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: Kevin Jensen, Ph.D., Elise E. DeVito, Ph.D., Joel Gelernter, M.D., Suprit Parida, M.D.

Nursing Staff: *Ellen Mitchell, RN, Angelina Genovese, R.N., Elizabeth O'Donnell, R.N., Jane Weiner, R.N.

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

3. Title of Project: Nicotine Delivery Rate and Its Abuse Potential: Impact of Menthol

4. Purpose, hypothesis and key questions:

The FDA can regulate tobacco products to reduce their harmful effects and to limit their addictive potential. Benowitz and Henningfield proposed that gradually reducing the nicotine content of cigarettes to an amount below the addictive threshold could prevent the development of addiction (1). More recently, Shihadeh and Eissenberg made a similar proposal for minimizing the addictive potential of electronic cigarettes (EC) (2). After considering several EC variables, the nicotine delivery rate, or "nicotine flux", was considered by Shihadeh and Eissenberg as the most critical factor for evaluating the abuse potential of a given EC(2). An EC with low nicotine flux might have low abuse potential and minimal negative health effects; in contrast, an EC with high nicotine flux might have high abuse potential and high risk for causing negative health effects. Indeed, newer generation ECs that deliver nicotine rapidly are rated as more satisfying compared to older generation ECs that deliver nicotine slowly (3), suggesting that higher flux ECs might have greater abuse potential. However, the nicotine delivery rate that is essential for the addictive effects of nicotine has yet to be empirically determined by carefully controlled human studies.

The primary aim of this project is to establish a dose-effect curve for nicotine reinforcement as a function of nicotine delivery rate. We will use the dose-effect curve to estimate a threshold delivery rate for reinforcement. IV nicotine, in contrast to ECs, can deliver precise, reproducible dosing, which is necessary for accurately assessing dose-response and threshold effects. The estimated threshold for reinforcement will establish a benchmark for evaluating the addictive potential of ECs and other inhaled nicotine products.

Specific Aim: To determine the dose-effect curves for positive subjective effects (drug liking and good drug effects) and alleviation of smoking urges, as a function of nicotine delivery rate in menthol and non-menthol cigarette preferring smokers.

Hypothesis #1A: Positive subjective effects of nicotine will gradually decline with slower delivery rates, with minimal effects of nicotine delivery rate on urges to smoke.

Hypothesis #1B: Non-menthol smokers, compared to menthol smokers, will have greater responses to nicotine for these outcomes as reflected by a leftward shift in dose-effect curves.

Exploratory Aim #1: To explore the dose-effect curves of nicotine delivery rate for a) reinforcement (assessed with the monetary value of the dose from the Multiple-Choice Procedure (6), b) heart rate and blood pressure, and c) tobacco withdrawal severity (assessed with self-report symptoms and cognitive performance).

Exploratory Aim#2: To examine the impact of plasma nicotine levels and rate of nicotine metabolism (measured by plasma hydroxycotinine/cotinine ratio) on the dose-effect curves for the main study outcomes.

Exploratory Aim#3: To examine sex differences for the main study outcomes.

5. Background:

Nicotine control policies to reduce the addictive potential of tobacco products:

Benowitz and Henningfield (1) proposed that the gradual reduction of nicotine in cigarettes to an amount below the addiction threshold could prevent the development of nicotine addiction among young smokers. The Benowitz and Henningfield proposal and its subsequent appraisal by the American Medical Association Council on Scientific Affairs (6) have provided the foundation for the current nicotine control approaches being considered by the FDA (7). More recently, Shihadeh and Eissenberg made a similar proposal for minimizing the potential risks of using EC (2). After considering several EC variables including liquid composition, puff behavior and electrical power, they focused on nicotine flux, the rate of nicotine delivery, as the key factor in determining the abuse potential of a given EC. Accordingly, an EC yielding no nicotine flux will not maintain usage. In contrast, an EC with high nicotine flux may have high addictive potential and lead to negative health effects. If the nicotine flux does not produce dependence but is sufficient to attenuate craving for cigarettes and withdrawal symptoms among those that are already dependent, the product may have limited abuse potential while retaining potential benefits (e.g. as a smoking cessation aid). In the "nicotine flux" model, the delivery rate of nicotine, rather than the amount of nicotine in the products, is more likely to reflect the addictive potential of the product. The proposed central role of nicotine flux in determining the addictive potential of nicotine remains to be tested in controlled human studies. Policies to reduce the addictive potential of tobacco product would be strengthened if the thresholds for nicotine's addictive properties were empirically defined by carefully controlled studies.

Nicotine pharmacokinetics: Cigarette smoking and EC use:

Among nicotine delivery products, cigarette smoking produces the most rapid delivery of nicotine to the brain (10 to 20 sec) and has the highest abuse potential. The peak nicotine concentrations in blood, ~15 ng/ml, occur approximately 5 minutes after starting to smoke a cigarette (4). The pharmacokinetics of nicotine delivered by EC devices was characterized in a recent study.

The peak nicotine concentration in blood, ~8 ng/ml, was approximately 12 minutes after the initiation of 15 puffs, with one puff every 30s (5). ECs deliver nicotine slower than tobacco cigarettes, probably because EC vapor does not penetrate as deeply into the lung tissue as cigarette smoke for most users. High "nicotine flux" ECs can overcome some of these limitations, so that nicotine is delivered at a rate that is closer to the rate of delivery from tobacco cigarettes. Indeed, newer generation ECs that deliver nicotine more rapidly are rated as more satisfying compared to older generation ECs that deliver nicotine slowly (3).

Nicotine pharmacokinetics: IV administration protocols to study nicotine reinforcement:

Nicotine administered by IV infusion can be carefully modulated to resemble the nicotine intake from tobacco product use. For example, Rose et al. showed that the pharmacokinetics of nicotine delivered by IV infusion resembles the pharmacokinetics of nicotine delivered via cigarette smoking (8). In this study, the arterial plasma nicotine concentrations from smoking one cigarette was first determined for each subject, then the equivalent dose of nicotine was delivered by IV infusion over a similar time course as smoking a cigarette. Both smoking and IV infusion produced similar arterial (20.5 vs. 20.4 ng/ml) and venous (6.9 vs. 7.8 ng/ml) plasma concentrations of nicotine. The rapid time to peak arterial nicotine concentrations following smoking was also observed for IV infusion (8). Multiple studies have demonstrated that rapid IV nicotine infusion (i.e., less than 60 seconds) elicits positive drug effects (e.g., good effects and

drug liking) similar to smoking, and reinforcing effects (i.e., self-administration) in dependent adult smokers (9-13).

Because cigarette smoke and EC vapor contain many compounds in addition to nicotine and the amount of nicotine delivered via smoking or EC puffing cannot be reliably controlled in an experimental setting, cigarettes and ECs are not suitable tools for examining dose-dependent effects of nicotine on reinforcement (14-17). It is also important to note that the IV infusion protocol models the nicotine delivery of tobacco products in the absence of sensory stimuli (e.g. taste, olfactory or visual stimuli) that are present in tobacco products, including cigarettes (and EC) with modified nicotine levels. In contrast to other nicotine delivery systems, IV infusion can deliver precise and reproducible dosing, which is crucial for systematically examining the relationship between nicotine delivery rate and nicotine's effects in humans.

Preliminary Studies:

IV nicotine infusion protocols to study the addictive properties of nicotine in humans

We have developed IV nicotine infusion protocols to answer key questions regarding the addictive properties of nicotine and individual differences in tobacco smoking behavior that are mediated by nicotine (12, 18-21). Subjects in **Fig 1** were 187 adult male and female dependent smokers that had abstained from smoking for ~10 h (adapted from Jensen KP et al. 2015)(19). The average age was 36 years (SD = 8.9). Subjects received infusions of saline and doses of nicotine at 0.12 and 0.24 mcg/kg/second for 60s (0.5 and 1 mg nicotine per 70 kg body weight doses) in uniform order. The doses of nicotine delivered in this session approximate the total dose of nicotine delivered by smoking 0.5 and 1.0 tobacco cigarettes. **Fig 1** shows the subjective ratings of pleasurable and aversive effects after IV saline and nicotine infusion (19). IV nicotine increased ratings of pleasurable effects (main effect for dose: $F_{(2,3025)} = 124.7$, $P < 0.01$), while it had a more subtle effect on ratings of aversive effects (main effect for dose: $F_{(2,3025)} = 1.85$, $P = 0.16$). **Fig 1c** shows that nicotine withdrawal symptoms (M-NWS total score), the urge to smoke for reward (BQSU factor 1), and the urge to smoke to reduce negative symptoms (BQSU factor 2) were lower at the end of the IV nicotine infusion session relative to overnight-abstinence baseline (main effect of time for MNWS: $F_{(1,188)} = 64.85$, $P < 0.01$; BQSU Factor 1: $F_{(1,184)} = 116.6$, $P < 0.01$; BQSU Factor 2: $F_{(1,184)} = 66.6$, $P < 0.01$). Withdrawal symptoms were assessed by the Minnesota Nicotine Withdrawal Scale (M-NWS) and Brief Questionnaire on Smoking Urges.

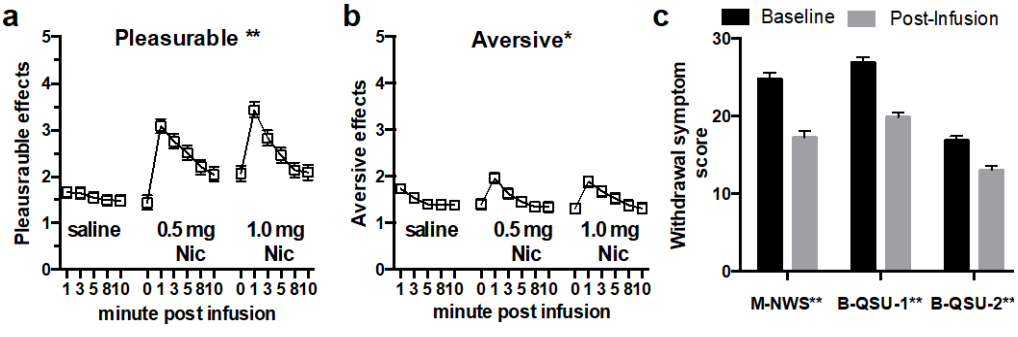

Fig 1. IV nicotine infusion to study the addictive properties of nicotine. IV nicotine induces (**A)** positive and **(B)** negative subjective effects and **(c)** suppresses nicotine withdrawal symptoms. n=187, dependent smokers, ** p<0.01, *p=0.16. (Jensen et al. *Neuropsychopharmacology*, 2015)

Summary of preliminary studies:

- We have developed IV nicotine infusion protocols to answer key questions regarding the addictive properties of nicotine and individual differences in tobacco smoking behavior that are mediated by nicotine (12, 18-20, 22).
- IV nicotine doses infused at 0.12 and 0.24 mcg/kg/second for 60s (~0.5 and 1 mg doses) induce pleasurable ("drug liking", "feels good") drug effects, aversive drug effects and suppresses withdrawal symptoms (**Fig 1)**, similar to smoking.
- Establishing a dose-response curve for nicotine reinforcement as function of nicotine delivery rate and estimating a threshold rate for reinforcement with the IV nicotine infusion protocol developed in this proposal is feasible.

6. Significance:

Nicotine is considered to be the main addictive ingredient in tobacco. Therefore, the nicotine content of tobacco products serves as a logical target in the development of effective tobacco-control policies. In an influential article, Benowitz and Henningfield proposed that the gradual reduction of the nicotine content of cigarettes to an amount below an addictive threshold could prevent the development of nicotine addiction among young smokers. More recently, a similar addictive threshold was proposed for electronic cigarettes (EC) based on their rate of nicotine delivery. Accordingly, if an EC delivers nicotine at rates above certain, yet undetermined threshold, they can be addictive and potentially harmful, especially in nicotine naïve users. In contrast, if the nicotine delivery rate of an EC is below a critical threshold, it may have low addiction liability but provide sufficient nicotine delivery to help smokers quit smoking by alleviating urges to smoke and reducing tobacco withdrawal symptoms. This proposed critical rate of delivery that underlies the addictive effects of nicotine, however, has yet to be empirically validated by controlled human studies. This knowledge gap is partly due to the difficulty in accurately controlling the rate of nicotine delivery with currently available nicotine delivery products like tobacco cigarettes or EC. To close this knowledge gap, we propose to use intravenous (IV) nicotine administration because the rate of nicotine exposure by the IV and inhaled routes are comparable. IV nicotine administration also allows for accurate dosing over a wide range of delivery rates.

7. Subjects:

One hundred smokers will be recruited from the New Haven area through newspaper, and radio advertisements and fliers. The study will be described over the telephone. Subjects will be given a brief tobacco use history and medical screening questionnaire. Eligible subjects will be invited to a single screening evaluation. The screening evaluation will include the following: a) obtaining an informed consent; b) tobacco use history via the Fagerstrom Test of Nicotine Dependence (FTND) and DSM-5 Tobacco Use Disorder (TUD) criteria; d) urine cotinine levels determined with a NicAlert test strip (info: www.nymox.com/default.action?itemid=47). If subjects are eligible 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-5 criteria; b) a complete physical and laboratory examination including CBC, TSH, ALT, AST, GGT, alkaline phosphatase, glucose, BUN and creatinine; c) urine analysis for drug screening, and for women, urine pregnancy test; and d) ECG

Inclusion criteria: 1) Female and male smokers, aged 18 to 35 years, who have been smoking tobacco cigarettes for at least a year; 2) smoke ≥ 5 and less than 20 cigarettes per day; 3) urine cotinine levels > 100 ng/mL consistent with nicotine intake of an active smoker (23); 4) not seeking treatment at the time of the study for nicotine dependence; 5) in good health as verified by medical history, screening examination, and screening laboratory tests; and 6) 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 or psychiatric disorders that the physician investigator deems as contraindicated for the subject to be in the study; 2) regular use of psychotropic medication (antidepressants, antipsychotics, or anxiolytics); 3) current alcohol or substance dependence for any other recreational or prescription drugs other than nicotine; 5) use of e-cigarettes more than 10 days in the past 30 days; 6) urine drug screening indicating recent illicit drugs use (with the exception of marijuana).

Justification for age criteria: The study will enroll young adults between the ages of 18 to 35 to minimize variation in the number of years of smoking that can influence measures of dependence including withdrawal severity (24, 25).

Justification to include smokeless tobacco and e-cigarette users: We will include users of smokeless tobacco or e-cigarettes given the high prevalence of e-cigarette and smokeless tobacco products among young adults (26-28). As noted above, urine cotinine will be used to biochemically confirm the subjects' nicotine consumption and smoking status and used as a covariate in all analyses if required.

Subject sampling to account for sex, race, dependence symptoms, and smoking frequency: We are planning to enroll Caucasian and African-American smokers while maintaining an approximately equal male-to-female ratio.

We will create dichotomous variables to be used as a covariate to match groups on race (European American and African American) and sex because of the potential influence of race and sex on smoking behavior and nicotine pharmacokinetics (22, 29). We will also control for differences in the frequency of smoking because it may influence the behavioral effects nicotine (30-32); a continuous variable based on the reported smoking frequency (cigs per day) will be used as a covariate in the analyses if required.

8: Recruitment:

Hundred subjects will be recruited. The goal is to have 70 completers, 35 menthol and 35 non-menthol cigarette smokers.

9. Research Plan:

A. Overview

Experimental Summary: Subjects will complete five separate experimental sessions on five separate days with each session beginning around 8 AM (**Fig 2**). Each session (1 to 15 days apart) will include one infusion condition that will be either saline or nicotine at four different infusion rates. The five infusion conditions will be assigned randomly to one of the five experimental sessions. All infusions will last 10 minutes and be controlled by two infusion pumps, a saline infusion pump and a nicotine infusion pump. The 0.24 mcg/kg/s condition will include a 1 minute infusion of nicotine followed by a 9 min infusion of saline; the 0.096 mcg/kg/s condition will include a 2.5 minute infusion followed by a 7.5 min infusion of saline; the 0.048 mcg/kg/s condition will include a 5 min infusion of nicotine followed by a 5 min infusion of saline; the 0.024 mcg/kg/s condition will include a 10 min infusion of nicotine and no infusion of saline; and the placebo condition will include a 10 min infusion of saline and no

infusion of nicotine. The most rapid nicotine infusion rate, 0.24 mcg/kg/s, has proven to be well tolerated in our prior studies (12, 18-20, 22). The total weight-adjusted nicotine dose will not exceed 1.2 mg. Subjects will complete baseline assessments after an indwelling catheter is inserted into an antecubital vein for safety and drug administration. Immediately prior to the start of the infusion (@ min 0) and at intervals following the start of the infusion, heart rate, blood pressure, DEQ and withdrawal assessments will be taken as shown in **Fig 2**. At the end of the session (min 120), subjects will complete the Drug versus Money Multiple-Choice Form ($MCP) (23).

Session procedures: Before the experimental session, subjects will be required to abstain from smoking for 10h, which will be verified by expired air CO levels ≤ 10 ppm (33, 34). Subjects will also be asked to refrain from consuming alcoholic beverages and drugs prior study participation, which will be verified by urine drug screening and breathalyzer before the sessions. Subjects that are non-compliant will be rescheduled or, if they are repeatedly non-compliant, discharged from the study. Subjects will be instructed to drink their typical amount of caffeinated beverages to minimize caffeine withdrawal, which could confound the study measures. Subjects will be instructed not to eat for 4 h before the sessions to minimize heartburn or nausea that can be associated with IV nicotine administration. A light meal will be provided at the end of each session.

Fig # 2 Schedule of Events: Experimental Session*

Time point	Measures and Events
Baseline	CO, urine and blood samples, HR/BP, M-NWSC, BQSU, SAFTEE, CPT
0 min	IV Infusion starts
1 min	HR/BP, DEQ, plasma nicotine
3 min	HR/BP, DEQ, plasma nicotine
5 min	HR/BP, DEQ, plasma nicotine
8 min	HR/BP, DEQ, plasma nicotine
10 min	IV Infusion ends, plasma nicotine
11 min	HR/BP, DEQ, BQSU, plasma nicotine
12 min	HR/BP, plasma nicotine
13 min	HR/BP, DEQ, plasma nicotine
15	HR/BP, DEQ, plasma nicotine
18	HR/BP, DEQ, plasma nicotine
20	HR/BP, DEQ, plasma nicotine
25	HR/BP, DEQ,
30 min	HR/BP, DEQ, BQSU, CPT, M-NWSC
40	HR/BP, DEQ, plasma nicotine
50	HR/BP, DEQ, plasma nicotine
60	HR/BP, DEQ, BQSU, M-NWSC CPT, plasma nicotine
90	HR/BP, DEQ, BQSU, M-NWSC CPT, plasma nicotine
120 min	End of session, HR/BP, DEQ, plasma nicotine, M-NWSC, BQSU, SAFTEE, CPT
180 min	Discharge

*Saline infusions will follow each nicotine infusion to maintain a 10 min total infusion time for each experimental session. Abbreviations: CO: Alveolar carbon monoxide; HR/BP: Heart rate/Blood pressure; M-NWSC: Minnesota Nicotine Withdrawal Symptom Checklist; BQSU:

Brief Questionnaire of Smoking Urges; CPT: Continuous Performance Test; DEQ: Drug Effects Questionnaire; SAFTEE: Systematic Assessment for Treatment Emergent Events.

B. Medical Monitoring And Safety

Subjects will be given a thorough physical examination as part of the post-consent screening procedures. For nicotine administration sessions, a physician or a nurse will present be and subjects will be attached to a cardiac monitor as well as a blood pressure and heart rate monitoring device. One IV catheter will be in place in each arm or only one if two IV's can't be placed. Subjects will be administered nicotine only if the systolic blood pressure is < 150 mmHg and heart rate is < 90 beats/minute. The procedures will terminate if blood pressure at any time is >170/110 mm Hg, the heart rate is >130 beats/min, or if they develop signs and symptoms of nicotine toxicity. Subjects will remain in the laboratory for at least an hour after the experimental infusion administration. These procedures have been developed as part of our IND application for IV nicotine.

C. Measures

C.1 Intake Measures

1) 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)

2) Center for Epidemiologic Studies Depression (CES-D) scale: The CES-D is a 20-item self-report measure of depressive symptoms (35). This scale will be used at intake to control for baseline differences in depressive symptoms.

3) The Holmes-Rahe Life Stress Inventory (HRLSI): This is a self-report measure of 43 common life events related with some degree of stress on an individual's life. The scale was developed by the psychologists T.J. Holmes and R. Rahe, who reported that serious physical disorders, such as myocardial infarction, peptic ulcer, infections, and a variety of psychiatric disorders were positively associated with the number of life stressors scale within a period of 1 year. This scale will be used at intake to control for baseline differences in life stress.

4) **PTSD Checklist for DSM-5 (PCL-5):** The PCL-5 is a 20-item self-report measure that assesses the 20 DSM-5 symptoms of PTSD(36). This scale will be used at intake to control for baseline differences in PTSD symptoms

C.2 Study outcome measures

1) Physiological:

1.1 Heart rate (HR) and blood pressure (BP) readings will be taken at intake during screening. During the sessions HR and BP will be monitored continuously for medical/safety reasons. The HR and BP responses will help us determine the effect of delivery rate on some of the acute cardiovascular health risks associated with nicotine (36). In our preliminary studies, the peak effect of nicotine versus saline has a large effect size. We will analyze the peak effect

(determined from assessments at min 0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20) in our primary analysis because with the peak effect we have the greatest statistical power (>0.8) to detect infusion rate differences. Additional responses will be included in secondary analyses.

2) Biochemical:

2.1 Cotinine levels: Urine cotinine levels will be determined at baseline with a semi-quantitative test strip.

2.2 Alveolar carbon monoxide: CO readings ≤ 10 ppm will be used to verify compliance with overnight smoking abstinence. In our prior work we have used plasma nicotine levels to confirm that smokers with CO ≤ 10 ppm were overnight abstinent with exceptional concordance (95% had plasma nicotine < 10 ng/ml; mean = 2.8 ng/ml (SD 3.1), N=201). The 10 ppm cutoff is higher than cutoffs recommend for distinguishing smokers from non-smokers (~5 ppm) but consistent, albeit slightly more conservative, than a suggested cutoff (12 ppm) for distinguishing 8 h abstinent smokers from non-abstinent smokers (25).

3) Drug reinforcement and subjective effects:

3.1 Drug versus Money Multiple-Choice Procedure ($MCP): This procedure was developed and validated by Roland Griffiths and colleagues as an efficient tool to assess drug reinforcement in humans, including nicotine reinforcement (23, 37-39). For each experimental session (5 total), subjects will have choices between forfeiting or receiving escalating sums of money on a scale of values between -$20.00 and $20.00 or re-receiving that trial's infusion. The monetary scale will include values at $0.25 increments between $2.00 and -$2.00 and values at $0.50 increments from $2.00 to $20.00 and from -$2.00-$20.00. A crossover point, the value at which the subject chooses money rather than infusion, will be determined for each session.

3.2 Subjective Drug Effects: 1) Drug Effects Questionnaire (DEQ): Smokers will rate on a 100 mm scale, from "not at all" to "extremely, 12 items that are related to nicotine's effect," The items are 1) feel the "drug strength," 2) feel "good" drug effects, 3) feel "bad" drug effects, 4) like the drug effects, 5) feel high, 6) feel stimulated, 7) feel anxious, 8) feel down, 9) want more. This instrument is for a rapid detection of nicotine's effects and is adapted from a VAS (40). The DEQ will be administered at minute 0 (pre-infusion) and at minute 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 30, 60, 120 post-infusion initiations. The timing of these measurements is based on our previous studies to optimally capture the physiological and subjective effects of nicotine. The peak effect of nicotine versus saline (e.g. Fig 1a, 1 minute post-infusion) has a large effect size. We will analyze the peak effect in our primary analysis because with the peak effect we have the greatest statistical power (>0.8) to detect infusion rate differences. Additional responses will be included in secondary analyses.

3.3**Cognitive Performance: CPT** (Continuous Performance Test): Cognitive performance will be assessed with the continuous performance test (CPT) from the ANAM battery (University of Oklahoma): This test was chosen because of its sensitivity to tobacco withdrawal (52, 53). CPT assesses sustained attention, concentration, and working memory. For CPT, the main outcome measures will be percent correct responses and reaction time (52).

4) Withdrawal syndrome symptoms:

4.1 Minnesota Nicotine Withdrawal Symptom Checklist (M-NWSC): Smokers will be asked to rate several nicotine withdrawal symptoms on a 100 mm scale, from "not at all" to

"extremely." The items are derived from the M-NWSC (41) and have been used in previous human studies (42), including our own (18-20, 43).

4.2 Brief Questionnaire on Smoking Urges (BQSU): This 10-item scale, originally developed by Tiffany and Drobes (44), is a reliable and sensitive assessment of craving and withdrawal symptoms (45, 46).

5) Measures of Dependence (Tobacco and other drugs) and Axis I psychiatric disorders:

5.1 DSM-5 Tobacco Use Disorder (TUD) criteria will be used to assess nicotine dependence (yes/no). The Fagerstrom Test of Nicotine Dependence (FTND) will be used as a second measure of dependence. The FTND assesses the degree of nicotine dependence on a 10 point scale (0-10) based on a self-report of symptoms related to heaviness of smoking, withdrawal and interference (47).

5.2 Tobacco and Other Drug Use: At intake self-reports of cigarette smoking, other tobacco products, drug and alcohol use (cocaine, alcohol, opioids, marijuana, and other illicit drugs) will be assessed with Time Line Follow-Back, which is a reliable and valid instrument for monitoring substance use (48-50).

5.3 Structured Clinical Interview for DSM-5 (SCID) for Axis I disorders, a semi-structured interview based on DSM criteria (51), will be performed to diagnose Axis I psychiatric disorders.

6) Adverse events

6.1 SAFTEE: In order to monitor adverse events from the study medications, the SAFTEE will be administered before and after each session. The SAFTEE, which has been used in a number of pharmacotherapy trials (52), and includes possible side effects of study medications.

7) Genetics

7. 1 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 that encode nicotinic receptors and enzyme that metabolize nicotine. 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 administration: Nicotine 1mg/mL solution for injection obtained from US Specialty Formulations LLC, Bethlehem, Pennsylvania will be prepared for IV infusion by the research pharmacy at the West Haven VA. Nicotine and saline will be administered by infusion pump via a single IV catheter located in a forearm vein. We have followed these procedures in our previous studies, which were completed without any adverse effects attributed to nicotine or other safety concerns. Each session will include one 10-minute infusion that will be controlled by a combination of two infusion pumps. One infusion pump will control nicotine delivery and one infusion pump will control saline delivery. The infusions pumps will be operated silently and be toggled on and off to reach the 10-min total infusion time for the given condition. Subjects will be blinded to the infusion pump procedures. For this project, an amendment to Dr. Sofuoglu's IND for IV nicotine will be submitted.

Justification for the nicotine dose and nicotine delivery rate: For this proposal, we selected one active dose of nicotine, 1.0 mg per 70 kg body weight, and placebo (saline). The 1.0 mg per 70 kg body weight nicotine dose approximates the total nicotine dose delivered to a smoker when they smoke a typical tobacco cigarette (53). Our previous studies of ND smokers indicates that 1.0 mg per 70 kg body weight dose delivered in 1 min (0.24 mcg/kg/s) induces robust drug liking subjective effects and suppresses withdrawal symptoms. However, this dose deliver rate is faster than what a smoker might encounter while smoking a cigarette. The moderate delivery rates, 0.096 and 0.048 mcg /kg/s, that approximates the rates of intake encountered while a smoker smokes a typical cigarette over 5 min. The slowest nicotine delivery rate, 0.024 mcg/kg/s, was chosen to approximate the nicotine intake rate that an individual might encounter from a newer E-cig device (2,5). The total weight-adjusted nicotine dose will not exceed 1.2 mg.

10. Data analysis methods

Hypothesis #1: To determine the dose-effect curves for positive subjective effects (drug liking and good drug effects) and alleviation of smoking urges, as a function of nicotine delivery rate in menthol and non-menthol cigarette preferring smokers.

Positive subjective effects of nicotine will be assessed with the "Like the Drug Effects" and "Good Drug Effects" items from the DEQ, consistent with the FDA Guidance on abuse potential assessment (10). These items have been shown to correlate with the abuse potential of drugs of abuse. Effects will be tested using linear mixed effects models with peak change on each DEQ item as a response variable, cigarette-preference (menthol vs. non-menthol) as a between-subject factor, nicotine delivery condition as a within-subject factor and their interaction. Period effects will be controlled for by including test day as an additional predictor in the models. Random effects for subject and condition within subject will be used to account for correlations between repeated measures. Because of the potential skewed distributions of the subjective effects data, transformations will be applied as necessary. We will evaluate the main and interactive effects of menthol cigarette preference and delivery rate. We anticipate statistically significant main effects of menthol-preference, nicotine delivery rate and a significant interaction between menthol-preference and nicotine delivery rate. To better understand the dose effects of nicotine delivery rate and whether they are moderated by menthol preference, we will construct specific contrasts among the four doses to test whether there is an indication of significant linear, quadratic, linear on log-scale or sigmoidal dose-response relationships across, or within menthol-preference groups. The possible dose-response curves identified by the specific mean contrasts will be compared by fitting linear and non-linear (as necessary) dose-response models with continuous dose and selecting the best-fitting model by the Akaike's Information Criterion (AIC).

To test for an effect of delivery rate on smoking urges, assessed by the BQSU (Factor 1 and 2) scores, we will use similar modeling approach described for subjective effects but we will also include time as a within-subject factor with outcomes for minute 0, 10, 30, 60 and 120. We anticipate a significant main effect of menthol-preference, nicotine delivery rate and potentially a significant interaction between menthol-preference and nicotine delivery rate.

Rationale for sample size:

The study will have 80% power to detect large effect sizes (f=0.4) for the main effect of menthol with 31 individuals per group at 0.025 significance level (Bonferroni-adjusted for two outcome measures) for each hypothesis). Our previous study showed large magnitude effects of preference for mentholated cigarettes on feeling good and stimulated effects (d=0.8 or larger, corresponding to f=0.4 or larger). Our pilot data also showed large effects of nicotine (f>0.6) and the rate of infusion effects are expected to be similarly strong (42, 48, 54, 83). With 28

individuals per group we have 80% power to detect effect sizes of this magnitude (f>0.5) for the within-subject effect of nicotine delivery rate and in the interaction between nicotine delivery rate and menthol. To account for 20% drop-out rate, we will recruit 35 participants for each group.

11. 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, hypersalivation, 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 a 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 less than 300 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.

4) Protection of Subjects: 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.

12. 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 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 or a nurse 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 < 90 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 > 130 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.

12.1 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.

12.2 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.

12.3 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., Alec Buchanan, M.D., and Brian Kiluk, 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.

13. 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 review 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.

14. 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.

15. 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.

16. Payment: Subjects will be paid cash; $30 for participating in the screening and $150 for each of the 5 lab sessions. In addition, to help offset transportation costs, subjects will be paid $20 for each of the 5 lab sessions. If the subject is asked to return in addition to these 5 visits for any reason, they will be paid $20 for travel. 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 be paid up to $880.00 if all parts of the study are completed. Payment may exceed $880.00 if the subject is asked to return in addition to the screening visit and the 5 lab sessions for any reason. The payment will increase by $20 for each additional visit.

Visits	Amount paid	Total
Screening Visit	$30.00	30.00
Lab session	$150 each lab session	Five lab sessions $750
Travel to each lab session	20.00 each lab session	Travel for 5 session $100
	Max total	$880.00
If subject is asked to return for additional testing or visit	$20 for travel	$20.00

17. Funding Source: An R03 DA043004-01 grant from NIDA (pending).

18. Duration: The entire study will take approximately 2 years to complete.

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