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2. Co-Investigators (including VA and WOC staff): While all research staff is trained to provide informed consent, those identified with an asterisk (*) will provide informed consent for this study.

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3. Title of the project

Menthol's Effects on Nicotine Reinforcement in Smokers

4. Purpose, hypothesis and key questions

Purpose: To determine if menthol administered by inhalation via electronic cigarettes (e-cigarettes) changes the reinforcing effects of pure nicotine administered intravenously in cigarette smokers who smoke mentholated or non-mentholated cigarettes. The reinforcing drug effects will be measured with the drug effects questionnaire (DEQ).

Hypothesis: Hypothesis #1A: Concurrent menthol and nicotine administration will lead to greater reinforcement than control flavor and nicotine, menthol and saline, or control flavor and saline conditions. Hypothesis #1B: Enhancement of nicotine reinforcement by menthol will be greater in menthol smokers than in non-menthol cigarette smokers.

5-6. Background and Significance:

a) Introduction

Menthol is found as an additive in over 90 percent of cigarettes, although at widely different concentrations. Cigarettes that are specifically marketed as menthol cigarettes are disproportionately preferred by adolescent smokers and African-Americans. Evidence from epidemiological studies suggests that menthol is a potential contributor to the development and maintenance of tobacco addiction. Given that nicotine, compared to other drugs of abuse, is a weak reinforcer, it is possible that additional reinforcers, combined with nicotine, may facilitate the development and maintenance of addiction. The potential reinforcing properties of menthol have not been examined with systematic studies in controlled settings. Most importantly, human studies examining the reinforcing effects of menthol in combination with nicotine are lacking. One roadblock in determining the contribution of menthol to cigarette reinforcement has been the 'brand loyalty' of smokers: those who smoke non-mentholated cigarettes do not like menthol cigarettes and vice versa. This gap in our knowledge base regarding the basic behavioral pharmacology of menthol makes it difficult to develop science-based policies for menthol as an additive to tobacco products. To overcome this problem, novel approaches are needed that will minimize the influence of the conditioned association between nicotine and menthol formed when smoking mentholated cigarettes, or while using mentholated chewing tobacco.

b) *Why Study Menthol?*

Menthol is a flavoring agent that is used in a wide range of products. It has also commonly been used as an additive in cigarettes since the 1920s. Menthol has a well-described cooling and soothing action in the airways that may reduce the harshness of tobacco smoke. These effects make menthol cigarettes particularly appealing among youth who are experimenting with cigarettes. In fact, among 12 to 17 year-olds, 44.7% smoke menthol cigarettes, compared to 30.1% of adults aged 26 or older (Rock *et al*, 2010). Among young smokers, menthol cigarette use is especially common in African-Americans (71.9%), compared to Hispanic (47%) or Non-Hispanic Caucasians (41%) (Rock *et al*, 2010). In several cross-sectional studies, menthol cigarette smoking has been found to be a risk factor for development of dependence (Collins and Moolchan, 2006; Hersey *et al*, 2006; Muscat *et al*, 2009; Wackowski and Delnevo, 2007). More recently in a prospective study in smokers 17 or younger, initiation of smoking with menthol cigarettes was associated with higher risk of progression to established smoking and higher levels of nicotine dependence (Nonnemaker *et al*, 2012). A recent report by the Tobacco Product Scientific Advisory Committee (TPSAC) concluded that there was evidence to support the relationship between menthol cigarettes and smoking uptake, especially in African-Americans (Benowitz and Samet, 2011). Collectively, these studies demonstrate an association of menthol cigarettes with smoking initiation, higher levels of dependence, and difficulty in quitting smoking. Thus, there is a clear need for further research on the role of menthol in multiple aspects of nicotine dependence in order to provide guidance for developing policies regarding menthol.

c) *Menthol's Biological Effects - Published Studies*

Although menthol use is ubiquitous, beyond its local sensory actions, surprisingly little is known about menthol's biological and behavioral effects. The well-known cooling effect of menthol (Green, 1992;1985; Eccles, 2003) is likely mediated by a subtype of the transient receptor potential channels (TRPM8) that are found in the airways (Bautista *et al*, 2007). The TRP channels act like microscopic thermometers that sense hot and cold, as well as different tastes and pressure. Menthol, however, also acts on the other TRP channels, as well as other neurotransmitter receptors including opioid, $GABA_{A,}$ and nicotinic acetylcholine receptors (nAChR) (Corvalan *et al*, 2009; Zhang *et al*, 2008). Behaviorally, menthol has locomotor activating effects that are likely mediated by brain dopamine activation (Umezu, 2009). Most relevant to nicotine addiction is whether menthol interacts with nicotine. Only one published study in humans has demonstrated a significant desensitizing effect of menthol on sensations evoked by nicotine on the tongue (Dessirier et al., 2001). A few studies point to an interaction between menthol and nicotine, both at the nAChR (Hans *et al*, 2012) and the TRP channels (Hans *et al*, 2012). In a recent study using patch clamp technique, menthol blocked the activation of alpha4beta2 nAChR, suggesting a direct interaction of menthol and nicotine at the nAChR level. At the behavioral level, nicotine antagonized menthol-induced increases in body temperature in rats (Ruskin *et al*, 2007, 2008). However, virtually no published studies have examined the reinforcing effects of menthol, alone or in combination, with nicotine.

d) *Menthol's Effects in Humans - Tobacco Company Documents*

Given the lack of published literature on the reinforcing effects of menthol, an intriguing question is what led the tobacco companies to add menthol in varying concentrations to more than 90% of the cigarettes sold in the U.S. In fact, tobacco companies have conducted key studies examining the

reinforcing effects of menthol as an additive in cigarettes (Yerger, 2011). These studies are mentioned in brief memos that are included in tobacco company documents. For example, a study conducted by Philip Morris systematically examined the 'impact' of cigarettes containing different amounts of nicotine (0.08, 0.41, and 0.91 mg/cig) and menthol (0.0, 0.41, 0.85, and 1.95 mg/cig) in a factorial design. The study concluded that menthol increased the 'impact' or liking of cigarettes that had low nicotine. In all cigarettes, medium amounts of menthol produced the highest liking scores (Carchman and Southwick, 1990). Studies conducted by Philip Morris also supported the effectiveness of menthol in enhancing the impact of low-nicotine or low-tar cigarettes, suggesting that menthol has reinforcing effects independent of nicotine. Together, these studies clearly demonstrated that the addition of menthol enhances the appeal and likability of cigarettes.

e) *Research Need for Understanding the Effects of Menthol in Smokers*

One key question that remains to be answered is whether menthol increases the reinforcing effects of nicotine. Pure nicotine, similar to other drugs of abuse, is reinforcing. It is self-administered by cigarette smokers as well as by rodents and primates (Corrigall, 1999; Donny *et al*, 1995; Henningfield *et al*, 1983; Le Foll *et al*, 2007; Sofuoglu *et al*, 2008). However, the primary reinforcing effects of nicotine are less robust compared to other drugs of abuse like cocaine, amphetamines, or opioids. Thus, in addition to nicotine, other stimuli associated with smoking, including the sight, taste, and smell of cigarettes, may also serve as conditioned reinforcers (Sorge *et al*, 2009). Nicotine provides dual-reinforcement. In addition to its primary reinforcing effects, nicotine also enhances the motivational effects of these conditioned reinforcers (Caggiula *et al*, 2009). Thus, if environmental stimuli are intrinsically reinforcing, nicotine self-administration may reflect both the primary reinforcing, and the reinforcement-enhancing, effects of nicotine (Paterson, 2009). Accordingly, if menthol has intrinsic reinforcing properties, then its combination with nicotine may enhance reinforcement. Alternatively, if menthol has no intrinsic reinforcing properties, its combination with nicotine should not enhance reinforcement. To test the validity of these alternatives, carefully controlled studies delivering nicotine and menthol are needed.

Although there are established human laboratory methods to determine drug reinforcement, the study of the relative reinforcing effects of menthol and nicotine present some unique challenges. First, direct comparison of the reinforcing effects of menthol and non-menthol cigarettes in smokers is confounded by the strong conditioning of smokers for either menthol or non-menthol cigarettes. This 'brand loyalty' makes blinding unfeasible in a direct comparison study with smokers. Second, while the local sensory effects of menthol are well recognized, little is known about the behavioral effects of menthol in humans, and how these effects change with menthol dose. Third, it is unclear if menthol is uniquely different than other flavors that have been used in tobacco products. This gap in our knowledge allows tobacco companies to persistently claim that menthol is just a flavor without any behavioral effects of its own (Heck, 2010).

7. Subjects:

Male and female smokers will be recruited from the New Haven area through newspaper advertisements, radio advertisements, and fliers. Interested subjects will have the study described over the telephone, and they will be asked to answer a brief tobacco use history and medical screening questionnaire. If subjects pass the telephone screening, they will be invited to come to the West Haven VA clinic for a screening evaluation. This screening evaluation will be for the menthol / nicotine reinforcement study as described in detail in this project description. The dose-finding study was conducted at the John B. Pierce Laboratory. The dose finding study concluded with the establishment of the high and low dose of menthol that will be used in this protocol "Menthol's Effects on Nicotine Reinforcement in Smokers." The screening of these subjects used the same inclusion and exclusion

criteria as described below.

Inclusion criteria: 1) Female and male smokers, aged 18 to 30 years; 2) history of smoking for the past 12 months, at least one cigarette per day; smoking status is verified with urinary cotinine levels above 10 ng/ml; 3) not seeking treatment for nicotine dependence at the time of study entry; 4) in good health as verified by medical history, screening examination, and screening laboratory tests; 5) 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 patient to be in the study; 2) regular use of psychotropic medication (antidepressants, antipsychotics, or anxiolytics); 3) psychiatric diagnosis and / or treatment for Axis I disorders including major depression, bipolar affective disorder, schizophrenia or panic disorder in the past month and 4) abuse of alcohol or any other recreational or prescription drugs in the past 30 days. 5) Any allergy to propylene glycol or menthol.

8. Privacy: Participation in research may involve a loss of privacy. To prevent this, research records will be kept as confidential as possible. Only a code number will identify the individual research records. The code number will not be based on any information that could be used to identify the subject (for example, social security number, initials, birth date, etc.) The master list linking names to code numbers will be kept separately from the research data. All research information will be kept in locked files at all times. Subject identity will not be revealed in any reports or publications resulting from this study. Only authorized research staff will have access to the information gathered in this study.

While filling out study forms, or during the psychiatric examination, some participants may feel uncomfortable disclosing personal thoughts and feelings. Careful efforts aimed at maintaining confidentiality have been effective in previous research, and only participants' code numbers will be recorded on the forms themselves to protect confidentiality.

9. Selection: Interested subjects will have the study briefly described over the telephone, and if interested, they 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 be invited to come to the clinic for a screening evaluation. The screening evaluation will include the following: a) obtaining informed consent; b) smoking history and assessment of nicotine dependence using the Fagerstrom Test of Nicotine Dependence (FTND) c) complete physical and psychiatric examination including the structured clinical interview (SCID) for DSM-IV; d) laboratory examination including complete blood count, liver and kidney function tests, and glucose; e) urine analysis, drug screen, and for women, urine pregnancy test and f) urinary cotinine and urine menthol glucuronide levels.

10. Recruitment: A total of 210 smokers will be recruited from the New Haven area by newspaper advertisements and fliers.

11. Research Plan

A. Overview

We are proposing a double-blind, placebo-controlled study that enrolls young adult smokers stratified for menthol preference. Menthol and control flavor will be delivered via an e-cigarette just prior to saline or IV nicotine infusion. This menthol / nicotine reinforcement study was preceded by a separate menthol dose-finding study that determined the low and high doses of menthol to be administered by the e-

cigarette. For this dose-finding study, subjects were screened at the West Haven site where the IV nicotine study will be performed, but the dose-finding study was conducted at the John Pierce Labs located on the Yale Campus located about 2 miles from the West Haven site. The screening procedure for the dose-finding study was described in a separate screening consent form. The dose-finding study did not administer any nicotine.

For this menthol / nicotine reinforcement study, the Test Sessions will be conducted after an Adaptation Session is completed where subjects will learn to use the e-cigarette for delivering the menthol or control (tobacco) flavor. A short e-cigarette questionnaire will also be administered during the Adaptation Session. Then, the nicotine Test Sessions will be performed following overnight abstinence from tobacco. Smokers will be assigned a random sequence of three different e-cigarette conditions (low menthol (0.5% or 5 mg/mL) plus tobacco flavor, high dose menthol (3.5% or 35 mg/mL) plus tobacco flavor or tobacco flavor alone for each of the three Test Sessions (a different menthol condition for each session). The concentration of tobacco flavor will be identical across all conditions. The amount of menthol in high dose condition is the same as those found in commercially available mentholated e-cigarette solutions (AmericaneLiquidStore™). The low dose menthol solution contains menthol that is minimally perceptible by smokers and is in the range of e-cigarette solutions targeting smokers with a preference for low menthol impact in the e-cigarette vapor. In each Test Session, smokers will receive a random order of one intravenous delivery of saline, and two intravenous deliveries of nicotine (3.6 mcg/kg and 7 mcg/kg or 0.25 mg/70 kg and 0.5 mg/70kg), just after the menthol or control flavor is delivered via the e-cigarette. The intravenous dose deliveries will be one hour apart to provide sufficient time for the nicotine and menthol effects to wear off. The nicotine infusion sessions will be at least 24 hours apart to minimize carryover nicotine effects. The main outcome measure will be subjective drug effects as measured with the Drug Effects Questionnaire (DEQ). Other outcomes include cardiovascular measures, cognitive performance, and self-report measures of nicotine withdrawal. Cardiovascular measures include heart rate, systolic and diastolic blood pressure. Cognitive performance will be assessed with the Stroop test, mathematical processing test (MPT), and continuous performance test (CPT). Nicotine withdrawal measures will be measured with the Nicotine Withdrawal Symptom Checklist (NWSC) and the Brief Questionnaire on Smoking Urges (BQSU*).*

B. Dose Finding Study

Psychophysical testing of menthol delivered by an e-cigarette established the menthol concentrations for the e-cigarette delivery system (see below). After recruitment and initial screening for this dose-finding study at VACHS (see VACHS consent form), young-adult smokers were referred to the testing site at The John B. Pierce Laboratory (immediately adjacent to the Yale Medical School campus) for two, 90 min sessions performed on separate days. Subjects signed a separate Yale consent form for all procedures conducted at the John B. Pierce Laboratory. Smokers were required to abstain from smoking on the day of testing and were given a breath CO test (MicroDirect CO Monitor) to insure compliance.

The first dose-finding session began with a brief training period: (1) to teach participants how to inhale e-cigarettes in the way they will be sampled in the Test Sessions with IV nicotine (see Experimental Sessions below); and (2) to familiarize them with the sensory (gLMS) and hedonic (LHS) rating tasks. The same type of e-cigarette was used during psychophysical testing as will be used in the Test Sessions, because this type of device allows for the customization of flavor and menthol concentrations. During the practice session, a tobacco flavored commercial product without menthol or nicotine were used to avoid interfering with subsequent measurements of menthol sensitivity. Once subjects mastered the delivery system, they received standard instructions in, and practice with, the rating scales (Lim et al., 2009; Green

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et al., 1993). Testing with menthol began using an ascending method of limits (AMOL) that included a presentation of 5 concentrations of menthol plus a no-menthol control, which was sampled first. The menthol used in this study was prepared and dispensed according to John B. Pierce Laboratory protocol. The menthol concentrations tested were chosen from pilot tests so as to encompass the level of menthol experienced in popular menthol-flavored e-cigarettes. The AMOL, in which the stimuli are presented in increasing intensity across trials, were used to reduce possible effects of sensitization and desensitization of menthol's sensory irritation (Cliff & Green, 1991). After a concentration was sampled, subjects made 4 separate subjective ratings: overall intensity of the menthol sensation, using the general version of the Labeled Magnitude Scale [gLMS; (Green et al., 1996;1993;Bartoshuk et al., 2002)]; degree of liking/disliking of the menthol sensation, using the Labeled Hedonic Scale [LHS;(Lim *et al.*, 2009)]; and separate ratings of the perceived intensities of cooling and irritation (burning, stinging), also using the gLMS. There was a 5-min pause between trials to allow the menthol sensation to dissipate. After sampling the 5 concentrations and the no menthol control, there was a 15-min rest period before the subject sampled a popular commercial e-cigarette and rated it in the same manner as the test samples. Subjects returned for a second session in which the measurements were repeated to provide replicate ratings of the test stimuli and the commercial product.

Table 1. Schedule of Events: Dose-Finding Session

Time	Measures and Events
0	CO measurement
5 min	E-cigarette training and practice (reminder in session 2)
15 min	Psychophysical scale instructions and practice (reminder in session 2)
45 min	Ascending method of limits trials (6) with sensation intensity and hedonic ratings
60 min	15 min break
75 min	Commercial e-cigarette sensation intensity and hedonic ratings
85 min	Discharge

Determination of the menthol doses: Once a subject completed the testing, a grand log-means of the intensity ratings were used to determine the menthol test concentration that best matched the overall intensity of the popular commercial product. This was determined to be the high dose of menthol. The low dose was determined as the menthol concentration at which subjects could discriminate effects vs. the no menthol control condition, but at which clear differences in ratings were observed as compared to a

clustering of responses (response plateau) at higher menthol concentrations. In addition to these studies, Dr. Peter Jatlow's laboratory analyzed the concentrations of menthol in the commercially available mentholated e-cigarette solution (about 35 mg/cc). Based on these findings, we chose two menthol doses that will be used in our study. While the high dose will have the same concentrations of menthol in mentholated e-cigarette solution (AmericaneLiquidStore™), the low dose will be minimally recognizable by participants as menthol flavor (about 5 mg/cc).

Potential additional learning from sensory testing: In addition to establishing menthol concentrations for clinical testing, the psychophysical data will also enable preliminary evaluation of potential sex and/or race differences in sensory and hedonic responses to menthol. The discovery of such differences would yield novel data on how inhaled menthol is perceived differently between these groups, and how these differences might contribute to variations in the enhancing effects of menthol on nicotine reinforcement.

C. Medical Monitoring and Safety

Subjects will be given a thorough physical examination prior to entry in the study. A physician will be present at all test sessions. Subjects will be attached to blood pressure and heart rate monitoring devices throughout each test session. 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 <100 beats/minute. The subject's participation will be terminated if their blood pressure at any time is >170/110 mm Hg, the heart rate is >120 beats/min, or if the subjects develop signs and symptoms compatible with nicotine toxicity. Subjects will be monitored for two hours after the last nicotine administration.

D. Measures

a) Physiological:

- Heart rate and blood pressure: Heart rate and blood pressure readings will be taken at screening, and during each test session to measure nicotine's effect on these parameters

b) Biochemical:

- Serum estradiol and progesterone analysis (females only): Serum estradiol and progesterone levels will be measured before each test session, and will be used as covariates in our analysis, since female sex hormones may contribute to sex differences in nicotine responses (Lynch *et al*, 2010).

- Plasma cotinine and 3-hyroxycotinine (3HC): Plasma cotinine and 3-hydroxycotinine levels will be measured from blood obtained during the three Test Sessions to to determine the nicotine metabolite ratio (NMR). The NMR is the ratio of 3HC, the main metabolite of cotinine, to cotinine and reflects the activity of cytochrome P450 (CYP) 2A6 and thus, the rate of nicotine clearance (Dempsey *et al*, 2004). NMR has been shown to be stable in smokers during ad-lib and reduced smoking (Mooney *et al*, 2008). Plasma samples for 3HC will be obtained at baseline, and the NMR will be included as a covariate in our analyses.

Plasma nicotine: Plasma nicotine levels will be measured from blood that was obtained prior to the first experimental vaping session on each test day to characterize the subject's smoking status.

- Urinary cotinine levels: A semi-quantitative rapid urine level of cotinine will be obtained at screening to confirm smoking status at study entry.

- Urine menthol glucuronide levels: Urine samples will be obtained at intake to confirm the menthol preference of smokers (Benowitz *et al*, 2010). Additional urine samples will be collected throughout each test session to measure the amount of menthol exposure (via urinary menthol glucuronide levels) that was provided by the e-cigarette.

- Plasma menthol glucuronide levels: Blood samples will be obtained throughout each test session to determine the amount of menthol delivered by the e-cigarette. Blood samples will be obtained prior to each of the three experimental vaping sessions, and at 2 time points after each nicotine or saline infusion. An additional blood sample will be obtained just prior to removing the IV. Plasma levels of menthol glucuronide will be measured at different time points to generate concentration-time curves for each menthol delivery. This will help characterize the pharmacokinetics of menthol and account for differing menthol exposure between subjects.

- Alveolar carbon monoxide: CO readings ≤ 10ppm will be used to verify overnight abstinence from smoking as recommended by the SRNT Subcommittee on Biochemical Verification (Benowitz *et al*, 2002).

c) Subjective:

- Measures of Dependence:
- 1) DSM-IV Nicotine Dependence Questionnaire.
- 2) Structured Clinical Interview for DSM-IV (SCID) for Axis I disorders: SCID is a semi-structured interview based on DSM-IV (American Psychiatric Association, 1994) and will be performed to diagnose Axis I psychiatric disorders.
- 3) 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).
- Measures of withdrawal and negative affect:
- 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 (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, originally developed by Tiffany and Drobes (Tiffany and Drobes, 1991), 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) 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.
- 5) E-cigarette Questionnaire: This 10 item self-report questionnaire was developed by our research group to characterize the study populations' experience with, and attitude about, e-cigarettes. This questionnaire will be administered during the Adaptation Session.

- Subjective Drug Effects: 1) Drug Effects Questionnaire (DEQ), adapted from Soria (1996), will be used during the test session to measure acute drug responses including: liking, cooling, irritation, strength, feeling, stimulation, good effects, bad effects, head rush, and want more. Each item is rated on a 100 mm scale, from 'not at all' to 'extremely'.

- Drug Use: Self-reports of cigarette smoking and use of other tobacco products, alcohol and drugs (cocaine, alcohol, opioids, marijuana, and other illicit drugs) will be assessed with Time Line Follow-Back, which has been shown to be a reliable and valid method for monitoring substance use and other outcomes in longitudinal studies (Miller and DelBoca, 1994; Sobell and Sobell, 1992). The Time Line Follow-Back will be administered at intake.
- Adverse events: SAFTEE: In order to monitor adverse events from the study medications, the SAFTEE will be administered before and after each session. The SAFTEE has been used in a number of pharmacotherapy trials (Levine and Schooler, 1986).
- Cognitive Performance: Cognitive performance will be assessed with 3 tests from the ANAM battery (University of Oklahoma): the Stroop test, the mathematical processing test (MPT), and the continuous performance test (CPT). These 3 tests were chosen because of their sensitivity to tobacco withdrawal and nicotine administration (Heishman *et al*, 2010; Mancuso *et al*, 1999; Myers *et al*, 2008).
  - Stroop Test: This test assesses processing speed, selective attention, and interference (Spreen, 1991). It is a computerized version of the classical Stroop Test (Reeves, 2002). The Stroop design consists of one three-minute block of congruent stimuli, and one three-minute block of incongruent stimuli.
  - Mathematical Processing Test (MPT): This test assesses basic computational skills, concentration, and working memory. An arithmetic problem involving three single-digit numbers and two operators is displayed (e.g., "5 - 2 + 3 ="). The subject presses buttons to indicate whether the answer to the problem is less than five or greater than five.
  - Continuous Performance Test (CPT): This test assesses sustained attention, concentration, and working memory. A target character is displayed for memorization. Then, as individual characters are displayed in sequence, the user presses a designated button only when the displayed character is the target letter.

d) Other measures: We recognize the value of banking biological samples for future genetic, epigenetic and biomarker analyses given the care taken to establish clinical phenotypes. For example, results from genetic studies may confirm the importance of certain TRP-ion channels, or sweet taste receptors, in the effects of menthol and sweeteners on initiation and maintenance of nicotine addiction. These findings would provide a rationale for examining established and novel

genetic variants of TRP-channels, and sweet taste receptors, as determinants of responses to flavors in the laboratory. Whenever specific hypotheses are generated in the future that require targeted genetic analysis, this protocol will be amended to describe the purpose and use of such analyses.

E. Drugs

a) Nicotine: Nicotine bitartrate will be obtained from Interchem Corporation, Paramus, NJ. Nicotine samples will be prepared by the research pharmacy at the West Haven VA. Common side effects of nicotine include nausea, vomiting, heartburn, elevated heart rate and increased blood pressure.

- IV Nicotine Administration: Nicotine will be administered over 30 seconds using a syringe that is attached to an IV catheter located in a forearm vein. We have followed these procedures in our previous studies (Sofuoglu et al. 2005, 2006b; 2008a, 2009a,b,c) which were completed without any serious and unexpected adverse effects attributed to nicotine, or with other safety concerns. After the last dose, the subjects will stay in the lab for at least three hours, which has been shown to be sufficient for the nicotine-induced changes in heart rate, blood pressure, and subjective effects to return to baseline (Sofuoglu *et al*, 2005; Sofuoglu *et al*, 2006).
- Justification for the nicotine doses: For this proposal, we chose two dose sequences of nicotine (3.6 mcg/kg and 7 mcg/kg or 0.25mg/70 kg and 0.5mg/70kg) with a maximum dose of 0.3 mg and 0.6 mg, respectively, regardless of body weight. In a previous study, these nicotine doses produced robust subjective and physiological responses (Sofuoglu *et al*, 2009a). However, the doses used in this study are much lower than doses used in previous studies with addicted smokers (up to 3 mg per injection). Therefore, we believe that the doses used in this study represent an appropriate balance between being sufficiently high to answer the research questions, but low enough to reduce risks to the study participants.

b) Menthol: USP grade natural menthol flavor (Flavor Savor™) will be dissolved in the AmericaneLiquidStore™ menthol e-cigarette solutions.. Menthol is widely used in a range of healthcare products and is classified by the FDA as compound that is Generally Recognized as Safe (GRAS). Menthol has mild anesthetic, antiseptic and counter irritant properties and is safe for oral ingestion, topical administration and inhalation.

- Menthol Administration: Participants will be instructed to take 6 inhalations, one inhalation every 15 seconds, for 90 seconds just prior to each nicotine / saline infusion (Vansickel *et al*, 2012). As described more fully below in the section Adaptation Session, subjects will be asked to use light, long inhalations of 3-4 seconds with vapor being pulled into the mouth initially then into the lungs at the end of the inhalation.

  The menthol / e-liquid base mixture used in the e-cigarette will be prepared just prior to each Test Session by the VA Research Pharmacy. Each e-cigarette cartridge will contain 2mL of the e-liquid. The cartridge will then be loaded into the e-cigarette device by the study physician.

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a. Justification for menthol doses: For this proposal, the concentrations of the high and low dose menthol solutions were determined in the dose-finding study. These concentrations were found to be different in overall sensory impact from the control flavor and from each other. The target concentrations for this study are 3.5% (35 mg/mL) and 0.5% (5 mg/mL). Human toxicity only occurs at concentrations well above those to be used in this study. The concentrations of menthol in the stock solutions procured from the AmericaneLiquidStore™ will be verified using an assay developed by Dr. Peter Jatlow.

F. Electronic Cigarette

- Hardware: The Joyetech eGo-C™ e-cigarette will be used since it appears to be among the most widely used e-cigarette type and allows for flexibility in the type of e-liquid administered.
- Ingredients: E-liquid will be obtained from AmericaneLiquidStore™, which prepares all its products in Wauwatosa, Wisconsin. They claim to be the first e-liquid manufacturer in the United States to obtain International Organization for Standardization (ISO) 9001:2008 and Current Good Manufacturing practices (cGMP) certification.
  The components of the e-liquid - propylene glycol (PG) USP, vegetable glycerin (VG) USP, and tobacco flavor – are premixed as commercially available solutions for e-cigarettes. The e-liquid will consist of the concentrated flavor liquid (menthol and tobacco flavor) added to a base liquid. The e-liquid base will itself consist of a mix of 70% PG and 30% VG. While e-liquids on the market range in their relative proportions of PG and VG, this 70/30 ratio is widely used. PG is the original base liquid for which most e-cigarettes were designed and remains the most common ingredient in e-liquid bases. PG is also credited with producing a throat sensation ('throat hit') that mimics the feel of smoking a cigarette. VG is commonly included in e-liquid bases to enhance the volume of vapor production, giving a greater sensory illusion of smoking.
  The menthol-flavor liquid was added at different concentrations to produce the low and high dose menthol conditions. As the control flavor, we chose 'Burley' tobacco flavor from the AmericaneLiquidStore™, rather than other flavors (e.g., cherry or chocolate), because the Burley tobacco flavor mimics the taste and smell of tobacco products to which smokers are accustomed. The concentration of tobacco flavor and the ratio of PG/VG will be the same across all three e-liquid conditions while the concentration of menthol will be different for the low and high dose menthol condition. The non-menthol condition will consist only of tobacco flavor concentrate plus PG/VG base liquid.

G. Genotyping

DNA Extraction and Genotyping

DNA will be extracted from peripheral blood using a commercial kit (Paxgene™; PreAnalytiX Qiagen/BD, Germany). We will genotype variations that may modulate nicotine responses using a 384-well plate format. Briefly, DNA samples will be genotyped in 2 µl-reactions. Each reaction will be prepared with 1 ng genomic DNA, 0.05 µl of 20× (or 0.025 µl of 40×) minor groove binder (MGB) probes and primers (hCV8950074 and hCV26000428) (supplied as pre-validated SNP Assays on Demand, Applied Biosystems) 1 µl of 2× TaqMan Universal PCR Master Mix, and 0.004 µl of 100× bovine serum albumin (New England Biolabs). Reactions will be genotyped for an initial denaturation at 95°C for 10 min followed by 60 cycles of 92°C for 15 s and 60°C for 1 min. Discrimination will be performed using ABI PRISM 7900 Sequence Detection System (Applied Biosystems). All samples will be genotyped in duplicate. We will also examine messenger RNA (mRNA) levels extracted from the peripheral blood. We are especially interested to examine the changes in mRNA levels related to opioid and related genes. About 2.5 ml of blood will be drawn into the PAXgene tubes at baseline, prior to each IV infusion, and end session. Extracted mRNA samples will be stored at -80°C in the Genetics Laboratory.

Study Procedures

For 24 hours prior to each Test Session, subjects will be asked to avoid using products containing menthol such as mint teas, cough lozenges, and mint-flavored gum. If needed, a trial-size tube of toothpaste that does not contain menthol will be provided for use the morning of each Test Session.Subjects will be required to abstain from smoking for 10 hours. Abstinence will be verified by expired air CO levels ≤10 ppm (Benowitz et al. 2002) measured on the morning of testing. Subjects will also be asked to refrain from consuming alcoholic beverages and drugs during study participation. Abstinence will be verified by urine drug screening and Breathalyzer measurements before the test sessions. If results indicate non-compliance with these study procedures, the session will be rescheduled. Repeatedly non-compliant 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 interpretation of study measures. Subjects will be given a light breakfast before each test session and a lunch will be provided at the end of each test session.

a) Adaptation Session: This session will take place at the West Haven VA prior to the nicotine Test Sessions. The goal of the Adaptation Session is to familiarize participants with the study procedures including the use of e-cigarettes, and to gather information regarding participants' use and knowledge about e-cigarettes. Participants will be given an e-cigarette and instructed to inhale more softly, but for a longer duration (3-4 seconds), than is customary for a standard cigarette. Additionally, a two-phase inhalation is suggested where the vapor from this elongated inhalation is initially gathered in the mouth (as one might do with pipe tobacco) but then further inhaled into the lungs. The e-cigarette used in this Adaptation Session will not contain nicotine, or menthol, only the control flavor (tobacco) mixed with the base liquid. Although the familiarization with this technique will occur during the Adaptation Session, participants will be reminded of this technique during each test session prior to the e-cigarette administration. In total, the e-cigarette vaping procedure consists of 6 inhalations at a rate of one inhalation every 15 seconds.

b) Test Sessions: Experimental sessions will start around 8:30 AM. As summarized in Table 2, baseline biochemical measures will include urine menthol glucuronide and cotinine, plasma nicotine, and for women, estradiol and progesterone levels. These samples will be obtained followed by cognitive testing. Subjects will then start inhaling from the e-cigarette with the assigned menthol condition (control flavor, low or high dose of menthol), one inhalation every 15 seconds as practiced in the adaptation session. It will take approximately 90 seconds to complete 6 inhalations. Subjects will then receive the first delivery of the assigned injection (saline, 0.25 mg/70 kg or 0.5 mg/70 kg nicotine). Each study injection will be 60 minutes apart to provide sufficient time for the physiological and subjective nicotine effects to return to baseline. Following each injection, physiological, subjective, and cognitive outcome measures will be obtained.

c) Medical monitoring: For nicotine test sessions, a physician will be present and subjects will be attached to 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 three hours after the last nicotine administration. These procedures have been developed as part of our IND application for IV nicotine.

Table 2. Schedule of Events: Experimental Session*

Time	Measures and Events
8:00 AM	(Building 36) CO level, urine sample, BAL, and light standard breakfast
9:00 AM	(Biostudies Unit) blood sample. HR/BP, EKG, M-NWSC, BQSU, SAFTEE, Cognitive Testing
11:00 AM	Menthol (control, high or low Menthol) DEQ + IV Saline or Nicotine (0.25 or 0.5mg/70 kg) blood draw
11:01	HR/BP, DEQ
11:02	HR/BP
11:03	HR/BP, DEQ,
11:05	HR/BP, DEQ
11:08	HR/BP, DEQ blood draw
11:10	HR/BP, DEQ
11:15	HR/BP, DEQ, Cognitive Testing
11:30	HR/BP, DEQ blood draw
11:45	HR/BP, DEQ
11:55	HR/BP, DEQ, BQSU
12:00	Menthol (control, high or low Menthol) DEQ + IV Saline or Nicotine (0.25 or 0.5 mg/70 kg), blood draw
13:00	Menthol (control, high or low Menthol) DEQ + IV Saline or Nicotine (0.25 or 0.5 mg/70 kg), blood draw
14:00 noon	HR/BP, EKG, M-NWSC, BQSU, SAFTEE

15:00 PM	End of session, collection of urine sample, blood draw Discharge

*The same measures 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; M-NWSC: Minnesota Nicotine Withdrawal Symptom Checklist; BQSU: Brief Questionnaire of Smoking Urges; DEQ: Drug Effects Questionnaire; SAFTEE: Systematic Assessment for Treatment Emergent Events.

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.

Specific Aim #1: To determine if menthol administered via e-cigarettes will change the reinforcing effects of pure nicotine administered intravenously in cigarette smokers who smoke mentholated or non-mentholated cigarettes. The reinforcing drug effects will be measured with the DEQ.

Hypothesis #1A: Concurrent menthol and nicotine administration will lead to greater reinforcement than nicotine and control flavor, saline and menthol, or saline and control flavor conditions.

Hypothesis #1B: Enhancement of nicotine reinforcement by menthol will be greater in menthol smokers than in non-menthol cigarette smokers.

The main outcome measures will be the "good effects" and "drug liking" items of the DEQ. Mixed effects models will include the within-subject factors nicotine dose (0.25, 0.5 mg nicotine or placebo), menthol dose (low dose of menthol, high dose of menthol, or control flavor) and time of measurement (+1,+3,+5,+10,+15,+30,+45,+55 minutes); the between-subject factor types of cigarette preference (menthol vs. non-menthol) and all possible interactions among these factors. We will also consider order effects for nicotine dose (1, 2 or 3) and session day (1, 2 or 3) and the effects of potential covariates (race, sex, baseline plasma NMR, FTND scores). These effects will be included in the final model if significant. Estradiol and progesterone levels will also be considered as potential covariates in a secondary analysis of women only. We will consider several different correlation structures for the repeated measures within individuals, and will select the best fitting one based on Schwartz Bayesian information criterion. SAS PROC MIXED will be used for the mixed-model analyses.

A significant interaction between nicotine dose and menthol dose, with significantly higher response to menthol + nicotine compared to control flavor + nicotine or control flavor + saline will be considered supportive of hypothesis 1A. A significant interaction between nicotine dose, menthol dose, and group (menthol vs. non-menthol smokers), with significantly stronger effects in menthol smokers than in non-menthol smokers, will be considered supportive of hypothesis 1B. We expect significant effects for both doses and will also test for dose-dependent effects.

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Rationale for sample size: Both studies have 80% power to detect large effect sizes (f=0.4) for the interactions of interest in repeated measures analysis with 30 subjects per group for a total of 60 subjects at 0.05 significance level. Our preliminary data for both outcome measures show larger magnitude effects for the nicotine effects (all f>0.5) and menthol effects are expected to be similarly strong (Sofuoglu et al. 2009a). Applying Bonferroni correction and using an alpha level of 0.01 we have 80% power to detect post-hoc comparisons of medium to large effect size d'=0.7 for the menthol and nicotine effects. Our previous study shows larger effects of nicotine d'>1.0. To account for up to 70% dropout we will recruit 160 subjects for a target of 60 completers.

13. Risks and benefits

Potential risks:

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

1) Common side effects of nicotine include nausea, vomiting, heartburn, and elevated heart rate and blood pressure. Toxic doses of nicotine may cause 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 40 to 50 times that which will be used in our study. Other potential risks from this study include administering a drug that has addictive potential.

2) Menthol administration produces typical sensory effects in the mouth and throat. The doses of menthol that are found in cough drops, ranging 1 to 10 mg are regarded to be safe (Sweetman, 2011). In spite of ubiquitous use of menthol in a wide range of products, only few cases of menthol poisoning has been described in the literature following very high doses of menthol ingestion, 200 mg or more. Menthol poisoning reported to cause ataxia, confusion, coma, nausea, and vomiting. However, these toxic effects occur at doses 20 to 30 times that which will be used in our study.

3) The combination of intravenous nicotine and inhaled menthol has not been previously examined to the best of our knowledge. We have no reason to believe there is an increased risk from the combination used in this study as compared to the widespread use of mentholated cigarettes, menthol e-cigarettes or smoking while using a menthol-containing lozenge. However, the administrations will be closely monitored for any signs that this unique combination of IV nicotine and inhaled menthol results in unexpected findings.

4) Propylene glycol and vegetable glycerin are categorized by the FDA as compounds that are Generally Recognized As Safe (GRAS). They are both commonly used ingredients in food products, pharmaceuticals and in personal care products. They are also now used as the base liquid ingredient for e-cigarettes. Although e-cigarettes are reported to be helpful for smoking cessation, the FDA has not approved e-cigarettes for this use, and the long-term consequences of e-cigarette use have not been assessed.

5) Blood Drawing: Subjects will have approximately 260 mL of blood drawn as a result of their participation in the study. Blood drawing can cause some pain and may result in bruising.

6) Study procedures: On the test days, subjects will not be able to smoke for more than 10 hours. During this cigarette abstinence period, subjects may experience symptoms of nicotine withdrawal such as craving for cigarettes, anxiety, restlessness, irritability, difficulty concentrating, loss of energy, and excessive hunger. In addition, smokers will be exposed to e-cigarettes and e-cigarette mixtures.

7) Loss of confidentiality: It is possible that participation in the study may make others, including friends or family members, be aware of the participant's tobacco use status. The potential participants will be told that if they do not feel comfortable with this then they should not participate in the project. Participants will also be told that if they report any information to us about abuse or homicidal/suicidal behavior we will be required to report this information to the appropriate authorities. There is also a potential risk of loss of confidentiality due to data sharing.

Protection of Subjects:

Before initiating any research activity, each subject must give informed consent that will detail the risks of study participation. They will also be advised of the data sharing plans for the study. Eligibility will be determined by the medical and psychiatric history, drug use history, the physical examination, and the laboratory studies done prior to beginning this research protocol. This study will enroll young adults between the ages of 18 to 30 because of the high prevalence of use of mentholated tobacco products in this age group (Rock *et al*, 2010). To minimize the risks associated with administering a drug that has addictive potential, we will only enroll those individuals with a consistent history of cigarette smoking as verified by a careful history, questionnaires, and urinary cotinine levels. Additionally, we will not enroll treatment-seeking smokers.

As mentioned previously, the most common expected adverse events related to IV nicotine administration are nausea, vomiting, heartburn, and elevated heart rate and blood pressure. To prevent nausea, subjects will be asked not to eat before the sessions. In addition, subjects will sit in a comfortable reclining chair during the test sessions that minimizes heartburn. Subjects will also be carefully screened for the presence of high blood pressure or other cardiovascular disorders to minimize cardiovascular adverse events.

During the test sessions, a physician will closely monitor all subjects. 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. Stopping rules are in place as described above (Study Procedures - Test Session).

Over the last 6 years, we have administered nicotine intravenously to more than 400 smokers and have not encountered any adverse events attributed to nicotine administration. The most common adverse events, or reasons for discharges, in these studies include difficulty finding venous access, high blood pressure before nicotine administration, the use of drugs of abuse or alcohol during study participation,

and adverse events attributed to other study medications. Similar to our experience, in a genetic study, Gary Swan et al. administered IV nicotine to 278 smokers and non-smokers via slow infusion (Cancer Epidemiol Biomarkers Prev 2007;16(6):1057–64). This procedure was also well tolerated in both smokers and non-smokers, further supporting the safety and tolerability of IV nicotine administration.

The close monitoring of subjects during the sessions will also help to detect any adverse events from menthol or e-cigarette administration.

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. The potential risk of loss of confidentiality due to data sharing will be minimized by creating de-identified data sets that exclude direct and indirect identifiers. In addition, researchers requesting data will be required to enter into a data sharing agreement.

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.

The risk associated with participating in this study is moderate, because the 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 the Center's 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. The Yale TCORS Independent Data Safety Monitoring Board includes experts in the field of tobacco use behaviors and challenge studies (Chair: Dr. Tony George, FRCPC, Professor and Co-Director, Division of Brain and Therapeutics, Dept. of Psychiatry, U of Toronto; Dr. Thomas Brandon, Professor and Chair, Department of Health Outcomes & Behavior, H. Lee Moffitt Cancer Center & Research Institute) and in statistics (Dr. Hanga Galfalvy, Assistant Professor of Neurobiology, Columbia University). 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.

We will report recruitment, follow-up, and adverse events to this panel on a bi-annual basis with serious adverse events reported within 48 hours. Prior to study initiation, critical parameters for collection of side effects and for study discontinuation will be recommended to the DSMB who may use these or other measures to monitor safety of the ongoing trial. The DSMB will be available to convene outside of scheduled meetings, if necessary, due to concerns regarding a particular subject or due to any troublesome developments in subjects' experiences during the study. The DSMB will make appropriate recommendations for changes in the study protocol, if needed.

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 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, Yale IRB, the DSMB, and NIH. We will directly report to the FDA, whenever their magnitude or frequency exceeds expectations.

15. Informed consent: Recruitment will be by word-of-mouth, referrals from area programs and by advertisement. A research staff member will interview individuals that are interested in participating in the study over the phone. If subjects pass the initial screening for the study, they will then come into the clinic for a full screening evaluation. Upon arrival, 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.

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16: Confidentiality: Only authorized research personnel will have access to the information gathered in this study. Patient names or other identifiers such as social security number, initials, and birthdate will not be used to identify paper or electronic records. Only a code number will identify the individual research records. The code number will not be based on any information that could be used to identify the subjects (for example, social security number, initials, birthdate, etc.). The master list linking names to code numbers will be kept separately from the research data and will be stored in an electronic file behind the VA firewall. All research information obtained will be kept in locked cabinets located on VA property or in electronic files behind the VA firewall. The names of the subjects will be used in VA electronic records, CPRS.

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 $30.00 for attending the screening visit and $30 for the Adaptation Session. Subjects will be paid $200 for each of the three Test Sessions. In addition, subjects will be paid $20 to cover transportation costs for the Adaptation Session and for each Test Session. Completing all components of the study will result in a maximum total payment of $740. In addition, 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 be given for transportation if a subject needs to be re-contacted. We will not share any information with the subject who makes the referral about the ultimate enrollment status or study outcome of the referred individuals.

19. Funding Source: Provided by an NIH Center Grant.

20. Duration: The entire study will take approximately two and a half years to complete.

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