Published in final edited form as:

J Psychopharmacol. 2018 September ; 32(9): 986–994. doi:10.1177/0269881118773972.

Acute effects of inhaled menthol on the rewarding effects of intravenous nicotine in smokers

Gerald W Valentine^{1,2}, Elise E DeVito^{1,2}, Peter I Jatlow^{1,3}, Ralitza Gueorguieva⁴, and Mehmet Sofuoglu^{1,2}

¹Department of Psychiatry, Yale School of Medicine, New Haven, CT, USA

²VA Connecticut Healthcare System, West Haven, CT, USA

³Department of Laboratory Medicine, Yale School of Medicine, New Haven, CT USA

⁴Department of Biostatistics, Yale School of Public Health, New Haven, CT, USA

Abstract

Objective: This double-blind, placebo controlled study examined whether menthol inhaled from an electronic cigarette (e-cigarette) would change subjective and withdrawal alleviating effects of intravenous nicotine in young adult smokers.

Methods: A total of 32 menthol-preferring smokers and 25 non-menthol-preferring smokers participated in the study that consisted of a random sequence of 3 different inhaled menthol conditions (0.0%, 0.5% and 3.2%) across 3 test sessions (a single menthol condition per session). In each test session, a random order of saline, and 2 nicotine infusions of 0.25 mg and 0.5 mg / 70 kg of bodyweight were administered, one hour apart, concurrent with menthol inhalation.

Results: While menthol did not alter the positive subjective effects of nicotine, menthol significantly enhanced aversive effects of nicotine in non-menthol-preferring smokers and reduced smoking urges in menthol-preferring smokers. In addition, menthol-preferring smokers reported blunted positive subjective responses to nicotine and less severe nicotine withdrawal after overnight nicotine deprivation. Finally, compared to non-menthol-preferring smokers, menthol-preferring smokers had a significantly lower baseline nicotine metabolite ratio indicating slower nicotine metabolism within our sample of menthol-preferring smokers.

Conclusions: Our findings did not support an enhancement of nicotine's positive subjective effects from inhaled menthol. However, as compared to non-menthol-preferring smokers, menthol-preferring smokers had blunted positive subjective responses to nicotine and reduced overnight withdrawal severity that may be partly due to inhibition of nicotine metabolism from chronic exposure to inhaled menthol. Collectively, these results reveal a more complex and nuanced role of inhaled menthol in smokers than previously recognized.

Correspondence to: Gerald Valentine, Yale University School of Medicine, Department of Psychiatry, 300 George Street, Suite 901, New Haven, CT, 06510, USA, gerald.valentine@yale.edu.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

INTRODUCTION

Menthol has been an enduring additive in cigarettes since the 1920s and is present at variable levels in nearly all combustible cigarettes, including varieties marketed as non-menthol (Ai et al., 2016; Gordon et al., 2011). Accumulating evidence indicates that menthol is not merely a ‘flavor’ in tobacco products, but a bioactive compound with diverse effects including inhibition of nicotine metabolism (Benowitz et al., 2004), activation of nociceptive and thermal receptors (Farco and Grundmann, 2013), and allosteric modulation of nicotine acetylcholine receptors (nAChRs) (Ashoor et al., 2013; Hans et al., 2012; Ton et al., 2015). In addition, menthol can impact behavior through its action as conditioned sensory (Rose and Behm, 2004) and interoceptive cues (Harrison et al., 2017) and when co-administered with nicotine, menthol can alter operant behavior consistent with enhanced nicotine reinforcement (Biswas et al., 2016; Hans et al., 2012; Wang et al., 2014). Collectively, these various actions of menthol likely influence the initiation, maintenance and cessation of tobacco product use, but an integrated understanding of menthol’s role in tobacco use has not yet been established.

The potential reinforcing effects of menthol are especially relevant for tobacco addiction, given that nicotine, the main addictive component of tobacco, is a relatively weak reinforcer when compared to other drugs of abuse (Caggiula et al., 2009; Sorge et al., 2009). Consequently, the development and maintenance of tobacco addiction may require exposure to a combination of nicotine and additional positive reinforcers. While menthol may play such a role, direct experimental demonstration of such an interaction in humans is complicated by the ‘brand loyalty’ to either menthol or non-menthol cigarettes among smokers (Dawes, 2014). Committed menthol and non-menthol smokers have different associative learning histories to smoking cues that confound interpretation of differences in subjective and behavioral responses to menthol and non-menthol cigarettes (Rose et al., 2010). Furthermore, the quantitative measurement of menthol and nicotine interactions in smokers requires precise control over nicotine dose, a challenging experimental condition due to the marked variability in smoking topography among smokers (Guyatt et al., 1989; Kolonen et al., 1992).

To address these challenges, we developed a human laboratory method that uses concurrent intravenous (IV) nicotine and inhaled menthol. The precisely dosed IV nicotine produces both dose- and time-dependent subjective drug effects similar to smoking (Mello et al., 2013; Rose et al., 1999), but without the chemosensory cues provided by mainstream smoke. The inhaled menthol from an e-cigarette, while producing characteristic chemosensory cues, is devoid of other cues present in tobacco smoke, including nicotine. Using this design, we sought to measure the interactive effects of nicotine and menthol on acute positive subjective effects when administered concurrently to young smokers stratified by smoking preference. The positive drug effects were operationalized by the *good effects* factor of the Drug Effects Questionnaire (DEQ) that is comprised of the items “like drug effects” and “would like more of the drug” (Morean et al., 2013). Two primary hypotheses were tested; (a) concurrent menthol and nicotine administration would lead to greater self-reported positive drug effects than either menthol or nicotine alone, or placebo, and (b) the enhancement of nicotine’s

positive subjective effects by menthol would be greater in menthol-preferring smokers (MSs) than in non-menthol-preferring smokers (NMSs).

METHODS

Subjects

A total of 116, young adult (aged 18–30 years), non-treatment-seeking cigarette smokers were recruited from the New Haven, Connecticut area. Young adults were chosen as the study population because of the persistently high prevalence of mentholated tobacco product use in this age group (Giovino et al., 2015; Rock et al., 2010; Villanti et al., 2016). Since the safety of IV nicotine administration has not been examined in smokers younger than 18, only individuals over 18 were included. Potential participants were required to smoke at least one cigarette/day for the past year (verified by a screening urine cotinine > 100 ng/mL as measured by rapid immunochromatographic assay (NicAlert, Nymox Corporation, Hasbrouck Heights, New Jersey, USA)) to ensure that both dependent and non-dependent smokers would be enrolled. Both MSs and NMSs were recruited and prior e-cigarette experience was not required. Participants were medically healthy and did not meet criteria for current Axis I psychiatric disorders, including alcohol or dependence (other than nicotine), as determined by the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (First et al., 1996) and urine drug screening for cocaine, amphetamines, benzodiazepines, barbiturates, and opioids. Seventy participants were eligible for participation with 63 participants randomized after completion of the training session. Of these, six participants with repeated no-shows were excluded before any test session participation. There were 57 participants in the first test session, 48 in the second, and 43 in the third test session. The protocol was approved by the Yale University and United States Department of Veterans Affairs (VA) Connecticut Healthcare institutional review boards. Written informed consent was provided prior to participation, for which participants were compensated.

Experiment Design

This outpatient, double-blind, placebo-controlled study consisted of an adaptation session followed by three test sessions. As shown in Figure 1, all participants were randomized to a test session order and received control menthol (0.0% + tobacco flavor), low menthol (0.5% + tobacco flavor), and high menthol (3.2% + tobacco flavor) by standardized inhalation from an e-cigarette just prior to each nicotine infusion (a single menthol condition for each test session). Within each test session, all 3 IV nicotine conditions were tested by delivering saline, nicotine at 0.25 mg nicotine/70 kg and nicotine at 0.5 mg nicotine/70 kg, one hour apart, in a random order just after the last inhalation. For a given participant, the randomized nicotine infusion sequence was fixed across the three test sessions, each performed at least 24 h apart.

Adaptation Session.

To reduce variability in menthol delivery, participants were introduced to the operation of the test e-cigarette, then coached on inhaling more softly, but for longer (3–4 s) than is typical for combustible cigarettes (Farsalinos et al., 2013; Vansickel and Eissenberg, 2013).

The practice e-cigarette contained tobacco flavor and 0.0% menthol. Participants were instructed to avoid exposure to menthol products (other than their usual brand of cigarettes) for 24 h prior to each test session, and to abstain from smoking and eating after midnight prior to test sessions. Typical morning caffeine intake was encouraged to avoid withdrawal symptoms that might confound interpretation of study measures.

Test sessions.

Test sessions started between 08:00 and 09:00. Participants were first evaluated for exclusionary drug use and pregnancy by urine testing, as well as for recent smoking (breath CO₁₀ ppm; Vitalograph, Inc., Lenexa, Kansas, USA) and recent alcohol use (breathalyzer, Alco-Sensor IV, Intoximeters, Inc., St. Louis, MO, USA). After a light breakfast, an indwelling 20-gauge, flexible catheter with multiple ports was inserted into an antecubital vein for blood sampling and infusions. Heart rhythm was monitored with a three-lead electrocardiogram and blood pressures were acquired using an arm cuff placed opposite to the catheterized arm.

Drugs

Menthol Administration.—Each e-liquid (prepared by Pace Engineering Concepts (Delafield, Wisconsin, USA)) contained the same concentration of “Burley” tobacco flavor (Tobacco Technology Inc., Eldersburg, Maryland, USA) in a 75% propylene glycol/ 25% vegetable glycerin base mixture (both from Hydrite Chemical Co., Brookfield, Wisconsin, USA). Tobacco flavor was used as the control condition because of its familiarity to all smokers and its relatively neutral appeal profile as an e-cigarette flavor (Goldenson et al., 2016). The menthol doses utilized were pre-determined in a dose-finding study (Rosbrook and Green, 2016). The low dose contained the minimally perceptible menthol concentration (0.5%), and is within the range of e-liquids marketed to consumers with a preference for low menthol impact. The high dose menthol (3.2%) produced the same cooling sensation as commercially available mentholated e-liquids and is within the range of e-liquids marketed to menthol-preferring consumers (i.e. as from the AmericaneLiquidStore). After menthol content and the absence of nicotine in the stock e-liquids were verified by laboratory analysis (PJ), the solutions were aliquoted into sealed, 5 mL amber glass vials and stored at 70–75 degrees F°. Just prior to each test session, 1.5 mL of the assigned e-liquid was transferred to the e-cigarette tank (2 mL capacity) of a Joyetech eGo-C configured with a single coil atomizer (2.2 ohm) and a 650-mAH battery operating at 3.7 volts (6.2 W). Participants took six inhalations, one every 15 s, over 90 s just prior to each of the three infusions. As reported elsewhere, this procedure resulted in highly significant, dose-dependent increases of venous plasma menthol glucuronide (a biomarker of menthol exposure) after inhalation that was proportional to the menthol solution concentrations inhaled by our study sample (Jatlow et al., 2018).

Nicotine Administration.—Nicotine bitartrate (Interchem Corporation, Paramus, New Jersey, USA) infusates were prepared by the research pharmacy in 5 mL of physiological saline. Just after the last of six inhalations, a 30-s infusion was administered by a study physician, each given one hour apart. The nicotine doses chosen (0.25 and 0.5mg/70 kg) are equivalent to the nicotine delivered by smoking half of a cigarette, which ranges from 0.25–

1 mg (Benowitz, 2008). In our previous studies, these doses of nicotine were well tolerated, yet produced robust positive and negative subjective effects (Sofuoglu et al., 2009, 2012; Sofuoglu and Mooney, 2009; Sofuoglu et al., 2008, 2011). The infusions were given one hour apart because this interval allows subjective responses to return to baseline (Sofuoglu et al., 2005, 2008, 2012).

Outcome Measures

The outcome measures assessed subjective and biochemical domains. In addition, heart rate and blood pressure were assessed for safety reasons and will not be discussed further.

Subjective.—Measures included the DEQ, the Brief Questionnaire of Smoking Urges (BQSU), and the Minnesota Nicotine Withdrawal Scale (MNWS). The DEQ captured acute drug effects by recording participants' response intensity on a 100 mm visual analogue scale ranging from "not at all" to "extremely." The DEQ consisted of 11 items: cooling effect, dislike the sensation, any sensations (in mouth, throat or chest), feel a drug effect, high, feel stimulated, feel a head rush, like drug effect, dislike any effects, craving a cigarette, and would like more of the drug. The DEQ items were clustered into four different factors. The DEQ items of "like drug effect" and "would like more of the drug" were averaged to obtain a summary score representing a *good effects* factor, which was the main study outcome. The items "feel a drug effect," "high" and "feel stimulated" were averaged to represent a composite *stimulatory effects* factor (Morean et al., 2013). The "dislike any effects" and "cooling effect" items were analyzed as independent factors. The DEQ was administered just before and after inhalations, and then at 1, 3, 5, 8 and 10 min post-infusion.

The BQSU is a 10-item scale with two factors rated on a seven-point Likert scale: Factor 1 reflects urges to smoke for stimulation, and Factor 2 reflects urges to smoke to relieve negative mood and withdrawal (Cox et al., 2001; Tiffany and Drobes, 1991). BQSU ratings were collected at baseline with an additional BQSU assessment after each infusion. The MNWS is a widely used eight-item scale measuring symptoms of tobacco withdrawal (Hughes and Hatsukami, 1986). It was collected after overnight nicotine deprivation (just prior to the start of each test session), and again at the end of each test session.

Biochemical.—Baseline plasma samples were collected for determination of plasma nicotine, cotinine and 3'-hydroxycotinine (3HC) concentrations for derivation of the nicotine metabolite ratio (NMR=3HC/cotinine) of each participant (Sofuoglu et al., 2012). The NMR, which reflects the activity of cytochrome (CYP) P450 2A6 and thereby, the rate of nicotine metabolism (Dempsey et al., 2004), was calculated to assess for potential differences in nicotine metabolism between participants stratified by menthol preference. Previous studies have demonstrated that menthol may inhibit nicotine metabolism, and compared to NMSs, MSs have been shown to have lower NMR values (Benowitz et al., 2004; Fagan et al., 2016). Unconjugated plasma nicotine and 3HC concentrations were assayed using high-performance liquid chromatography interfaced with tandem mass spectrometry (LC/MS/MS) as previously described (Sofuoglu et al., 2012).

Statistical analysis

The main outcome was the *good effects* factor of the DEQ, which included the average peak values of the “like drug effect” and “would like more of the drug” items of the DEQ. In addition, the *stimulatory effects* factor, and dislike and cooling items were analyzed separately as secondary outcomes. The DEQ measures were assessed for normality. Since the DEQ measures were skewed, square root transformations were used to bring the variables more in line with the normal distribution. We used a mixed effects model with a between-subject effect of cigarette preference (MSs vs. NMSs), within-subject effects of menthol dose (0.0%, 0.5%, 3.2%) and nicotine dose (saline, 0.25 mg/70 kg, 0.5 mg/70 kg), and all possible interactions. Session (test session 1, 2, or 3; menthol condition randomized across sessions) and period (order of nicotine or saline conditions within sessions) were also added to the models to account for period effects in menthol and nicotine administrations, respectively. Random effects for subject, menthol dose and nicotine dose within subject were used to model the correlations among repeated measures on the same individual. This was the best-fitting structure identified based on Akaike’s and Schwartz-Bayesian Information Criteria (AIC and BIC respectively) for all outcomes. Least square means and standard errors were calculated to describe the patterns of means for each outcome. Effect slices were tested to explain significant interactions in the models. Pairwise comparisons of least square means were used to describe significant main effects.

A mixed model approach was also used for the analysis of MNWS and BQSU outcomes. Analysis of BQSU subscales (Factor 1, Factor 2) included nicotine infusion as a within-subject factor with 5 levels (pre-nicotine infusion, dose during first, second and third infusion and post-infusion). MNWS and BQSU analyses included cigarette preference (MSs vs. NMSs) as a between-subject factor. The MNWS model did not include a within-subject effect of nicotine because it was only administered before and after each test session.

MSs and NMSs were compared on baseline demographics, biochemical and self-reported tobacco product use indicators, and NMR values, using t-tests for continuous variables and chi-square or Fisher’s exact tests for categorical outcomes (Table 1).

RESULTS

Selected statistical results for primary outcomes are described below and full statistical results are presented in the Supplementary Material Table 1.

Baseline Variables

MSs were more likely to be female and African American than NMSs ($p<0.001$) and have less experience with e-cigarettes ($p<0.05$). There were no significant differences on other baseline variables (Table 1).

Nicotine Metabolite Ratio

The baseline NMR value of 0.29 (0.19) in MSs was significantly lower than the NMR value of 0.55 (0.35) in NMSs ($p<0.05$, Table 1) indicating slower nicotine metabolism within the MSs of our study population.

Subjective Effects

DEQ. For the *good effects* factor, NMSs reported higher ratings compared to MSs (main effect for cigarette preference: $F(1,162)=8.20$, $p=0.005$) (Figure 2(a)). The responses were nicotine dose-dependent (main effect for nicotine: $F(2,105)=15.06$, $p<.0001$); high dose>low dose> placebo (all p -values <0.05)).

For the *stimulatory effects* factor, NMSs, compared to MSs, reported greater effects from high ($p<0.001$) and low dose ($p<0.05$) nicotine, but not saline ($p>0.05$) (cigarette preference by nicotine interaction: $F(2,163)=7.95$, $p=0.0005$), and greater overall “stimulated” effects (main effect for cigarette preference: $F(1,163)=5.28$, $p=0.02$)) (Figure 2(b)). The ratings for the *stimulatory effects* factor were nicotine dose-dependent (main effect for nicotine: $F(2,105)=73.61$, $p<0.0001$; high dose>low dose> placebo, all p -values <0.0001).

For the *dislike effects* item, a significant effect of menthol was observed in NMSs, but not in MSs, in response to high ($p<0.05$) and low nicotine ($p<0.01$) doses, but not to saline ($p>0.05$) (three-way interaction between cigarette preference, menthol and nicotine: $F(4,162)=2.47$, $p=0.05$; high dose>low dose>placebo menthol) (Figure 2(c)). The rating of “dislike any effects” was greater in response to high dose menthol than in the no menthol condition ($p<0.01$) (main effect of menthol: $F(2,85)=4.57$, $p=0.01$; high menthol>no menthol).

For the *cooling effect* of menthol (Figure 2(d)), a significant dose-dependent increase was observed within both MSs and NMSs (main effect for menthol: $F(2,85)=40.87$, $p<.0001$; high dose>low dose> no dose (all p -values <0.0001)).

Measures of Craving and Withdrawal

BQSU Factor 1 scores after high dose nicotine were lower than baseline and pre-infusion scores (main effect of nicotine: $F(4,209)=2.85$, $p=0.02$) and were lower in MSs as compared to NMSs (main effect of cigarette preference: $F(1,325)=3.83$, $p=0.05$). Compared to the no menthol condition, BSQU Factor 2 scores were lower after high and low dose menthol in MSs (all p -values <0.05) (menthol dose by cigarette preference interaction: $F(2,325)=5.20$, $p=0.006$), but the difference was in the opposite direction and not significant for NMSs (Figure 3(a)). The mean total MNWS scores, collected just prior to the start of each test session, are displayed by cigarette preference in Figure 3(b). Compared to MSs, NMSs reported higher withdrawal symptoms after overnight nicotine deprivation (main effect of cigarette preference: $F(1,223)=14.37$, $p=0.0002$).

DISCUSSION

This study produced several key findings. First, inhaled menthol did not alter the positive subjective effects of nicotine (i.e., *good effects* factor), or of saline, in either MSs or NMSs but enhanced the “dislike” of nicotine, but only in NMSs. Second, inhaled menthol reduced urges to smoke in MSs, but not in NMSs. Third, compared to NMSs, MSs reported less positive subjective responses to IV nicotine and less severe withdrawal following overnight nicotine deprivation. Lastly, MSs had a lower NMR than NMSs, suggesting that MSs cleared nicotine more slowly than the NMSs in our study sample of young smokers. Collectively,

these findings do not support our primary study hypotheses regarding menthol's positive subjective effects but, as discussed below, provide additional insight into other possible mechanisms by which menthol impacts tobacco use behavior.

As positive drug effects correlate with drug reinforcement, the absence of positive subjective effects from inhaled menthol, alone or in combination with nicotine, suggests that menthol may not be a simple primary positive reinforcer in smokers, or one that adds to the reinforcing effects of nicotine. These findings are in general agreement with several preclinical and clinical studies. For example, in a recent animal study, oral menthol administration, as compared to oral sucrose or saccharin, did not induce either phasic dopamine release in the nucleus accumbens or an alteration in IV nicotine self-administration, suggesting the absence of menthol reinforcement (Wickham et al., 2018). Further, in a recent study of 32 adult smokers, inhalation of e-liquids with a range of nicotine (0, 6, 12, 18, 24 mg/ml) and menthol (0%, 0.5% and 3.5%) concentrations were only "slightly liked" and the degree of liking did not vary across nicotine or menthol concentrations (Rosbrook and Green, 2016). In contrast, we observed that inhaled menthol dose-dependently enhanced the rating of "dislike" after nicotine administration, but only in NMSs. Furthermore, in the absence of nicotine (i.e. saline infusion only), neither the low, nor the high dose of menthol produced more disliking as compared to the control (tobacco) flavor without menthol, while menthol inhalation did produce dose dependent "cooling effects" that did not differ between MSs and NMSs. Collectively, these results suggest that the enhanced dislike ratings in NMSs observed after menthol plus nicotine administration are not simply due to the disliking of inhaled menthol, but rather represent menthol's enhancement of nicotine disliking. Since nicotine was given intravenously, this effect is independent of chemosensory interactions between menthol and nicotine in the respiratory tract.

In addition, our finding that both high and low doses of menthol, as compared to the no menthol control, alleviated urges to smoke to relieve negative mood and withdrawal (BQSU Factor 2) in MSs, but not in NMSs, is consistent with other studies demonstrating that with habitual menthol inhalation, menthol becomes a conditioned cue that alleviates smoking urges (Ahijevych and Garrett, 2004; Wang et al., 2014). In addition, the high dose of nicotine, as compared to both the low dose of nicotine, and saline, reduced smoking urges as measured by both Factor 1 and Factor 2 of the BQSU, but inhaled menthol did not enhance nicotine's effect on smoking urge reduction. These two findings suggest that menthol and nicotine's effects on reducing urges to smoke are not additive, but are due to differing stimulus properties of drug and conditioned cues that become manifest when they are experimentally dissociated in MSs. Collectively, these findings suggest that within MSs, menthol can function as a conditioned cue that predicts nicotine delivery thereby providing negative reinforcement through the acute alleviation of urges to smoke that develop during abstinence. However, in a preclinical nicotine self-administration model, a conditioned menthol stimulus has been shown to reinstate extinguished nicotine-seeking behavior, a finding consistent with the possibility that menthol may also serve as a conditioned cue that triggers nicotine craving and relapse (Harrison et al., 2017). To the best of our knowledge, studies examining the influence of menthol on smoking urges in MSs and NMSs have not been conducted. An improved understanding of how conditioned responses to menthol cues

impact complex behavior during different states of nicotine deprivation may guide more effective interventions for smoking cessation.

The differences between MSs and NMSs in the positive subjective responses to nicotine (i.e. blunted sensitivity in MSs) and in nicotine withdrawal intensity (decreased in MSs after overnight abstinence), are consistent with findings from our previous IV nicotine study conducted in an independent sample of dependent smokers that used higher nicotine doses (0.5 and 1.0 mg) without concurrent menthol administration (DeVito et al., 2016). In that study, when compared to NMSs, MSs did not show separation between the 0.5 and 1 mg nicotine doses for the rating of “good effects”, and showed a trend for less severe withdrawal following overnight abstinence (DeVito et al., 2016). Together, the findings from these two independent samples of smokers suggest that long-term, but not acute, exposure to menthol may change sensitivity to nicotine. This possibility is consistent with the finding of a blunted sensitivity to nicotine’s acute effects in MSs, as indexed by a higher nicotine threshold dose than in NMSs, for behavioral discrimination of the nicotine content in combustible cigarettes (Perkins et al., 2017). As pointed out in a recent commentary, the duration of menthol exposure may be critical to its effects on nicotine reward (Lallai and Fowler, 2017). For example, an 11-day infusion of menthol in mice inhibited nicotine reward, as assessed with the conditioned place paradigm (CPP) (Henderson et al., 2016), while menthol and nicotine acted synergistically to enhance reward in the CPP following acute exposure (Henderson et al., 2017). These preclinical differences suggest that clinically relevant effects of habitual mentholated cigarette smoking may be better modeled by a longer duration of menthol exposure, rather than the acute menthol treatments utilized in the majority of preclinical studies (Ashoor et al., 2013; Hans et al., 2012; Ton et al., 2015).

The striking group differences we observed in nicotine reinforcement and withdrawal between NMSs and MSs may be partly due to menthol’s inhibitory effects on nicotine metabolism (Benowitz et al., 2004; Fagan et al., 2016). A slower rate of nicotine metabolism, as assessed by a lower NMR in our MS sample, is associated with lower urges to smoke and less severe withdrawal symptoms following nicotine deprivation, as well as reduced positive subjective effects to IV nicotine (Falcone et al., 2016; Sofuoglu et al., 2012). Because our study sample of MSs were predominantly African-American males and females, while most of the NMSs were male Caucasians, we cannot exclude the possibility that the group differences in NMR we observed are due to sex and ethnicity. It has been difficult to experimentally separate the influence of menthol, from racial and ethnicity effects on nicotine metabolism, because MSs are so frequently overrepresented by African-Americans. However, a recent study of 186 young menthol and non-menthol smokers found that the lower NMR in menthol smokers remained significant after controlling for race/ethnicity (Fagan et al., 2016). This finding supports the possibility that a significant amount of the between group variance in our experimental findings is due to decreased nicotine clearance in participants with chronic exposure to inhaled menthol, rather than the racial or cultural disparity in our study sample. Further studies that carefully balance sex and race across cigarette preference will be needed to more precisely quantitate the relative contributions of habitual menthol inhalation, sex and race to the interaction between nicotine metabolism and smoking behavior.

In addition to inhibition of nicotine metabolism (Benowitz et al., 2004; MacDougall et al., 2003), menthol may modulate nicotine's pharmacological effects through additional mechanisms. Menthol's cooling and anti-irritant effects, which are likely mediated through TRP (transient receptor potential) channels, can counteract nicotine's local aversive effects (Liu et al., 2013; Peier et al., 2002). For example, in a two-bottle choice task, oral menthol reversed oral nicotine aversion (Wickham et al., 2018). Furthermore, a variation in the gene for TRPA1, a cold-sensitive cation channel modulated by menthol, mediates individual differences in menthol preference among smokers (Uhl et al., 2011). Finally, menthol's direct allosteric inhibition, and enhanced desensitization, of nAChRs represent additional biological mechanisms by which menthol modulates nicotine's effects, including a reduction in the positive rewarding effects nicotine (Ashoor et al., 2013; Hans et al., 2012; Ton et al., 2015). It is presently unclear if such effects represent peripheral or central effects of menthol. Although these peripheral effects occur prior to menthol's systemic absorption, there is experimental evidence suggesting that menthol may also have central nervous system (CNS) effects. For example, after intraperitoneal menthol administration in mice, brain levels of menthol rise rapidly over five minutes (Pan et al., 2012) and inhibit chemically induced seizures (Zhang et al., 2008). Similarly, menthol administered intravenously in mice rapidly increases locomotor activity (Umezu et al., 2001). Whether central mechanisms also contribute to menthol's typical effects following tobacco product use in humans remains to be determined.

A strength of our model is the ability to examine dose-dependent effects of menthol and nicotine, alone or in combination, on multiple subjective measures in smokers stratified by cigarette preference. Furthermore, by using an e-cigarette to deliver menthol, local sensory cues of menthol similar to those produced by menthol cigarette smoking were maintained, but disassociated from the chemosensory cues provided by nicotine and other chemicals present in mainstream smoke. While both IV nicotine and nicotine inhaled from a cigarette result in similarly rapid subjective effects, nicotine administered intravenously provides precise dosing and minimizes conditioned responses to the harshness, bitter taste and other oral and respiratory tract effects of nicotine. Our observation of the anticipated, dose-dependent increases in the ratings of the *stimulatory effect* and *good effect* factors after IV nicotine, and dose-dependent enhancement of "coolness" ratings by menthol, represent positive control conditions within our model that increase the validity of our novel findings.

As discussed above, a primary limitation of this study was the unbalanced ethnicity and sex distribution across MSs and NMSs that may confound interpretation of several study outcomes, including withdrawal severity and subjective effects of nicotine (Bello et al., 2016; DeVito et al., 2014; Robinson et al., 2014). Another limitation is the narrow age range of the study participants, 18 – 30 years, which may limit the generalizability of these findings to other age groups. Finally, an additional limitation is the possibility that the group differences in prior e-cigarette experience contributed to the differences between MSs and NMSs, as experience with e-cigarettes may improve the efficiency of vaping (Farsalinos et al., 2013; Vansickel and Eissenberg, 2013). However, following menthol inhalation, MSs and NMSs did not differ in their ratings of "coolness", or in their plasma levels of menthol glucuronide (Jatlow et al., 2018), making it less likely that the differences in e-cigarette

experience between groups resulted in variable menthol delivery that contributed to the differences we observed.

Our results have important implications for tobacco regulatory science because they identify potential mechanisms by which menthol may impact tobacco use that are separate from the established direct interactions between menthol and nicotine in the respiratory tract, and therefore, highlight significant gaps in our knowledge of menthol's effects in relation to tobacco use. These gaps include (a) the neurobiological, behavioral and metabolic impact of chronic exposure to inhaled menthol, (b) a clarification of central vs. local effects of menthol following inhalation, and (c) the development of models that will capture the effects of menthol in different stages of tobacco use (e.g., initiation, established smoking, and cessation). Future studies could utilize a cross-over design (e.g., switching menthol smokers to non-menthol cigarettes) to reduce the impact of individual variation (e.g., race, sex, age) on study outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors wish to thank Mercedes Coffman, Lance Barnes, Stacy Minnix, Christopher Cryan and Ellen Mitchell for their important contributions to this study including the execution of laboratory sessions, recruitment, administrative support, and data collection/management. The authors also acknowledge the contributions of VA Connecticut Healthcare System Biostudies Unit support staff including Elizabeth O'Donnell, Angelina Genovese, Margaret Dion-Marovitz and Willie Ford.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Research reported in this publication was supported by the New England Mental Illness Research, Education and Clinical Centers (MIRECC), Department of Veterans Affairs, and the National Institute on Drug Abuse of the National Institutes of Health under Award Number P50DA036151. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The authors have no conflicts of interest to declare.

References

- Ahijevych K and Garrett BE (2004) Menthol pharmacology and its potential impact on cigarette smoking behavior. *Nicotine Tob Res* 6 Suppl 1: S17–28. [PubMed: 14982706]
- Ai J, Taylor KM, Lisko JG, et al. (2016) Menthol content in US marketed cigarettes. *Nicotine Tob Res* 18(7):1575–1580. [PubMed: 26259988]
- Ashoor A, Nordman JC, Veltri D, et al. (2013) Menthol binding and inhibition of alpha7-nicotinic acetylcholine receptors. *PloS one* 8(7): e67674. [PubMed: 23935840]
- Bello MSs, Pang RD, Cropsey KL, et al. (2016) Tobacco withdrawal amongst African American, Hispanic, and white smokers. *Nicotine Tob Res* 18(6): 1479–1487. [PubMed: 26482061]
- Benowitz NL. (2008) Clinical pharmacology of nicotine: implications for understanding, preventing, and treating tobacco addiction. *Clin Pharmacol Ther* 83: 531–541. [PubMed: 18305452]
- Benowitz NL, Herrera B and Jacob P, 3rd (2004) Mentholated cigarette smoking inhibits nicotine metabolism. *J Pharmacol Exp Ther* 310(3): 1208–15.
- Biswas L, Harrison E, Gong Y, et al. (2016) Enhancing effect of menthol on nicotine self-administration in rats. *Psychopharmacology (Berl)* 233(18): 3417–3427. [PubMed: 27473365]

- Caggiula AR, Donny EC, Palmatier MI, et al. (2009) The role of nicotine in smoking: a dual-reinforcement model. *Nebr Symp Motiv* 55: 91–109. [PubMed: 19013940]
- Cox LS, Tiffany ST and Christen AG (2001) Evaluation of the brief questionnaire of smoking urges (QSU-brief) in laboratory and clinical settings. *Nicotine Tob Res* 3(1): 7–16. [PubMed: 11260806]
- Dawes J (2014) Cigarette brand loyalty and purchase patterns: an examination using US consumer panel data. *Journal of Business Research* 67(9): 1933–1943.
- Dempsey D, Tutka P, Jacob P, 3rd, et al. (2004) Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. *Clin Pharmacol Ther* 76(1): 64–72. [PubMed: 15229465]
- DeVito EE, Herman AI, Waters AJ, et al. (2014) Subjective, physiological, and cognitive responses to intravenous nicotine: effects of sex and menstrual cycle phase. *Neuropsychopharmacology* 39(6): 1431–1440. [PubMed: 24345818]
- DeVito EE, Valentine GW, Herman AI, et al. (2016) Effect of menthol-preferring status on response to intravenous nicotine. *Tob Regul Sci* 2(4): 317–328. [PubMed: 28890914]
- Fagan P, Pokhrel P, Herzog TA, et al. (2016) Nicotine metabolism in young adult daily menthol and nonmenthol smokers. *Nicotine Tob Res* 18(4): 437–446. [PubMed: 25995160]
- Falcone M, Cao W, Bernardo L, et al. (2016) Brain responses to smoking cues differ based on nicotine metabolism rate. *Biol Psychiatry* 80(3): 190–197. [PubMed: 26805583]
- Farco JA and Grundmann O (2013) Menthol--pharmacology of an important naturally medicinal "cool". *Mini Rev Med Chem* 13(1): 124–131. [PubMed: 23061635]
- Farsalinos KE, Romagna G, Tsiapras D, et al. (2013) Evaluation of electronic cigarette use (vaping) topography and estimation of liquid consumption: implications for research protocol standards definition and for public health authorities' regulation. *Int J Environ Res Public Health* 10(6): 2500–2514. [PubMed: 23778060]
- First MB, Spitzer RL, Gibbon M, Williams JB (1996) Structured Clinical Interview for DSM-IV Axis I Disorders--Patient Edition Biometrics Research Department, New York State Psychiatric Institution: New York.
- Giovino GA, Villanti AC, Mowery PD, et al. (2015) Differential trends in cigarette smoking in the USA: is menthol slowing progress? *Tob Control* 24(1): 28–37. [PubMed: 23997070]
- Goldensen NI, Kirpatrick MG, Garrington-Trimis JL, et al. (2016) Effects of sweet flavorings and nicotine on the appeal and sensory properties of e-Cigarettes among young adult vapers: Application of a novel methodology. *Drug Alcohol Depend* 1(168):176–180.
- Gordon SM, Brinkman MC, Meng RQ, et al. (2011) Effect of cigarette menthol content on mainstream smoke emissions. *Chem Res Toxicol* 24(10): 1744–1753. [PubMed: 21888394]
- Guyatt AR, Kirkham AJ, Baldry AG, et al. (1989) How does puffing behavior alter during the smoking of a single cigarette? *Pharmacol Biochem Behav* 33(1): 189–195. [PubMed: 2780776]
- Hans M, Wilhelm M and Swandulla D (2012) Menthol suppresses nicotinic acetylcholine receptor functioning in sensory neurons via allosteric modulation. *Chem Senses* 37(5): 463–469. [PubMed: 22281529]
- Harrison E, Biswas L, Ramachandram A, et al. (2017) Effects of menthol and its interaction with nicotine-conditioned cue on nicotine-seeking behavior in rats. *Psychopharmacology* 234(23–24): 3443–3453. [PubMed: 28918457]
- Henderson BJ, Wall TR, Henley BM, et al. (2016) Menthol alone upregulates midbrain nAChRs, alters nAChR subtype stoichiometry, alters dopamine neuron firing frequency, and prevents nicotine reward. *J Neurosci* 36(10): 2957–2974. [PubMed: 26961950]
- Henderson BJ, Wall TR, Henley BM, et al. (2017) Menthol enhances nicotine reward-related behavior by potentiating nicotine-induced changes in nAChR function, nAChR upregulation, and DA neuron excitability. *Neuropsychopharmacology* 42(12):2285–2291. [PubMed: 28401925]
- Hughes JR and Hatsukami D (1986) Signs and symptoms of tobacco withdrawal. *Arch Gen Psychiatry* 43(3): 289–294. [PubMed: 3954551]
- Jatlow P, Valentine G, Gueorguieva R, et al. (2018) Plasma menthol glucuronide as a biomarker of acute menthol inhalation. *Tob Regul Sci* 4: 586–591. [PubMed: 29479558]
- Kolonen S, Tuomisto J, Puustinen P, et al. (1992) Puffing behavior during the smoking of a single cigarette in a naturalistic environment. *Pharmacol Biochem Behav* 41(4): 701–706. [PubMed: 1594637]

- Lallai V and Fowler CD (2017) More than just chillin': interactive effects of menthol and nicotine in drug reward. *Neuropsychopharmacology* 42(12):2283–2284. [PubMed: 28553838]
- Liu B, Fan L, Balakrishna S, et al. (2013) TRPM8 is the principal mediator of menthol-induced analgesia of acute and inflammatory pain. *Pain* 154: 2169–2177. [PubMed: 23820004]
- MacDougall JM, Fandrick K, Zhang X, et al. (2003) Inhibition of human liver microsomal (S)-nicotine oxidation by (–)-menthol and analogues. *Chem Res Toxicol* 16(8): 988–993. [PubMed: 12924926]
- Mello NK, Peltier MR and Duncanson H (2013) Nicotine levels after IV nicotine and cigarette smoking in men. *Exp Clin Psychopharmacol* 21(3): 188–195. [PubMed: 23647094]
- Morean ME, de Wit H, King AC, et al. (2013) The drug effects questionnaire: psychometric support across three drug types. *Psychopharmacology (Berl)* 227(1): 177–192. [PubMed: 23271193]
- Pan R, Tian Y, Gao R, et al. (2012) Central mechanisms of menthol-induced analgesia. *J Pharmacol Exp Ther* 343: 661–672. [PubMed: 22951274]
- Peier AM, Moqrich A, Hergarden AC, et al. (2002) A TRP channel that senses cold stimuli and menthol. *Cell* 108: 705–715. [PubMed: 11893340]
- Perkins KA, Kunkle N and Karelitz JL. (2017) Threshold dose for behavioral discrimination of cigarette nicotine content in menthol vs. non-menthol smokers. *Psychopharmacology (Berl)* 234(8): 1255–1265.
- Robinson CD, Pickworth WB, Heishman SJ, et al. (2014) The acute tobacco withdrawal syndrome among black smokers. *Psychol Addict Behav* 28(1): 173–181. [PubMed: 23528199]
- Rock VJ, Davis SP, Thorne SL, Asman KJ, Caraballo RSCINNTRE, author reply P (2010). Menthol cigarette use among racial and ethnic groups in the United States, 2004–2008. *Nicotine Tob Res* 12 Suppl 2: S117–124. [PubMed: 21177368]
- Rosbrook K and Green BG (2016) Sensory effects of menthol and nicotine in an e-cigarette. *Nicotine Tob Res* 18(7): 1588–1595. [PubMed: 26783293]
- Rose JE and Behm FM (2004) Extinguishing the rewarding value of smoke cues: pharmacological and behavioral treatments. *Nicotine Tob Res* 6(3): 523–532. [PubMed: 15203786]
- Rose JE, Behm FM, Westman EC, et al. (1999) Arterial nicotine kinetics during cigarette smoking and intravenous nicotine administration: implications for addiction. *Drug Alcohol Depend* 56(2): 99–107. [PubMed: 10482401]
- Rose JE, Salley A, Behm FM, et al. (2010) Reinforcing effects of nicotine and non-nicotine components of cigarette smoke. *Psychopharmacology (Berl)* 210(1): 1–12. [PubMed: 20358364]
- Sofuoglu M, Herman AI, Nadim H, et al. (2012) Rapid nicotine clearance is associated with greater reward and heart rate increases from intravenous nicotine. *Neuropsychopharmacology* 37(6): 1509–1516. [PubMed: 22334123]
- Sofuoglu M, Mitchell E and Mooney M (2009) Progesterone effects on subjective and physiological responses to intravenous nicotine in male and female smokers. *Hum Psychopharmacol* 24(7): 559–564. [PubMed: 19743227]
- Sofuoglu M, Mooney M (2009) Subjective responses to intravenous nicotine: greater sensitivity in women than in men. *Exp Clin Psychopharmacol* 17(2): 63–69. [PubMed: 19331482]
- Sofuoglu M, Mouratidis M and Mooney M (2011) Progesterone improves cognitive performance and attenuates smoking urges in abstinent smokers. *Psychoneuroendocrinology* 36(1): 123–132. [PubMed: 20675057]
- Sofuoglu M, Mouratidis M, Yoo S, et al. (2005) Effects of tiagabine in combination with intravenous nicotine in overnight abstinent smokers. *Psychopharmacology (Berl)* 181: 504–510. [PubMed: 15983800]
- Sofuoglu M, Yoo S, Hill KP, et al. (2008) Self-administration of intravenous nicotine in male and female cigarette smokers. *Neuropsychopharmacology* 33(4): 715–720. [PubMed: 17534380]
- Sorge RE, Pierre VJ and Clarke PB (2009) Facilitation of intravenous nicotine self-administration in rats by a motivationally neutral sensory stimulus. *Psychopharmacology* 207(2): 191–200. [PubMed: 19756529]
- Tiffany ST and Drobes DJ (1991) The development and initial validation of a questionnaire on smoking urges. *Br J Addict* 86(11): 1467–1476. [PubMed: 1777741]

- Ton HT, Smart AE and Aguilar BL (2015) Menthol enhances the desensitization of human $\alpha 3 \beta 4$ nicotinic acetylcholine receptors. *Mol Pharmacol* 88(2): 256–264. [PubMed: 25964258]
- Uhl GR, Walther D, Behm FM, et al. (2011) Menthol preference among smokers: association with TRPA1 variants. *Nicotine Tob Res* 13: 1311–1315. [PubMed: 21719896]
- Umezu T, Sakata A and Ito H. (2001) Ambulation-promoting effect of peppermint oil and identification of its active constituents. *Pharmacol Biochem Behav* 69: 383–390. [PubMed: 11509195]
- Vansickel AR and Eissenberg T (2013) Electronic cigarettes: effective nicotine delivery after acute administration. *Nicotine Tob Res* 15(1): 267–270. [PubMed: 22311962]
- Villanti AC, Mowery PD, Delnevo CD, et al. (2016) Changes in the prevalence and correlates of menthol cigarette use in the USA, 2004–2014. *Tob Control* 25(Suppl 2): ii14–ii20. [PubMed: 27729565]
- Wang T, Wang B and Chen H (2014) Menthol facilitates the intravenous self-administration of nicotine in rats. *Front Behav Neurosci* 8: 437. [PubMed: 25566005]
- Wickham RJ, Nunes EJ, Hughley S, et al. (2018) Evaluating oral flavorant effects on nicotine self-administration behavior and phasic dopamine signaling. *Neuropharmacology* 128: 33–42. [PubMed: 28943284]
- Zhang XB, Jiang P, Gong N, et al. (2008) A-type GABA receptor as a central target of TRPM8 agonist menthol. *PLoS One* 3: e3386. [PubMed: 18852885]

Figure 1.

Schematic depiction of experimental design. Following the adaptation session, participants were randomized to one of three menthol conditions (0.0%, 0.5% or 3.2%) for each test day. Within each test session, participants received a randomized IV nicotine sequence consisting of all three nicotine conditions: placebo (saline), low dose nicotine (0.25 mg/70 kg) and high dose nicotine (0.5 mg/70 kg), each given one hour apart. Each infusion was preceded by six puffs from the electronic cigarette (e-cigarette). For a given participant, the same randomized IV nicotine infusion sequence was used on each test day. The red arrows reflect an exemplar randomization of a participant for menthol condition (low menthol, high menthol, then tobacco flavor) and IV nicotine sequence (saline, high nicotine, then low nicotine).

Figure 2.

The average subjective drug effects scores from the Drug Effects Questionnaire (DEQ) for (a) good effects factor, (b) stimulatory effects factor (c) dislike effects and (d) cooling effect, after menthol and nicotine (NIC) administration in menthol and non-menthol smokers. The columns represent the peak post-dose values with standard error of the mean (SEM) error bars. Symbols indicate significant differences ($p < 0.05$). *Main effect of usual cigarette preference (menthol vs. non-menthol); §main effect of menthol dose; †main effect of NIC dose; cigarette preference by NIC interaction; #cigarette preference by NIC interaction by menthol dose. See text for further details.

Figure 3.

The average rating for the Brief Questionnaire of Smoking Urges (BQSU) Factor 2 (reflecting urges to smoke to relieve negative mood and withdrawal), and the total score on the Minnesota Nicotine Withdrawal Scale (MNWS; reflecting nicotine withdrawal intensity). Error bars give the standard error for the mean (SEM). Symbols indicate significant differences ($p < 0.05$). (a) Within menthol-preferring smokers (MSs), the high and low menthol conditions were associated with lower BQSU Factor 2 scores compared to the placebo condition. ^φmenthol by cigarette preference interaction. (b) For the MNWS, MSs had significantly lower baseline scores after overnight nicotine deprivation compared to NMS. *main effect of cigarette preference.

Table 1.

Comparison of baseline demographics, smoking severity, tobacco product use history and biochemical smoking indices between menthol-preferring (MSs) and non-menthol-preferring (NMSs) smokers.

	Combustible Cigarette Preference		
	Menthol-Preferring (N=32)	Non-Menthol-Preferring (N=25)	Statistics
	<i>N (%)</i>	<i>N (%)</i>	<i>P</i>
Demographics			
Race / Ethnicity			<0.001
African American/Black	24 (75.0)	1 (4.0)	
Non-African American	8 (25.0)	24 (96.0)	
Hispanic Ethnicity	3 (9.4)	1 (4.0)	ns
Sex			<0.001
Male	20 (62.5)	24 (96.0)	
Female	12 (37.5)	1 (4.0)	
Other Demographics	<i>Mean (SD)</i>	<i>Mean (SD)</i>	
Age, years	24.2 (3.6)	24.0 (3.6)	ns
Body mass index (BMI)	29.3 (8.2)	25.9 (5.1)	ns
Weight (lb)	178.2 (40.0)	181.76 (38.51)	ns
Smoking Severity and Tobacco Product Use History			
Fagerström Test of Nicotine Dependence (FTND)	4.34 (2.01)	4.0 (2.2)	ns
Average cigarette consumption/day	13.1 (7.4)	14.5 (6.7)	ns
Age of onset for regular smoking	16.1 (3.5)	16.5 (2.0)	ns
Longest Period of Abstinence (months)	3.1 (4.5)	4.3 (8.7)	ns
No history of e-cigarette use	22 (68.8)	9 (36.0)	<0.05
E-cigarette use in past month	8 (25.0)	14 (56.0)	<0.0001
Blend own e-liquids	1 (4.2)	1 (4.0)	ns
Baseline Biochemical Smoking Indices			
Plasma Cotinine, ng/ml	205.56 (115.46)	158.32 (96.80)	ns
Plasma 3'-hydroxycotinine (3HC)	66.83 (49.33)	76.00 (54.43)	ns
Nicotine Metabolite Ratio (NMR; 3HC/cotinine)	0.29 (0.19)	0.55 (0.35)	<0.05
Plasma Nicotine, ng/ml	2.60 (1.82)	2.22 (2.94)	ns