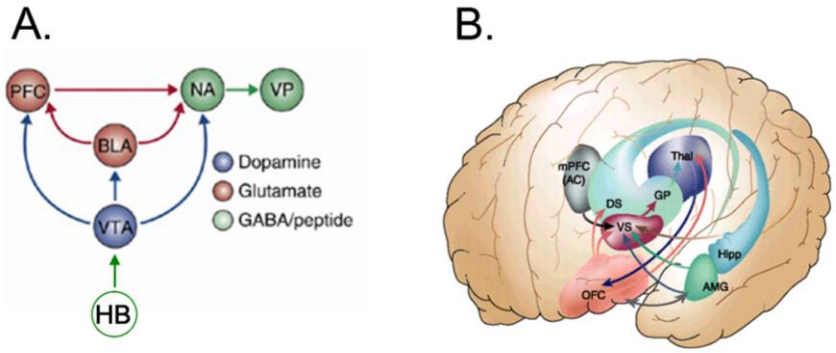

Figure 1. Schematic overview of the MCL circuit of interest. Selected task paradigms are intended to probe different nodes within this network to further elucidate DA functioning related to nicotine addiction and cessation: (region---task) Habenula---*Motion Prediction Task*, amygdala---*Reward Reversal-Learning* and *Amygdala Reactivity Tasks*, nucleus accumbens---*Monetary Incentive Delay Task*, OFC---R*eversal-Learning Task*, and ACC---*Flanker* and *Go-NoGo Task*. A) Adapted from Kalivas and O'Brien (2008) Figure 2a. HB, habenula; VTA, ventral tegmental area; BLA, basolateral amygdala; PFC, prefrontal cortices; NA, nucleus accumbens. B) Taken from Everitt and Robbins (2005) Figure 1A. Thal, thalamus; VS, ventral striatum; DS, dorsal striatum; amg, amygdala; Hipp, hippocampus; mPFC(AC) medial prefrontal cortex (Anterior cingulate); OFC, orbitofrontal cortex.

Pharmacology of Nicotine.

General consensus suggests that people use tobacco primarily to experience the neuropharmacological effects of nicotine (Stolerman and Jarvis, 1995). In humans, nicotine produces positive reinforcing effects that may include memory facilitation, anti-nociception, mild calming, mild euphoria, reduced stress, reduced anxiety, reduced negative affect, increased energy, heightened arousal, and reduced appetite (Aceto and Martin, 1982; Warburton, 1990; Pomerleau and Pomerleau, 1992; Benowitz, 1996). Cigarette smokers consistently report reinforcing effects from smoking (Etter et al., 2000) and nicotine shares behavioral reinforcing properties with other drugs of abuse, notably the psychostimulants (Henningfield and Goldberg, 1983; Pontieri et al., 1996). Users report IV nicotine as pleasant, preferring it to cigarettes (Johnston, 1942) and cocaine abusers identify IV nicotine as similar and, in many cases as identical to IV cocaine (Henningfield et al., 1983). Although nicotine and cocaine have very different mechanisms of action, they appear to share many behavioral properties and neuroanatomical loci. For example, self-administration of both nicotine and cocaine in the rat increase glucose metabolism in identical limbic regions (Pich et al., 1997). Nicotine can substitute for self-administered cocaine in the rat (Tessari et al., 1995), while the nicotine patch increases cue-induced cocaine craving in cocaine addicts (Reid et al., 1998). The positive reinforcing effects consequent to nicotine administration likely play a critical role in the maintenance of tobacco use that ultimately leads to physical dependence and tolerance (Watkins et al., 2000).

The primary effects of nicotine on the central nervous system (CNS) are exerted through nicotinic acetylcholine receptors (nAChRs) by opening sodium channels and inducing neuronal depolarization (Kelly and

Rogawski, 1985). While multiple nAChRs subtypes[1] are expressed in the CNS, the addiction propensity of nicotine is thought largely due to its quick but short agonistic effect on α4β2 nAChRs located on the presynaptic membrane of DA neurons (Tapper et al., 2004). Like cocaine, as well as most drugs of abuse, nicotine is thought to interact with DA in MCL pathways. Although the neurobiological mechanisms of nicotine reinforcement are less well-understood (Corrigall, 1991; Corrigall et al., 1992) cholinergic presynaptic modulation of catecholaminergic neurons have been reported (Wonnacott et al., 1989). Notably, somatodendritic nicotinic receptors are localized on VTA DA cells and in such MCL terminal fields as the NAcc and olfactory tubercle (Clarke and Pert, 1985; Deutch et al., 1987). Nicotine excites VTA DA cells in vitro (Calabresi et al., 1989), and increases the concentration of extracellular DA in the NAcc (Imperato et al., 1986), via presynaptic modulation of DA cells (Wonnacott et al., 1989). In humans, ventral striatal DA release following cigarette smoking has been inferred from displacement of the radiotracer ^{11}C-raclopride (Brody, 2004). Infusion of a nicotinic agonist into the VTA has been shown to significantly reduce the number of nicotine self-administrations in rats (Corrigall et al., 1994). Thus, α4β2 nAChRs located in the VTA appear to mediate some aspects of the rewarding effects of nicotine via DA release in the NAcc and prefrontal cortex, and may play a role in craving and withdrawal resulting from low DA levels in the absence of nicotine due to the drug's short half-life.

Nicotine abstinence after chronic administration produces an aversive withdrawal syndrome in human smokers and laboratory animals (Kenny and Markou, 2001). In humans, aversive symptoms including increased anger, irritability, frustration, anxiety, depression, difficulty concentrating, impatience, insomnia, and restlessness are commonly reported during periods of abstinence (Hughes, 2007a). It has been proposed that the maintenance of tobacco smoking depends not only on the positive reinforcing actions of nicotine but also on the alleviation of aversive consequences (negative reinforcement) of nicotine withdrawal (Kenny and Markou, 2001). In laboratory rodents, abstinence from nicotine results in a significant decrease in brain reward function as assessed by elevations in intracranial self-stimulation brain reward thresholds (Epping-Jordan et al., 1998). More recently, it has been demonstrated that previously neutral stimuli repeatedly paired with nicotine withdrawal can be conditioned to elicit decreased brain reward function through Pavlovian processes (Kenny and Markou, 2005). Avoidance and alleviation of abstinence-induced reward deficits has been considered to be a major factor contributing to continued drug use and relapse during quit attempts (Ahmed et al., 2002). A perturbation of DA reward circuitry produced by chronic nicotine administration and particularly apparent during periods of abstinence may be associated with a subjective state of dysphoria and/or anhedonia that may lead individuals to self-medicate.

[1] nAChRs are ligand-gated ion channels that are opened by the endogenous neurotransmitter acetylcholine as well as the exogenous alkaloid nicotine. Each receptor consists of 5 subunits which are arranged symmetrically around a central pore. A receptors subunit composition dictates its electrophysiological and agonist-binding properties. Currently 12 neuronal subunits have been identified (α2 to α10 and β2 to β4) and therefore many nAChRs subtypes exist. Of particular interest, the α4 subunit is thought to play a major role in tolerance, reward, and the modulation of dopamine function, all being critical for the etiology of nicotine dependence (Tapper et al., 2004).

Current neurobiological models of drug abuse emphasize the importance of brain reward pathways consisting of DA projections from the VTA to NAcc, amygdala, OFC and ACC for the initiation and maintenance of addiction (Goldstein and Volkow, 2002). Increasing evidence suggests that tonic DA-levels may be reduced in chronic drug-users possibly leading to decreased responsivity to primary and secondary reinforcers (Volkow et al., 2004; Volkow et al., 2007). It has been proposed that frequent phasic increases in dopaminergic activity during drug consumption may produce a down regulation of DA receptors and, in turn, reduced activity in certain brain areas (Garavan and Stout, 2005). Positron emission tomography (PET) and single photon emission computed tomography (SPECT) studies have indicated that abstinent drug-addicted individuals have lower striatal D2 receptor availability (Volkow and Li, 2004) and decreased striatal DA release (Volkow et al., 1997) relative to non-drug using controls. In smokers, reduced D1 receptor binding in the NAcc has been reported (Dagher et al., 2001) and decreased activation in brain reward circuitry has been observed when using PET to measure regional cerebral blood flow during tasks involving monetary incentives (Martin-Soelch et al., 2001; Martin-Soelch et al., 2003). Also suggestive of reduced DA activity, abstinent smokers have been noted to: 1) show reduced responsiveness to financial incentives during simple card sorting tasks in comparison to non-smokers and satiated smokers (al-Adawi and Powell, 1997; Powell et al., 2002; Dawkins et al., 2006); 2) experience less enjoyment from a range of ordinarily pleasurable events and activities as assessed by self-report measures (Dawkins et al., 2006); 3) experience less emotional "uplift" after viewing positively valenced film clips (Dawkins et al., 2007a); and 4) show impairments during response inhibition tasks that appear dependent on prefrontal DA-activity (Dawkins et al., 2007b). Thus, the brains of smokers appear to react to rewarding stimuli differently than those of non-smokers and this difference may be a manifestation of altered DA transmission.

It has been suggested that chronic exposure to nicotine may not produce any overt changes in behavior or brain function until the organism is deprived of the drug (Dawkins et al., 2006; Besson et al., 2007). Accordingly, nicotine cessation is considered necessary to reveal latent modifications of brain circuitry resultant from chronic nicotine exposure (e.g., Rahman et al., 2004). Recently, Dawkins and coworkers (2006: pp. 356) articulated a similar perspective related to nicotine and DA functioning in the human brain: "…regular smokers may simultaneously possess a hypo-functioning tonic DA system but experience DA-enhancing effects of cigarette smoking. Their hypo-functioning DA system would thus be disguised as long as they are smoking but "unmasked" during periods of acute abstinence—for example at the start of a quit attempt, manifesting in blunted motivational and emotional responses to natural reinforcers. The pharmacological "boost" achieved via smoking a cigarette or taking nicotine replacement therapy, would reinstate "normal" behavioral and emotional reactivity." Hypo-functioning DA activity has also been proposed as a mechanistic account for reduced ACC activity and poor response inhibition in cocaine users (Garavan and Stout, 2005). Chronic exposure to a drug may result in the homeostatic down regulation (opponent process) of DA activity such that the use of the drug is necessary for "normal" functioning. Dawkins and coworkers have shown an association between acute smoking abstinence and

impaired reward motivation (Dawkins et al., 2006) as well as impaired response inhibition (Dawkins et al., 2007b) which can be reversed by nicotine administration. Their findings suggest that nicotine may return hypo-dopaminergic states to a "normalized" level in abstinent smokers possibly via α4β2 nAChRs in the VTA.

Varenicline Mechanisms of Action.

The negative and positive reinforcing effects of nicotine make smoking cessation difficult and, as such, both aspects should ideally be addressed by pharmacological interventions. Varenicline (Coe et al., 2005) may reduce behaviors associated with nicotine dependence by ameliorating abstinence-induced aversive consequences leading to negative reinforcement and dampening transient, smoking-induced dopaminergic responses mediating positive reinforcement (Rollema et al., 2007). Varenicline is thought to selectively bind to α4β2 nAChRs acting as a partial agonist/antagonist theoretically leading to 1) a sufficient and sustained DA-tone providing relief from craving and withdrawal by its agonistic quality and 2) a simultaneous attenuation of DA responses to nicotine during smoking by its antagonistic property. The efficacy and safety of varenicline have been demonstrated in several smoking cessation trials (Gonzales et al., 2006; Jorenby et al., 2006; Nides et al., 2006; Oncken et al., 2006; Tonstad et al., 2006), suggesting that the compound is able to break the reward-craving-reinforcing cycle leading to and maintaining addiction. The primary question of interest in the current protocol is: does varenicline, like nicotine, elevate DA levels thereby increasing activity in brain reward structures and prefrontal cortices.

Varenicline increases extracellular DA levels in the NAcc via selective high-affinity binding to α4β2 nAChRs on DA cells (Coe et al., 2005). In comparison to nicotine, varenicline: 1) has approximately 20-fold higher affinity for human α4β2 receptors (Rollema et al., 2007); 2) evokes only 45% of nicotine's maximal intracellular current as assessed by patch clamp studies using cells expressing human α4β2 receptors (Rollema et al., 2007); 3) produces an increase in extracellular DA levels in rat NAcc that is ~60% the maximal effect of nicotine (Coe et al., 2005; Rollema et al., 2007); and 4) produces slower onset and longer duration DA release in rat NAcc (Coe et al., 2005; Rollema et al., 2007). Importantly, varenicline is able to effectively block nicotine-induced DA increase in NAcc and significantly decreases nicotine self-administration by 50% in the rat (Rollema et al., 2007). Taken together, the pharmacodynamic profile suggests varenicline is a potent partial agonist acting at α4β2 nAChRs (Mihalak et al., 2006) yielding a lower level of sustained DA release in the NAcc relative to nicotine. Varenicline receptor occupation at α4β2 nAChRs in the VTA prevents nicotine from binding and precipitating large amounts of DA release thereby limiting the reinforcing and addictive properties of nicotine. The compound's dual action as an antagonist and agonist appears to "stabilize" DA activity in reward pathways both in the presence and absence of nicotine without producing its own dependence syndrome.

Overall the pharmacokinetic profile of varenicline is relatively straightforward, which may simplify the use of this compound in clinical practice and experimental research. Single-doses of orally administered varenicline up to 3mg in smokers and 1mg in nonsmokers are well tolerated, with nausea and vomiting being

dose-limiting factors (Faessel et al., 2006a). With respect to multiple-dose oral administration, 2mg/d appears to be the maximum tolerated dose (Faessel et al., 2006b). Dose-limiting factors can be decreased when varenicline is given as a divided dose (1mg twice a day vs. 2mg once a day) or when the dose is given with food (Faessel et al., 2006b). No safety concerns have been identified when assessing single- or multiple-dose pharmacokinetics in participants with normal renal function. Varenicline is virtually completely absorbed after oral administration and absorption is not affected by food or time of day (Faessel et al., 2006b). Maximum plasma concentrations are typically achieved within 3-4 hrs post-dose (Burstein et al., 2006). The mean elimination half-life of varenicline is approximately 24 hrs and systemic steady-state conditions are reached within 4 days of repeated administration (Burstein et al., 2006; Faessel et al., 2006b). Renal secretion is the major route of varenicline clearance from the body. A substantial portion of the drug-related material excreted in urine is unchanged varenicline (>90% in humans) indicating that the drug undergoes limited hepatic metabolism (Obach et al., 2006). Individuals with moderate or severe renal impairment will experience higher levels of varenicline exposure than those with normal or mildly-impaired renal function (Chantix pharmacological Insert, Pfizer). Pfizer states that the use of varenicline by patients with renal impairments warrants caution. Therefore, only individuals with normal kidney function will be recruited into the present study. Systemic exposure does not appear to differ between smokers and non-smokes and pharmacokinetics are not affected by smoking restrictions (Faessel et al., 2006a).

Based on a recent meta-analysis, varenicline appears to be associated with a 3-fold increase in the odds of successful smoking cessation relative to unassisted quit attempts (Cahill et al., 2007). In addition, more participants in clinical trials remain smoke-free with varenicline relative to buproprion (see: Doggrell, 2007; Glover and Rath, 2007 for review). Across multiple Phase II and III trials, varenicline administration has been consistently associated with higher short-term (7-12 weeks) quit rates (44-49%) in comparison to either placebo (11-17%) or buproprion (29-33%) (Gonzales et al., 2006; Jorenby et al., 2006; Nides et al., 2006; Oncken et al., 2006). In addition, long-term (52 week) quit rates are higher following varenicline treatment (22-23%) relative to placebo (4-10%) or buproprion (14-16%) treatments (Gonzales et al., 2006; Jorenby et al., 2006; Oncken et al., 2006). Taken together clinical trials and preclinical research indicate that varenicline is an efficacious, safe, and well tolerated pharmacotherapy for smoking cessation presumably due to its impact on the DA system at $\alpha4\beta2$ nAChRs in the VTA.

Reward Processing.

It has been postulated that nicotine reinforces smoking behavior via activation of nAChRs in midbrain dopaminergic centers, regions also necessary for reward processing (Mansvelder et al., 2003). For example, impairment of DA transmission, via either lesion or receptor antagonist, results in alterations in nicotine's discriminative stimulus properties, nicotine-induced facilitation of self-stimulation, intravenous nicotine self-administration, nicotine mediated place preferences, and nicotine-induced disruption of latent inhibition (Di

Chiara, 2000). In vivo monitoring studies have shown that the areas most sensitive to a DA-stimulant effect following acute nicotine are the shell of the NAcc (Pontieri et al., 1996) and the bed nucleus of the stria terminalis (Carboni et al., 2000a; Carboni et al., 2000b).

Monetary Incentive Delay Task.

Both animal and human models of reward processing have documented the role of DA neurotransmission. It has been asserted that the mesencephalic DA system contributes to reinforcement learning by mediating feelings of satisfaction experienced upon intake of a rewarding stimuli (e.g., Wise et al., 1978). More recently, however, it has been suggested that the role of DA in reward processing relates to the anticipation of rewards, rather than to the hedonic experience associated with receipt of that reward (Phillips et al., 1991; Richardson and Gratton, 1998). Berridge and Robinson (1998; 2003) have suggested that DA plays a role in the attribution of incentive salience to a conditioned stimulus or its representation. In this account, incentive salience consists of both perceptual and motivational elements, thus salient events incur attention and evoke goal-directed behaviors. Conversely, Schultz and colleagues (e.g., Schultz et al., 1997; Schultz, 1998, 2002) suggest that midbrain DA neurons respond to changes in the temporal prediction of the "goodness" of ongoing events, such that a phasic increase in DA activity is elicited when an event is better than predicted and a transient cessation of DA activity occurs when an event is worse than expected. While these two accounts, i.e. the incentive salience and temporal difference error models, come from different theoretical backgrounds and employ different terminologies, from a computational standpoint they are almost the same and likely are both subserved by the same neural system (Egelman et al., 1998). In the current protocol, we will use the *Monetary Incentive Delay Task* (MID: Knutson et al., 2000; Knutson et al., 2001), a measure of incentive salience, to examine brain responses to rewards and reward anticipation, particularly in the NAcc.

The two lines of experimental evidence outlined above (i.e. the interplay between nicotine and DA and the role of DA in multiple aspects of reward processing) suggest a role for nicotine in reward processing via modulation of midbrain DA systems. In addition, the smoking cessation medication varenicline, like nicotine, is also thought to modulate DA activity, although in a less efficacious manner. As such, we will explore DA activity related to reward processing during performance of the MID task in the presence and absence of nicotine and varenicline, both in abstinent-smokers and non-smokers. Specifically, we anticipate that acute abstinence from nicotine (~12hrs) will be associated with weakened incentive motivation in smokers (putatively reflecting low DA levels) which will be "normalized" by the administration of nicotine and varenicline. If activity in the DA reward pathway is compromised in chronic smokers then abstinence should be associated with reduced neural, behavioral, and subjective responses to reinforcers. Furthermore, responsivity in the reward system should be increased by triggering DA release with nicotine or varenicline in both smokers and non-smokers.

Probabilistic Reversal-Learning Task.

Reversal-learning tasks involve the alteration of behavioral responses according to changes in stimulus-reward contingencies through feedback-guided learning (Cools et al., 2002; Budhani et al., 2007; Hampton et al., 2007; Hampton and O'Doherty, 2007; Cohen et al., 2008). In this paradigm, participants first learn which of two alternatives is the more rewarding, and then flexibly switch their choices when contingencies change. The ability to adapt behavior when previously rewarded behaviors are no longer advantageous is likely dependent on prefrontal and limbic brain structures such as OFC, ACC, ventral striatum, and amygdala. Activations in these regions predict behavioral adjustments according to changing reinforcement contingencies (Cools et al., 2002; O'Doherty et al., 2003; Cohen and Ranganath, 2005; Hampton et al., 2006; Cohen and Ranganath, 2007), possibly even before overt behavioral responses are executed (Hampton and O'Doherty, 2007). Interactions between striatal and orbitofrontal regions are important for optimal reversal-learning and are thought to be modulated by serotonin and DA neurotransmitter systems (Clark et al., 2004; Frank and Claus, 2006). In the rodent, experimental manipulations of the DA system via amphetamine administration (Idris et al., 2005), D2 receptor genetic knockout (Izquierdo et al., 2006), pharmacological blockade of D2/D3 receptors (Lee et al., 2007), or surgically induced DA depletion (O'Neill and Brown, 2007), have been shown to impair initial-learning and/or reversal-learning. Furthermore, repeated cocaine administration in both non-human primates (Jentsch et al., 2002) and rats (Calu et al., 2007) has been shown to produce severe reversal-learning deficits, presumably through a disruption of DA mediated striatal-OFC interactions. In addition to striatum and OFC, the amygdala is also an important structure involved in the learning of stimulus-reward associations (Hampton et al., 2007; Cohen et al., 2008). Recently, Hampton and coworkers (2007) have compared the performance of two subjects with focal, bilateral amygdala lesions with healthy controls in this paradigm. Their results indicated that the amygdala plays a critical role in establishing reward-related activity in PFC and ultimately behavioral choice.

It has been suggested that impairments in the capacity for reward-learning and/or reversal-learning may be related to breakdowns in fronto-striatal and amygdala-striatal anatomical and functional connectivity (Cohen et al., 2008). Such a breakdown may be particularly apparent in participants with a chronic history of psycho-stimulant abuse. In the current protocol, we will use a reversal-learning paradigm to interrogate OFC and amygdala activity, two important nodes in the MCL network. Given the modulatory role of DA in this paradigm, we expect that nicotine abstinence as well as varenicline and nicotine administration will affect DA-tone and, in turn, brain responses and task performance.

Amygdala Reactivity Task.

The amygdala, a key brain region involved in the perception of fearful and threatening stimuli, is also involved in reward-related learning and decision making (Hampton et al., 2007). Specifically, converging animal and human research indicates that this region participates in the processing of positive and negative reinforcers

and in the learning of cue-outcome associations (LeDoux, 2000; Holland and Gallagher, 2004). In the current protocol, we will probe amygdala functioning and assess potential physiological consequences of DA modulation using an fMRI paradigm known to robustly activate this region (Hariri et al., 2002b; Hariri et al., 2005; Pezawas et al., 2005). Previous studies indicate that DA may modulate the response of the amygdala during the perceptual processing of angry and fearful facial expressions (Hariri et al., 2002a; Tessitore et al., 2002). For example, patients with Parkinson's disease fail to show amygdalar responses when in a relatively hypo-dopaminergic state (i.e., >12hrs after their last dose of DA treatment) but display partially restored activity following DA treatment (Tessitore et al., 2002). In addition, the DA agonist dextro-amphetamine has been shown to increase amygdala activity during task performance (Hariri et al., 2002a). We will use an amygdala reactivity task to specifically probe this brain region, which represents one node in a network involved in reward-learning and decision making. We expect smokers to be in a hypo-dopaminergic state following acute abstinence and thus show reduced amygdala activity relative to non-smokers. Administration of nicotine and varenicline is hypothesized to increase DA-tone and, in turn, increase amygdalar reactivity and functional connectivity with other prefrontal regions. Using subtle manipulations of the DA system via smoking abstinence, nicotine patch, and varenicline administration we hope to further elucidate the role of the amygdala in human reward processing.

Cognitive Control/Performance Monitoring.

Cognitive control processes typically refer to high-level executive functions that direct attention and information processing in the service of flexible goal-directed behavior. In the laboratory, cognitive control processes typically explored include performance monitoring, conflict detection, working memory, inhibitory control, attention switching, and decision making. Convergent evidence across a range of neuroscience methodologies associate these control processes with a network of prefrontal structures including ACC, OFC, and dorsal lateral prefrontal cortex (dl-PFC). During acute abstinence, we hypothesize smokers will show abnormal PFC activity reflecting altered MCL DA functioning, similar to deficits seen following chronic abuse of other psychostimulants (Garavan and Hester, 2007), that can be reduced following nicotine and varenicline administration.

Relatively recent advances in hardware and data processing techniques have made it feasible to simultaneously acquire multi-channel EEG and fMRI data. These two major non-invasive tools used in cognitive neuroscience each possess complementary advantages in terms of temporal and spatial resolution for assessing brain activity. While EEG offers superb temporal resolution for the assessment of neural dynamics on the order of milliseconds its spatial resolution is limited by the blurring and mixing of different electrical signals. In contrast, fMRI offers excellent spatial resolution allowing for the identification of potentially activated brain regions; however, the blood-oxygenation level dependent (BOLD) signal recorded by fMRI is temporally sluggish and separated from neural activations by orders of magnitude. Combining these two modalities during a

simultaneous recording session has been suggested to "…provide a major improvement that will advance considerably our understanding of how cognitive functions are implemented in the brain" (Debener et al., 2006, pp. 558). In the current protocol, simultaneous EEG/fMRI data will be acquired to assess the neural correlates of cognitive control and their possible interactions with DA modulations induced by nicotine and varenicline.

Error/Performance Monitoring.

Flexible goal-directed behavior necessitates a system that is able to monitor actions and outcomes to detect information processing breakdowns and performance errors. A psychophysiological index of performance monitoring is the error-related negativity (ERN), a negative deflection in the event-related brain potential (ERP) with a frontocentral scalp distribution that peaks ~50-100ms after an erroneous response. The ERN is often assessed in paradigms such as the Flanker or Stroop tasks designed to induce a suitable number of performance errors. The neural locus of the ERN is thought to be in/near the ACC, particularly the dorsal aspect. According to a recent hypothesis (Holroyd and Coles, 2002; Holroyd et al., 2005), ACC error-related activity is a manifestation of the same MCL DA system that generates ventral striatal responses related to (un)expected rewards and losses (e.g., Schultz, 1997; Schultz et al., 1997). The theory of Holroyd and Coles posits the ERN is elicited by a negative reinforcement learning signal conveyed from the mesencephalic DA system to the ACC indicating that events are worse than expected. Specifically, a phasic decrease in dopaminergic neuronal activity following error commission disinhibits the apical dendrites of motor neurons in the ACC giving rise to the ERN. The ACC then utilizes this signal to modify task performance via interactions with other prefrontal areas (e.g., dl-PFC) to optimize behavioral output. In contrast, following correct responding, no ERN is produced because the ACC apical dendrites are inhibited by the phasic increase of dopaminergic activity. Thus, according to this framework, the ERN is viewed as a negative reinforcement-learning signal conveyed from the mesencephalic DA system to the ACC in the service of optimizing behavioral output.

Psychopharmacological manipulations as well as assessment of DA-deviant populations provide support for DA's role in the elicitation of the ERN. Administration of the indirect DA agonist d-amphetamine leads to larger ERNs (de Bruijn et al., 2004) while the DA antagonist haloperidol attenuates ERN amplitude (Zirnheld et al., 2004; de Bruijn et al., 2006). Reduced ERN amplitude has also been observed after ethanol intake (Ridderinkhof et al., 2002), which may reflect the impact of alcohol on DA receptors (Holroyd and Yeung, 2003). In contrast, caffeine consumption has been shown to yield larger ERNs, possibly by blocking inhibitory adenosine receptors and thereby increasing DA levels (Tieges et al., 2004). Altered DA functioning may partly explain differences in ERN amplitude observed between healthy controls and some neuropsychiatric populations. For example, error-related activity is larger in patients with obsessive-compulsive disorder (Gehring et al., 2000; Johannes et al., 2001) and Tourette syndrome (Johannes et al., 2002), and reduced in patients with schizophrenia (Alain et al., 2002; Bates et al., 2002; Kerns et al., 2005), Parkinson's disease (Stemmer et al., 2007), anorexia

nervosa (Pieters et al., 2007), and attention deficit/hyperactivity disorder (Fallgatter et al., 2004). Supported by computational modeling efforts and assessments of ERN amplitude in older-aged participants, it has been argued that reduced error-related activity reflects weakened phasic activity of the mesencephalic DA system (Nieuwenhuis et al., 2002).

Given DA's putative role in the generation of the ERN, the amplitude of this ERP component is expected to be modulated by manipulations of the DA system. More specifically, boosting the MCL DA system is expected to result in the strengthening of the error signal carried to the ACC and, in turn, a larger amplitude ERN. Conversely, a hypo-functioning DA system, possibly seen in abstinent smokers, is expected to be associated with reduced ERN amplitudes. A recent report describing reduced ERN-amplitudes in cocaine dependent participants (at least one month abstinent) provides support for the notion that a drug-induced deficiency in the DA system can be detected using this ERP component (Franken et al., 2007). Currently, we are not aware of any reports investigating the effects of nicotine or nicotine abstinence on the ERN or error-related hemodynamic responses. In the present study, error-related activity, assessed using both ERP and fMRI modalities, is expected to be reduced in abstinent smokers (presumably reflecting reduced DA-tone) in comparison to non-smoking controls and in comparison to when abstinent-smokers are administered nicotine or varenicline. Furthermore, nicotine and varenicline administration are anticipated to increase error-related activity in non-smokers.

External Feedback and Error Monitoring.

According to the model of Holroyd and Coles (2002), dorsal ACC (dACC) uses reward prediction errors conveyed via the MCL DA system to promote optimal behaviors. The model contends that dACC is activated by unexpected error information indicating the non-occurrence of reward, regardless of whether the source of that information is "internal" (self-detection of errors) or "external" (negative performance feedback in the outside environment). While the ERN has been related to the self-detection of errors, an ERP component with similar scalp topography has been described following negative external feedback and termed the feedback ERN (fERN: Miltner et al., 1997). This medial frontal negativity peaking ~250ms after participants receive negative performance feedback (Ruchsow et al., 2002; Luu et al., 2003; Nieuwenhuis et al., 2004) or stimuli indicating monetary loss or non-reward (Gehring and Willoughby, 2002; Yeung and Sanfey, 2004; Hajcak et al., 2005; Yeung et al., 2005) is thought to originate in/near the dACC. Functional neuroimaging studies have also demonstrated increased dACC activation in response to negative feedback (Knutson et al., 2000; Monchi et al., 2001; O'Doherty et al., 2001; Bush et al., 2002; Delgado et al., 2003; Ullsperger and von Cramon, 2003; Holroyd et al., 2004). The non-occurrence of reward indicated by either self-detection of errors or negative performance feedback is thought to result in decreased midbrain DA activity. This decreased DA activity representing a negative prediction error signal (Schultz and Dickinson, 2000) is conveyed to the medial prefrontal cortex putatively giving rise to the ERN and fERN.

The transient cessation of DA activity in the midbrain (i.e., VTA) encodes a prediction error signal, but how these DA neurons are inhibited in the absence of expected rewards is less obvious. Recent studies have suggested that the habenula, an epithalamic structure on the dorsomedial surface of the caudal thalamus (Lecourtier and Kelly, 2007), is likely involved in reward-related modulation of DA activity (Matsumoto and Hikosaka, 2007). Cells in the lateral habenula (LHb) project to the substantial nigra (SN), raphe nuclei, VTA, and locus ceruleus and modulate monoamine and cholinergic transmission (Lecourtier and Kelly, 2007). Electrical stimulation of LHb transiently inhibits the activity of ~97% of the DA neurons in the SN and VTA via GABAergic mechanisms (Ji and Shepard, 2007). Conversely, habenula lesions in rodents enhance DA turnover in medial prefrontal cortex, NAcc, and striatum (Nishikawa et al., 1986). Electrophysiological recordings in non-human primates indicate that LHb neurons are excited by non-reward-predicting stimuli and inhibited by reward-predicting stimuli. Conversely, DA neurons show an opposite pattern, being excited by reward-predicting and inhibited by non-reward-predicting stimuli (Matsumoto and Hikosaka, 2007). Furthermore, when an organism is presented with non-reward-predicting stimuli, LHb excitation appears to precede the inhibition of DA cells. Regarding human neuroimaging research, the role of the habenula in the reward processing MCL circuit has been relatively neglected and only two reports appear available. Ullsperger and von Cramon (2003) utilized a task involving positive and negative performance feedback to probe activity in reward-related brain structures. Their results clearly showed that positive feedback was associated with increased activity in the NAcc, while negative feedback was associated with increased habenula, dACC, and insula activity. In another study, the habenula was robustly activated following informative negative feedback in healthy but not schizophrenic participants (Shepard et al., 2006). The emerging view is that the habenula is a critical structure involved with reward processing, performance monitoring, and cognitive control. Increased LHb activity following the non-occurrence of reward appears to increase the inhibition of midbrain dopaminergic nuclei resulting in decreased DA output and, in turn, activation of dACC.

In the current protocol, brain activity in the dACC, LHb, and the striatum following informative negative and positive feedback will be assessed. Similar to predictions derived for error-related activity, feedback-related activity is expected to be modulated by DA tone. In addition, assessment of habenula activity in both smokers and non-smokers may provide additional insight into the functional role of this relatively unexplored structure. A major strength of the current protocol is that it will allow us to probe several brain regions including the habenula, striatum, amygdala, OFC, and ACC that likely form a network of brain regions involved in outcome monitoring, reward prediction, and behavioral optimization that may be altered by drugs of abuse.

Response Inhibition.

The inability to inhibit a prepotent response tendency is a common characteristic seen in numerous pathologies that loosely fall under the rubric of impulsivity disorders. Chronic drug abuse, pathologic gambling,

obsessive-compulsive disorder, attention deficit/hyperactivity disorder, and Tourette syndrome share symptoms of disinhibition and loss of self-control which may be related to a dysfunctional DA system (Kaufman et al., 2003; Muller et al., 2003; Roth et al., 2007; Suskauer et al., 2007). While successful inhibitory control likely involves the recruitment of medial prefrontal structures, such as supplemental motor area and ACC, hypoactivity of these regions in cocaine-using participants has been associated with reduced response inhibition in a Go-NoGo task relative to controls (Kaufman et al., 2003). One possible mechanistic account for ACC hypoactivity in drug users is that frequent phasic increases in ACC DA activity during drug consumption may produce a down regulation of DA receptors in the ACC (Garavan and Stout, 2005). This down regulation could result in a basal hypo-dopaminergic state possibly related to poor response inhibition. Like cocaine, nicotine self-administration is thought to lead to phasic increases in DA throughout the MCL system and presumably may also result in a hypo-dopaminergic state. This hypo-dopaminergic state in abstinent smokers, indexed by reduced ACC activity and poor behavioral performance, is expected to be reduced by nicotine and varenicline administration.

Relation between Reward Processing and Cognitive Control.

Although little data are available concerning how brain reward regions interact with prefrontal networks involved in cognitive control, it has been theorized that striatal reward pathways modulate the recruitment of frontal cognitive resources (Holroyd and Coles, 2002; Ullsperger and von Cramon, 2003; Montague et al., 2004). Understanding the interaction between a reward system that responds to outcome expectations and a prefrontal network that responds to needs for cognitive control may provide insight into reinforcement learning mechanisms (Montague et al., 2004), incentive salience/attentional allocation (Garavan and Hester, 2007), and decision making processes (Satterthwaite et al., 2007). The intersection of these two dissociable systems, i.e. a limbic reward system and a neocortical control network, may involve the paralimbic anterior cingulate regions. Recently, in a gambling paradigm, a frontal network (including ACC) showed increased activity in response to losses that violated reward expectations, whereas dorsal striatal activity was unaffected or even suppressed by such worse-than-expected events (Satterthwaite et al., 2007). This pattern of activity is consistent with Holroyd and Coles (2002) ERN-generative model, which proposes that phasic suppression of DA neuronal firing disinhibits ACC neurons, which in turn promotes the recruitment of cognitive control. Furthermore, it has been proposed that a similar dopaminergic gating mechanism may be a fundamental reinforcement learning signal (Montague et al., 2004) that could be usurped by drugs of abuse. The current protocol provides a novel approach, using nicotine and varenicline to mildly alter DA-tone, to elucidate the interaction between these two dissociable systems.

Genetic Association.

We plan to implement imaging genetics as secondary analyses. Imaging signals (BOLD, DTI, and Connectivity z score) will be imaging phenotypes. Genotyping will be done using the Addiction Gene Array and

other genotyping methods. The DA system is thought to be involved in the common final pathways for several substances of abuse. We are interested in the effects of dopaminergic genes especially DRD2 on the BOLD signals in dorsolateral frontal lobe, ACC, and ventral striatum, as well as cingulate-ventral striatum connectivity. For nicotine related genes, we are interested in genes associated with the nicotinic receptors such as nicotinic receptor genes CHRNA4 and CHRNB2 that have been shown to be associated with nicotine addiction. Phenotype-genotype data analyses will use association study design to compare genotype by group interactions; and regression analysis to explore the additive effect of candidate allele on imaging phenotypes. The specific analytic plan will in part depend on genes and polymorphisms discovered in the future. For instance, if specific polymorphisms in α4β2 subunits are identified in the future as functional and related to nicotine addiction, we will genotype these polymorphisms. Given the rapid advance in genomic discovery, it is reasonable to finalize the genotyping plan at the end of the study.

C. Study Objectives/Hypotheses

Primary goal: To investigate neurophysiological and behavioral changes related to nicotine and varenicline administration during *reward processing* and *cognitive control* tasks in abstinent-smokers and non-smokers. This protocol will assess the degree to which potential abstinence-induced performance deficits in deprived smokers can be ameliorated by nicotine or varenicline. Smokers are expected to show abnormal functioning in limbic and paralimbic regions following acute (12hr) abstinence, reflecting deficit DA activity in MCL circuitry. In non-smokers, the effects of nicotine and varenicline will be assessed to dissociate the reversal of nicotine-abstinence effects from pure cognitive enhancement effects.

Secondary goal: To use nicotine and varenicline to probe the MCL DA system to relate reward-based striatal activity to medial prefrontal activity associated with the psychological construct of cognitive control. The neural mechanisms thought to underlie reward processing and cognitive control are theoretically likely to be affected by DA modulations related to nicotine abstinence, as well as nicotine and varenicline administration.

Aim 1: To compare brain activation patterns and behavioral performance associated with *reward processing* in deprived-smokers to that observed in non-smoking controls both in the presence and absence of *nicotine*. We hypothesize that habitual smokers possess a hypo-functioning dopaminergic system and experience DA-enhancing effects from nicotine. Thus, when regularly smoking, a smoker's hypo-functioning DA system is "masked" and appears "normal" due to the pharmacological "boost" from nicotine. Hypo-functioning during periods of acute abstinence is expected to be manifest in the form of blunted responses to reinforcers (MID task), impaired reversal learning, and reduced amygdala reactivity.

Hypothesis 1.1: (brain activation) In the absence of nicotine, abstinent-smokers will display hypoactivity in brain regions previously associated with reward processing (e.g., NAcc, amygdala, OFC) in comparison to non-smokers.

Hypothesis 1.2: (brain activation) Nicotine administration will increase activation in reward processing regions in abstinent-smokers and possibly in non-smokers.

Hypothesis 1.3: (behavioral) In the absence of nicotine, abstinent-smokers are expected to find normally rewarding stimuli (i.e., money, MID task) less salient, take longer to switch behavioral responses during reversal-learning, and respond more slowly in the amygdala reactivity task.

Hypothesis 1.4: (behavioral) Nicotine administration will lead to increased DA release in both smokers and non-smokers and will be associated with enhanced reward salience, improved reversal learning, and faster responses in the amygdala reactivity task.

Aim 2: To compare brain activation patterns and behavioral performance in task paradigms associated with *cognitive control/performance monitoring* (Flanker Task: error-processing, Motion Prediction Task: negative feedback, and Go-NoGo Task: response inhibition) in non-smoking controls with that seen in abstinent-smokers both in the presence and absence of nicotine. Smokers may show reduced functioning in prefrontal cortex during acute abstinence, reflecting a deficiency in MCL circuitry. Cognitive correlates of this deficiency will be manifest as impaired response inhibition and online error detection. Neurophysiological manifestations of this deficiency may be identified using ERP, fMRI, MRS, and connectivity assessments.

Hypothesis 2.1: (brain activation) In the absence of nicotine, abstinent-smokers will display hypoactivity in brain regions previously associated with performance monitoring (frontal midline structures such as dACC and supplemental motor area) in comparison to non-smokers. Following errors, abstinent-smokers will show reduced amplitude (f)ERNs and reduced error-related fMRI signals.

Hypothesis 2.2: (brain activation) Nicotine will increase activation of these brain regions and (f)ERN amplitudes in abstinent smokers and possibly in non-smokers.

Hypothesis 2.3: (behavioral) In a hypo-dopaminergic state abstinent-smokers will show decreased behavioral adjustments following errors and reduced response inhibition. Nicotine administration will improve behavioral measures.

Hypothesis 2.4: Smokers will show altered biochemistry (MRS) in dorsal ACC, and altered functional connectivity between reward processing and cognitive control brain regions (resting) relative to non-smokers.

Aim 3: To determine if varenicline administration increases activity in regions associated with reward processing and cognitive control. In addition, the ability of varenicline to ameliorate deprivation-induced hypoactivity in task specific brain regions will be assessed. Finally, due to varenicline's partial antagonist properties, increased brain activity following nicotine administration is expected to be blocked following a full varenicline dosing regime.

Hypothesis 3.1: (brain activation) In both groups, varenicline will increase activity in brain regions previously associated with reward processing and cognitive control.

Hypothesis 3.2: (brain activation) Regional hypoactivity observed in nicotine-free, abstinent-smokers before varenicline dosing will be reduced after varenicline.

Hypothesis 3.3: (brain activation) Nicotine-induced increases in brain activity observed before varenicline will be blocked after varenicline administration due to the compounds partial antagonistic properties.

Aim 4: To relate cognitive control/performance monitoring activity in the medial prefrontal cortex with striatal reward-processing since both are considered to be mediated by DA. Mild pharmacological manipulations of the DA system are expected to modulate medial prefrontal cortex and striatal activity in a similar fashion.

Hypothesis 4.1: ERN amplitude, frontal midline error-related fMRI signals, and the intensity of striatal reward-related activities are expected to be positively correlated.

Aim 5: To determine the relationship between: 1) cholinergic genetic polymorphisms that may mediate nAChR binding properties (e.g., CHRNA4 α4 subunit gene), and 2) monoaminergic polymorphisms that may be related to the rewarding effects of nicotine (e.g., DRD2), on the magnitude of nicotine and varenicline effects. In addition, we may genotype additional genes which in the interim between the beginning of this study and its conclusion may have been discovered to alter nicotine response. Genotyping of such loci would be performed as necessary to insure the integrity of positive findings.

Hypothesis 5.1: Previously, a single-nucleotide polymorphism (SNP) on the gene that codes for the α4 subunit of nAChRs has been associated with α4β2 binding properties and sensitivities to the acute effects of nicotine in human participants (Hutchison et al., 2007). A relationship between this SNP and the magnitude of nicotine and/or varenicline effects is anticipated.

Hypothesis 5.2: Nicotine and varenicline effects are expected to be related to polymorphisms previously associated with the DRD2 gene (Klein et al., 2007).

D. Subjects

Study Population.

There will be two study populations: 1) healthy nicotine-dependent adult participants who smoke 10 or more cigarettes per day; and 2) healthy non-smoking, non-drug dependent controls. Participants will not be discriminated against on the basis of gender, race, or ethnicity. Subjects must be generally healthy, right-handed, male or non-pregnant/non-lactating females between the ages of 18-55. A "participant eligibility checklist" for this protocol can be found in Appendix 1.

Smoker group. The current protocol aims to assess possible deficits in reward motivation and cognitive processing related to acute nicotine deprivation in dependent smokers. More importantly, this study is intended to assess the potential amelioration of such deficits via nicotine and varenicline intake. Withdrawal from nicotine produces negative mood and attentional/cognitive impairments (Kenny and Markou, 2001; Hughes, 2007a, b) that can be reversed by nicotine administration (Rezvani and Levin, 2001; Newhouse et al., 2004; Levin et al., 2006). The alleviation of withdrawal-related symptoms through nicotine may be responsible for relapse and failed quit attempts. The efficacy of varenicline as a smoking cessation intervention may lie in its ability to counteract withdrawal deficits in reward motivation and cognitive processing via DA mechanisms. Thus, it is important to assess participants with an extended history of smoking behavior during periods of acute nicotine deprivation.

Non-smoker group. It is important to assess the effects of nicotine and varenicline administration in healthy non-smokers to provide additional insight into these compounds' mechanisms of action. Further elucidation of the neurobiological effects of nicotine could lead to novel treatments for such pathological states as attention deficit/hyperactivity disorder, mild cognitive impairment, Alzheimer's disease, Parkinson's disease, and schizophrenia (Newhouse et al., 2004; Sacco et al., 2004). Furthermore, when studying the effects of nicotine in only participants with a history of tobacco use, it is difficult to separate the direct cognitive benefits attributable to nicotine from the reversal of withdrawal effects (Hughes, 1991). Nicotine-withdrawal in smokers may cloud the interpretation of nicotine and varenicline effects on reward and cognitive processing. Thus, normal non-smoking adults provide an important group for understanding nicotine and varenicline effects on reward and cognitive processing.

Inclusion criteria.

All participants must be:

(1) between the ages of 18-55. *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. Therefore, older individuals, defined as those over 55, will be excluded from the present study. *Assessment tool(s)*: driver's license, birth certificate, or other government-issued forms of identification.

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

(3) 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.

(4) 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 may not have any current use (past 6 months) of the substance on which they were dependent. *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) and the possibility that varenicline may causes changes in mood and behavior, frequent users, defined as those using twice or more per week within the last month, will be excluded from participation.

(5) 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.

In addition, <u>smokers</u> must:

(1) smoke 10 or more cigarettes per day and have smoked for more than 2 years. *Justification:* The present protocol is interested in how the brain may be altered by nicotine consumption as well as assessing how varenicline administration leads to increased smoking cessation efficacy. *Assessment tool(s):* Self-report, Fagerstrom, Nicotine Dependence History questionnaire.

(2) be able to refrain from smoking for up to 12hrs (at 6 different time points) during the study. *Justification:* The present protocol will investigate possible withdrawal related deficits in task performance and nicotine's effect on brain functioning. Thus, it becomes important to control the level of plasma nicotine levels throughout the study session using the nicotine patch. *Assessment tool(s)*: Self-report confirmed with expired CO levels.

(3) be able to tolerate the nicotine patch. *Justification*: The nicotine patch may be poorly tolerated by a small percentage of individuals, possibly producing nausea. *Assessment tool(s)*: Nicotine patch tolerance test before the imaging visits.

In addition, non-smokers must:

(1) Not have a history of daily cigarette smoking lasting more than a month and no smoking within the past 2 years.

Exclusion criteria.

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 RPR+ (>1:8 without history of adequate 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, or a history of, 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.

(6) have any current, or a history of, major psychiatric disorders, substance-induced psychiatric disorders, suicidal ideations and/or suicide attempts, or currently under antidepressant or antipsychotic medication treatment. *Justification:* Psychiatric disorders may be accompanied by alternations in brain structure and/or function. 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 examination and Wechsler Abbreviated Scale of Intelligence (WASI). IQ estimate must be over 85.

(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 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 pharmokinetics were unchanged in subjects 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.

(10) 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.

(11) 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:

a. Hemoglobin < 10 g/dl
b. White Blood Cell Count < 2400/µl
c. Liver Function Tests > 3X normal
d. Serum glucose > 200 mg/dl
e. Urine protein > 2+
f. Serum creatinine > 2 mg/dl
g. Estimated creatinine clearance <60ml/min

The MRP will retain discretion to exclude based on less extreme lab results. After the screening process has been completed, the MRP 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.

(12) 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.

E. Study Design and Methods

E1) Study Overview.

This study will require 10 visits to NIDA-IRP (one screening, one orientation, 6 fMRI imaging visits, and 2 neurocognitive assessments) over approximately a 5-6 week period. After screening and being admitted into the study, an orientation visit will be devoted to the consenting process and task training. Subsequently, each of the 6 scanning visits will consist of two separate imaging sessions (a morning and afternoon scan) each lasting ~2hrs. During the AM-sessions, data will be collected during performance of three cognitive paradigms (detailed below) selected to assess cognitive control functions, including the self-detection of errors (Flanker task), external-feedback error processing (Motion Prediction task), and response inhibition (Go-NoGo task). During the PM-sessions neurophysiological data will be acquired while participants perform reward processing tasks (i.e., MID, reversal-learning, and amygdala reactivity tasks; detailed below).

All participants will be administered varenicline and placebo-pill over 2 separate two-week periods. Neurophysiological and performance data will be collected during 6 separate imaging visits. The 6 visits are

conceptually grouped into sets of 2 according to the varenicline/placebo-pill dosing periods (baseline, post-varenicline, post-placebo-pill). One set of 2-scan visits (baseline-1 and baseline-2) will take place before either the two-week varenicline or placebo-pill periods. A set of visits will take place after the two-week varenicline cycle (varenicline-1, varenicline-2), and another after the two-week placebo-pill period (placebo-1, placebo-2). Each set of visits involves a cross-over nicotine challenge utilizing a randomized, double blind administration of nicotine transdermal patch (Nicoderm; GlaxoSmithKline, Moon Township, PA) or placebo-patch (on separate days). A schematic overview of the protocol can be found in Figure 2.

Prior to scanning sessions, smokers and non-smokers will be administered either a nicotine or placebo patch to assess the effects of nicotine on task performance. Participants will be blinded as to which sessions involve nicotine- or placebo-patches. Within each subject the order of nicotine or placebo-patch will be kept constant across each set of visits (baseline, varenicline, placebo-pill) but will be counterbalanced across participants. The order of the varenicline or placebo-pill two-week dosing periods will be counterbalanced across participants. Smokers will be asked to abstain from smoking for 12hrs before each scanning visit.

In addition to imaging measures, participants will also be asked to complete several psychological questionnaires (see detailed list below) and to give a blood sample for genotyping. Two additional visits, one taking place during the varenicline period and one during the placebo-pill period, will involve the collection of standard neurocognitive and ERP measures.

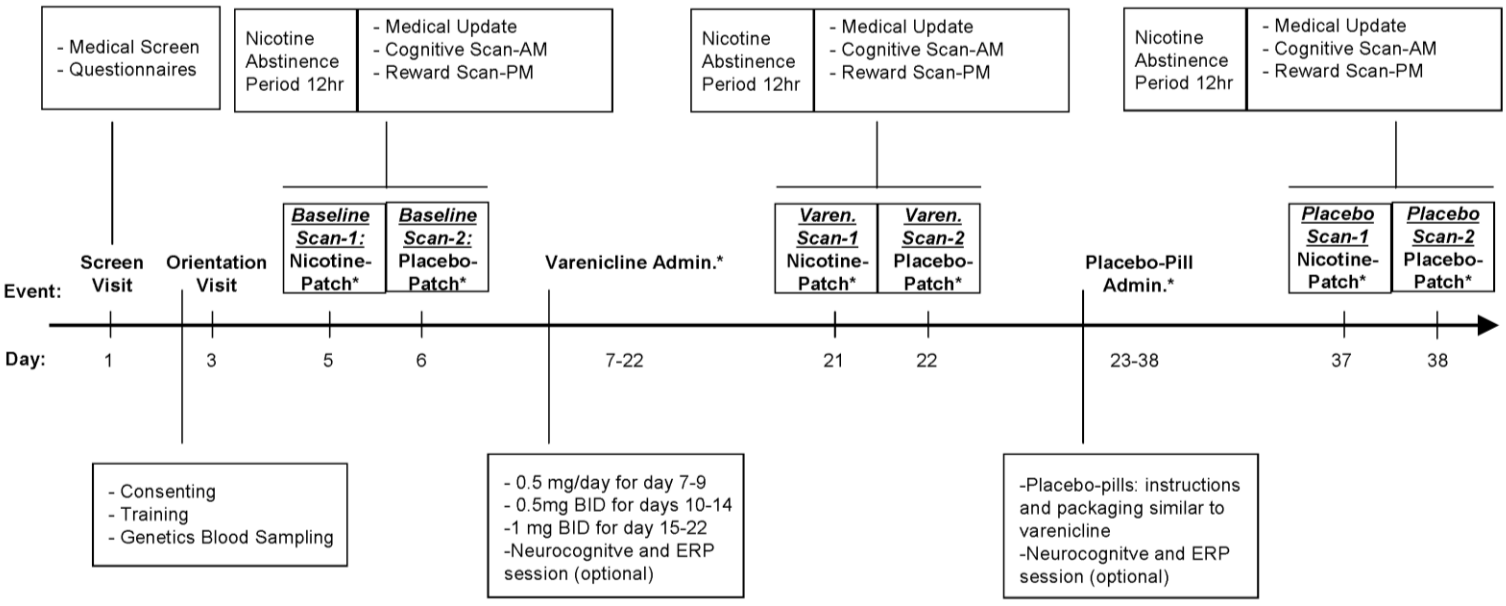

Figure 2. Schematic overview of the current protocol. Please see section E5) Study Procedures for additional details. Note, days indicated are for an ideal scenario. Actual days for each of the study procedures may vary by 1-7 days due to scanner-scheduling, participant work schedules, sick days, holidays and weekends. * indicates study procedures that will be counterbalanced across participants.

E2) Recruitment.

Participants will be recruited from the general population through advertising in city, regional or campus newspapers, flyers, via referrals, or radio and television advertisements (See Appendix 2 for recruitment advertisements). Participants will not be discriminated on the basis of gender, ethnicity, or race.

E3) Screening Methods.

Candidate participants will be screened for participation under an IRB approved NIDA-IRP screening protocol. During screening participants will be given some initial information regarding the study (see Appendix 6 "Fact Sheet") do determine if they are interested in possible participation.

E4) Study Design.

This protocol is a randomized, placebo-controlled, double-blind, repeated-measures varenicline crossover administration study comparing the effects of nicotine (vs. placebo-patch) administration between smoking-deprived cigarette smokers and non-smokers. Neurophysiological and performance data will be collected during 6 separate imaging visits. The 6 visits are conceptually grouped into sets of 2 according to the varenicline/placebo-pill dosing periods (baseline, post-varenicline, post-placebo-pill). Each set of two scans will involve the randomized, double blind administration of a nicotine transdermal or placebo-patch. Please note, the Lead Investigator, Research Assistants, and Technicians running the study visits as well as the participant will be blind to both nicotine/placebo and varenicline/placebo administration. The MRP will be responsible for maintaining the study randomization tables and submitting pharmacy orders and will not be blind. Please refer back to Figure 2 for a schematic overview.

Nicotine Administration During Scanning Visits.

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. Smokers will be instructed to abstain from smoking/nicotine for 12hrs before each of the two separate scans (please see "*Nicotine Abstinence Period*" below for justification). During the smoker's nicotine scans, 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*" for justification). The minimum dose for smokers consuming 10-15 cigarettes per day will be a 21mg patch and this minimum 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 (Zevin et al., 1998; Ebbert et al., 2007). Non-smokers will receive a 7mg patch to assess the absolute effects of nicotine (as opposed to the amelioration of abstinence-induced deficits) on task

performance. Transdermal nicotine has been previously used in studies exploring the effects of nicotine with non-smokers (e.g., Inami et al., 2005; Giessing et al., 2007) and 7mg is considered a low-dose for the purposes of the present study (Matta et al., 2007, pp. 280). The use of higher doses in non-smokers may lead to side effects (e.g., nausea, headache) that could interfere with task performance. The likelihood of strong reinforcing properties leading to potential abuse liability from this dose of transdermal nicotine is considered to be low. The abuse liability of a drug depends on the kinetics of drug delivery, such that reinforcing properties are greater for delivery routes associated with faster absorption/administration (Johanson and Fischman, 1989). Since nicotine plasma levels peak approximately 3-4hrs after patch application (Gore and Chien, 1998), the protracted absorption of nicotine is unlikely to produce strong reinforcing properties. A large body of literature indicates that nicotine transdermal patches are safe and posses very low abuse liability (Pickworth et al., 1994; Joseph et al., 1996).

Nicotine Abstinence Period.

The elimination half-life of nicotine is approximately 2hrs (Benowitz, 1990), but at least 8hrs of abstinence may be required in order for nicotine plasma levels to decline, allowing for the detection of physiological effects related to drug administration (Matta et al., 2007). Accordingly, many studies interested in exploring the effects of nicotine deprivation/administration have smokers abstain from smoking overnight. Previous literature reviews suggest that a general nicotine withdrawal syndrome begins within the first 1-2 days after deprivation/cessation, peaks in the first week, and lasts 2-4 weeks (Hughes, 2007a). Individual withdrawal symptoms (e.g., anger, anxiety, depression, difficulty concentrating) may peak within the first 1-3 days. In the current protocol, 12hrs is accepted as a reasonable period of nicotine deprivation to assess abstinence effects while maximizing non-smoking compliance. Given that the current protocol requires several smoking cessation periods, we are concerned that non-smoking compliance may become an issue for longer duration abstinence periods.

Given that 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), robust effects are also anticipated in the current study. 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). Regarding electrophysiological indices of brain functioning, nicotine administration after abstinence has been associated with: 1) decreased EEG power in lower frequency and increased power in higher frequency bands (Knott et al., 1999); 2) increased amplitudes in the P300 ERP component (Knott et al., 1999; Gilbert et al., 2007); and 3) an augmentation in the mismatch negativity component (Baldeweg et al., 2006). 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). In the present study, a 12-hour abstention is anticipated to produce adequate effect sizes for the detection of statistically significant behavioral, electrophysiological, and hemodynamic differences between placebo and drug conditions.

Individually Tailored Nicotine Dose.

Most previous studies exploring the effects of nicotine on performance have used a standard dose across subjects despite individual differences in smoking behavior. In the current experiment, we consider individual differences in nicotine intake to be an important factor related to pharmacological responses (Hughes et al., 1999). Chronic nicotine administration in animals (Marks et al., 1986) and humans (Breese et al., 1997) elicits a dose-dependent increase in the number and/or binding affinities of neuronal nicotinic receptors as well as receptor sensitivity states (Buisson and Bertrand, 2002). Nicotine binding levels, assessed in postmortem human brain tissue, have been shown to be positively correlated with the amount of daily nicotine intake (packs/day) before death (Breese et al., 1997). Given a potential dose-dependent modulation of nicotinic receptors we have opted for an individualized dose of nicotine, as opposed to a standard dose across all participants. Individually tailored nicotine patch doses have been used in smoking cessation trials (Hurt et al., 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.

Varenicline Administration.

Varenicline dosing will start the day following the designated imaging visit (i.e., baseline-2 or placebo-pill-2) and will continue for the subsequent 15 days. The varenicline-1 and varenicline-2 visits will take place at the end of this 2-week period. Varenicline will be titrated as follows: ***Week 1)*** 0.5 mg/d for study days 1 to 3, 0.5 mg twice per day for days 4 to 7; and ***Week 2)*** 1mg twice per day for days 8 to 15. 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. Smokers typically are able to better tolerate varenicline than nicotine-naïve individuals. Dose limiting factors are decreased when varenicline is given in a divided dose (i.e., 1mg twice daily vs. 2mg once daily) (Faessel et al., 2006b). A varenicline dosing regime involving titration (identical to that in the current protocol) has been shown to significantly improve short- and long-term smoking cessation rates while reducing the incidence of nausea (Oncken et al., 2006). Given that non-smokers may present more varenicline related side effects than smokers, the daily dose of varenicline may be reduced (on a case-by-case basis) to alleviate such symptoms and increase retention rates if necessary.

A similar 2-week period using placebo-pills will also be employed in the current protocol to control for placebo and practice effects. Placebo-pills will look and be packaged in similar ways as the varenicline pills. The instructions for the placebo-pills will be the same as for varenicline in order to maintain the blind. Placebo-pill administration will begin the day following the designated imaging visit (i.e., baseline-2 or varenicline-2) and will continue for the subsequent 15 days. The order of varenicline or placebo-pills will be counterbalanced across participants.

E5) Study Procedures.

The current protocol will require 8-10 visits over the course of approximately 5-6 weeks (1 screening, 1 orientation, 6 scanning visits, and 2 neurocognitive assessments). The screening visit described above will last ~3 hours, as will the orientation visit. Each scanning visit will last ~8.5hrs. The neurocognitive/ERP sessions will last ~4hrs. The total time commitment requested from each participant over the duration of the entire study is about 65hrs. Figure 2 indicates a timeline for an idealized scenario which requires ~38 days. Actual days for each of the study procedures may vary by 1-7 days due to scanner-scheduling, participant work schedules, sick days, holidays and weekends. Participants may miss appointments on ideal dates due to personal reasons or weekends/holidays. To prevent unnecessary dropout and increase completion rate, imaging visits can be completed within a range from 2 days prior to 7 days after the ideal dates stated in Figure 2. If each of the relevant study procedures was delayed by the maximum 7 days, the maximum duration of the entire procedure period would be ~11 weeks from the first to the last imaging visit.

E5.A) Screen Visit.

During the screen candidates will sign a screening consent and undergo a targeted medical history and physical examination to determine study eligibility.

E5.B) Orientation Visit.

After being admitted into the study, a visit to NIDA-IRP will involve protocol consent procedures, task training in the mock scanner, blood draw for genetics testing, baseline questionnaire completion, and instructions on a nicotine patch tolerance test.

After obtaining signed, informed consent (see also section "M. Consent Documents/Process") participants will complete a training session where they will be instructed on and practice the experimental tasks in the mock scanner. The mock scanner training session will familiarize the participants with study procedures and the scanner environment, and allow for the collection of baseline behavioral data that will be used to individually tailor task levels for each participant. Participants will be trained using a set number of trials as opposed to being trained to

a criterion level. Given that training and scanning will take place on separate days, individuals will receive training refreshers during subsequent visits.

Venous blood will be drawn during this visit for genotyping and assessment of baseline nicotine and cotinine plasma levels. Blood samples will be stored indefinitely under appropriate NIH guidelines in the NIAAA laboratory of Dr. David Goldman where the samples will be used for genotyping. Blood samples for assaying nicotine and cotinine levels will be stored on ice and laboratory tests will be conducted in the NIDA-IRP laboratory of Dr. Marilyn Huestis. Cotinine is widely used as a biomarker of daily nicotine intake, because cotinine concentrations in the plasma and urine of smokers are more stable than nicotine levels throughout the day as a result of its longer half-life. No personal information will be attached to the samples.

During the orientation visit a series of questionnaires (see below) related to nicotine use and dependence, withdrawal symptoms, psychological state and mood inventories will be administered. Participants will also complete the alcohol/drug use section of the Addiction Severity Index (ASI) to characterize previous alcohol/drug use behavior.

At the end of this visit, Nicoderm patches of appropriate dose will be distributed to the participants for a patch toleration test. Subjects will be instructed to remove the patch after 8hrs or at any time bothersome side effects develop. Subjects will be provided with instructions on how to apply the patch (see Appendix 3). Subjects will be given a number to call and speak with a protocol investigator 4hrs after patch application to assess any adverse side effects. If participants do not call, a protocol investigator will contact them. If the subject develops significant side effects, particularly nausea, the participant may be excused from the study depending on severity.

E5.C) Scanning Visits.

Each of the 6 scanning visit (baseline-1 and 2, varenicline-1 and 2, and placebo-pill-1 and 2) will be essentially identical. Each visit will consist of two 2hr scanning sessions, one taking place in the morning (AM) and one in the afternoon (PM). The AM-scanning session will involve tasks assessing aspects of cognitive control (referred to as the cognitive-session) and the PM-session will involve reward processing tasks (referred to as the reward-session). Participants will be instructed to abstain from nicotine (12hrs), alcohol (24hrs), caffeine (12hrs), and marijuana (24hrs) before the visit. Smokers will not be able to smoke during the visit. All imaging visits will take approximately 8.5hrs to complete. A general timeline for a given imaging visit can be found in Figure 3 and further details regarding each component follow.

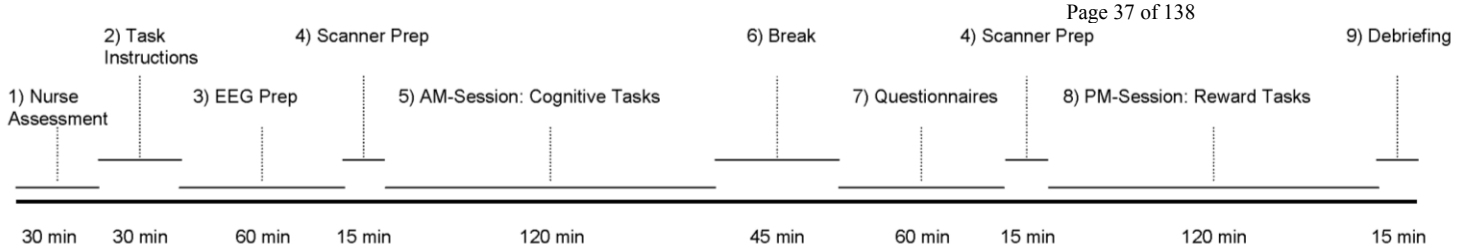

Figure 3. Imaging visit general timeline. Note: all times are approximate.

1) Nurse Assessment.

Upon arrival at NIDA-IRP participants will undergo a medical update which will include vital sign assessment, 12-lead ECG, a breathalyzer test for expired ethanol, an expired CO test for smoking-abstinence compliance, a drug screening (TRIAGE®, Biosite Diagnostics), and a metal/MRI safety screening. 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. Participants will not be permitted to complete the visit if there is admission of marijuana use in the 24hrs preceding the visit or acute signs of intoxication. The medical assessment will last approximately 30 min. The nurse will also apply the study patch (nicotine or placebo-patch) to the participant's back. To allow nicotine plasma levels to reach stable levels, functional brain scanning will begin ~2hrs after patch placement.

Nicotine/Placebo Patch Application. Each set of 2-imaging visits (baseline-1 and 2, varenicline-1 and 2, and placebo-pill-1 and 2) involves a double-blind, placebo controlled, cross-over nicotine challenge. Researchers in the Neuroimaging Research Branch have successfully administered nicotine via transdermal patch in previous fMRI studies (Hahn et al., 2007). In the current protocol, participants will wear either a nicotine or placebo-patch. The placebo-patch is a square, two layer-thick boxing tape that is the same size, thickness, and stickiness as the nicotine patch. We are also in the process of obtaining a specially made placebo-patch from the Nicoderm suppler that would look indistinguishable from the active patch. We will use this commercially available placebo if and when it becomes available. The order of nicotine and placebo will be counterbalanced across subjects. Patches will be applied to a non-hairy part of the upper back or upper arm under a large bandage in order to insure that subjects are kept blind to drug condition. A small amount of blood will be obtained after each scanning session to assess nicotine and cotinine plasma levels. Venous blood samples drawn from an arm will be obtained for both placebo and nicotine patch conditions in order to maintain the blind.

Analysis of blood nicotine and cotinine levels. Samples will be stored on ice and centrifuged within 2 hrs of collection. Plasma will be frozen at -20ºC for subsequent electron impact gas chromatography/mass spectrometry (GC/MS) analysis by the Chemistry and Drug Metabolism Section. Deuterated internal standards (nicotine-d_3, cotinine-d_3, and *trans*-3-hydroxycotinine-d_3) will be utilized to optimize quantification. Nicotine and metabolites will be extracted from plasma at a pH of 5.5 using solid phase extraction with 200mg Clean Screen DAU columns

(United Chemical Technologies, Inc.). Extracts will be derivatized with N,O-bis(trimethyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA + 1% TMCS) to form trimethylsilyl derivatives. Derivatization will improve analyte volatility and chromatography, further optimizing quantification and sensitivity. Derivatized extracts will be injected onto a Hewlett-Packard 6890 gas chromatograph interfaced with a Hewlett-Packard 5973 mass selective detector operated in splitless mode. Separation of analytes will be achieved with an HP-5 (12m x 0.2 mm I.D., 0.33 μm film thickness) fused-silica capillary column with helium as carrier gas at a flow rate of 1.0 mL/min. The initial column temperature of 70°C will be held for 1.0 min, followed by temperature ramps of 30°C/min to 226°C and 7.5°C/min to 280°C. Three ions will be monitored for each analyte and two ions for each internal standard.

2) Task Instructions/Practice.

After the nurse assessment, participants will be reacquainted with the task instructions and will complete brief training runs. During the training runs behavioral data will be collected and used to individually tailor task difficulty during the imaging sessions. The task instruction/practice period will last ~30 min.

3) EEG Preparation.

Simultaneous EEG and fMRI data will be collected during the cognitive-session, while only fMRI data will be collected during the reward-session. Therefore, an EEG preparation period will always precede the cognitive- and a questionnaire period will always precede the reward-session. Both the EEG prep and questionnaire period will last approximately 60min.

Participants will be encouraged to wash their hair just before, or alternatively, upon arrival at NIDA-IRP. During the preparation process an investigator will first 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 below the left eye and another on the upper back to monitor eye blinks and cardiac activity, respectively. 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) Scanner prep.

Approximately 15min will be needed to position the participant in the scanner bed, connect the EEG, setup subject response pads, and prepare other equipment for the recording session.

All scanning experiments will be performed on a Siemens 3T Allegra scanner, located at the NIDA-IRP. 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.

5) Cognitive-Session.

The cognitive-session will consist of three paradigms designed to probe cognitive control functions: *Flanker Task: error-monitoring, Motion Prediction Task: feedback monitoring, and Go-NoGo Task: response inhibition.* During the session simultaneous EEG/fMRI data will be collected. An overview of the cognitive-session can be seen in Figure 4. This session will last ~2hrs.

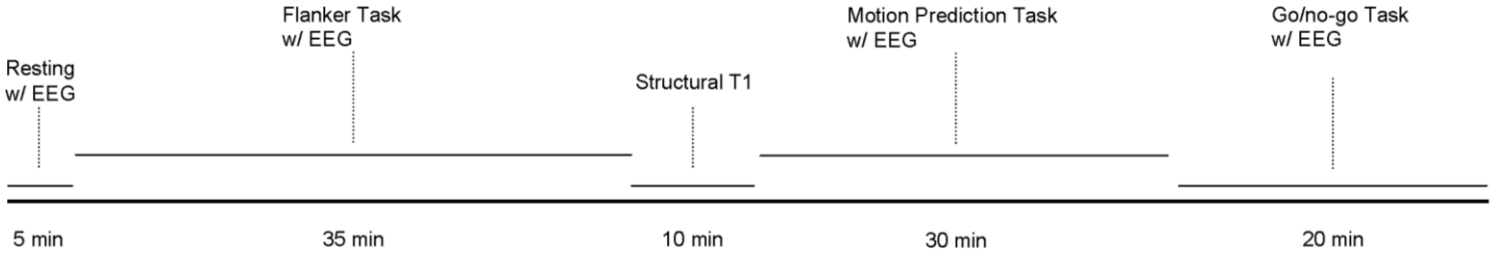

Figure 4. Cognitive-scan timeline. Note: all times are approximate.

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: ~5 min.

Flanker Task: Error Monitoring. Identifying and characterizing neural correlates of performance monitoring has been important for recent theories regarding the functional role of various prefrontal brain structures, such as dACC and lateral prefrontal cortex (Botvinick et al., 2001; Holroyd and Coles, 2002). Current views contend that medial prefrontal cortex serves a performance monitoring function signaling the need for behavioral adjustments in the service of outcome optimization (Ridderinkhof et al., 2004). The need for behavioral adjustments is particularly high following an erroneous action and as such neural activity following errors has been intensively studied using EEG and fMRI (Debener et al., 2005; Taylor et al., 2007).

Participants will complete a modified Flanker task designed to yield sufficient error rates to study the ERN and fMRI signatures of error processing (de Bruijn et al., 2004). In this task participants respond with the left or right index finger according to the central letter (e.g., H or S) of a congruent (HHHHH or SSSSS) or

incongruent (HHSHH or SSHSS) stimulus array. Participants will be presented with a fixation mark at the center of the screen, after which the stimulus array will appear. Participants will be told to respond as fast as possible to avoid feedback indicating that their response was too slow according to a preset reaction time (RT) deadline (see below). Whenever participants respond too slowly, feedback (e.g., "!") will be present after the stimulus array indicating that they need to respond faster on future trials. The RT deadline and feedback are intended to adjust the difficulty level of the task such that a suitable and comparable number of errors are produced across participants and visits. During training in the mock scanner and at the beginning of each imaging day, participants will familiarize themselves with the task in a practice block. After completion of the practice block the mean RT and standard deviation (SD) will be computed for the correct-response trials and used to determine a RT deadline for each individual participant to be used during scanning. The total time needed to complete the flanker task will be ~35 min.

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: ~10 min.

Motion Prediction Task: Performance Feedback. To assess habenula activity that may mediate midbrain DA neuronal responses and neural activation associated with performance feedback, a motion prediction task based on that used by Ullsperger and von Cramon (2003) will be used in the current protocol. During each trial, participants will briefly observe the motion of two balls that are traveling at different speeds from different starting positions towards a finish line on the opposite side of the screen. After a short period the balls, still rather far from the finish, disappear and participants are asked "Which Ball?". The task is to predict which of the two balls will reach the finish first. During the task, difficult levels (operationally defined as the time difference of arrival between the two balls at the finish) will be dynamically adjusted so that error rates are held constant. A constant error rate attempts to ensure that participants will generally remain uncertain about the correctness of their predictions. Feedback about the correctness of the prediction (smiley faces) will be presented after the response. Completion time: ~30 min.

Go/No-go Task: Response Inhibition. Response inhibition may be dependent on prefrontal DA activity. Participants will perform multiple blocks of a Go-NoGo task where the goal will be 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. 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.

6) Break.

Following completion of the AM-session, participants will take a break. During this time participants will be able to have lunch, relax, and spend time in the day room at their leisure. The break will last ~45 min.

7) Questionnaires.

A questionnaire period will always precede the reward-session. This period will last ~60min. The following set of questionnaires will be administered to the participants over the course of their 6 imaging visits. Each item can be found in Appendix 4.

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. 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. 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. 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. 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. Completion time: ~2-3min.

6) 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. 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. Completion time ~10 min.
8) A general smoking history questionnaire will also be administered. To be completed once. Completion time: ~5 min.
9) 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. Completion time: ~15min.
10) 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. Completion time: ~25min.
11) 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. Completion time: ~5-10min.
12) 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. Completion time: ~5-10min.
13) 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. Completion time ~5min.

14) 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. To be completed once. Completion time: ~5min.
15) The Snaith-Hamilton pleasure scale (SHAPS, Snaith et al., 1995) is a 14-item self-report scale designed to measure hedonic-tone/anhedonia. To be completed each visit. Completion time: ~5 min.
16) The Revised Social Anhedonia Scale (RSAS, Eckblad et al., 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). To be completed each visit. Completion time: ~10 min.
17) The Physical Anhedonia Scale (PAS, Chapman and 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). To be completed each visit. Completion time: ~15 min.
18) 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. Completion time: ~5 min.
19) Beck Anxiety Inventory (Beck et al., 1988): a brief inventory similar to the BDI-II that assesses symptoms of anxiety. Completion time: ~5 min.
20) Toronto Alexithymia Scale (TAS-20): a 20-item self report questionnaire to assess emotional awareness. Completion time: ~5min.

8) Reward-Session.

The reward-session will last ~2hrs and an overview of the session can be found in Figure 5 with further details following.

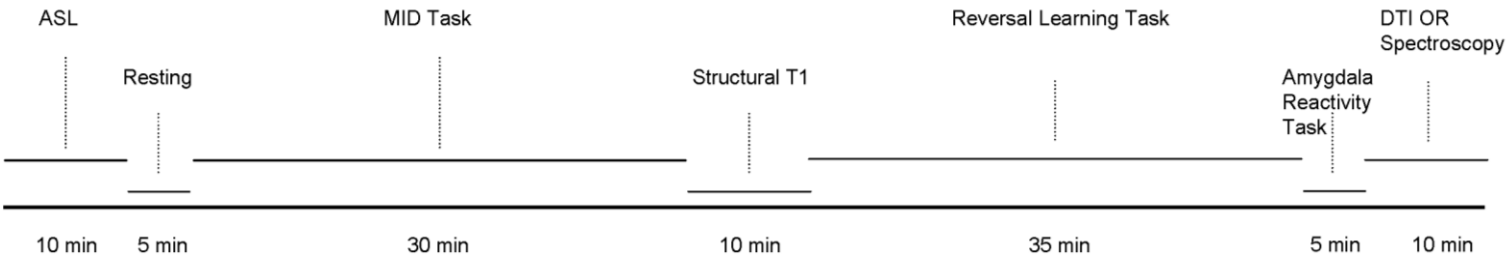

Figure 5. Reward-scan timeline. Note: all times are approximate.

ASL scan. We will acquire arterial spin labeling (ASL) images to assess potential changes in hemodynamic activity resulting from the study drugs. During ASL acquisition participants will be asked to perform basic sensory or motor tasks (e.g. visual stimulation, finger tapping). A recent report assessing ASL images has indicated that the smoking abstinence state (vs. satiated state) is associated with increased regional cerebral blood flow (CBF) in ACC, medial and left OFC (Wang et al., 2007). These brain regions are also implicated in reward processing and cognitive control operations. Assessing different hemodynamic parameters may provide additional insight into abstinence-induced modulations in brain functioning and varenicline's precise mechanism of action. Completion time: ~10 min.

Resting-state fluctuations (RSF) scan. Resting scans, as described above, will precede the rewarding processing tasks. Completion time: ~5 min.

Monetary Incentive Delay (MID) Task. During the reward-session, participants will complete tasks designed to interrogate brain structures related to reward processing, reward-learning, and associated DA functioning. The first task deals with the salience of rewarding stimuli (MID Task), the second task (Reversal-Learning Task) assesses the ability to flexibly adapt to changing reward contingencies, and the third (Amygdala Reactivity Task) probes the activity of one specific brain region thought to be important for decision making.

The MID task was designed to identify brain circuitry involved with anticipation and receipt of monetary gains and losses (Knutson et al., 2000; Knutson et al., 2001; Knutson et al., 2003; Bjork et al., 2004). The aim of the task is for participants to maximize overall winnings by minimizing losses and maximizing gains. There are three trial types in the task: win, loss, and neutral (i.e. neither win nor loss). A significant advantage of the MID task is that it allows one to examine multiple aspects of the reward process, including both reward anticipation and receipt. For purposes in this study, a number of revisions have been made to the published MID task to enhance and extend the original design. In the original implementation, it was possible for participants to avoid losses. Thus, during loss trials, it was difficult to distinguish loss avoidance from loss anticipation. In the present study, to address this we have ensured that all loss trials will incur a minimum loss and that all win trials will

incur a minimum gain. Prior to revealing the monetary value of a given trial, participants will be informed whether the trial is win, loss, or neutral by the appearance of one of three primary stimulus types (square = loss; circle = gain; triangle = neutral). This stimulus item will be followed by a picture depicting the amount of money available for loss or gain on that trial. After the second stimulus, participants will be presented with a target item (a cross). Participants can minimize loss and maximize gain only by responding to the target within an allotted time window. The time window will vary both within experimental trials and between participants. Based on the performance of participants on a practice version of the task, the range of target intervals will be adjusted so that participants are able to optimize performance on ~2/3 of trials. Using this modified design we will be able to observe three different aspects of reward processing, relating to both losses and gains, i.e., anticipation, strategic planning, and reward receipt. It is likely that the salience of rewarding stimuli in this type of measure is increased if the outcome is associated with an actual (rather than abstract) reward that participants will receive on completion of the study. Therefore, participants will receive a performance based payment associated with this version of the MID task. This payment will be equal to 10% of the theoretical total that they score on the task. Therefore, the maximum bonus payment will be $53. Completion time: ~30min.

Structural Images. High resolution structural images as described above will be collected.

Probabilistic Reversal-Learning Task. A monetary probabilistic reversal-learning task will be used to probe reward-related learning, behavioral decision-making, and brain responses. The task used in the current protocol will be based on that from Hampton and colleagues (Hampton et al., 2007; Hampton and O'Doherty, 2007), Cools and coworkers (Cools et al., 2002), and Cohen et al., (2008) previously used to explore striatal, OFC, and amygdala activity. The participant's task is to determine, through trial-and-error, which stimulus is "correct" and to keep track of this when a reversal occurs. At the beginning of each trial participants will chose one of two easily-distinguishable, abstract color patterns presented randomly to the left or right of a fixation cross with a button press. Immediately after a stimulus is selected, a green square will highlight the selected response and feedback indicating reward or loss will be displayed. At the start of the task one stimulus is randomly designated as the "correct" stimulus and its selection results in a monetary reward most of the time (e.g., 75%) and a loss a small percentage of the time (e.g., 25%). Selection of the "incorrect" stimulus leads to a loss most of the time and reward a small percentage of time. After a random number of trials the contingencies will be reversed. The participant needs to determine when a reversal has occurred and thus switch their pattern of responding. It is likely that the salience of rewarding stimuli in this type of measure is increased if the outcome is associated with an actual (rather than abstract) reward that participants will receive on completion of the study. Therefore, participants will receive a performance based payment based on the total amount of money accumulated during this task (max: $56.25). Completion time: ~35 min.

Amygdala Reactivity Task. Participants will complete a simple perceptual task previously shown to produce robust bilateral amygdala activity (Hariri et al., 2002b; Pezawas et al., 2005). This blocked fMRI paradigm consists of two experimental conditions, an emotion task and a sensorimotor control task. During the emotion task, participants view an array of 3 faces and their task is to select (via button press) one of the two facial expressions (angry vs. afraid, bottom of the array) that matches the simultaneously presented target expression (top of the array). 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 emotion" 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: ~5min.

Diffusion tensor imaging (DTI) scan. A DTI scan will be performed once for participant allowing for the assessment of white matter microstructure in both smokers and non-smokers. Previous research has suggested that some addiction-related deficits in cognitive task performance can be related to impaired anatomical connectivity between different brain regions (Pfefferbaum et al., 2000; Lim et al., 2002; Madden et al., 2004; Moeller et al., 2005). DTI data may reveal a significant relationship between white matter integrity, reward/cognitive task performance, and nicotine addiction in smokers. Given the nature of the current investigation the integrity of white matter tracks between ACC, amygdala, striatum, and OFC is of particular interest. Completion time: ~5-10min.

Spectroscopy scan. A proton magnetic resonance spectroscopy (MRS) scan will be used to probe dorsal anterior cingulate cortex (dACC) biochemistry. The dACC has been identified as a critical brain region for the recruitment of cognitive control and behavioral regulation (Botvinick et al., 2001; Holroyd and Coles, 2002) that may be dysfunctional in drug-addicted populations (Garavan and Stout, 2005; Yucel et al., 2007). Previous reports in healthy control populations have used MRS to demonstrate an association between concentrations of the neural metabolite N-acetylasparate (NAA, a marker of neuronal density and function) in dACC and individual performance (RT) differences during a cognitive interference task (Grachev et al., 2001). In the present protocol, NAA as well as other metabolites routinely detected by MRS (e.g., choline and creatine) will be assessed to investigate differences between smokers and non-smokers, to test for metabolic changes resulting from varenicline administration, and to demonstrate an association between neural metabolites and task performance. If a link between biochemistry and task performance can be established this could provide a useful biomarker that can be assessed in the absence of task performance. The identification of such biomarkers would be useful for

assessing participants who are unwilling or unable to comply with task instructions. Assessment of biochemistry through MRS may help explain group and drug effects. Completion time: ~5-10 min.

Reproducibility of Task paradigms. One potential concern related to the present design pertains to the stability of the imaging and behavioral outcomes across repeated assessments. The design is sufficiently robust that it can be used to establish, at least in part, test-retest reliability (i.e., the initial pair of baseline scans). However, this is not fully extensible across an additional 4 visits. Some of the task paradigms chosen are believed to be relatively resistant to practice effects. For example, in interference tasks such as the Flanker or Stroop, hundreds-to-thousands of trials (or several hours of training) are generally needed before practice effects in behavioral measures can be observed (Dulaney and Rogers, 1994; MacLeod, 1991; MacLeod and Dunbar, 1988). If we assume that behavioral measures remain stable across repeated assessments, particularly error rates, it is also reasonable to assume that neurophysiological correlates of error processing are likely to be unaffected as well. Furthermore, in order to maintain the stability of error rates across assessments, RT deadlines are calculated for each subject before each scan session. In the motion prediction task, we also expect behavioral and physiological outcomes to be relatively unaffected by practice. In this task, difficulty levels are dynamically adjusted so that error rates are held constant. A constant error rate attempts to ensure that participants will generally remain uncertain about the correctness of their predictions. In the Go-NoGo task, a time pressure deadline is imposed to maintain stable error rates. There is some evidence to indicate that practice effects in the Go-NoGo task can be observed in behavioral and ERP measures (Schapkin et al., 2007). However, in that study participants completed the task twice a day over a period of 3-weeks. In the current study, participants will have considerably less experience with the task.

The possibility of practice effects in the MID task may be problematic. Learning or practice effects could mask the drug and group effects that are of interest. To determine if unintended effects such as learning or practice effects are present and if larger sample sizes are needed we will conduct an interim analysis after 8-10 participants in each group have successfully completed. Please see "Power analysis" section, particularly the "Interim analysis" subsection (pp.67, lines 1-16). To help mitigate practice effects in the MID task, RT deadlines are adjusted so that participants are able to optimize performance on only ~2/3 of the trials. We believe that the reversal learning task is less likely to show practice effects given the nature of the task. It will take participants several trials to determine if a true reversal has taken place or if an incorrect response is simply related to a probabilistic error. Finally, we also believe practice effects to be less of a concern in the amygdala reactivity task. The response to fearful faces is believed to be robust particularly if different faces are used across runs.

In addition, it should also be noted that participants will receive extensive and repeated training on all tasks. This training, taking place on multiple days, should help mitigate the impact of practice effects on the

measures of interest. Nonetheless, the potential confound of practice effects may have to be kept in mind as a potential limitation of the current study.

Number of tasks. Given the substantial financial, human effort, and imposition on the participants associated with the current protocol we have tried to seek the appropriate balance between collecting as much data as possible and over-taxing participants. Nonetheless, the number of tasks to be performed in a single scan visit is high in comparison to most imaging experiments and this could be viewed as a potential confound. To mitigate the effects of this confound and participant fatigue we have attempted to carefully design the current study. We have divided the scan visits into two 2-hour scan sessions. A 2-hour scan session is typical of other imaging protocols. We have developed carefully timed tasks to maintain this 2-hour schedule. Between tasks and scan session there are also times for breaks. The amount of tasks to be performed will have to be kept in mind as a potential limitation of the study. We have successfully used this strategy in protocol 357, wherein subjects are scanned five times over a 16 day period twice/day, and thus believe that it is both logistically doable and not unduly burdensome for our participants.

By collecting as much data as possible from individuals, using several task paradigms and multiple imaging modalities in a single large study, we are able to gain a more complete understanding of varenicline's mechanism of action in comparison to a scenario involving multiple smaller studies exploring individual task paradigms. Using the cross-over, within-subjects design proposed, we are able to ask more questions using few participants. Furthermore, we are able to ask questions that would otherwise not be possible. For example, many studies have looked separately at reward and cognitive control in addiction, but few have examined the interaction of these systems. Thus, the ambitious nature of the protocol is intended to maximize the amount of data collected while minimizing the number of people exposed to the potential risks/discomforts associated with the proposed study.

9) Debriefing (end of day).

At the end of the second scanning session there will be a debriefing period for discussion of questions/issues related to the day's tasks, scheduling/reminder of subsequent visits, and drug compliance instructions (non-smoking, varenicline compliance). At the end of the study, participants will be given a complete debriefing at which time they will be told which sessions/periods involved active study drugs or placebos.

E5.D) Neurocognitive battery.

We will obtain additional standard neurocognitive battery and ERP measures outside the scanner under placebo and varenicline conditions but without nicotine or placebo-patch application. This will be implemented during the window between 2 days after the full study dose and prior to the date of the first imaging visit. These

tasks will require ~3-4hrs to complete and will be done on a separate day prior to the imaging visit. Smokers are allowed to smoke in their usual smoking pattern, and stop smoking for 1 hour prior to the baseline testing. These tests will examine varenicline effects on behavioral and ERP cognitive performance without nicotine patch or withdrawal manipulation and will enhance our ability to interpret imaging findings during nicotine patch and withdrawal conditions. The baseline neurocognitive battery will test speed of processing, attention/vigilance, working memory, verbal learning, visual learning, reasoning and problem solving, and social cognition, done as paper and pencil and computerized tasks, and will take ~1.5-2 hours to complete. The baseline EEG/ERP will include double click sensory gating, oddball P300, and resting EEG. The EEG preparation process will be the same as described above, however data will be acquired outside of the scanner. EEG preparation and tasks will require about ~1.5 hrs. Basic assessment including breath CO will be done prior to behavioral testing. The Tobacco Craving Questionnaire, Tobacco Craving Scale, and The Minnesota Nicotine Withdrawal scale will be taken for smokers at the end of the behavioral testing. As a whole this study requires many visits. In case there is a scheduling conflict and a participant may only come on restricted times, priority may be given for the imaging visits over these behavioral sessions. We will not consider a participant as a dropout as long as the scheduled imagining visits are completed.

E5.E) Varenicline Administration/Compliance.

Varenicline administration will ideally start the day following the designated imaging visit (baseline-2 or placebo-2 scans) and will continue for the subsequent 15 days. Varenicline will be titrated as follows: 0.5 mg/d for days 1-3, 0.5 mg twice per day for days 4-7, then 1mg twice per day for days 8-15. Participants will be encouraged 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 7 for study pill instructions). One week's worth of varenicline will be distributed to participants at the conclusion of the designated imaging visits. The first week's supply of varenicline will be distributed in blister packages supplied by the pharmacy and participants will be asked to return the package to verify compliance. If a participant misses a dose they will be asked to leave the corresponding pill in its blister, however participants will be strongly encouraged to remember all doses. The second week's supply of varenicline will be prepared by the pharmacy and distributed to participants in person at the end of week-1. Participants will be asked to return the second week's blister package for compliance.

A similar two-week period using placebo-pills will also be employed in the current protocol to control for placebo and practice effects. Placebo-pills will look and be packaged in similar ways as the varenicline pills. The instructions for the placebo-pills will be the same as for varenicline in order to maintain the blind. Placebo-pill administration will begin the day following the designated imaging visit (i.e., baseline-2 or varenicline-2) and will continue for the subsequent 15 days. The order of varenicline or placebo-pills will be counterbalanced across participants. Placebo-pill packages will also be collected and distributed in one-week supplies.

Potential Problems and Alternatives. Participants may miss appointments on scheduled dates due to personal reasons or weekends/holidays. To prevent unnecessary dropout and increase completion rate, imaging visits can be completed within a range of days, i.e., from 2 days prior to 7 days after the ideal dates stated above. Participants will be given spare (backup) study pills containing varenicline or placebo, packed in the same blister package as their main study pills. They will be instructed to use these back-up study pills only if they absolutely cannot come to the scheduled visit so that they will not abruptly discontinue the study. Regardless if all the pills are used, the doses in the pack will be counted at the end of the study for compliance. In addition, as stated above, to reduce potential dropout, varenicline daily-doses may be reduced on a case-by-case basis to alleviate side effects (particularly in non-smokers).

E6) Follow-up and Termination Procedures.

Per the clinical monitoring plan [see section "G) Subject Monitoring" for details], telephone assessments to monitor for changes in mood, behavior, and suicidal ideation will continue for a two-week period following the completion of the last scanning visit. The last scanning visit marks the final day of study pill (varenicline or placebo) administration. According to the recent Pfizer safety update and FDA advisory (2/1/2008 FDA) some patients have experienced neuropsychiatric symptoms not only while being treated with varenicline but also after varenicline treatment was discontinued.

If there is a problem with data quality that is noted during or immediately following the completion of an imaging visit (most likely due to head motion or equipment malfunction), the investigators reserve the right to invite participants in for repeat or additional testing. Ideally these repeat sessions would take place the day after (and no more than 5 days after) the original scan. Participants will be instructed to continue taking study pills (which may be varenicline or placebo) until the repeat session is completed (max 5 days). As per the original session, during these repeat sessions participants will be asked to wear a nicotine or placebo patch. If problems with data quality are noted after the participant has passed the medication cross-over point or been discharged from the study, they will **not** be recalled for repeat testing. Given the complex nature of the study procedures and the time needed (two-weeks) for administration of the placebo or active study pills, participants will **never** be asked to undergo a repeat medication trial. By ensuring the collection of optimal data from each participant we hope to minimize the total number of participants exposed to the risks/discomforts associated with the current protocol. The consent informs participants that repeat or additional testing may be required in order to meet data quality requirements (e.g. if there is a problem with data quality that is noted after the completion of an imaging visit).

Individual participation will be terminated when all study sessions have been completed or if circumstances arise that make continued participation unsafe or ill-advised [see section "G) Subject Monitoring" for details]. For example, if there is a change in the individual's eligibility for participation or if the participant

experiences an adverse event [see section "F) Risks/Discomforts" for details] that would make study continuation inappropriate. The study will also be terminated if a subject decides that they no longer wish to participate. Irrespective of the reason for study termination (e.g. study completion, participant withdrawal, or involuntary termination), the study clinician will determine whether the participant is eligible for study discharge (e.g. an AE has been reported, or continued participation is unsafe or inappropriate). Once the participant is cleared for discharge, they will be advised that their participation has now ended and they may leave the NIDA-IRP facility. At the time of discharge participants will receive compensation in accordance with current guidelines for participant remuneration (see section "S) Compensation" for details). If the participant is interested in continuing to use varenicline to quit smoking, we will refer the participant to his/her physician or provide a referral.

Typically, no data obtained during the course of this study will be shared with the individual participant or their health care providers.

E7) Research and Clinical Care Procedures.

All study procedures are considered to be research-related and no explicit clinical care will be provided to participants under this protocol. All participants entering into the study will be advised during the consent process that varenicline is a smoking cessation drug and that it is being used in the present study strictly for research purposes. Smokers will not be explicitly asked to stop smoking while taking the study drug. Thus, this protocol does not involve clinical care per se. If however, participants express an interest in smoking cessation they will be referred to a smoking cessation program or health care provider for further information regarding varenicline after study completion.

E8) Medications/Devices Requiring IND/IDE.

Varenicline may require an IND since it will be given to non-smoking participants in the current protocol. As per our understanding, the FDA needs an approved protocol to consider an IND application. However, the paperwork is being submitted in parallel to this submission to expedite the process. The current protocol intends to use two study drugs, one an over-the-counter product (nicotine patch) and the other a prescription medication (varenicline). Once the IND application has been approved, we will forward that information to the IRB along with documentation.

E9) Relationship to other Protocols.

The MID reward paradigm and the reversal-learning task to be used in this study are also used in other protocols involving the NIDA-IRP NRB [i.e. #372(C), #388, #398, #399, and #423]. These studies are exploring reward processing in nicotine-dependent adults, cocaine-dependent adults, and adult marijuana users. Moreover, all of these studies involve acute administration of a drug of interest (e.g. nicotine, cocaine, or Sativex®). Also, the response inhibition task to be used is employed in other protocols involving the NIDA-IRP NRB (e.g., #406).

It is expected that data[2] obtained under this protocol may be compared to data acquired in these other protocols, in order to allow us to better understand commonalities and differences in reward processing and response inhibition across different drug using cohorts and healthy controls.

Individuals who participate in this study will not be required to participate in any other protocol. Furthermore, individuals who participate in this study will not participate in other studies involving the use of the same reward paradigms, even if they meet eligibility criteria for those studies. The reason for this exclusion is that we aim to keep the number of exposures to each of the reward measures constant between participants, within studies.

E10) Storage of Data and Samples.

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. 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.

Genetics blood samples are shipped by trained NIDA research associates to Dr. David Goldman's laboratory at NIAAA for genetics testing. Samples are sent with only the ARC number and date of blood draw as identifiers. Other identifiers, such as name or date of birth, are not included with these samples. At NIAAA the samples receive a code consisting of the study abbreviation and a number designating the sample. Genetics data will be stored on a password protected computer in a locked room. The data files are also password protected. Two permanent NIAAA scientists (system administrator, Lisa Moore, and database manager, Pei-Hong Shen) have access to the database files, and another permanent scientist, Longina Akhtar, performs the initial processing of samples, including making DNA and cell lines.

Other biological samples that are obtained from participants will be disposed of once their analysis is complete.

[2] Primarily, we plan to compare fMRI, cognitive and behavioral data, although other data acquired may be used either as factors of interest or regressors in data analyses.

F. Risks/Discomforts

F1) Known Risks and Discomforts.

Risks and discomforts associated with this study include those related to: a) questionnaires and characterization measures, b) nicotine patch administration, c) varenicline administration, d) blood draw, e) MRI scanning, f) EEG recording, g) MRI tasks, h) acoustic noise, i) head foam, j) mock scanner training, and k) genotyping. Risk and discomforts are described further below.

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.

Nicotine Patch Administration.

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

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

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.

Risk Minimization. In order to minimize risks associated with nicotine, only individuals who meet certain medical history criteria will be recruited into the study. Nicotine patch toleration tests are always performed outside the MRI prior to scanning to monitor for any idiosyncratic reactions. During imaging visits, all subjects receiving a nicotine patch will be monitored for blood pressure, respiratory rate, and pulse rate changes prior to scanning sessions and compared to their admission baseline values. Drug administration will be terminated immediately upon participant's request.

Varenicline Administration.

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 [section "G) Subject Monitoring"] consists of 1) weekly in-person assessments at NIDA-IRP during scanning visits or study-pill pickups, and 2) phone call assessments every 2-3 days during, and the one-week period following, study pill administration and a call 10-14 days after last dose.. 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 involvement in the study and the possibility that they may be taking varenicline.

Simultaneous varenicline and nicotine administration.

According to the Pfizer insert regarding the co-administration of varenicline and nicotine: "Although co-administration of varenicline (1 mg BID) and transdermal nicotine (21 mg/day) for up to 12 days did not affect nicotine pharmacokinetics, the incidence of nausea, headache, vomiting, dizziness, dyspepsia and fatigue was greater for the combination than for NRT [nicotine replacement therapy] alone. In this study, eight of twenty-two (36%) subjects treated with the combination of varenicline and NRT prematurely discontinued treatment due to adverse events, compared to 1 of 17 (6%) of subjects treated with NRT and placebo."

While the Pfizer insert does state that NRT+varenicline increases the incidence of side effects in comparison to NRT+placebo, it is unclear whether the combination of NRT+varenicline increases side effects in comparison to the administration of varenicline alone. The difference between NRT+varenlicine and NRT+placebo may be a result of the "typical" varenicline side effects. It is not clear if the concomitant use of NRT and varenicline exacerbates the side effects typically associated with varenicline administration.

Nonetheless, the possibility of increased side effects such as nausea, vomiting, headache, and dizziness related to simultaneous nicotine and varenicline administration can not be ruled out. Such effects may be more likely to occur in non-smoking controls. Participants will be informed of the possibility of increased side effects in the consent

Risk Minimization. Nicotine patch toleration tests will be conducted to identify individuals who may be susceptible to side effects. Daily doses of varenicline may be reduced on a case-by-case basis to alleviate side effects. Drug administration procedures will be terminated immediately upon participant request.

Blood draw.

AE related to blood draw may include:

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

Risk Minimization. Blood draws will only be performed by experienced medical or nursing staff.

MRI Scanning.

When used on appropriately qualified individuals, MRI presents virtually no 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 field of the 3T scanner is higher than that of most clinical magnets and there is a remote chance of other risks associated with the stronger magnetic field that might include temporary dizziness with nausea and flickering light sensations. 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.

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 (> 6hrs) wearing the EEG cap may produce tension headaches. Participants will need to wash the conductive gel from their hair at the end of the recording session.

Risk Minimization. EEG preparation will be conducted by investigators that have extensive experience and new investigators will be well trained in the procedure if needed. Participants will not wear the EEG cap for more than 6 hrs. Participants will be informed of the EEG clean-up process before enrolling.

MRI Tasks.

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

Risk Minimization. In order to reduce discomforts associated with performing the reward and cognitive tasks, scanning session 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.

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.

Head Foam.

The polyurethane foam used for head restraint gets warm for a few minutes while it expands. It is possible that the level of heat generated by the foam could make the participant feel uncomfortable. There is also a risk that the chemicals that are used to make the foam could cause skin or eye irritation if they come into contact with a participant.

Risk Minimization. All study investigators are trained in how to safely make the foam head restraint. Moreover, the foam is always placed inside a double layer of plastic, which is partially sealed before the expansion of the foam begins and before the participant is asked to place their head upon it.

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).

Genotyping.

Potential loss of confidentiality is a particular risk of studies involving genotyping. Under some circumstances, it could be a risk for genetic information about an individual to be made known. For example, genetic information could be interpreted by an insurance company or employer to mean that the person was at increased risk for a problem.

Risk Minimization. Genetics blood samples are shipped by trained NIDA research associates to Dr. David Goldman's laboratory at NIAAA for genetics testing. Samples are sent with only the ARC number and date of blood draw as identifiers. Other identifiers, such as name or date of birth, are not included with these samples. At NIAAA the samples receive a code consisting of the study abbreviation and a number designating the sample. Genetics data will be stored on a password protected computer in a locked room. The data files are also password protected.

G. Subject Monitoring

G1) Parameters to be Monitored.

After patch application, nursing will monitor blood pressure and heart rate every 30 minutes for two hours. During placebo-patch and nicotine scans heart rates will be monitored via pulse oxymeter. After completion of the scan, nursing will remove the patch and measure blood pressure and heart rate.

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 ideation, and suicidal behavior. 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 both 1) weekly in-person assessments, and 2) telephone assessments taking place every 2-3 days will be implemented. During the study, participants will be seen weekly at NIDA-IRP either for scanning visits or to

receive their week's supply of study pills. During these visits, participants will be administered the Beck Depression and Anxiety inventories, as well as brief clinical interviews with the MRP. Participants with suicidal ideations, atypical changes in behavior, those scoring >20 on the BDI 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.

During telephone assessments participants will be asked questions (see Appendix 5), some derived from the BDI, to determine if there if there is a significant level of distress possibly related to the study drug. The MRP or Ph.D. level research associates will conduct these calls after receiving extensive training from the MRP. Research associates designated by the PI to conduct these telephone assessments include Dr. Betty Jo Salmeron, the study MRP and Dr. Matthew T. Sutherland, the study lead associate investigator. Dr. Salmeron is Board-Certified in psychiatry and Dr. Sutherland is an experimental psychologist who is familiar with psychiatry evaluation tools such as the BDI. Furthermore, learning to perform such assessments is an important aspect of Dr. Sutherland's training at NIDA. If the participant reports an increase in neuropsychiatric or physical symptoms they will be transferred to the MRP for further evaluation. The participant will also be transferred to the MRP if any questions or issues arise. At the end of the call participants will be reminded to call if any issues should arise between assessments. Participants will be asked to give two telephone numbers and the best time to be contacted for these monitoring calls. The telephone assessments will continue for a one-week period following the completion of the last scanning visit and a final follow up call will occur 10-14 days after last study medication. The last scanning visit marks the last day that study pills (varenicline or placebo) are administered. According to the recent Pfizer safety update and FDA advisory (2/1/2008 FDA) some patients have experienced neuropsychiatric symptoms not only while being treated with varenicline by also after varenicline treatment was discontinued.

G2) Toxicity.

Acute nicotine poisoning produces nausea, vomiting, abdominal pain, diarrhea, headaches, sweating, and pallor. Burning in the throat, nausea, and vomiting occurring quickly after ingestion may be initial indicators. More severe toxic effects 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.

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 the study 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.

The Common Toxicity Criteria (CTC) from the National Cancer Institute (NCI) will be used to provide an objective measure related to the severity of adverse events associated with the study drugs. The CTC will be used for collecting, monitoring, and reporting of adverse effects. The table below contains a list of adverse effects that are pertinent to the drugs used in the current protocol. The table was adapted from the "Common Toxicity Criteria Document" (PDF) [Publish Date April 30, 1999 CTC v2.0, http://ctep.cancer.gov/forms/CTCv20_4-30-992.pdf.

During telephone and in-person assessments participants will be directly asked about the most commonly anticipated adverse effects (e.g., nausea, vomiting, dizziness, headache) and potential mood changes (e.g., agitation, irritability, depression, suicidal ideation). Open ended questions will be used to assess for the presence of other physical adverse effects. During in-person assessments, the presence and severity of physical adverse effect (e.g., cardiovascular/blood pressure) will be assessed. A severity grade of 2 or greater on a mood alteration or behavioral adverse event will result in automatic withdrawal. A score of 3 or greater on other expected adverse events in the table will also result in automatic withdrawal from the study. The MRP may also withdraw a participant at less extreme levels if clinical assessment indicates this would be in the best interest of the participant.

Severity Grade	0	1	2	3	4
Allergy/Immunology					
Allergic reaction/hypersensitivity	None	transient rash, drug fever <38°C (<100.4°F)	urticaria, drug fever ≥38°C (≥100.4°F), and/or asymptomatic bronchospasm	symptomatic bronchospasm, requiring parenteral medication(s), with or without urticaria; allergy-related	anaphylaxis

				edema/angioedema	
Auditory/Hearing					
Auditory/Hearing - Other (Specify, _ringing in ears__)	Normal	Mild	Moderate	Severe	Life-threatening or disabling
Cardiovascular (arrhythmia)					
Nodal/junctional arrhythmia/dysrhythmia	none	asymptomatic, not requiring treatment	symptomatic, but not requiring treatment	symptomatic and requiring treatment	life-threatening (e. arrhythmia associated with CHF, hypotension, syncope, shock)
Sinus bradycardia	none	asymptomatic, not requiring treatment	symptomatic, but not requiring treatment	symptomatic and requiring treatment	life-threatening (e. arrhythmia associated with CHF, hypotension, syncope, shock)
Sinus tachycardia	none	asymptomatic, not requiring treatment	symptomatic, but not requiring treatment	symptomatic and requiring treatment	life-threatening (e. arrhythmia associated with CHF, hypotension, syncope, shock)
Cardiovascular (general)					
Hypertension	none	asymptomatic, transient increase by >20 mmHg (diastolic) or to >150/100* if previously WNL; not requiring treatment	recurrent or persistent or symptomatic increase by >20 mmHg (diastolic) or to >150/100* if previously WNL; not requiring treatment	requiring therapy or more intensive therapy than previously	hypertensive crisis
Hypotension	none	changes, but not requiring therapy (including transient orthostatic hypotension)	requiring brief fluid replacement or other therapy but not hospitalization; physiologic consequences	requiring therapy and sustained medical attention, but resolves without persisting physiologic consequences	shock (associated with acidemia and impairing vital organ function due to tissue hypoperfusion)
CONSTITUTIONAL SYMPTOMS					
Fatigue (lethargy, malaise, asthenia)	none	increased fatigue baseline, but not altering normal activities	moderate (e.g., decrease in performance status by 1 ECOG level or 20% Karnofsky or *Lansky*) or causing difficulty performing some activities	severe (e.g., decrease in performance status by ≥2 ECOG levels or 40% Karnofsky or *Lansky*) or loss of ability to	bedridden or disabling

				perform some activities	
Sweating (diaphoresis)	normal	mild and occasional	frequent or drenching	-	-
DERMATOLOGY/SKIN					
Rash/desquamation	none	macular or papular eruption or erythema without associated symptoms	macular or papular eruption or erythema with pruritus or other associated symptoms covering <50% of body surface or localized desquamation or other lesions covering <50% of body surface area	symptomatic generalized erythroderma or macular, papular or vesicular eruption or desquamation covering ≥50% of body surface area	generalized exfoliative dermatitis or ulcerative dermatitis
ENDOCRINE					
Hot flashes/flushes	none	mild or no more than 1 per day	moderate and greater than 1 per day	-	-
GASTROINTESTINAL					
Constipation	none	requiring stool softener or dietary modification	requiring laxatives	obstipation requiring manual evacuation or enema	obstruction or toxic megacolon
Diarrhea	none	increase of <4 stools/day over pretreatment	increase of 4-6 stools/day, or nocturnal stools	increase of ≥7 stools/day or incontinence; or need for parenteral support for dehydration	physiologic consequences requiring intensive care; or hemodynamic collapse
Dyspepsia/heartburn	None	Mild	Moderate	Severe	-
Flatulence (gas)	None	Mild	Moderate	-	-
Mouth dryness	Normal	Mild	Moderate	-	-
Nausea	None	Able to eat	oral intake significantly decreased	no significant intake, requiring IV fluids	-
Stomatitis/pharyngitis (oral/pharyngeal mucositis)	none	painless ulcers, erythema, or mild soreness in the absence of lesions	painful erythema, edema, or ulcers, but can eat or swallow	painful erythema, edema, or ulcers requiring IV hydration	severe ulceration or requires parenteral or enteral nutritional support or prophylactic intubation
Vomiting	none	1 episode in 24 hours over pretreatment	2-5 episodes in 24 hours over pretreatment	≥6 episodes in 24 hours over pretreatment; or need for IV fluids	requiring parenteral nutrition; or physiologic consequences requiring intensive care; hemodynamic

					collapse
NEUROLOGY					
Dizziness/lightheadedness	None	not interfering with function	interfering with function, but not interfering with activities of daily living	interfering with activities of daily living	bedridden or disabling
Insomnia	Normal	occasional difficulty sleeping not interfering with function	difficulty sleeping interfering with function, but not interfering with activities of daily living	frequent difficulty sleeping, interfering with activities of daily living	-
Mood alteration-anxiety, agitation	Normal	mild mood alteration not interfering with function	moderate mood alteration interfering with function, but not interfering with activities of daily living	severe mood alteration interfering with activities of daily living	suicidal ideation or danger to self
Mood alteration-depression	Normal	mild mood alteration not interfering with function	moderate mood alteration interfering with function, but not interfering with activities of daily living	severe mood alteration interfering with activities of daily living	suicidal ideation or danger to self
Mood alteration-euphoria	Normal	mild mood alteration not interfering with function	moderate mood alteration interfering with function, but not interfering with activities of daily living	severe mood alteration interfering with activities of daily living	danger to self
Personality/behavioral	Normal	change, but not disruptive to patient or family	disruptive to patient or family	disruptive to patient and family; requiring mental health intervention	harmful to others or self; requiring hospitalization
Syncope (fainting)	Absent	-	-	Present	-
OCULAR/VISUAL					
Vision-blurred vision	Normal	-	symptomatic and interfering with function, but not interfering with activities of daily living	symptomatic and interfering with activities of daily living	-
PAIN					
Abdominal pain or cramping	none	mild pain not interfering with function	moderate pain: pain or analgesics interfering with function, but not interfering with activities of daily living	severe pain: pain or analgesics severely interfering with activities of daily living	disabling
Chest pain (non-cardiac and nonpleuritic)	none	mild pain not interfering with function	moderate pain: pain or analgesics interfering with function, but not interfering with activities of daily living	severe pain: pain or analgesics severely interfering with activities of daily living	disabling
Headache	none	mild pain not interfering with function	moderate pain: pain or analgesics interfering with function, but not	severe pain: pain or analgesics severely interfering with	disabling

			interfering with activities of daily living	activities of daily living	
Myalgia (muscle pain)	none	mild pain not interfering with function	moderate pain: pain or analgesics interfering with function, but not interfering with activities of daily living	severe pain: pain or analgesics severely interfering with activities of daily living	disabling
PULMONARY					
Cough	absent	mild, relieved by nonprescription medication	requiring narcotic antitussive	severe cough or coughing spasms, poorly controlled or unresponsive to treatment	-
Dyspnea (shortness of breath)	Normal	-	dyspnea on exertion	dyspnea at normal level of activity	dyspnea at rest or requiring ventilator support

G3) Criteria for Withdrawal.

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. Less severe expected adverse events will be evaluated using the NCI tables provided above. A severity score of 2 or greater on a mood alteration or behavioral adverse event will result in automatic withdrawal. A score of 3 or greater on other expected adverse events in the table will also result in automatic withdrawal from the study. The MRP will determine on a case-by-case basis whether a subject needs to be withdrawn from the study for unexpected adverse events. The MRP may also withdraw a participant at less extreme levels if clinical assessment indicates this would be in the best interest of the participant.

Participants may be withdrawn from the study and advised to immediately stop taking the study pills if changes in neuropsychiatric symptoms become apparent. The MRP and PI can decide to remove the participant if there is a clinical assessment of suicidal or homicidal ideation, increased depression, or indications of atypical behavior. The MRP and PI can decide to remove the participant from the protocol based on the following criteria: The development or exacerbation of current depression symptoms beyond threshold (i.e., BDI > 20) ***or*** suicidal ideation, behavior and/or attempts ***or*** indications of atypical behaviors.

H. Outcome Measures

The Primary outcome measures in this study are: a) percent change in BOLD signal related to task performance, between groups and drug conditions, b) ERN and fERN amplitudes, c) behavioral performance on

each of the reward and cognitive tasks (e.g., reaction time, error rate, hit rate, etc.) and d) variations in genes related to nicotinic receptors and DA functioning.

Secondary outcome measures include: a) ratings and scores on questionnaires and characterization methods assessing mood, b) feelings toward tobacco use and craving, c) smoking attitudes/behavior, d) spectroscopy and DTI data.

I. Statistical Analysis

I1) Data Analysis.

The imaging data will be pre-processed and analyzed 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). The specific statistical analyses to be performed on the fMRI 3-D datasets will be dictated by the nature of the factors involved and the specific scientific question addressed. For example, ANOVA models (*3dANOVA, 3dANOVA2, 3dANOVA3*) can be used to test for differences between participant groups or drug condition, while linear and multiple linear regression models (*3dRegAna, 3dLME*) can be used to probe for dose dependent drug effects and to predict individual responses.

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 and fERN) will be assessed for differences across participant groups and experimental conditions. BESA (Brain Electrical Source Analysis, MEGIS, Germany) will also be used to identify the putative neural generators of ERP components using dipole source models.

Behavioral performance measures (e.g., reaction time, error rate, and hit rate) on each of the reward and cognitive tasks as well as questionnaire data (e.g., feelings related to smoking and craving, and mood inventories) from each of the experimental conditions will be compared both within and between experimental groups using mixed, repeated-measures ANOVA and linear mixed models.

Metabolite concentrations derived from MRS will be quantified for the ACC 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. Differences in metabolite concentrations between smokers and non-smokers will be assessed using between group comparisons (e.g., independent samples t-tests). Neural metabolite changes resulting from

varenicline administration will be assessed using within group comparisons (e.g., dependent samples t-tests). Correlation and multiple regression approaches will be used to test for an association between metabolite levels and task performance (e.g., correlation between NAA levels and RT).

Genotyping results will be incorporated into the analysis of other datasets by providing a new grouping (independent) variable based on polymorphisms. In other words, the results will be used to place individuals into different groups based on their genetic makeup. These new groups will then be compared in ANOVA models to assess for differences in behavioral and neurophysiological measures. In addition, genotyping results may also be included as regressors or covariates in analyses to examine the contribution of a particular polymorphism to the observed BOLD signals and/or behavioral performance or to account for unexplained variance in the data.

Primary analyses. Each of the task paradigms were chosen to tap specific psychological constructs and neural substrates that have been shown to be associated with dopaminergic activity. These tasks were selected to provide evidence consistent with the hypothesis that alterations in dopaminergic tone will affect task performance and associated brain activity. The selected paradigms have been published and in many cases replicated across or within labs; one of them the Go-NoGo task, by the PI's own group. Since the modifications to these tasks are minor, the primary analyses associated with behavioral, fMRI and EEG/ERP data will be similar to those outlined in previous reports (please see the task descriptions and associated references in section "E5.C Scanning visits" for description). Therefore, details are omitted here and a general presentation of primary analyses is presented.

For the primary analyses each of the task paradigms is conceived of essentially as a separate study from the point of view of analysis and interpretation. Across each paradigm there are multiple dependent variables (i.e., RT, error rates, BOLD signal in a region of interest, ERP component amplitude) that will be examined to assess for drug (nicotine and varenicline) and group (smoker vs. non-smoker) effects. The initial independent variable of interest is the group variable, used to assess for differences between smokers and non-smokers. A simple independent samples *t*-test can be used to detect differences. Paired samples *t*-tests can be used to determine differences between nicotine and placebo conditions, as well as varenicline and placebo conditions. Ultimately, more advanced factorial designs will be used to explore how the different groups respond to the study drugs of interest. For example, one analysis that will provide important information about the effects of varenicline and nicotine across groups is a 2 (group: smoker vs. non-smoker) x 2 (nicotine vs. placebo) x 3 (varenicline: baseline vs. placebo vs. varenicline) repeated measures, mixed model ANOVA. We would expect smokers and non-smokers to respond differently in both behavioral and neurophysiological measures which would manifest as a possible three-way interaction in this analysis. Given the large number of dependent variables of interest, MANOVAs may also be used to uncover differences between group and drug conditions.

The current study involves the use of simultaneously recorded EEG and fMRI to asses for changes in brain activity consistent with our main hypothesis regarding dopaminergic tone. These two imaging methods

provide complementary advantages in terms of temporal and spatial resolution for assessing brain activity. While EEG offers superb temporal resolution for the assessment of neural dynamics on the order of milliseconds its spatial resolution is limited by the blurring and mixing of different electrical signals. In contrast, fMRI offers excellent spatial resolution allowing for the identification of potentially activated brain regions; however, the fMRI signal is temporally sluggish and separated from neural activations by orders of magnitude. These two methods can be used to provide complementary evidence consistent with our main DA hypothesis. For example, the ERN is expected to be decreased in amplitude when dopamine levels are low, as is the BOLD signal in ACC (the likely neural generator of the ERN). A reasonable prediction that follows is ERN amplitude and ACC BOLD activity should be correlated.

Secondary analyses. The selected paradigms have overlapping neural circuitries. While the primary analyses are conceived of as separate studies the secondary analyses will integrate the results from each of the paradigms. These secondary analyses will be used to gain a more complete understanding of the neural circuitries involved in goal directed behavior and decision making in control populations as well as factors leading to addiction, drug seeking behavior, continued abstinence, and possible relapse in drug-addicted populations. For example, integrating the results from the Motion Prediction task with those from the MID task will allow us to assess not only ventral striatal activity but also the input to that region, the habenula. It is difficult at the current time to outline a detailed analysis plan due to the limited knowledge regarding varenicline effects. Nonetheless, given the substantial financial, human effort, and imposition on the participants we imagine that such a rich dataset will give rise to numerous secondary analyses using data mining techniques such as independent component analysis (ICA), principal component analysis (PCA), and advanced modeling techniques such as exploratory and confirmatory factor analysis and structural equation modeling. Because the paradigms tap related psychological constructs and overlapping neural substrates, the resultant dataset will allow researchers to ask many questions, test many models, and ideally gain much insight. Such an exhaustive assessment has not been done previously.

I2) Criteria for Significance.

When assessing the statistical results from the EEG/ERP, behavioral, and questionnaire data a standard α-level of 0.05 will be used. Alpha-levels for multiple comparison follow-up tests will be corrected using an appropriate method (e.g., Bonferroni, Tukey, Scheffe).

Regarding the fMRI data, the primary technique for multiple comparisons is designed to compensate for the overly stringent nature of standard correction methods encountered when applied to imaging data. The currently proposed correction approach is especially important when dealing with individual voxels, where a standard Bonferroni correction would be so severe that even highly active voxels would go undetected. For

example, using a Bonferroni correction when making a number (n) of simultaneous inferences, the corrected α-value is determined by dividing the family-wise α by the number of inferences made (i.e. α/n). In a typical fMRI 3D data set, the number of inferences becomes rather high (e.g. in a data set based on 16 slices using a 64 x 64 matrix n = 16 x 64 x 64 = 65536), therefore the Bonferroni correction can be overly conservative, resulting in a loss of statistical power and a decreased likelihood of detecting areas of 'true' activation.

The alternative method employed in the current protocol is to use probability thresholding in conjunction with cluster size thresholding, as opposed to using only the individual probability threshold. The principle underlying this approach is that regions of true activation will tend to occur over contiguous voxels (i.e. clusters), whereas noise has much less tendency to form clusters of activated voxels. By combining the two thresholds the power of the statistical test is greatly enhanced. Although tables of false detection probability vs. cluster size have been published, they are limited to 2D images and are not directly applicable. Moreover, even if published tables not limited to 2D images were available, they would not be able to cover every possible combination of experimental parameters. Therefore, we have empirically investigated the trade-off between probability and cluster thresholds 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, simulations using this program 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.

I3) 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.

Smokers vs. non-smokers power. According to the current protocol, smokers will be instructed to remain abstinent for at least 12 hrs before testing. This manipulation is intended to initiate a state of mild withdrawal in smokers that will putatively produce a large difference between smokers and non-smokers in behavioral, fMRI, and EEG/ERP measures. Thus, we anticipate a medium-large effect size (Cohen's $d = 0.65$) when comparing smokers and non-smokers across each of these dependent measures. Furthermore, according to our hypothesis regarding decreased dopaminergic tone in abstinent smokers (i.e, impaired task performance and brain function in smokers) a directional test was used to calculate power. In other words, a one-tailed *t*-test model was used to calculate power since we expect smokers to perform worse than non-smokers. A power level ($1-\beta$) 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 vs. placebo power. Both smokers and non-smokers will be administered nicotine and placebo patches. Given previous work conducted in the PI's lab assessing nicotine's impact on task performance (Hahn et al., 2007) we anticipate a medium effect size ($d = 0.50$) associated with behavioral, fMRI and EEG/ERP dependent measures. The direction of the nicotine effect is not easy to predict across each of the task paradigms, thus a two-tailed dependent samples t-test will be used. Approximately 34 participants will be required to achieve power of 0.8 when assessing the effect of nicotine vs. placebo at an α level of 0.05 in a two-tailed dependent *t*-test assuming a medium effect size.

Varenicline vs. placebo power. Although no studies have been performed assessing the effects of varenicline administration on behavioral or neurophysiological measures in humans, based on clinical reports regarding the effectiveness of this compound associated with smoking cessation as well as animal research (see

"Varenicline Mechanisms of Action" section) we anticipate a medium effect size. Approximately 34 participants will be required to achieve power of 0.8 when assessing the effects of varenicline vs. placebo at an α level of 0.05 in a two-tailed dependent t-test assuming a medium effect.

Smokers vs. non-smokers across all repeated (6) sessions power. As discussed in Maxwell and Delaney (2004, pp.639-645), "Calculating statistical power and choosing an appropriate sample size is more complicated in *within-subjects* designs than in between-subjects designs. The additional complication is that effect size in repeated-measures designs depends not only on population means and population variances but also on population covariance. Although the mathematics necessary to calculate power is relatively straightforward, in practice it is difficult to specify accurate values of all parameters that influence power…. How many subjects do we need? The answer depends on three factors: the power we desire, the anticipated effect size, and the correlation between scores at each level…In the real-world there is no single "correct" value for the number of subjects needed in a study…" However, tables and power calculation tools can be used as general guidelines to help identify reasonable sample sizes.

To gain a general idea of the number of participants needed to assess for group differences (between-factor: smokers vs. non-smokers) across repeated testing sessions (6: baseline 1 and 2, varenicline 1 and 2, placebo 1 and 2) a power analysis was conducted using the G*Power software. Again, a medium effect size was assumed (this time using $f = 0.25$, as an effect size estimate). A total sample size of 66 participants would be required to reach power of 0.8 when considering two separate groups and 6 repetitions, using an α level of 0.05, and a minimal correlation across scores of 0.4 (the lower the score the more conservative). In other words, 33 participants per group would be needed.

We expect abstinent smokers, in comparison to non-smokers, to respond differently to the study drugs (nicotine and varenicline) that are administered across the different scan sessions. A test of the interaction between group and scan session is a general method to test for such differential responding. A power analysis was conducted to determine the number of participants needed to detect such an interaction. A total sample size of 36 is needed to reach power of 0.8 assuming a medium effect size ($f = 0.25$), an α level of 0.05, a minimum correlation between scores of 0.4, and assuming a conservative non-sphericity correction of 0.5 (this corrects for violations of the heterogeneity of variance assumption related to the *F*-test). This is equivalent to 18 participants per group.

Interim analysis. As stated above a priori power analyses for within-subject, repeated measure analyses (like that proposed here) are difficult and yield only general guidelines for sample size estimates. All of the task paradigms used in the present study have been published and the literature provides a good estimate of the effect sizes. However, what is not known is the varenicline effect size. Thus, further complicating the issue is the lack

of information available regarding varenicline-related effect sizes. As far as we are aware, no studies exploring the cognitive or neurophysiological consequences of varenicline administration in humans are available. However, based on animal and human clinical research we assume that the varenicline effect is relatively robust and have assumed a medium effect size.

Given the substantial financial, human effort and imposition on the participants associated with the current protocol an interim analysis will be conducted to determine if the estimated effect sizes remain appropriate. An interim analysis will be performed after 8-10 participants in each group have successfully completed. The information derived from this interim analysis will allow us to determine if the effect size estimates are reasonable or if unintended effects such as learning or practice effects are present that may mask drug or group effects requiring an increase in sample size. We feel that an independent pilot study is not logistically feasible. The results of this interim analysis will be used to determine if an increase in sample size is required. We will not use the results of the analysis to decrease the proposed sample size.

Genetics power. A power analysis was run for an ANOVA assuming 3 genetics groups (representing the 3 possible CHRNA4 and DRD2 polymorphisms). Based on previous studies by Dr. Goldman and his colleagues addressing brain imaging correlations with single nucleotide polymorphisms (Heinz et al., 2000; Egan et al., 2001; Hariri et al., 2002b; Egan et al., 2003), it can be predicted that means from imaging data will differ by one standard deviation for each genotype (i.e., a large effect size is expected). A power analysis was run under two assumptions: first, using a conservative approach where the genotype frequencies are not in Hardy-Weinberg equilibrium (Table 1); and second, using a more liberal approach where the genotype frequencies are the expected Hardy-Weinberg frequencies (Table 2). Standard deviations in the tables below are estimated based on previous studies and the expertise of Dr. Goldman and his colleagues.

Based on Tables 1 and 2, approximately 19-29 participants per group (smokers, non-smokers) would be needed to achieve an acceptable power level (> 0.8). This estimate is roughly consistent with the power analysis for the fMRI data above.

Table 1: Conservative estimation of genotype frequencies: 0.10, 0.52, 0.38 - Numeric Results

Power	Average n	k	Total N	Alpha	Beta	Std Dev of Means (Sm)	Standard Deviation (S)	Effect Size
0.14	3.00	3	9	0.01	0.86	0.82	0.96	0.85
0.31	3.00	3	9	0.05	0.59	0.82	0.96	0.85
0.41	4.33	3	13	0.01	0.69	0.79	0.96	0.82
0.62	4.33	3	13	0.05	0.38	0.79	0.96	0.82
0.64	6.33	3	19	0.01	0.36	0.83	0.96	0.86
0.87	6.33	3	19	0.05	0.13	0.83	0.96	0.86
0.77	8.00	3	24	0.01	0.23	0.79	0.96	0.83
0.93	8.00	3	24	0.05	0.07	0.79	0.96	0.83
0.91	9.67	3	29	0.01	0.09	0.83	0.96	0.86

0.98	9.67	3	29	0.05	0.02	0.83	0.96	0.86
0.95	11.33	3	34	0.01	0.05	0.81	0.96	0.84
0.99	11.33	3	34	0.05	0.01	0.81	0.96	0.84
0.98	13.00	3	39	0.01	0.02	0.83	0.96	0.86
1.00	13.00	3	39	0.05	0.00	0.83	0.96	0.86

Table 2: Assuming genotype frequencies according to HWE: 0.16, 0.48, 0.36 - Numeric Results

Power	Average n	k	Total N	Alpha	Beta	Std Dev of Means (Sm)	Standard Deviation (S)	Effect Size
0.12	2.67	3.00	8.00	0.01	0.88	0.86	0.96	0.90
0.37	2.67	3.00	8.00	0.05	0.63	0.86	0.96	0.90
0.46	4.67	3.00	14.00	0.01	0.54	0.88	0.96	0.91
0.76	4.67	3.00	14.00	0.05	0.24	0.88	0.96	0.91
0.74	6.33	3.00	19.00	0.01	0.26	0.90	0.96	0.94
0.92	6.33	3.00	19.00	0.05	0.08	0.90	0.96	0.94
0.87	7.67	3.00	23.00	0.01	0.13	0.91	0.96	0.95
0.97	7.67	3.00	23.00	0.05	0.03	0.91	0.96	0.95
0.93	9.33	3.00	28.00	0.01	0.07	0.88	0.96	0.91
0.99	9.33	3.00	28.00	0.05	0.01	0.88	0.96	0.91
0.98	11.00	3.00	33.00	0.01	0.02	0.89	0.96	0.93
1.00	11.00	3.00	33.00	0.05	0.00	0.89	0.96	0.93
0.99	13.00	3.00	39.00	0.01	0.01	0.89	0.96	0.93
1.00	13.00	3.00	39.00	0.05	0.00	0.89	0.96	0.93

I4) Accrual Number Request.

Based on power analyses above we anticipate that between 25-30 completers in each group (smokers, non-smokers) would provide a reasonable level of statistical power allowing for the detection of effects of interest in the present study. We will strive for a minimum of 25 completers in each experimental group. However, since drug administration may increase head movement and given the potential for unforeseeable errors during data collection, an estimated 20-25% of the participants who complete all phases of the study will likely be removed from the final analyses. Thus, a total of 30 completers in each group is requested.

In order to attain this number of completes three factors must be considered when estimating how many participants are to be recruited. First, given the complex nature of this study and the number of visits that are required, we anticipate that a portion of individuals who are consented into this study will fail to complete all experimental visits. Second, drug tolerance issues may be a source of attrition such that participants may be discontinued from the study if they are unable to tolerate the nicotine patch or experience anticipated adverse effects during varenicline administration. Third, drug compliance issues that could complicate data interpretation, such as a failure to abstain from nicotine or sub-optimal varenicline compliance, could arise. Taking into account these possible sources of attrition, we expect that the recruitment of 50 participants for each experimental group should result in a sample of completers within the desired range.

J. Human Subjects Protection

J1) 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. Smokers and non-smoker controls will be carefully matched with respect to gender, age, handedness, general IQ, years of education, race/ethnicity, and socioeconomic status.

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). The demographic distributions (http://quickfacts.census.gov/qfd/states/24/24510.html) of interest are as follows:

	Percentage						
	White	Black/ African American	American Indian & Alaskan Native	Asian	Native Hawaiian & other Pacific Islander	Hispanic or Latino	Female
Baltimore City	31.7	64.9	0.3	1.9	0.1	2.2	53.4
Baltimore County	70.5	24.0	0.3	3.9	0.0	2.4	52.4
Anne Arundel County	80.5	14.7	0.3	2.8	0.1	3.6	50.1
Howard County	70.6	16.1	0.3	10.9	0.1	4.0	50.8
Average	**63.3**	**29.9**	**0.3**	**4.9**	**0.1**	**1.5**	**51.7**

Based on these distributions we will aim have the following numbers of participants from each demographic cohort complete the study:

Gender	**White**	**Black/ African American**	**American Indian & Alaskan Native**	**Asian**	**Native Hawaiian & other Pacific Islander**	**Hispanic or Latino**	**Total**

Male	19	9	0	1	0	1	30
Female	19	9	0	1	0	1	30

J2) Inclusion/Exclusion of Children.

Children will not be included in the present study. This study focuses on the effects of nicotine and varenicline. Given that nicotine and varenicline are controlled substances (must be 18 or older to purchase nicotine containing products, and must have a prescription to receive varenicline) children under the age of 18 will not be recruited.

J3) Inclusion/Exclusion of Vulnerable Subjects.

Individuals in the following vulnerable groups will be excluded from study participation:

-HIV-positive individuals, since HIV infection and the development of AIDS produce abnormalities in brain function

-Individuals with any significant psychiatric or neuropsychological history

-Cognitively impaired individuals

-Pregnant or lactating females

J4) Safeguards for Vulnerable Populations.

The following measures will be used to identify and exclude individuals in vulnerable populations:

-HIV testing, including pre- and post-test counseling, in accordance with NIDA clinical policy on HIV

-DSM-IV criteria will be used to determine current and/or past psychiatric history

-IQ assessment using the Weschler Adult Intelligence Scale

-Urine and/or blood serum pregnancy testing

J5) Sensitive Procedures.

The administration of nicotine in the present study may be considered a sensitive procedure given the potential abuse liability of stimulant drugs especially with regard to non-smoking participants. The likelihood of strong reinforcing properties leading to potential abuse liability from the dose of transdermal nicotine used in the current protocol (7mg) is considered to be low. The abuse liability of a drug depends on the kinetics of drug delivery, such that reinforcing properties are greater for delivery routes associated with faster absorption/administration (Johanson and Fischman, 1989). Since nicotine plasma levels peak approximately 3-4 hours after patch application (Gore and Chien, 1998), the protracted absorption of nicotine is unlikely to produce strong reinforcing properties. Furthermore, when studying the effects of nicotine in only participants with a history of tobacco use, it is difficult to separate the direct cognitive benefits attributable to nicotine from the

reversal of withdrawal effects (Hughes, 1991). Nicotine-withdrawal in smokers may cloud the interpretation of nicotine and varenicline effects on reward and cognitive processing. Thus, normal non-smoking adults provide an important group for understanding nicotine effects on reward and cognitive processing. Elucidation of the neurobiological effects of nicotine in non-smokers could lead to novel treatments for such pathological states as attention deficit/hyperactivity disorder, mild cognitive impairment, Alzheimer's disease, Parkinson's disease, and schizophrenia (Newhouse et al., 2004; Sacco et al., 2004). In regards to smokers, the amount of nicotine delivered transdermally to participants is considered to be relatively low in comparison to what a smoker would encounter in their normal un-impinged environment, on par with about 3-4 cigarettes.

The administration of varenicline may be considered a sensitive procedure particularly with respect to non-smoking adults. As outlined above, assessment of the effects of nicotine and varenicline in non-smokers allows one to disambiguate the direct cognitive benefits of these compounds from alleviation of nicotine-withdrawal related effects. Given that varenicline is reasonably well tolerated, has an acceptable safety profile, and is associated with low abuse liability (Glover and Rath, 2007) the use of this compound in the present study may provide additional insight related to the functioning of the DA system. A more complete and less clouded understanding of the neuropharmacological effects of varenicline may spur the use of this compound for the treatment of other conditions associated with DA deficiencies such as schizophrenia and cocaine addiction.

J6) Qualifications of Investigators.

Elliot A. Stein, Ph.D. (Principal Investigator), Chief, Neuroimaging Research Branch, NIDA-IRP, is a behavioral neuroscientist. He has more than 25 years of experience in the neurophysiology and neuropharmacology of drugs of abuse, including research on heroin, cocaine, nicotine, and marijuana. His background in motivation and reinforcement began as a NIDA postdoctoral fellow with Dr. James Olds at the California Institute of Technology. His laboratory at the Medical College of Wisconsin was one of the first to apply fMRI to the study of human drug abuse. For the proposed project, he will provide close supervision and guidance for all aspects of the study, including, but not limited to, fMRI data acquisition, analysis and interpretation, and manuscript preparation.

Betty Jo Salmeron, M.D. (Medically Responsible Physician) has more than 8 years experience performing pharmacological fMRI studies, including nicotine, marijuana, and cocaine administration. She is Board-Certified in psychiatry. She trained at the Massachusetts General Hospital where she was a chief resident. As the MRP on this project, she will be primarily responsible for the safe and responsible conduct of all participant interactions, including participant recruiting, screening, and experimental drug administration of nicotine. Together with other clinicians in the Branch, she will perform psychiatric and chemical dependence histories and interpret laboratory tests. She will be responsible for the development of emergency procedures and assure that all members of the project are well versed in their implementation. She will assist in the development of experimental paradigm and

perform experimental manipulations regarding drug administration and interpretation of results. This investigator has been designated by the PI to obtain informed consent.

Matthew T. Sutherland, Ph.D. (Lead Associate Investigator) is a psychologist with a research doctorate in the field of cognitive neuroscience. He currently holds the position of postdoctoral fellow with the Neuroimaging Research Branch at NIDA-IRP. He has more than 5 years of experience working with EEG/ERPs and associated signal processing techniques to investigate electrical activity associated with sensory and cognitive processing. His responsibilities will include protocol development, data collection, coding and analysis, as well as manuscript preparation. This investigator has been designated by the PI to obtain informed consent.

L. Elliot Hong, M.D. (Associate Investigator) is a Board-Certified psychiatrist by the American Board of Psychiatry and Neurology, and a researcher in neurophysiology and neuroimaging in schizophrenia. He is currently affiliated with the Maryland Psychiatric Research Center, Department of Psychiatry, University of Maryland School of Medicine. He is a Special Volunteer to NIH/NIDA. He is currently the PI and co-investigator on several projects funded by NIMH and private foundations. Dr. Hong has recently obtained the approval for patient care privileges at NIH. Dr. Hong is currently conducting an off-label study of varenicline with schizophrenic patients. He will collaborate on the design of experimental paradigms, and in preparation and execution of fMRI data collection and analyses.

Thomas Ross, Ph.D.(Associate Investigator), earned his doctorate in experimental plasma physics and holds the position of Staff Scientist at NIDA-IRP. He has spent the last 10 years involved in the acquisition and modeling of noisy signals. He also spent 2 years as a professional computer programmer and has extensive hardware and instrumentation experience. He will hold primary responsibility for all aspects of the acquired fMRI data, including the development and application of novel analysis techniques. He will serve as systems administrator on the computationally demanding fMRI aspects of the protocol and will be responsible for the integrity of all data storage, hardware and software implementations, and programming of new scanner-compatible tasks.

Loretta Spurgeon, B.A., (Associate Investigator), is a Research Associate in the Neuroimaging Research Branch. Her educational background includes a Bachelor's of Arts in Psychology and continuing education classes at the graduate level. Her responsibilities will include consenting participants, data collection, as well as administration and scoring of screening instruments.

David Goldman, M.D., (Associate Investigator), is a Board Certified psychiatrist with more than 10 years of research experience in psychiatric genetics, including prior experience in integrating functional brain imaging and psychiatric genetics. This work has included contributions to seminal publications showing the effect of single nucleotide polymorphisms in monoaminergic genes on cognitive-affective processing. For the proposed project he will assume responsibility for the storage of genetics samples, and for their appropriate genotyping. He will also participate in data interpretation and manuscript preparation.

Yihong Yang, Ph.D., (Associate Investigator), is Chief of the MRI Physics Unit, Neuroimaging Research Branch. Dr. Yang has extensive experience in the development of functional and structural MRI techniques. He, with his colleagues, have developed fast spiral BOLD imaging, multi-slice arterial spin labeling (ASL) perfusion imaging, event-related ASL perfusion imaging, silent fMRI for auditory studies, a novel diffusion tensor imaging method, and methods for reduction of susceptibility artifacts. Dr. Yang will be responsible for the development and implementation of functional and structural MRI techniques employed in this protocol.

Kimberly Modo, B.A., (Associate Investigator), earned a degree in psychology from the College of Notre Dame of Maryland. She started working at NIDA-IRP in 2003 as a Clinical Recruiter, and was primarily responsible for the recruitment and screening of potential participants for neuroimaging studies. Her duties, among others, included obtaining consent and the administration of various psychological assessments. She began managing recruitment for the NRB in February of 2005, and in October 2006, she became a Research Associate for the NRB. For this protocol, Ms. Modo will assist with the administration of research survey instruments and with coordination of the protocol, including organization of study days and conducting experimental manipulations.

Mary R. Lee, M.D., (Associate Investigator), is a Staff Clinician at NIDA IRP. She completed medical school and internal medicine residency at Columbia Presbyterian Medical Center in New York and psychiatry residency at George Washington University Medical Center. She is board-certified in internal medicine and psychiatry. Her research interests are the social and emotional antecedents of substance abuse and she has extensive experience in combined psychosocial and pharmacologic treatment of psychiatric disorders.

Allison Carroll, B.A., (Associate Investigator), earned a degree in biopsychology from Monmouth College in December, 2009. Following graduation, she joined Dr. Beverly Davidson's microbiology lab working as a Research Assistant at the University of Iowa. She joined Dr. Stein's lab at NIDA-NRB as a post-bac IRTA in July, 2010 where she has been working with clinical research subjects, and training for administration of the experimental manipulations related to this protocol. For this protocol, Ms. Carroll's roles include scheduling and coordinating participant study visits, conducting data collection procedures during study visits, and organizing and pre-processing collected data. Scheduling and coordinating visits can be done without direct supervision. Data collection during study visits involving preparation for EEG recording, running neuroimaging scan sessions, and administration of computer and paper-based survey instruments are also done without constant direct supervision. Finally, data organization and pre-processing duties involve assisting other investigators with the analyses of large amounts of data collected. Such responsibilities necessitate supervision interactions. Ms. Carroll will not conduct any of the telephone assessments.

K. Benefits

Since all experimental measures are for research purposes only, there are no *direct* benefits anticipated for individuals who participate in the present study. Although this study does not offer direct benefit to participants, it is likely to yield generalizable knowledge regarding the (dys)function of the MCL DA system in control and drug-addicted populations. It is hoped that the data obtained will aid in the understanding of brain function involved in cognitive and reward processing, and how these functions are influenced by subtle manipulations of the DA system and genetic polymorphisms. Elucidating the neurobiological and behavioral consequences of varenicline administration will not only aid in the development of future (possibly individually-tailored) smoking cessation regimes but may also demonstrate the utility of this compound as a tool for improving the DA system in pathological conditions (e.g., cocaine addiction, schizophrenia).

An *indirect* benefit of study participation is a possible reduction in the number of cigarettes smoked or the precipitation of a cessation-attempt in smokers who would otherwise not be interested in quitting. By occupying α4β2 nicotinic binding sites, varenicline is thought to block the rewarding aspects of tobacco consumption. Thus, smokers may find smoking less pleasurable ultimately leading to a reduction in intake and drug seeking behaviors. A reduction in smoking behaviors is expected even if smokers do not intend to cut back. Tobacco-dependent individuals may also benefit from contact with health professionals who can discuss smoking related issues and, if requested, provide them with referrals. Another indirect benefit of study participation is access to medical screening. Screening measures can provide participants with general health information, of which they were not previously aware.

L. Summary/classification of risk

L1) Risk Classification.

In accordance with current OHSR guidelines (http://ohsr.od.nih.gov/info/sheet3.html), this study is classified as "more than minimal risk" to subjects, since it will involve exposing participants to procedures that may result in possible discomforts (outlined in section "F. Risks/Discomforts") beyond those ordinarily encountered in their normal, everyday life.

L2) Risk/Benefit Ratio.

The procedures described in this protocol provide a unique opportunity to collect large amounts of data from both smokers and non-smokers. These data will aid in understanding the neurobiological mechanisms underlying cognitive and reward processes related to tobacco use, dependence, and possible cessation. The use of multiple imaging modalities (EEG, ERPs, MRS, fMRI), genetics analyses, and pharmacological manipulations to study two different, but theoretically linked, psychological domains (reward processing, cognitive control) may

lead to increased understanding of individual differences related to drug-abuse susceptibility and the development of psychological and pharmacological therapies for tobacco-dependence. Given that smoking is the leading preventable cause of death in the US, responsible for more than 430,000 annual deaths (http://www.cdc.gov/tobacco/data_statistics/Factsheets/adult_cig_smoking.htm), studies leading to increased and/or improved smoking cessation therapies are a priority. While there are known risks associated with the planned procedures, previous studies have suggested that these risks are low and that the proposed procedures are safe and generally well tolerated by individuals in both experimental groups. Therefore, we consider the risk/benefit ratio to be favorably low for this study.

M. Consent Documents/Process

Since the procedures for both non-smoking and smoking participants are identical, a single consent form will be used for this study. Included in the consent document are details regarding all study procedures (e.g. fMRI, EEG, and genotyping). The consent informs participants that repeat or additional testing may be required in order to meet data quality requirements (e.g. if there is a problem with data quality that is noted after the completion of an imaging visit), therefore, this document will be considered as the consent should a participant be recalled.

Written informed consent will be obtained from each subject at entry into the study. Informed consent is obtained by the following process: Subject reviews the study consent form; PI or co-investigator[3] then meets with the subject 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, 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 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 3 copies of 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. The participant is given a copy of the consent form for his/her own records, the second copy is attached to their medical record, and the third copy is stored with other protocol consents in a binder. The consent quiz is attached to and stored with this latter copy. Once the signed consent has been obtained, the investigator will note the participant's enrolment in the study in the CDW database. Only after this last step has been completed will study procedures begin.

[3] Please see 'Investigator Qualifications' for details of investigators designated to obtain consent.

N. Data and Safety Monitoring

Data and safety will be monitored by a temporary board until such time as the NIDA-IRP DSMB is established. Drs Epstein and Schwartz have graciously agreed to serve as members of this board and will assume responsibility for the review of all aspects of this investigation, with particular attention to the safety of varenicline administration. The board will review all information for evidence of the medical safety of protocol procedures every 4 months during the first year of the study and every 12 months thereafter. Furthermore, they will be required to report on any significant trends in the data that are indicative of negative or adverse events to the NIDA-IRP IRB and the office of the CD. At such time as the NIDA-IRP DSMB is established all responsibilities will transfer to the NIDA-IRP DSMB.

O. Adverse Event Reporting

The current plan for the collection, monitoring, and analysis of adverse events and the description of expected events, is presented in accordance with Interim Guideline for Reporting Adverse Events to NIH IRBS (http://ohsr.od.nih.gov/info/). The Guidelines for reporting adverse events will be followed for any AE.

Expected adverse events for this protocol are defined in section "F. Risks and Discomforts" and will be gathered as described in section "G.1 Parameters to be monitored" and "G.2 Toxicity". Adverse events will be reported to the PI, Clinical Director, IRB and FDA in accordance with NIH and NIDA policies. Adverse events will be reported to the DSMB (or IRB subcommittee, until the DSMB is functional) as described in section "N. Data Safety And Monitoring."

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.

P. Alternatives to Participation

Smokers entering into the study will be advised during the consent process that varenicline is a smoking cessation drug and that it is being used in the present study strictly for research purposes. Smokers will not be explicitly asked to stop smoking while taking the study drug. Thus, subjects do not receive any treatments in this study or forego any treatment in order to participate in this study. The alternative, therefore, is not to participate.

Q. Confidentiality

Strict subject confidentiality will be maintained throughout. Participants will be assigned a code number 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.

Potential loss of confidentiality is a particular risk of studies involving genotyping. Under such circumstances, it could be a risk for genetic information about an individual to be made known. For example, genetic information could be informative to determine parentage or could be interpreted by an insurance company

or employer to mean that the person was at increased risk for a problem. As a result, a number of additional safeguards against transmission of this information to unauthorized individuals have been established. Genotyping will be done at the Intramural Program of the National Institute of Alcohol Abuse and Alcoholism. Subject ID numbers and not subject names will accompany blood samples sent to Dr. Goldman's laboratory for DNA analyses. No genotype data will be entered into the clinical record of subjects participating in the study and no genotype data will be released to subjects under any circumstance. No CLIA (Clinical Laboratory Improvement Act) certified genotyping acceptable for a clinical diagnostic or insurance purpose will be performed. In the study, there will be no identification of genetic diseases, and consequently, no relevant information to report to subjects.

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.

Research Records/Data.

Each participant will be assigned a research record folder upon entry to the first NRB study that they enroll in. This folder is kept in a locked cabinet, in a locked room, which has limited access. Participant folders remain in this room at all times, apart from when required for study sessions. At the completion of each session the folder 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.

Stored Samples.

The only biological samples that will be stored will be blood samples obtained for genetics testing. Blood samples for genetics will be identified only by NIDA ARC number and date on which the blood was drawn and will be sent for genetic testing to the NIAAA Laboratory of Neurogenetics (LNG) in Rockville, Maryland. Identifying names are never sent along with the samples. At the Laboratory of Neurogenetics, the DNA is extracted and stored in refrigerators there under a separate NIAAA code in a locked facility. Data from genotyping must be analyzed with the imaging and clinical data acquired from each participant. Therefore, data from genetic analysis will not be completely anonymized. Specifically, data from genotyping as well as information linking NIDA ARC number with the NIAAA code are stored on a computer in a locked room within a suite of rooms that is itself locked. The computer is password protected. The data files are also password protected. To maximize protection of confidentiality, access to this data

will be restricted to the PI and the LNG data coordinator. No clinically usable genotype data will be generated during the course of this study and no CLIA (Clinical Laboratory Improvement Act)-certified genotyping acceptable for diagnostic or insurance purposes will be performed. Samples will be stored indefinitely at NIAAA as additional polymorphisms relevant to addiction may develop, and, in that case, stored samples would be analyzed for those polymorphisms as well. Of course, any use of stored samples for future genetic testing will be brought to the IRB for approval prior to testing. Also, subjects will give consent for future genetics testing on the blood sample obtained in the course of this study.

Special Precautions.

There are no special precautions necessary for the protection of participant confidentiality in this study.

R. 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.

S. Compensation

All participants will be compensated for their participation in this study. Participants who voluntarily withdraw before completing the study will only be paid for that part of the study they have completed. However, if medical reasons preclude completion of the study, participants will be paid the full stipend for that day. Participants will be compensated according to the current NIDA-IRP policy and according to the following rates for various study procedures (http://www.nida.nih.gov/intranet/AdminSupp/clinicalpolicies/Remuneration.html).

Procedures	Remuneration
Study related procedures > 30 minutes	$20/hr
Scanning Visits	$20/hr + $25 per visit
Medical Clearance	$10/day
Performance Related Pay	Up to $109 per reward scan
Travel	$15 per round trip
Genetics Testing	$40

Screening and Orientation Visits. All participants will be paid $20/hr for these two visits which are anticipated to last ~3 hrs each for a total of $60/visit. Total = $120.

Scanning Visits. For each of the 6 imaging visits (~8.5 hrs each) participants will be paid $20/hr ($170/visit). In addition to the basic payment for experimental visits, performance incentives are also available to participants. For example, for each testing visit that is successfully completed by the participant and during which they have followed all of the investigator's instructions they will receive an additional $25 for that visit. Participants will also receive a bonus payment each time they complete the MID and reward reversal task during scanning (up to $53 and $56, respectively). Total = 6 visits * ($170 hourly rate + $25 bonus + $106 performance) = $1806.

Medical Clearance. Participants will receive $10 for completion of each of the 6 medical clearance assessments before the scanning visits. Total = $60.

Travel. Participants will receive $15/round-trip to compensate for travel costs. Eight visits are required. Total = $120.

Genetics testing. Participants will be paid $40 for participation in this study procedure.

Additional Neurocognitive Visits. Participants will be paid $20/hr for these two visits lasting about 4 hours each, participants will also be compensated for travel ($15/round-trip *2). Total: $160 + $30 = $190.

Completion of all phases of testing will also incur additional financial incentive of participants (i.e., 10% * $2336). It is estimated that for participants who complete all experimental measures (i.e., training, orientation, 6 scanning visits, genetics, and 2 neurocognitive visits) the maximum total payment will be $2570. Please note, however, that this amount could vary depending upon supplementary procedures that may be deemed necessary, such as repeat testing.

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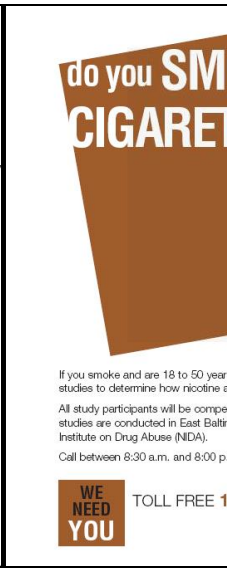
Appendix 1: Participant Eligibility Checklist

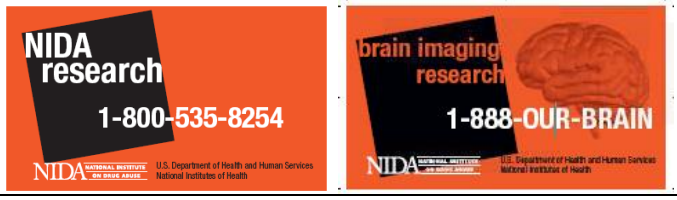
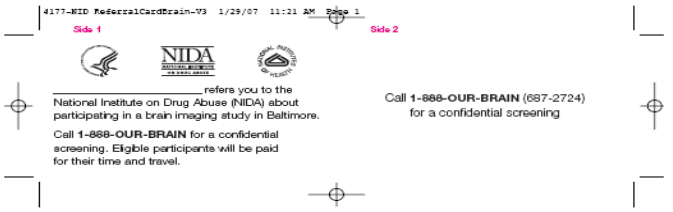
To be considered for participation, individuals must meet the following eligibility criteria:

	Yes	No
1. Age 18 – 55 years	☐	☐
2. In good physical health	☐	☐
3. Right-handed	☐	☐
4. Free of substance dependence (other than nicotine)	☐	☐
5. Right-handed	☐	☐
6. Smokers: smoke more than 10 cigarettes/day	☐	☐
7. Smokers: able to be abstinent for 12hrs	☐	☐
8. Non-smokers: no history of daily smoking	☐	☐
9. Suitable for MRI	☐	☐
10. No history of psychological/neurological illness	☐	☐
11. No history of suicidal ideation/behavior	☐	☐
12. Not diabetic	☐	☐
13. No indication of renal impairment	☐	☐
14. Not HIV Positive	☐	☐
15. Not cognitively impaired	☐	☐
16. Not pregnant	☐	☐
17. Not lactating	☐	☐

Appendix 2: Recruitment Materials

Material	Target Audience	Intended Distribution	Duration	Snapshot
Print Ad	Smokers	Local Newspapers	On going	
Table top display	Smokers and controls	Through outreach efforts at approved locations	On going	

Material	Target Audience	Intended Distribution	Duration	Snapshot
NIDA Recruitment Website	Smokers and controls	Web outreach	On going	
On line Ad	Smokers	Web Outreach: Craigslist, Baltimore backpage.com	On going	**There might be a research study for you.** Do you smoke cigarettes? If so, you may be able to participate in rese smoking affects the body and brain. The studies take place in East Ba the National Institute on Drug Abuse (NIDA), National Institutes of H Health and Human Services. You will be compensated for your time Call 1-800-535-8254 from 8:30am to 8:00 pm for a confidential scree www.researchstudies.drugabuse.gov.
Flyers	Smokers and controls	Through outreach efforts at approved locations	Ongoing	 

Material	Target Audience	Intended Distribution	Duration	Snapshot
				
Magnets	Smokers and controls	Through outreach efforts at approved locations	While supplies last	
Referral Cards	Smokers and controls	Through outreach efforts at approved locations	Ongoing	
MVA Crawler Ad	Smokers	MVA Essex	5 months	Screenshots of sign:

Material	Target Audience	Intended Distribution	Duration	Snapshot
				

Appendix 3: Nicotine Patch Instructions

Read ALL the instructions before you begin.

Begin this test in the morning, preferably the day after you receive the patch. You can select another day, but you must complete this test before your next visit to NIDA. Smoke your first cigarette of the day as usual (if a smoker). Apply the patch about 30 minutes later. Do not smoke any more 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 a total of 8 hours.
7. After 8 hours, remove the patch you have been wearing. Fold the used patch in half with the sticky sides together. Carefully place the used patch in its original package and then throw the package away. Keep the nicotine patch away from children and pets. Wash your hands after disposing of the patch.

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 the test, please call **Dr. Betty Jo Salmeron at 443-740-2651 (office) or 410-283-1790 (pager)** and ask for a new patch.
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**. **After hours she can be reached on her pager at 410-283-1790. Also, the study nurses can be reached at 443-740-2294 (available 24 hours everyday).**

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 4: Questionnaires

1) 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. Completion time: ~>5min.

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

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

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

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

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

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

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

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. 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

3) 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

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. 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

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. Completion time: ~5min.

Directions: Please answer each of the following questions

1) 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) _____.

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

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

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

4) For you quitting smoking for good would be:
   a. Impossible
   b. Very difficult
   c. Fairly difficult
   d. Fairly easy
   e. Very easy

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

5) After a few hours without smoking, I feel an irresistible urge to smoke
   a. Totally agree
   b. Somewhat agree
   c. Neither agree nor disagree
   d. Somewhat disagree
   e. Totally disagree

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

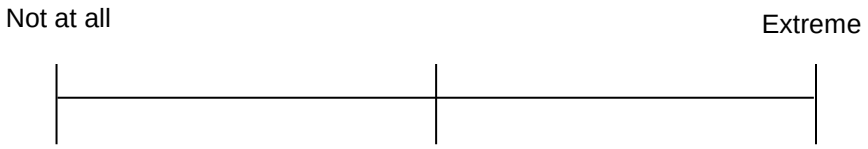
6) The Positive and Negative Affect Schedule is a 20-item scale composed of 10 items describing negative affect and 10 items describing positive affect. Completion time: ~5 min.

Directions: Please read each item and then circle the appropriate answer next to that word. Indicate to what extent you have felt this way during the past week. Use the following scale to record your answer:

(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

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. Completion time ~10 min.



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

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

1) Number of years smoking_____
2) age when first cigarette smoked_____
3) age when started daily smoking_____
4) average number of cigarettes/day_____
5) number of quit attempts_____
6) strategies used during quit attempts (nicotine replacement, buproprion, counseling, cold turkey),
7) longest period of time not smoking since smoking everyday,
8) Please rate your desire to quit smoking on a 0-100 scale (0= no desire to quit, 1= extreme desire to quit) _____.
9) 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)_____.
10) Please rate your level of addiction to cigarettes (0=not addicted at all, 100= Extremely addicted)

9) 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.

10) 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.

11) 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.

12) 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.

13) 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		Spiteful		Sluggish	
Angry		Sympathetic		Rebellious	
Worn Out		Uneasy		Helpless	
Unhappy		Restless		Weary	
Clear-headed		Unable to concentrate		Bewildered	
Lively		Fatigued		Alert	
Confused		Helpful		Deceived	
Sorry for things done		Annoyed		Furious	
Shaky		Discouraged		Efficacious	
Listless		Resentful		Trusting	
Peeved		Nervous		Full of pep	
Considerate		Lonely		Bad-tempered	
Sad		Miserable		Worthless	
Active		Muddled		Forgetful	
On edge		Cheerful		Carefree	
Grouchy		Bitter		Terrified	
Blue		Exhausted		Guilty	
Energetic		Anxious		Vigorous	
Panicky		Ready to fight		Uncertain about things	
Hopeless		Good-natured		Bushed	
Relaxed		Gloomy			
Unworthy		Desperate			

14) 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.

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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

15) 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 in the last few days. 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:

16) The Revised Social Anhedonia Scale (Eckblad et al., 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.

17) The Physical Anhedonia Scale (Chapman and 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.

18) *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. Completion time: ~5 min. This measure will also be used to characterize participants' level of dysphoria.

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

Continued on Back

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Roche

V 0477

Beck Depression Inventory

CRTN: _____ CRF number: _____ Page 15 patient inits: _____

Baseline

11. Agitation

0 I am no more restless or wound up than usual.
1 I feel more restless or wound up than usual.
2 I am so restless or agitated that it's hard to stay still.
3 I am so restless or agitated that I have to keep moving or doing something.

12. Loss of Interest

0 I have not lost interest in other people or activities.
1 I am less interested in other people or things than before.
2 I have lost most of my interest in other people or things.
3 It's hard to get interested in anything.

13. Indecisiveness

0 I make decisions about as well as ever.
1 I find it more difficult to make decisions than usual.
2 I have much greater difficulty in making decisions than I used to.
3 I have trouble making any decisions.

14. Worthlessness

0 I do not feel I am worthless.
1 I don't consider myself as worthwhile and useful as I used to.
2 I feel more worthless as compared to other people.
3 I feel utterly worthless.

15. Loss of Energy

0 I have as much energy as ever.
1 I have less energy than I used to have.
2 I don't have enough energy to do very much.
3 I don't have enough energy to do anything.

16. Changes in Sleeping Pattern

0 I have not experienced any change in my sleeping pattern.
1a I sleep somewhat more than usual.
1b I sleep somewhat less than usual.
2a I sleep a lot more than usual.
2b I sleep a lot less than usual.
3a I sleep most of the day.
3b I wake up 1–2 hours early and can't get back to sleep.

17. Irritability

0 I am no more irritable than usual.
1 I am more irritable than usual.
2 I am much more irritable than usual.
3 I am irritable all the time.

18. Changes in Appetite

0 I have not experienced any change in my appetite.
1a My appetite is somewhat less than usual.
1b My appetite is somewhat greater than usual.
2a My appetite is much less than before.
2b My appetite is much greater than usual.
3a I have no appetite at all.
3b I crave food all the time.

19. Concentration Difficulty

0 I can concentrate as well as ever.
1 I can't concentrate as well as usual.
2 It's hard to keep my mind on anything for very long.
3 I find I can't concentrate on anything.

20. Tiredness or Fatigue

0 I am no more tired or fatigued than usual.
1 I get more tired or fatigued more easily than usual.
2 I am too tired or fatigued to do a lot of the things I used to do.
3 I am too tired or fatigued to do most of the things I used to do.

21. Loss of Interest in Sex

0 I have not noticed any recent change in my interest in sex.
1 I am less interested in sex than I used to be.
2 I am much less interested in sex now.
3 I have lost interest in sex completely.

3 4 5 6 7 8 9 10 11 12 A B C D E

_____ Subtotal Page 2
_____ Subtotal Page 1
_____ Total Score

NR15645

19) Beck Anxiety Inventory (Beck et al., 1988): a brief inventory similar to the BDI-II that assesses symptoms of anxiety. Completion time: ~5 min. This measure will also be used to characterize participants' level of dysphoria.

Directions: 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.

0-Not At All 1-Mildly but it didn't bother me much. 2-Moderately – it wasn't pleasant at times 3-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

20) 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.

Appendix 5: Telephone Assessment

Telephone questions to assess for toleration of the study medication are included below. Participants will be contacted every 2-3 days from the start of study medication through one week after last dose, and a final call 10-14 days after last study dose. Research associates conducting the telephone assessments will be extensively trained by the MRP on question administration. If the interview reveals a possibility of significant side effects, the participant will be put in contact with the MRP for further evaluation. The MRP 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 6: Study 09-DA-N044 FACT SHEET

STUDY 09-DA-N044 FACT SHEET

Below is information about what will be asked of you if you volunteer for study 09-DA-N044.

Study 09-DA-N044 involves 9 visits over a 5-6 week period: a) 3 visits will require 4-5 hours, b) 6 visits will require 8-9 hours. You will be compensated for your time and travel at the end of each visit for the study procedures you completed that day.

During study 09-DA-N044 you will:

- take study pills that will contain Chantix (varenicline). Chantix is a FDA approved medication used to help people quit smoking.
- wear a nicotine skin patch or a placebo patch during your study visits.
- have your blood drawn for 1) genetics testing and 2) to test your nicotine levels throughout the study.
- complete multiple fMRI scanning sessions that last about 2 hours each.
- undergo EEG (brain waves) recording.
- be asked to NOT smoke for 12 hours before the study visits. If you are a smoker, you are NOT asked to completely quit smoking.
- answer questionnaires about how you think and feel.
- Receive multiple phone calls from study researchers to see how you are responding to the study pills.

Please consider the following items:

- Study 09-DA-N044 is long and involves multiple visits.
- The study involves drug administration of Chantix and nicotine.
- We take multiple blood samples throughout the study. If you have had problems giving blood in the past, this may not be a good study for you.
- During fMRI scans you will lay on your back without moving for about 2 hours at a time.
- You will wear an EEG cap and gel in your hair. This gel can be washed out with shampoo and water at NIDA after the session.
- If you are a smoker you CANNOT smoke for 12 hours before the visits. You will not be able to smoke during the visits.
- Consider your work schedule. Some visits require the full day, and other visits require a half day. Some of these visits CANNOT be put off for more than a few days if your schedule changes. Study visits will take place mainly on weekdays (Monday-Friday) and occasionally on Saturdays if possible. The study team will do the best they can to accommodate your schedule, but you will need to be available at least 1 weekday (preferably 2) per week during the 5-6 week study period.

During your next visit you will need to read and sign an informed consent document in order to enroll in this study. If you are interested in volunteering for this study a researcher will contact you to discuss more details and to schedule your first study visit.

Appendix 7: Take-home study pill instructions

You have been given two packages that contain study pills. These packages contain a week's supply of pills plus some backup pills. You will take two study pills everyday. Sometimes these pills will contain the medication varenicline (Chanitx©) and sometimes the pills will be a placebo. Varenicline is approved by the FDA to help people quit smoking. You will not be told if the pills you are given contain varenicline or placebo.

Take two study pills everyday, one in the *morning* and one in the *evening*:

Everyday you will take two pills, one in the morning and one in the evening. One of your packages is the morning package and the other is the evening package.

1) On the first morning, take the first pill from the morning package. The first pill is the one in the upper left corner of the package just under the "start" sticker.
2) On the first evening, take the first pill from the evening package.
3) The pills are numbered in descending order: Pill #1 is labeled 31, Pill #2 is 30, Pill #3 is 29, and so on. Continue taking the study pills in this (descending) order until your next study visit.

How to take the study 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. We will need to count and see if you have missed any pills.

If you cannot make it to a scheduled appointment:

Continue taking the study pills as you normally would. The packages contain extra "backup" pills. If you cannot make it to a scheduled appoint please contact **Dr. Matthew Sutherland at 443-740-2628** as soon as you can. You will need to reschedule your appointment and make sure that you have enough study pills.

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.

A researcher will call you every 2-3 days to ask about side effects and see how you are responding to the pills.

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) or 410-283-1790 (pager)**.

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.

Remember to bring your pill packages back to NIDA:

You must bring these packages with you to your next visit. Leave any unused pills in the package. At the next visit you will receive a new supply of pills.