1. Principal Investigator: Mehmet Sofuoglu, M.D., Ph.D.

2. Co-Investigators While all research staff are trained to provide informed consent, those identified with an asterisk (*****) will provide informed consent for this study. All the research staff will have access to the PHI

Co- Investigator: Suprit Parida, M.D.,

Nursing Staff: *Ellen Mitchell, RN, Angelina Genovese, R.N., Elizabeth O'Donnell, R.N., Maragaret Dion-Markovitz,R.N.

Research Staff: *Lance Barnes, *Stacy Minnix, BSW, and *Chris Cryan,

* Michael Stephens

3. Title of Project: Nicotine Reinforcement and Aversion in Young Adult Light Smokers

4. Purpose, hypothesis and key questions:

The proposed study will examine the threshold for nicotine self-administration (NSA) using five different nicotine doses in young adult male and female non-dependent smokers (light and intermittent smokers or LITS). We propose a double-blind, placebo-controlled study that will enroll 195 individuals, targeting a total of 72 completers (36 male and 36 females). In each of the five experimental sessions, smokers will be randomly assigned to one of the five doses of nicotine (0.0125, 0.025, 0.05, 0.1 and 0.2 mg/70 kg). The highest dose, 0.2 mg/70 kg, corresponds to nicotine delivered by about one or two puffs of a cigarette. At the beginning of each experimental session, smokers will sample the assigned both the nicotine dose for that experimental session, and the placebo (saline) dose, followed by the opportunity to choose between nicotine and placebo for a total of ten choices over a 165-minute period. The main outcomes will be threshold dose (the minimum dose of nicotine that is self-administered more than placebo) and the slope of dose-response for nicotine self-administration (changes in nicotine self-administration per unit change in nicotine dose). We will also collect measures of nicotine intake (cotinine), nicotine clearance (3-hydroxycotinine (3-HC) / cotinine), and self-report drug effects.

The following questions will be addressed:

Aim #1: To assess the threshold reinforcing dose and dose-effect curve for IV NSA at low doses in young adult LITS.

Hypothesis #1A: The threshold reinforcing doses for IV NSA will be between 0.0125 to 0.1 mg/70 kg.

Hypothesis #1B: The dose-effect curve for NSA will differ between males and females with relatively flat curve in female smokers.

Aim #2: To assess the threshold and dose-effect curve for the positive and negative/aversive subjective effects of IV nicotine at low doses and its relationship to nicotine reinforcement.

Hypothesis #2 A: The threshold for the positive effects will be between 0.0125 to 0.1 mg/70 kg, for the negative/aversive effect it will be ≥ 0.1 mg/70 kg.

Hypothesis #2B: Nicotine reinforcement will be positively correlated with the positive and negatively correlated with the negative/aversive subjective effects of IV nicotine.

Exploratory Aims: To examine the influence of nicotine clearance rate on nicotine reinforcement threshold and dose-effect curve.

5. Background:

Addictive threshold for nicotine

Similar to other drugs of abuse, cigarette smoking is rewarding (e.g., drug liking or good drug effects) and reinforcing (self-administered). Previous studies have shown that smoking a cigarette delivers approximately 1 to 1.5 mg of nicotine, inhaled over 10 to 12 puffs (Benowitz and Jacob 1984). A group of smokers known as light and intermittent smokers (LITS) or 'chippers', show few or no signs of addiction (Shiffman 1989). Based on the nicotine intake of the LITS, it has been suggested that blood cotinine levels over 50 to 70 mg are needed to sustain nicotine addiction in smokers (Benowitz and Henningfield 1994). This threshold roughly corresponds to a daily intake of 5 mg nicotine or approximately smoking 5 cigarettes/day. Based on these figures, Henningfield et al (1998) estimated that the threshold for nicotine reinforcement is approximately 0.2 mg of delivered nicotine. As will be summarized below, this estimated threshold for nicotine reinforcement has yet to be validated by carefully controlled nicotine administration studies.

Since cigarette smoke contains many other compounds in addition to nicotine and the amount of nicotine delivered via smoking cannot be reliably controlled, cigarette smoking is not suitable for examining dose-dependent nicotine effects (Hoffmann and Wynder 1986). Pure nicotine administration studies, especially via nasal spray, have provided crucial information for the threshold doses of nicotine that are reinforcing. Nasal spray provides quicker nicotine delivery, compared to nicotine gum or patch, and the amount of delivered nicotine can be reasonably controlled (Perkins 2009). In a series of studies, Perkins et al. (1994) have shown that smokers and non-smokers can reliably detect the interoceptive stimulus effects of intranasally administered nicotine using the drug discrimination procedure. The threshold for nicotine discrimination is approximately 3 mcg/kg (0.21 mg for a 70 kg individual) for smokers and 2 mcg/kg (0.14 mg for a 70 kg individual) for nonsmokers (Perkins et al. 2001). The threshold doses for nicotine discrimination are likely lower than the reinforcement threshold since smokers are unlikely to find nicotine reinforcing if the dose is below that for which they can detect its subjective effects (Perkins 2009). Importantly, smokers did not self-administer nicotine nasal spray doses, which were either above or below the threshold for nicotine discrimination, more than placebo (6.1 and 7.4, out of 16 choices) (Perkins et al. 2001). This lack of reinforcement might be due to the slower rate of nicotine delivery via spray, which does not match the rapid nicotine delivery via smoking. Thus, although the nicotine discrimination studies with nasal spray provide crucial information for the threshold of the interoceptive stimulus of nicotine, the threshold for nicotine reinforcement remains to be determined using more rapid delivery systems.

Dose-response curve for nicotine reinforcement

Characterization of the dose-response curve of a drug provides crucial information including the minimum effective or threshold dose as well as the shape and slope of the dose-response curve. While the shape of the dose-response curve indicates how the response changes at different dose levels (e.g., linear, U-shaped, or flat), a higher slope indicates greater response to dose increases (i.e., greater sensitivity). The dose-response curve for IV nicotine self-administration has been well characterized in preclinical studies (Corrigall et al. 2000; Donny et al. 2000; Fattore et al. 2002; Le Foll et al. 2007; Rose and Corrigall 1997). The typical pattern of nicotine self-administration behavior that has emerged from many preclinical studies is that the dose-dependent changes in drug use behavior are restricted to low and high dose ends of the dose-response curve, while the middle range is relatively insensitive to changes in nicotine doses (Rose and Corrigall 1997). Consistent with these findings, in our previous study with IV nicotine self-administration, we observed no change in nicotine self-administration between 0.4 and 0.7 mg of nicotine (Sofuoglu et al. 2008a). A similar insensitivity of smoking behavior has been observed when nicotine intake is experimentally manipulated (Benowitz and Jacob 1990).

Further characterization of the dose-response curve for nicotine reinforcement remains to be examined using rapid and accurate nicotine delivery systems.

IV nicotine administration

We propose to use IV NSA to determine the threshold for nicotine reinforcement due to its advantages over other routes of pure nicotine. First, the IV route produces fast nicotine delivery with peak plasma levels reached in 20 sec, which is comparable to smoking (Rose et al. 2003; Zins et al. 1997). Second, IV nicotine provides accurate dosing by delivering nicotine directly into the circulation and bypassing the absorption step, which shows significant individual variation. Third, IV nicotine is reinforcing in rodents, primates, and humans. Rats and monkeys self-administer IV nicotine (Corrigall et al. 2000; Donny et al. 2000; Fattore et al. 2002; Le Foll et al. 2007; Rose and Corrigall 1997). In humans, when administered rapidly (i.e., in less than 60 sec), IV nicotine elicits pleasurable subjective effects such as “good effects” and “drug liking” similar to those elicited by smoking and is self-administered by smokers (Harvey et al. 2004; Henningfield and Goldberg 1983; Henningfield et al. 1983). Thus, the rapid and accurate dose delivery, combined with reinforcing effects, makes IV nicotine an optimum route to examine the threshold for nicotine reinforcement.

A critical issue in IV NSA studies is the dose of nicotine. In the Harvey et al (2004) study, male smokers preferred nicotine injections over saline-administration for 0.75, 1.5, or 3.0 mg/injection of nicotine doses. These nicotine doses, however, are much higher than the typical nicotine intake of an average smoker, which is 1-4 mg nicotine/hour (Benowitz and Jacob 1990). To address this limitation, in a recent study, we examined self-administration of nicotine doses within the range of average intake by smokers (Sofuoglu et al. 2008a). We used a choice procedure in which male and female smokers were able to choose between various IV nicotine doses (0.1, 0.4, and 0.7 mg) or saline. Both the 0.4 and 0.7 mg/70 kg, but not the 0.1 mg, doses were preferred over placebo. In a more recent study, we examined self-administration of lower doses of nicotine (0.1, 0.2, 0.3 or 0.4 mg) using a more rapid, 30 sec, delivery rate 12 male and 14 female smokers (34). NSA was negatively correlated with nicotine dose in males who displayed choice preference for lower doses (0.1 and 0.2 mg doses) of nicotine over the highest tested dose (0.4 mg). However, no significant relationship between dose and choice preference was evident in females. The 0.1 and 0.2 mg nicotine doses also produced positive subjective effects, suggesting that nicotine reinforcement threshold is equal or less than 0.1 mg. To our knowledge, the dose-effect curve of nicotine in doses less than 0.1 mg has not been examined in humans.

Proposed Study

Although the presumption of an addictive threshold dose of nicotine is one of the pillars of nicotine reduction strategies, it has yet to be characterized in well-designed human studies. This project will address this gap by assessing the threshold and dose-effect curve for NSA over a low dose range that is expected to capture the reinforcement threshold. It is likely that the nicotine reinforcement threshold will display inter-individual differences. Older and dependent smokers, compared to young adult LITS, may likely have higher reinforcement threshold due to tolerance to nicotine’s behavioral effects as a result of long-term nicotine intake. We chose young adult LITS given that the threshold for addictive doses of nicotine was estimated based on the nicotine intake of LITS. As suggested by previous findings from our, and other research groups, male and female smokers may differ in their sensitivity to the behavioral effects of nicotine. In addition, there is evidence to suggest that the rate of nicotine clearance may affect nicotine dose-effect curve and reinforcement threshold. Therefore, the influences of both gender and nicotine clearance rate [assessed by the ratio of hydroxycotinine/cotinine or otherwise known as the nicotine metabolite ratio (NMR)] will be examined in this application. This project will also examine the relationship between NSA and the positive and negative/aversive subjective effects of IV nicotine.

These subjective measures are important because evidence suggests that while positive drug effects are associated with abuse liability and drug intake, sensitivity to the negative/aversive subjective effects of nicotine may prevent both the initiation of tobacco product use and the amount of nicotine intake in established tobacco users. Accordingly, nicotine reinforcement may be a function of the relative balance between nicotine's positive and negative/aversive effects. However, to what extent the individual's sensitivity to the negative/aversive effects of nicotine changes the threshold and dose-effect function for nicotine reinforcement has not been determined. Identifying factors that affect the reinforcement threshold of nicotine will be an important step in developing science-based policies for reducing nicotine in tobacco products.

Preliminary Studies

Study 1: Sex differences in nicotine self-administration of low doses of nicotine in smokers.

Aim: The goal of this study was to investigate the dose-response function for the reinforcing and subjective effects of intravenous (IV) nicotine in male and female smokers using nicotine doses that are estimated to be delivered by smoking one or two puffs of cigarettes.

Method: Twenty-six smokers (12 male and14 female) participated in a double-blind, placebo-controlled, crossover study that included 4 experimental sessions. In each session, participants were randomly assigned to one of 4 doses of nicotine (0.1, 0.2, 0.3 or 0.4 mg). In each session, subjects first received the sample infusions of the assigned nicotine dose and placebo and then had the opportunity to choose between nicotine and placebo for a total of 6 choices spaced evenly over a 90-minute period. The main outcome measures were the number of nicotine choices, self-report drug effects and physiological responses. Results: A sex-by-dose interaction was observed in the nicotine choice paradigm (Fig 1). Nicotine self-administration rate was negatively correlated with nicotine dose in males (males displayed choice preference for low doses of nicotine over high doses of nicotine), but no significant relationship between dose and choice preference was evident in females. Overall, the number of nicotine choices was not greater than saline choices ($p>0.05$).

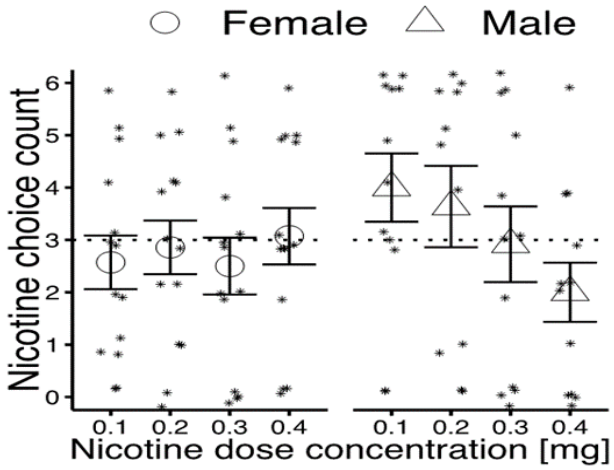

Figure 1. The mean (± SEM) nicotine infusion number (maximum 6) for male (n=12) and female (n=14) subjects at each dose is shown. Nicotine self-administration frequency was negatively correlated with nicotine dose among males but not females (dose by sex $p < 0.05$). Post hoc comparisons showed that both 0.1 and 0.2 mg doses were self-administered more than the 0.4 mg nicotine doses.

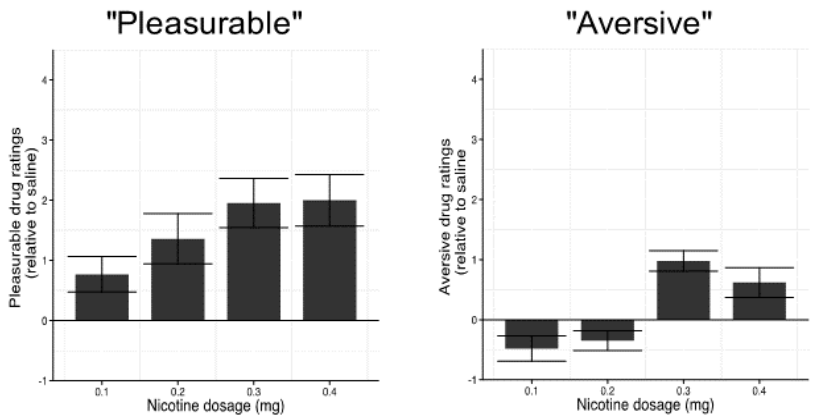

Figure 2. Subjective ratings of the pleasurable and aversive effects of intravenous nicotine in smokers. Shown is the mean value (± SEM) for 6 post-infusion ratings of drug effects for subjects (n=26). The subjective ratings for nicotine are relative to the ratings for saline, using the DEQ with a scale of 0-10.

Further, all nicotine doses (0.1, 0.2, 0.3, 0.4 mg) were rated as more pleasurable than saline (Figure 2). The aversive effects were significantly higher relative to saline in response to nicotine at 0.3 mg, but not at the other nicotine doses.

Conclusions: Among males, low doses of IV nicotine were preferentially chosen over high doses of nicotine. In contrast, among females no dose-sensitive choice preference was observed. The IV nicotine doses also induced subjective ratings of positive and negative drug effects that differed from saline. Notably, all doses tested, including the lowest tested dose (0.1 mg) were rated as more pleasurable than saline by both sexes, indicating abuse potential for all tested doses, although the number of nicotine choices were not greater than those for saline. This discrepancy could be due to the small sample size and the relatively few opportunities to self-administer nicotine at each dose (up to 6 choice trials per dose). These limitations will be addressed in the current study.

The dose-dependent sex differences in reinforcement behavior and the dose-dependent changes in positive and negative subjective ratings of drug effects are relevant to dependence vulnerability. These findings, which demonstrate sex differences in NSA for doses that are near to the reinforcement threshold, suggest that male and female smokers may respond differently to changes in nicotine doses available for self-administration. The negative correlation between nicotine dose and self-administration suggests partial compensation in males.

<u>Study 2: Higher 3-hydroxycotinine (3-HC)/cotinine is associated with greater reward and heart rate increases from intravenous nicotine:</u> In previous studies, high nicotine metabolite ratio (3-HC/cotinine) predicted poor outcomes for smoking cessation treatment with nicotine patch. The underlying mechanisms that associate metabolite ratio with treatment outcomes have not been fully elucidated. A total of 100 smokers were divided into quartiles based on their baseline plasma nicotine metabolite ratio. Following overnight abstinence, smokers received saline followed by escalating intravenous doses of nicotine (7 and 14 mcg/kg) given 30 min apart. Smokers in the first quartile (slower metabolizers) had lower FTND scores, suggesting lower levels of dependence. In contrast, smokers in the fourth quartile (faster metabolizers) reported greater craving for cigarettes following overnight abstinence from smoking and reported greater ratings of nicotine-induced good drug effects, drug liking, and wanting more drug. Higher nicotine metabolite ratio was also associated with greater heart rate increases in response to nicotine. These results suggest that nicotine metabolite ratio may influence the subjective rewarding and physiological effects from nicotine and withdrawal severity.

In summary, our previous work demonstrates the feasibility of IV nicotine self-administration to determine the reinforcement threshold for nicotine in LITS. Our findings point to a range of nicotine doses, 1.5 to 6.0 mcg/kg, that is well-tolerated by non-dependent smokers and can be used to determine the reinforcement threshold. Our group has unique expertise and a track record in conducting IV nicotine administration studies in both male and female smokers.

6. Significance:

The proposed study is highly significant. First, this study will be especially informative in determining the threshold doses of nicotine needed to maintain nicotine self-administration in male and female LITS smokers. Characterization of the shape and slope of dose-response curves for the reinforcing effects of IV nicotine will help to accurately determine how male and female smokers adapt their nicotine self-administration to reductions in nicotine doses that are available. Whereas the actual threshold and dose-response function may differ in cigarette smoke-delivered nicotine, the IV self-administration data should contribute to a better understanding of the potential threshold and dose-response function for nicotine reinforcement in general. Second, the proposed study will likely foster further preclinical and clinical research on nicotine reinforcement and its moderation by sex. Since the IV route is commonly used for nicotine self-administration studies in rodents and primates (Le Foll et al. 2007), findings from our study will allow translational comparisons of threshold and dose-response curves for nicotine reinforcement between human and animal studies. Finally, the goals of this proposal are also highly relevant to the FDA's new mission of setting standards for the evaluation of new tobacco products as well as the risks of initiation and dependence and consistent with the recommendations of the World Health Organization (WHO) for further evaluation of the importance of nicotine in the appeal, addiction potential, and harmful effects of cigarette smoking (WHO, 2007). Ultimately, the result of this study may provide information that is crucial for the development of science-based policies to control the addictive potential of cigarette smoking for both male and female smokers.

7. Subjects:

Inclusion criteria: 1) Female and male smokers, aged 18 to 35 years, who have been smoking for at least a year, and a life-time consumption of at least 100 cigarettes; 2) smoke more frequently than once a week and ≤5 cpd; 3) FTND score ≤3 indicating no or minimal evidence for nicotine dependence; 4) urine semi-quantitative cotinine levels >100 ng/mL indicating an active smoking status; 5) not seeking treatment at the time of the study for nicotine dependence; 6) in good health as verified by medical history, screening examination, and screening laboratory tests; 7) for women, not pregnant as determined by pregnancy screening, nor breast feeding, and using acceptable birth control methods.

Exclusion criteria: 1) history of major medical illnesses that the physician investigator deems as contraindicated for the subject to be in the study; 2) requirement of any form of regular psychotropic medication (antidepressants, antipsychotics, or anxiolytics) or psychiatric diagnosis and treatment for psychiatric disorders including major depression, bipolar disorder, schizophrenia in the past 6 months; and 3) current dependence to alcohol or any other recreational or prescription drugs and; 4) daily use of smokeless tobacco products or exclusive daily use of e-cigarettes (non-daily users will be included).

Justification for age criteria: The study will enroll young adults between the ages of 18 to 35 because this age group represents a critical time for development of nicotine addiction, with almost all progression to daily smoking completed by the age of 26.

Stratified sampling procedure for race and presence of daily smoking: The study will enroll 36 male and 36 female LITS. The two groups will be matched on race and presence of daily smoking. Race has been shown to influence smoking behavior and nicotine pharmacokinetics. We will create a race variable (0= Caucasian, 1= African-American, 2=Other) to match groups on race. We will also stratify for the presence of daily smoking because daily smoking may influence the behavioral effects from smoking by tolerance development (9-11). This approach will allow balancing the male and female smokers for light vs. intermittent smokers. A categorical variable will be created for the presence of daily smoking (1= present, and 0 = absent).

9. Selection:

Male and female smokers will be recruited from the New Haven area through newspaper advertisements and fliers. We will recruit both veterans and non-veterans since there are not enough veterans in our area who meet the inclusion criteria for the study. Interested subjects will have the study described over the telephone and will be asked to answer a brief tobacco use history and medical screening questionnaire. If subjects are eligible for the study based on the telephone screening, they will then be invited to come to the clinic for an initial screening evaluation. The initial screening evaluation will include the following: a) obtaining an informed consent; b) smoking history and assessment of nicotine dependence based on the Fagerstrom Test of Nicotine Dependence (FTND); and c) a urine semi-quantitative cotinine level to confirm active smoking status at study entry..

If subjects are eligible based on the stratification procedure, they will be invited to participate in a second evaluation, which will include: a) complete physical and psychiatric examination including the structured clinical interview (SCID) for DSM-V; b) laboratory examination including CBC, ALT, AST, alkaline phosphatase, glucose, BUN, creatinine c) DNA; d) urine analysis, drug screening, and for women, urine pregnancy test. The laboratory test results will be part of the patients' medical record at the VA Hospital located on the CPRS system, as well as in the subject's research chart; and e) ECG.

10: Recruitment:

A total of 300 subjects will be enrolled, targeting 72 completers (36 males and 36 females) under the new study design. To date (April 2016), 45 subjects have signed consents with 23 completers under the previously approved protocol design. These subjects will not be asked to return for participation in the new study design.

11. Research Plan:

A. Overview

This will be a double-blind, placebo-controlled study, with 72 LITS (36 male and 36 female smokers) participating in five experimental sessions. All sessions will be conducted following overnight abstinence to control for recent nicotine intake. The lab sessions will be at least 24 hours apart to minimize carryover nicotine effect between sessions In each experimental session, subjects will be randomly assigned to one of the five doses of nicotine (0.0125, 0.025, 0.0.5, 0.1 and 0.2 mg/70 kg or about 0.18, 0.36, 0.7, 1.4 and 2.8 µg/kg). At the beginning of each experimental session, subjects will first sample the assigned nicotine dose and placebo (saline) condition that are randomly labeled as A or B. Beginning 15 minutes after the second sample dose, subjects will have the opportunity to choose between drug A or B every 15 minutes, for a total of ten opportunities over a 165-minute period. Immediately after subjects make their choice, drugs will be administered over 30 seconds using an infusion pump. The main outcome measure will be the number of nicotine self-administrations for each nicotine dose. The parameters of this nicotine self-administration procedure were based on our previous study (Sofuoglu et al. 2008a).

B. Medical Monitoring and Safety

Subjects will be given a thorough physical examination prior to entry in the study. For nicotine administration sessions, a physician will be present and subjects will be attached to a cardiac monitor as well as a blood pressure and heart rate monitoring device. An IV catheter will be in place throughout the session. Subjects will be administered nicotine only if the systolic blood pressure is <150 mmHg and heart rate is <100 beats/minute. Subjects will be terminated from the study if the blood pressure at any time is >170/110 mm Hg, the heart rate is >120 beats/min, or if they develop signs and symptoms compatible with nicotine toxicity. Subjects will remain in the laboratory for at least an hour after the last nicotine administration. These procedures have been developed as part of our IND for IV nicotine.

C. Outcome Measures

Behavioral

Nicotine Self-Administration: Nicotine self-administration behavior will be measured with the number of nicotine administrations during the session.

Physiological

Heart rate and blood pressure: Heart rate and blood pressure readings will be taken at intake for screening, and during the lab sessions to monitor nicotine's effects.

Biochemical

1) Alveolar carbon monoxide: The CO measurement taken before the sessions will help to verify compliance with smoking abstinence. CO readings ≤ 8ppm will be used to verify compliance with overnight smoking as recommended by the SRNT Subcommittee on Biochemical Verification (Benowitz et al. 2002).

2) Serum estradiol and progesterone analysis (females only): Serum estradiol and progesterone levels will be measured before each lab session for use as covariates in our analysis, since female sex hormones may contribute to sex differences in nicotine responses (Smith et al. 2006; Sofuoglu et al. 2001; Zubieta et al. 2002).

4) Plasma/urine cotinine, 3-hyroxycotinine (3-HC), and nicotine levels: Plasma/urine samples of cotinine will be taken at intake to quantify the level of nicotine intake of the subjects. 3-HC is the main metabolite of cotinine and the ratio of 3-HC/cotinine, also known as the nicotine metabolite ratio, reflecting the activity of cytochrome P450 (CYP2A6) and thus, the rate of nicotine clearance. Plasma nicotine levels will also be measured before each session to ensure overnight abstinence from smoking. Assays for nicotine, cotinine, and 3-HC levels will be performed in Dr. Peter Jatlow's laboratory. Briefly, nicotine, cotinine and 3-HC are measured using HPLC coupled to tandem mass spectrometry (LC/MS/MS) employing stable isotope labeled internal standards as previously described.

Subjective

Intake Measures:

1) Structured Clinical Interview for DSM-5 (SCID) for Axis I disorders: SCID is a semi-structured interview based on DSM-5 (American Psychiatric Association, 2013) and will be performed to diagnose Axis I psychiatric disorders.

2) Fagerstrom Test of Nicotine Dependence (FTND): This self-report measure assesses the degree of nicotine dependence and has been used widely in smoking studies (Heatherton et al. 1991)

3) Center for Epidemiologic Studies Depression (CES-D) scale: The CES-D is a 20-item self-report measure of depressive symptoms (Radloff 1977). This scale will be used at intake to control for baseline differences in depressive symptoms.

Session Measures:

1) Minnesota Nicotine Withdrawal Symptom Checklist (M-NWSC): Smokers will be asked to rate several nicotine withdrawal symptoms from on a 100 mm scale, "not at all" to "extremely." The items are derived from the M-NWSC (Hughes and Hatsukami 1986) and have been used in previous human laboratory studies (Eissenberg et al. 1996). The items include cigarette craving, irritability/anger, anxiety/tension, difficulty concentrating, restlessness, increased appetite, depressed mood, and insomnia.

2) Positive and Negative Affect Schedule (PANAS): The PANAS is a 20-item scale that assesses both negative and positive affective states (Watson et al. 1988). This scale is sensitive to the affective symptoms of tobacco withdrawal and predicts relapse to smoking (Kenford et al. 2002).

3) Brief Questionnaire on Smoking Urges (BQSU): This 10-item scale has been found to be highly reliable and reflects levels of nicotine deprivation (Bell et al. 1999; Morgan et al. 1999). This scale will be used to monitor cigarette craving.

4) Drug Effects Questionnaire (DEQ): Smokers will rate 5 items that are related to nicotine effect on a 100 mm scale, "not at all" to "extremely." The items are feeling the “drug strength,” feel “good” drug effects, feel “bad” drug effects, liking the drug effects, and “head rush.” This instrument allows rapid detection of nicotine effects and is adapted from a VAS (Soria et al. 1996).

Genetics

Blood samples for DNA extraction will be collected to examine whether any of the genes modify the effects of nicotine. Candidate genes will be selected from a list of genes hypothesized to be involved in nicotine’s effects. The current list of genes includes but is not limited to genes for nicotinic and muscarinic receptors. To protect confidentiality, each subject’s blood sample will be encoded with a numeric designation and the name of the individual will be stored in a separate database. The samples will be transferred to the Genetic Laboratory at the VA medical Center for processing and storage.

D. Drugs

Nicotine: Nicotine bitartrate will be obtained from Interchem Corporation, Paramus, NJ. Nicotine solution for injection will be prepared by U.S. Specialty Formulations, Bethlehem, P.A.. Nicotine increases heart rate, peripheral vascular resistance, myocardial contractility, cardiac output, and blood pressure. Nicotine may cause nausea, vomiting, and diarrhea. Acute toxicity of nicotine occurs with high doses; 40-60 mg orally is considered lethal. Signs and symptoms of acute toxicity include nausea, vomiting, abdominal pain, hypersalivation, diarrhea, dizziness, confusion, hearing and vision problems, syncope, seizures, hypotension, irregular pulse, and death (Benowitz and Gourlay 1997; Kobayashi et al. 1999).

Nicotine administration: In our proposed study, nicotine will be administered over 30 seconds, via an IV catheter located in a forearm vein, using an infusion pump. We have followed these procedures in our previous studies (Sofuoglu et al. 2003, 2005, 2006b; 2008a, 2009a, b,c; Sofuoglu and Mooney 2009), which were completed without any adverse effects or safety concerns. For each experimental sessions, two IV infusions will be prepared in randomized, double-blinded fashion. The two infusions will be marked as either "A" or "B" on identical-looking IV labels. Depending on the randomization, one of the infusion will contain an active nicotine dose (0.0125, 0.025, 0.05, 0.1 and 0.2 mg/70 kg) and the other will contain saline. The total amount of nicotine solution in the active infusion will contain enough for 1 sample dose of 5 mL, 10 optional doses of 5mL each, and additional volume to prime the infusion line. The matching placebo infusion will also contain enough for 1 sample dose of 5mL, 10 optional doses of 5mL each, and additional volume to prime the infusion line.

The matching placebo infusion bags will also contain enough for 1 sample dose and 10 optional doses and for the line flush of 0.9% NaCl. The infusion bags will be labeled and dispensed to the study staff by the research pharmacy. Dr. Sofuoglu holds an IND for IV nicotine administration.

Justification for the nicotine doses: For this proposal, we chose five doses of nicotine that are in low dose range including 0.0125, 0.025, 0.05, 0.1 and 0.2 mg/70 kg. The highest dose, 0.2 mg, is equivalent to the amount of nicotine delivered from one or two puff of a cigarette, which ranges from 0.05 to 0.2 mg/puff or 0.5 to 2.4 mg/cigarette 1.0 - 2.4 mg, delivered via 10 to 13 puffs (33, 81, 82). In our previous study, 0.2 mg/70 kg dose of nicotine produce positive subjective drug effects and were self-administered by male dependent smokers (34). The lowest dose will be 0.0125 mg/70 kg, lower than the amount estimated to be the addictive threshold for nicotine by B&H, 0.17mg nicotine/cigarette or 0.014 mg nicotine/ puff. We also included 3 additional doses of nicotine 0.025, 0.05, 0.1 mg, in between the 0.2 and the 0.0125 mg dose to provide a more detailed assessment of dose-effect function. These five doses of nicotine and placebo should allow for a detailed examination of individual differences in NSA at a low dose range

E. Study Procedures

Session procedures: Before the test sessions, smokers will be required to abstain from smoking for 10 h, which will be verified by expired air CO levels ≤8 ppm (Benowitz et al. 2002). Subjects will be asked to refrain from consuming alcoholic beverages and drugs during study participation, which will be verified by a urine drug quick test. If results indicate non-compliance with these study procedures, subjects will be discharged from the study. Subjects will be instructed to drink their typical amount of caffeinated beverages in the morning to minimize caffeine withdrawal, which could confound the study measures. Subjects will be instructed not to eat for four hours before the lab sessions to reduce the chance of vomiting. A light lunch will be provided at the end of each experimental session.

Medical monitoring: Subjects will be given a thorough physical examination prior to entry in the study. For nicotine administration sessions, a physician will be present and subjects will be attached to a cardiac monitor as well as a blood pressure and heart rate monitoring device. An IV catheter will be in place throughout the session. Subjects will be administered nicotine only if the systolic blood pressure is <150 mmHg and heart rate is <100 beats/minute. Subjects will be terminated from the study if the blood pressure at any time is >170/110 mm Hg, the heart rate is >120 beats/min, or if they develop signs and symptoms compatible with nicotine toxicity. Subjects will remain in the laboratory for at least an hour after the last nicotine administration. These procedures have been developed as part of our IND for IV nicotine.

TABLE 1. SCHEDULE OF EVENTS: EXPERIMENTAL SESSION*

Minutes	Time point	Measures and Events
0 Min	1	HR/BP, DEQ, IV nicotine or saline administration A (sampling) CO, HR/BP, EKG, M
1 Min	2	HR/BP, DEQ
2 Min	3	HR/BP,
3 Min	4	HR/BP, DEQ
5 Min	5	HR/BP, DEQ
15 Min	6	HR/BP, DEQ I V nicotine or saline administration B
16 Min	7	HR/BP, DEQ
17 Min	8	HR/BP
18 Min	9	HR/BP, DEQ
20 Min	10	HR/BP, DEQ
30 Min	11	HR/BP, DEQ IV Choice Sampling Option 1
31 Min	12	HR/BP, DEQ
32 Min	13	HR/BP
33 Min	14	HR/BP, DEQ
35 Min	15	HR/BP, DEQ
45 Min	16	HR/BP, DEQ IV Choice Sampling Option 2
46 Min	17	HR/BP, DEQ
47 Min	18	HR/BP
48 Min	19	HR/BP, DEQ
50 Min	20	HR/BP, DEQ
60 Min	21	HR/BP DEQ IV sampling Option 3
61 Min	22	HR/BP, DEQ
62 Min	23	HR/BP
63 Min	24	HR/BP, DEQ
65 Min	25	HR/BP, DEQ
75 Min	26	HR/BP, DEQ IV sampling Option 4
76 Min	27	HR/BP DEQ
77 Min	28	HR/BP,
78 Min	29	HR/BP, DEQ
80 Min	30	HR/BP , DEQ
90 Min	31	HR/BP, DEQ IV sampling Option 5

91 Min	32	HR/BP DEQ
92 Min	33	HR/BP,
93 Min	34	HR/BP, DEQ
95 Min	35	HR/BP, DEQ
105 Min	36	HR/BP, DEQ IV sampling Option 6
106Min	37	HR/BP DEQ
107Min	38	HR/BP,
108 Min	39	HR/BP, DEQ
110 Min	40	HR/BP, DEQ
120 Min	41	HR/BP DEQ IV sampling Option 7
121 Min	42	HR/BP DEQ
122 Min	43	HR/BP,
123 Min	44	HR/BP, DEQ
125 Min	45	HR/BP, DEQ
135 Min	46	HR/BP DEQ IV sampling Option 8
136 Min	47	HR/BP DEQ
137 Min	48	HR/BP,
138 Min	49	HR/BP, DEQ
140 Min	50	HR/BP, DEQ
150 Min	51	HR/BP DEQ IV sampling Option 9
151 Min	52	HR/BP DEQ
152 Min	53	HR/BP,
153 Min	54	HR/BP, DEQ
155 Min	55	HR/BP, DEQ
165 Min	56	HR/BP DEQ IV sampling Option 10
166 Min	57	HR/BP DEQ
167 Min	58	HR/BP,
168 Min	59	HR/BP, DEQ
170 Min	60	HR/BP, DEQ
180-240 Min	61	HR/BP, DEQ, EKG, SCF M-NWSC, BQSU, PANAS, SNACK and Discharge

*Same measure will be obtained following saline and each nicotine administration. For brevity, only the measures after saline are shown.

Abbreviations: CO: Alveolar carbon monoxide, HR/BP: Heart rate/Blood pressure; EKG: Electrocardiogram; SCF: Symptom Checklist form; M-NWSC: Minnesota Nicotine Withdrawal Symptom Checklist; BQSU: Brief Questionnaire of Smoking Urges; PANAS: Positive and Negative Affective Schedule; DEQ: Drug Effects Questionnaire.

Experimental sessions: The schedule of events during the experimental sessions is shown in Table 1. Before the session begins, subjects will have an IV catheter placed and the baseline measures will be taken. Subjects will begin NSA as described below.

Nicotine self-administration procedure:

Apparatus: Saline and nicotine will be administered with two separate infusion pumps, which will be prepared with IV infusion bags randomly labeled as Drug A or B by the research pharmacist. Both the researchers and the subjects will be blind to the randomization. The infusion pumps will deliver saline and nicotine at a precise rate and volume.

Procedure: The NSA procedure will consist of two phases: Sampling and Nicotine Choice.

Sampling: In each of the five test sessions, subjects will be randomly assigned to one of the five doses of nicotine: 0.0125, 0.025, 0.05, 0.1 and 0.2 mg/70 kg. Subjects will first receive saline and the assigned nicotine dose in a randomized order and double-blind fashion. Subjects will be informed that they will be receiving drug A or B, which may be nicotine or saline. This procedure will allow subjects to sample the nicotine and saline that will be available during that session. In addition, subjective and physiological responses to the sample nicotine dose and saline will be assessed.

Nicotine Choice procedure: Beginning 15 minutes after the sampling phase is completed, subjects will be asked to make a choice between drug A or B every 15 minutes for a total of ten choices over 150 minutes. The drug chosen by the subjects will be immediately delivered with the infusion pump over 30 sec in 5 ml of saline. This choice procedure has been used in our IV nicotine self-administration study as well as in many previous studies with various drugs of abuse (Sofuoglu et al. 1999, 2000, 2001; 2009c). One particular advantage of this model, compared to the ad lib models used in previous IV nicotine self-administration studies (Harvey et al. 2004; Rose et al. 2003), is the ability to control amount of nicotine delivered without interfering with the self-administration behavior.

12. Data analysis methods:

Data analyses: The primary analyses will be intent-to-treat and will include all available data on subjects who complete at least one test day. Mixed-effects models will be used to test the study hypotheses. These models allow for different numbers of observations per subject, use all available data on each subject, and are unaffected by randomly missing data. They also provide flexibility in modeling the correlation structure of the data. All data will be checked for normality and transformations will be applied as necessary. Significance level of 0.05 will be used for the main hypotheses and Bonferroni correction will be applied for post-hoc tests and secondary analyses.

Specific Aim #1: Aim #1: To assess the threshold reinforcing dose and dose-effect curve for IV NSA at low doses in young adult LITS. Hypothesis #1A: The threshold reinforcing doses for IV NSA will be between 0.0125 to 0.05 mg/70 kg. Hypothesis #1B: The dose-effect curve for NSA will differ between males and females with relatively flat curve in female smokers.

Aim #1 Analysis: The reinforcing effects of nicotine will be determined by the number of IV NSAs in each session, ranging from 0 to 10. The threshold dose for NSA will be determined by the lowest dose of nicotine for which the number of NSAs is significantly greater than those for placebo (saline) (i.e., proportion of NSA significantly higher than 0.5). For assessing the dose-response relationship, the parameters of interest will be the slope and shape of the dose-response curve. The slope indicates the degree to which self-administration changes with an increase in nicotine dose (in other words, sensitivity of NSA to changes in nicotine dose).

The analysis of Aim #1 will conducted in several steps. First, we will use descriptive statistics and figures to summarize the data. In particular, the number of NSAs for each dose of nicotine will be plotted individually, for the entire sample, and for male vs. female and smokers. This step will allow a visual inspection of both individual and group differences for estimating the threshold dose and the shape of the dose-response curve for nicotine reinforcement. Second, we will fit mixed effects logistic regression models with the proportion of NSAs per session (out of 10) as the dependent variable, dose as a fixed categorical predictor and subject as a random effect. This approach allows us to take into account the correlations between repeated observations on the same individual and to estimate individual deviations from the overall group thresholds.

It also does not assume a specific form of the dose-response relationship and allows us to use all available data on a subject in the unlikely event that a subject fails to complete a session. If a subject fails to complete a session, the total possible number of self-administrations for this subject and session will be adjusted.

We expect to observe a significant dose effect and will compare the proportion of NSAs at each dose to 0.5 across subject groups. The lowest nicotine dose that has a significantly higher proportion of NSA than 0.5 will be selected as the threshold dose (Hypothesis 1A). To assess the dose-response relationship in the preference for nicotine, polynomial effects of dose (linear, quadratic, cubic) and change-point linear models will be tested in the mixed effects logistic regression model. We anticipate that there will be a significant linear relationship between dose and response rate at lower doses with flattening of the relationship at higher doses. We will use the slope of the dose effect to assess the linear dose effect. Alternative non-linear dose response relationships will be considered if residual plots reveal significant deviations of the models from linearity. Gender differences in the shape of the curve for NSA will be examined in the logistic regression model by including gender main effect and its interactions with dose (Hypothesis 1B). Significant interactions between the group factors and dose will be considered supportive of systematic group differences in the dose-response relationships between groups. If significant interactions are observed, then separate thresholds and curves will be estimated within each subject group.

Specific Aim #2: To assess the threshold and dose-effect curve for the positive and negative/aversive subjective effects of IV nicotine at low doses and its relationship to nicotine reinforcement. Hypothesis #2 A: The threshold for the positive effects will be between 0.0125 to 0.1 mg/70 kg, for the negative/aversive effect it will be ≥ 0.1 mg/70 kg. Hypothesis #2B: Nicotine reinforcement will be positively correlated with the positive and negatively correlated with the negative/aversive subjective effects of IV nicotine.

Aim 2 Analysis: The subjective-positive effects of nicotine which will be determined by the main dependent variables of scores on the “like the drug” and “want more drug” items of the Drug Effects Questionnaire, ranging from 0 to 100. The negative aversive effects of nicotine will be determined by the “dislike the drug” and “lightheaded/dizzy” item of the DEQ. The analysis for Specific Aim #2A will be similar to the analysis described for Specific Aim #1A except that the dependent variables will be continuous and thus we will use random effects linear regression. Gender effects will be explored also as outlined for Specific Aim #1B. Prior to analysis, we will also assess the distribution of the DEQ items and apply transformations as necessary. Furthermore, the mean ratings at each dose will be compared to 0. To assess the

relationship between nicotine reinforcement and positive subjective effects, and between nicotine reinforcement and negative subjective effects (Specific Aim #2B), we will use mixed effects models with the proportion of NSAs per session as the dependent variable, positive (negative) subjective effects in response to the sample dose as the main predictor of interest (in separate models for positive and negative effects). Nicotine dose, gender and the interactions among subjective effects, dose and gender will also be assessed. Non-significant interactions will be dropped from the models via backward elimination subject to the constraint that the models will be hierarchically well-formulated at each step. Statistically significant associations between subjective effects and NSA across doses and gender are expected but we will also explore whether these relationships vary by gender and dose.

Exploratory Aim #1: To examine the association between baseline 3HC/cotinine ratio and nicotine reinforcement threshold. To test this hypothesis, correlation analysis including 3 HC/cotinine ratio and nicotine reinforcement threshold will be performed in the total sample as well as in subgroups (male vs. female smokers).

Rationale for sample size: Power for Specific Aims 1 and 2 is based on our preliminary studies comparing nicotine doses of 0.1, 0.2, 0.3 and 0.4mg to saline (34). We consider a difference between 50% (half of infusions in favor of nicotine) and 67% (two-thirds in favor of nicotine) as clinically meaningful. With our sample of 72 subjects we can detect such a proportion (67%) as statistically different from 50% with 80% power assuming two-sided test and alpha=0.05 (Specific Aim 1). Also with our sample of 72 subjects, we can detect medium effects (d'=0.34) for the threshold tests on "good effects" and "drug liking" under the same assumptions (Specific Aim 2).

13. Risks and benefits:

Potential risks

There are potential risks, discomforts and inconveniences associated with the participation in this study. These may be due to nicotine administration, blood drawing, and other study procedures.

1) The administration of nicotine may cause cardiovascular, autonomic, and gastrointestinal complications. Large doses of nicotine may cause nausea, vomiting, abdominal pain, hyper salivation, diarrhea, dizziness, confusion, hearing and vision problems, syncope, seizures, hypotension, irregular pulse, and death. However, these toxic effects occur at doses 10-20 times that which will be used in our study. Other potential risks from this study include administering a drug that has addictive potential. However, since only subjects with an extensive history of cigarette use are to be included, we will not be exposing subjects to the risks of nicotine for the first time. Additionally, we are not enrolling subjects who are seeking treatment to quit smoking. Over the last 5 years, we have administered nicotine intravenously to more than 100 smokers and have not encountered any adverse events from nicotine.

2) Blood Drawing: Subjects will have approximately 200 ml of blood drawn as a result of their participation in the study. Blood drawing can cause some pain and may result in bruising.

3) Study procedures: On the test days, subjects will not be able to smoke for 10 hours. During this cigarette abstinence period, subjects may experience symptoms of nicotine withdrawal such as craving cigarettes, mild anxiety, restlessness, irritability, difficulty concentrating, loss of energy, and excessive hunger.

Protection of Subjects

In order to participate in a study, each subject must give informed consent. All potential risks will be described in detail to the subjects in the consent form. The personnel in the laboratory have

been certified in either Advance Cardiac Life Support (ACLS) or Basic Life Support. If a problem arises, the subject will be treated immediately.

Confidentiality in this study is of the utmost importance to us. All information obtained will be stored in coded form. The names of the subjects will be used in hospital records.

14. Safety

Prior to initiating any research activity, each subject must give informed consent. Before the study, the subjects will be informed about all potential risks of the study. Our inclusion and exclusion criteria will be applied by experienced professionals who will be carefully trained and monitored in order to accept only appropriate subjects into the study. Thus, effective screening will exclude subjects who would be placed at a greater risk. Eligibility is determined by the medical and psychiatric history, drug use history, the physical examination, and the laboratory studies done prior to beginning this research protocol.

For nicotine administration sessions, a physician will be present and subjects will be attached to a cardiac monitor as well as a blood pressure and heart rate monitoring device. An IV catheter will be in place throughout the session. Subjects will be administered nicotine only if the systolic blood pressure is <150 mmHg and heart rate is <100 beats/minute. Subjects will be terminated from the study if the blood pressure at any time is >170/110 mm Hg, the heart rate is >120 beats/min, or if they develop signs and symptoms compatible with nicotine toxicity. Subjects will remain in the laboratory for at least an hour after the last nicotine administration. These procedures have been developed as part of our IND for IV nicotine.

Confidentiality will be protected by having records identified by code number only with the master list including names kept in a sealed envelope in a locked file in the Principal Investigator's office and by the pharmacy. Subjects will be given telephone numbers to call in case of emergency, 24 hours a day.

14.1 Potential benefits of the proposed research to the subjects and others. There will be no direct benefit to subjects participating in this study. However, subjects will receive complete medical and psychological evaluation. Cigarette smokers will be given anti-smoking literature and treatment resources will be provided for smoking cessation.

14.2 Importance of the knowledge to be gained. This proposed study may help to develop new and more effective tobacco control policies. We believe that the risk/benefit ratio for this study is acceptable, and that the benefits of the proposed studies outweigh the potential risks to subjects.

14.3 Data safety and monitoring plan: The Principal Investigator will conduct a review of all adverse events and determine the attribution and grade of severity of the adverse event by using the following scales:

Attribution of Risk Categories:

Definite: Adverse event(s) will clearly be related to investigational agent(s) or other intervention

Probable: Adverse event(s) will likely be related to investigational agent(s)

Possible: Adverse event(s) may be related to investigational agent(s)

Unlikely: Adverse event(s) will doubtfully be related to investigational agent(s)

Unrelated: Adverse event(s) will clearly not be related to the investigational agents(s)

Grades of Risk:

0: No adverse event or within normal limits

1: Mild adverse event

2: Moderate adverse event

3: Severe adverse event resulting in hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect

4: Life-threatening or disabling adverse event

5: Fatal adverse event

Serious adverse events (SAEs) include any untoward medical occurrence that at any dose results in death or the immediate risk of death, hospitalization or the prolonging of an existing hospitalization, persistent or significant disability/incapacity or a congenital anomaly/birth defect. Subjects will be terminated from participation if the investigator feels that subjects' health or well-being may be threatened by continuation in the study. Serious unanticipated and anticipated adverse events will be reported within 48 hours to the VA Hospital and Yale IRB, and NIDA. We will directly report to the FDA, whenever their magnitude or frequency exceeds expectations.

The risk associated with participating in this study is moderate, because nicotine administered may be associated with mild side effects. Serious side effects associated with this treatment are not expected. This project will be monitored by a Data and Safety Monitoring Board (DSMB) because the study involves double-blind treatment of smokers with nicotine. This board is composed of persons not otherwise affiliated with the clinical study who are experienced in various aspects of the conduct of clinical trials for the treatment of addictive disorders. We propose three investigators located here in Connecticut who are not directly involved in this study – Declan Berry, Ph.D., Sherry McKee, Ph.D., and David Fiellin, M.D. as the members of the DSMB. The members of the DSMB and all study Investigators will complete Conflict of Interest forms created by Yale's IRB in accordance with NIH guidelines.

In order for the DSMB to fulfill its mission of assuring the safety of human subjects and the scientific integrity of the studies conducted, the Board will have access to accumulating study outcome data in a manner that will protect its confidentiality and preserve its statistical integrity. The Board will examine accumulating data to assure that the risks and benefits of participation remain acceptable and that the results of the study will be considered scientifically reliable. The conditions under which the Board will examine this data are described below. This monitoring will be consistent with NIH policy regarding the protection of human subjects in research, and FDA guidance on statistical practices for clinical trials (ICH E9) and good clinical practices (ICH E6). In general, the data to be reviewed will include screening data, baseline data, efficacy data, and safety data.

The study will be monitored for safety in an ongoing way as well as three times each year formally by the DSMB. The P.I. will attend an initial part of this meeting to present the study's adverse events and ongoing subject accrual, as well as any potential study design changes under consideration. The remainder of the meeting will not include any direct study personnel until the end of the meeting, when the DSMB will convey directly to the P.I. any safety or study conduct concerns, as well as requests for potential interim analyses.

Following each DSMB meeting written minutes will be prepared and distributed summarizing any recommendations. These written reports will insure timely communication with the study P.I. with preparation of any protocol amendments necessary. After each DSMB meeting, this written report will describe all recommendations including additional safety steps. The FDA adverse drug experience reporting timelines will be utilized as timelines to disseminate feedback from the DSMB to the principal investigator and sub investigators. That is, three days for acute circumstances and ten days for non-acute circumstances.

15. Informed consent: Subjects will be recruited from the New Haven area by newspaper advertisements and fliers. Interested subjects will be informed about the study over the telephone and asked for current use of drugs and medical problems. If subjects are interested, they will then come into the clinic for a full screening evaluation. Upon arrival, a research assistant will read the detailed consent form and will ask questions to make sure that the subjects understand the procedure and their rights and informed consent will be obtained.

16. Information Security/Confidentiality: Confidentiality in this study is of the utmost importance to us. All information obtained will be stored in coded form.

Personally identifiable information (PII) and protected health information (PHI) that is obtained from subjects or about them will be protected by the research team who will follow all guidance provided in the following VHA HANDBOOKS and DIRECTIVE:

- VHA HANDBOOK 1605.1, PRIVACY AND RELEASE OF INFORMATION Dated May 17, 2006
- VHA HANDBOOK 1907.01, HEALTH INFORMATION MANAGEMENT AND HEALTH RECORDS Dated August 25, 2006
- VHA HANDBOOK 6500, INFORMATION SECURITY PROGRAM Dated September 18, 2007
- MEMORANDUM FOR UNDER SECRETARIES, ASSISTANT SECRECTARIES AND OTHER KEY OFFICIALS, SUBJECT: Protecting Information Security and Privacy Dated February 27, 2009
- VHA HANDBOOK 1200.12, USE OF DATA AND DATA REPOSITORIES IN VHA RESEARCH Dated March 9, 2009

All data collected will be the property of the Department of Veterans Affairs whether in paper or electronic form and will be secured utilizing the following methods:

Paper:

- All paper documents will be stored on VA property unless authorized by the Director, Information Security Officer, and Privacy Officer in writing.
- All paper documents will be locked in an approved file cabinet with only members of the research team having access.

Electronic:

- All information in electronic form will be stored on VA servers behind the VA firewall.
- All portable media will meet or exceed FIPS 140-2 compliance and encryption. And will be approved in writing by the Information Security Officer.

The location(s) where information will be stored is (are) Building 35, rooms 19 and 39

The people or agencies that will have access to the information are the study investigators.

No information related to this research will be released to any third party or disclosed outside of the VHA – except as required or permitted by law.

Research records will have identifiers removed and will be stored with a code number linked to subjects. The code will not be derived from any personal identifiers. The key to the code will be kept in a locked file cabinet, located in Bldg 35, room 19. Subjects' identity will not be revealed in any reports or publications resulting from this study.

17. Location of Study: This study will be conducted in Ward G9W (the Biostudies Unit) located in Building 1 at the West Haven VA Medical Center.

18. Payment: Subjects will be paid $30 for participating in the screening and $130 for each of the 5 lab sessions and $20 for transportation for each of the 5 test sessions. If the subjects choose to terminate a session prematurely, or a session is terminated early for medical reasons, they will receive full payment for that day. If they become ineligible to continue in the study due to non-compliance with study procedures, they will only be paid for the portions of the study in which they have participated. Subjects may also earn $20 for referring people they know who also smoke cigarettes and are eligible for study participation. A contingency payment of $20 will also be given for transportation for a visit that wasn't fulfilled in the outlined time frame within the protocol. Subjects may be paid up to $780 if all parts of the study are completed.

19. Funding Source: An RO1 grant from NIDA (pending).

20. Duration: The new study design will take approximately 3 years to complete.

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