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Smoking Behavior: An Analysis of Menthol's Effect on Nicotine

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By

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Abstract

Smoking cigarettes is the leading cause of preventable death in the United States. Menthol is well known to exacerbate tobacco addiction. It is unclear, however, if menthol directly effects dopamine release which may increase the reward associated with smoking or if menthol has any effect on environmental cues which act as reinforcers for smoking behavior. Study 1 used fast scan cyclic voltammetry to examine the effects of nicotine and menthol, administered alone and in combination, on phasic dopamine release in the nucleus accumbens of male Sprague-Dawley rats. Results confirmed that nicotine, but not menthol, enhances phasic dopamine release in the nucleus accumbens. Menthol added to nicotine did not enhance phasic dopamine release above that elicited by nicotine alone. Study 2 used an online survey to examine the relationship between menthol status and environmental cues on nicotine consumption in human smokers. Results determined that there is no significant relationship between menthol status and smoking behavior. Taken together these results suggest that nicotine is the driving force behind tobacco addiction and menthol may act as a facilitator to make nicotine consumption more appealing.

Keywords: addiction, environmental cues, menthol, nicotine

Smoking Behavior: An Analysis of Menthol's Effect on Nicotine

Smoking tobacco cigarettes is the leading cause of preventable death in the United States (Biswas et al., 2016). Nicotine is the primary psychoactive and addictive component in tobacco cigarettes and is a powerful reinforcer in both animals and humans (Ahijevych & Garrett, 2010; Wickham, 2015). Mentholated cigarettes have been shown to have greater addictive potential and are correlated with a lower quit rate than that associated with nonmenthol cigarettes (Fait et al., 2017; Henderson et al., 2017; Wickham, 2019).

Nicotine

Nicotine is inherently rewarding and has positive reinforcing effects which increase the probability of self-administration in animals, as well as negative reinforcing effects that relieve negative symptoms associated with withdrawal (Ikemoto & Bonci, 2014; Valentine & Sofuoglu, 2018). Nicotine is a nicotinic acetylcholine receptor (nAChR) agonist that binds to nAChRs, facilitating the release of dopamine (DA) from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) (Benowitz, 2009; Ikemoto & Bonci, 2014). nAChRs are comprised of five subunits with the most abundant receptor subtypes in the mammalian brain being α4β2, α3β4, and α7 (Benowitz, 2009). The rapidly desensitizing α4β2 and α6β2, and slowly desensitizing α4α6β2 and α7 nAChR subtypes are believed to be the main mediating receptors in nicotine dependence (Benowitz, 2009; Picciotto et al., 2008).

Smoking is the fastest route of administration, when a drug is inhaled it enters circulation rapidly and reaches the brain within seconds (Benowitz, 2009). Increased rates of absorption and drug entry into the brain are correlated with a greater “rush” and consequently the drug is more reinforcing (Benowitz, 2009). Nicotine is metabolized into cotinine in the liver by the enzyme

CYP2A6, cotinine has a longer half-life than nicotine and both are used as quantitative markers for nicotine exposure (Benowitz, 2009).

Menthol

Menthol is a naturally occurring cyclic monoterpene alcohol that has a pleasant mint flavor and creates a cooling sensation via transient receptor potential melanostatin 8 (TRPM8) receptors which masks the aversive taste of nicotine and provides relief from the irritation associated with inhaling tobacco smoke (Hans et al., 2012; Kamatou et al., 2013; Lehr et al., 2021; Wickham, 2019). Menthol has two potential stereoisomers depending on the method of production, (-)-menthol and (+)-menthol, however, (-)-menthol appears to be more relevant for nicotine abuse because chronic exposure to (-)-menthol upregulates nAChR expression and increases DA neuron excitability whereas (+)-menthol exhibits no such effect (Henderson et al., 2019). With this in mind, a recent study comparing these stereoisomers of menthol found that (-)-menthol and (+)-menthol may both effectively contribute to menthol-dependent exacerbation of tobacco dependence (Lehr et al., 2021).

Menthol is a common flavorant in chewing gum and oral care products, and has been an additive in cigarettes since the 1920s (Ahijevych & Garrett, 2010; Anderson, 2011; Kamatou et al., 2013). Even cigarettes that are not labeled as menthols contain small amounts of menthol (Ai et al., 2016; Anderson, 2011; DeVito et al., 2016; Fait et al., 2017; Kamatou et al., 2013; Lee & Glantz, 2011). Cigarettes marketed as mentholated products contain a range between 2.9 – 19.6 mg menthol/cigarette, while cigarettes marketed as nonmentholated products contain 1.8 – 73.5 µg menthol/cigarette (Ai et al., 2016; Harrison et al., 2017).

Nicotine and Menthol Interactions

Menthol exposure increases nicotine self-administration and produces a leftward shift of the inverted-U of nicotine's dose response curve, this facilitating effect of menthol on nicotine self-administration is dose dependent (Biswas et al., 2016). Menthol also widens the dose-response curve for nicotine's euphoric and addictive effects, indicating nicotine is the driving force behind addictive behaviors and menthol can act as a motivator for continued nicotine use (Wickham, 2019). Menthol sustains and reinstates nicotine seeking behavior in rats, independent of TRPM8 receptor activity (Biswas et al., 2016; Harrison et al., 2017). This suggests that menthol, labeled or unlabeled, in most tobacco products could potentially contribute to the high rates of continued use of and relapse to tobacco, though acute exposure to menthol has not been shown to alter nicotine sensitivity (Valentine et al., 2018). Menthol also enhances the rewarding effect of nicotine and may directly facilitate nicotine consumption (Biswas et al., 2016; Wickham, 2019).

Conversely, long term menthol treatment before nicotine exposure decreases nicotine reward-related behavior and the interactions between nicotine and menthol reduce nicotine's reinforcing and positive subjective effects (Henderson et al., 2016; Valentine et al., 2018). Extended exposure to nicotine before exposure to menthol may elicit different interactions between nicotine and menthol because acquisition did not occur simultaneously. This could explain why Nesil and colleagues (2019) found that menthol decreased nicotine reward in their study involving rats that were trained to lever press for nicotine alone before they were separated into test groups, some of which received no nicotine during testing. This indicates that nicotine and menthol may have different effects when present in the blood stream individually compared to the effects that are present when combined.

Nicotine menthol interactions indicate that reduced concentrations of nicotine in cigarettes increases smoking cessation more in nonmenthol smokers than menthol smokers, and menthol smokers experience stronger cravings than nonmentholated smokers (Denlinger-Apte et al., 2019; Fait et al., 2017). Menthol smokers also have a reduced likelihood of successful smoking cessation, and relapse rate is more common with female African American menthol smokers (Smith et al., 2014).

Sensory Effects

Menthol cigarettes have a characteristic cool minty taste which can make them more appealing than nonmentholated cigarettes (Ahijevych & Garrett, 2010). This may enable menthol to facilitate smoking initiation by providing positive early smoking experiences and the minty taste of menthol may contribute as a reinforcer for smoking behavior (Ahijevych & Garrett, 2010).

Menthol can have an analgesic effect whereby it alleviates the irritation in the throat and lungs of first-time smokers, this can lead someone who was merely experimenting with smoking to develop a habit because they do not experience the negative side effects commonly associated with nonmentholated cigarettes (Anderson, 2011; Fait et al, 2017; Lehr et al., 2021). Smoke intake in established smokers may be affected by cigarette mentholation, specifically menthol smokers appear to have higher levels of smoke inhalation than nonmenthol smokers, and menthol also decreases oral nicotine aversion in mice and is correlated with taste and flavor association in human smokers (Fan et al, 2016; Strasser et al., 2013; Watson et al., 2017).

The amount of menthol necessary to begin having slight sensory effects is 0.6 – 1.5 mg menthol/cigarette (Ai et al., 2016). Menthol masks the negative short-term physiological effects

of smoking including throat pain, burning, and cough which provides superficial relief to smokers; however, menthol does not reduce the stinging sensation produced by nicotine in the nose (Anderson, 2011; Renner et al., 2012). Menthol dramatically enhances pharmacological effects of nicotine including decrease in body temperature and analgesia and can act as a counterirritant directly at nAChRs (Alsharari et al., 2015; Hans et al., 2012).

Systemic Effects

Menthol smokers have poorer smoking outcomes, as well as higher expression of nAChRs in the VTA and other reward-related areas than nonmenthol smokers (Brody et al., 2013; Wickham, 2015). Thus, menthol may influence neural responses to nicotine and may play a role in the poorer cessation outcomes menthol smokers experience. Chronic exposure of nicotine and menthol in combination significantly promotes α4β2 nAChR expression in the hippocampus, striatum, and prefrontal cortex of mice, and chronic exposure to menthol alone causes upregulation of these nAChR subtypes in the same brain regions (Alsharari et al., 2015). Chronic nicotine exposure results in increased amounts of nAChRs with a higher affinity for nicotine which leads to changes in reward-related DA release (Henderson et al., 2016). Acute menthol exposure, however, has no effect on nAChR function (Henderson et al., 2016).

Chronic menthol exposure stabilizes lower sensitivity nAChRs and produces upregulation of midbrain α4* nAChRs on VTA DA neurons which may partially negate nicotine's increased phasic DA release (Henderson et al., 2016). Menthol and nicotine in combination produce a significant increase of α4α6β2 nAChRs in the midbrain which is correlated with increased frequency of DA-neuron firing and enhancement of reward related behavior (Henderson et al., 2017). When paired with nicotine, menthol attenuates α3β4 nAChRs in humans through augmented desensitization which has important implications for menthols'

analgesic effects in sensory nerves and may blunt or delay symptoms of nicotine withdrawal (Ton et al., 2015).

Menthol is associated with increased nicotine to cotinine metabolic conversion and inhibits nicotine absorption which could lead to subsequent higher consumption of mentholated cigarettes to achieve the same level of nicotine exposure (Abobo et al., 2011). Menthol smokers also have slower nicotine clearance than nonmenthol smokers which suggests an overall greater exposure to nicotine per cigarette (DeVito et al., 2016). Menthol has an inhibitory effect on nicotine metabolism which is associated with lower urges to smoke and less severe withdrawal symptoms because nicotine remains unmetabolized in the blood stream and can interact with more nAChRs which results in greater desensitization and more DA release (Benowitz et al., 2009; Valentine et al., 2018).

This inhibition of nicotine metabolism could be a possible explanation for the decreased nicotine infusion seeking seen in preclinical studies. Menthol exposed subjects may have less of an incentive to receive more nicotine infusions since there is a greater amount of nicotine in their blood stream compared to nonmenthol exposed subjects that would require more nicotine infusions to attain an equivalent amount of nicotine in the blood stream. When undergoing forced abstinence, menthol smokers also experience less alleviation of short-term abstinence-induced craving than nonmenthol smokers when given intravenous injections of nicotine (DeVito et al., 2016).

Dopamine Release and Nicotine Induced Reward

The mesolimbic DA system, extending from the VTA to the NAc, influences goal directed behaviors, and nicotine augments preexisting goal directed behaviors (Grimm et al.,

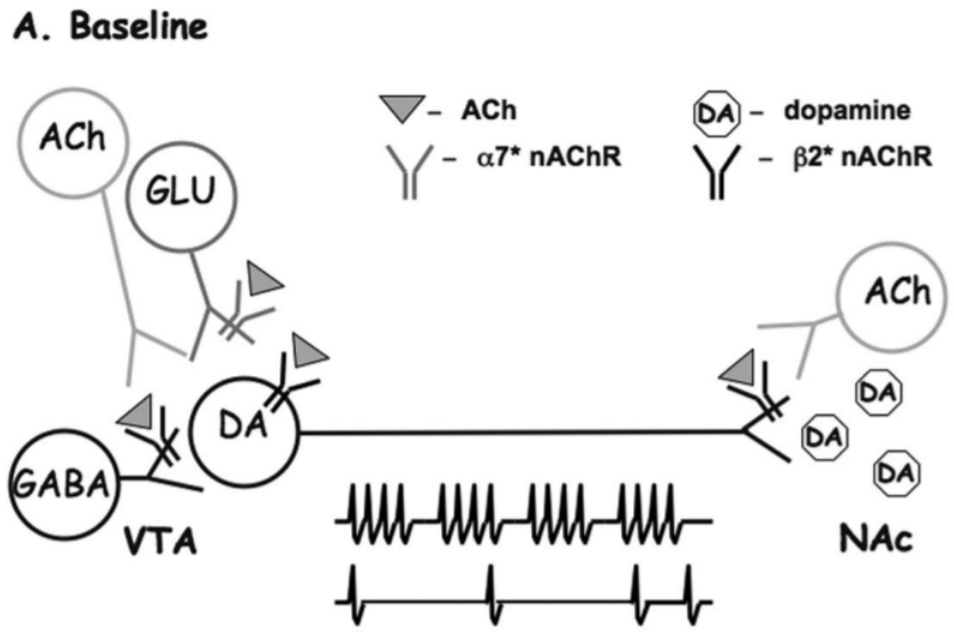
2012; Wickham et al., 2014). DA release results in a pleasurable feeling and is vital to the reinforcing effects of drugs of abuse including nicotine (Benowitz, 2009; Grieder et al., 2019). During tonic, baseline, firing, very little DA is released, however, phasic firing, where high rates of neuron firing occur, there is increased DA release which is typically caused by the activation of excitatory inputs to DA neurons (Picciotto et al., 2008).

Acute nicotine exposure elevates DA release in the NAc and hippocampus (Grimm et al., 2012). Chronic use of nicotine and menthol in combination significantly increases DA levels in the NAc and produces greater reward-related behavior compared to nicotine alone (Henderson et al., 2017; Wickham, 2019; Zhang et al., 2018).

In the aftermath of long-term exposure to nicotine, α4* nAChRs, and sometimes α6* nAChRs, upregulate and exhibit an increased sensitivity to nicotine (Akers et al., 2020). nAChRs on GABA neurons are rapidly desensitized due to their increased sensitivity to nicotine and this desensitization results in the disinhibition of VTA DA neurons and subsequently there is an increase in DA neurotransmission within the mesolimbic pathway (Akers et al., 2020). Excitatory inputs from medial VTA glutamate neurons, where α7 nAChRs are located, are also enhanced, and the combined changes in the inhibitory and excitatory transmission contributes to nicotine induced reward (Akers et al., 2020; Picciotto et al., 2008). β2* nAChRs in the VTA are necessary for GABA and dopaminergic signaling for reward and aversion respectively (Grieder et al., 2019) (see **Figure 1**).

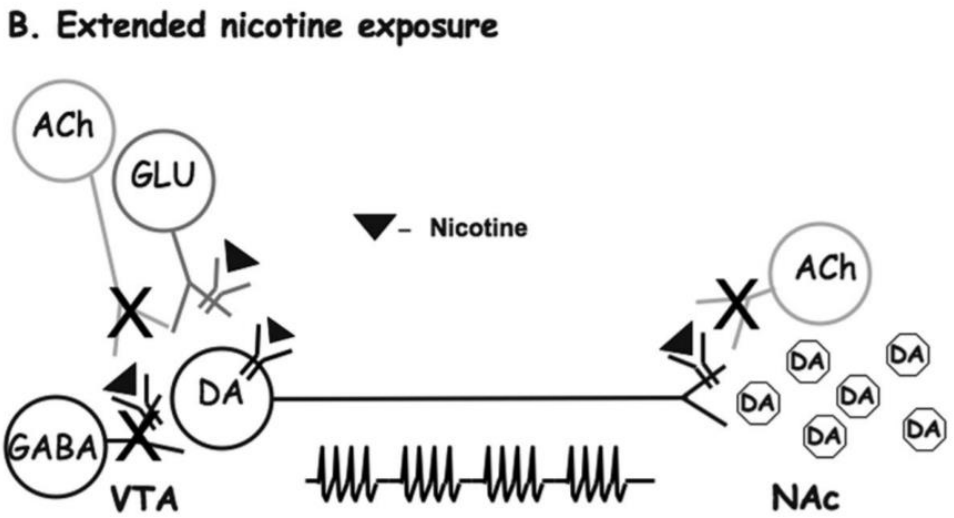
Figure 1

Baseline Firing of Nicotinic Acetylcholine Receptors



Note. Figure 1a shows DA firing before nAChR desensitization. The DA neuron receives excitatory input from β2* receptor activation on the DA neuron itself, as well as α7 receptor activation on glutamate neurons, and the DA neuron receives inhibitory input from β2* receptor activation on GABA neurons. Tonic and phasic firing both occur before nAChR desensitization. (Picciotto et al., 2008)

Firing of Nicotine Acetylcholine Receptors after Extended Nicotine Exposure



Note. Figure 1b shows DA firing after extended exposure to nicotine. β2* nAChRs are desensitized so there is no longer excitatory input from β2* receptor activation on the DA neuron itself and there is no longer inhibitory input from β2* receptor activation on the GABA neuron. DA release is still sustained due to the continued activation of excitatory input from α7 receptor activation on glutamate neurons since α7 nAChRs desensitize much slower than β2* nAChRs. (Picciotto et al., 2008).

Environmental Cues

Environmental cues are external stimuli associated with a certain behavior, such as drug use (Perry et al., 2014). Drug associated cues are important contributors to the effects of the associated drug and can also evoke drug seeking in times of abstinence (Palmatier & Bevins, 2008). These cues also increase DA release in the NAc and are acquired through conditioned reinforcement (Perry et al., 2014; Wickham et al., 2013). Desensitization of nAChRs may enhance responses to and increase the salience of environmental cues that are paired with smoking (Brunzell & Picciotto, 2009). Cues that predict the administration of a reward evoke an increase in phasic DA firing to a greater degree than the expected rewards themselves (Day et al.,

2010). Higher phasic:tonic DA firing ratios, such as those present when nicotine is in the brain, make cues more salient and easier to associate with smoking (Picciotto et al., 2008).

Cues can produce withdrawal symptoms independent of their associated drug in both humans and animals (Perry et al, 2014). Contextual cues are environmental stimuli that are not directly related to the drug taking behavior, but are present in the background of drug taking activities and are therefore important mediators of drug-seeking behavior because the environment has become associated with drug taking (Perry et al., 2014).

Smokers who experience simultaneous cues such as being in a smoking environment and seeing cigarettes burning in an ashtray experience cue-induced craving and increased smoking behavior (Conklin et al., 2019). Conditioned reinforcers such as flavorants, notably menthol, can act as orosensory cues which are important for the self-administration of tobacco products in humans (Palmatier et al., 2020; Wang et al., 2014; Wickham, 2019). Menthol can have an occasion setting effect wherein the presence of menthol can act as a cue for nicotine self-administration, independent of TRPM8 receptor activity (Harrison et al., 2017).

Smoking Demographics

Sex and age dependent factors may underlie the influence of menthol on nicotine intake. Fait and colleagues (2017) found that adult male mice significantly increased nicotine intake when it was paired with menthol, whereas adult female mice did not show this preference. The adult male mice in this study also exhibited decreased locomotion whereas this behavioral response to menthol was not observed in any of the adolescent mice. Ross and colleagues (2016) found that, in human smokers, puff characteristics of individual cigarettes are indicative of daily nicotine intake and factors including sex and menthol status were significant predictors of daily

nicotine exposure. African Americans, females, lower socioeconomic status individuals, and adolescents are more likely to use mentholated cigarettes (Ahijevych & Garrett, 2010; Foulds et al., 2010; Ross et al., 2016; Wickham, 2015; Wickham, 2019). Among menthol smokers, young adults and members of the LGBTQIA+ community are less likely to have an intention to quit smoking (Smiley, 2018).

Marketing for menthol cigarettes has historically targeted African Americans, women, and young populations (Lee & Glantz, 2011; Smith et al., 2020; Wickham, 2019). Younger populations may be drawn to menthol cigarettes and this may result in menthol cigarettes acting as a "starter product" which can lead to the development of a smoking habit (Ahijevych & Garrett, 2010; Lee & Glantz, 2011). Menthol smokers who are young and in a racial or ethnic minority have a lower quit rate than nonmenthol smokers (Ahijevych & Garrett, 2010; Foulds et al., 2010).

The Present Studies

The purpose of Study 1 was to examine if menthol alone alters phasic DA release given that menthol can influence nicotine receptor expression. Furthermore, this study examined whether menthol could influence nicotine's ability to drive DA release. The first hypothesis was that nicotine alone would significantly increase DA release relative to baseline, given that previous studies have shown similar results (Picciotto et al., 2008). The second hypothesis was that menthol alone would not increase DA levels relative to baseline given that acute menthol has no effect on nAChR function (Henderson et al., 2016). And the third hypothesis was that when combined with nicotine menthol would have an additive effect on DA release in the NAc relative to baseline, given that menthol increases nicotine self-administration in preclinical studies and widens the dose response curve of nicotine (Biswas et al., 2016; Wickham, 2019).

Cues are potent drivers of relapse to drugs, and menthol can act as a cue as well as enhance the rewarding value of other nicotine paired cues (Wickham, 2019). Study 2 aimed to examine if menthol increases nicotine's effect on the salience of environmental cues in human smokers. More specifically, it was hypothesized that menthol smokers would have stronger urges to smoke associated with environmental cues, menthol smokers would have a more difficult time quitting than nonmenthol smokers, and that menthol smokers would be more likely to use a smoking cessation tool than nonmenthol smokers.

Study 1

Methods

Subjects

Twenty male Sprague-Dawley rats (250-300g) from Charles River Laboratories were housed 2 to 3 per cage and provided *ad libitum* food and water on a 12-hour light/dark cycle beginning at 7 am. Four subjects were removed from analysis due to unreliable data recordings and an additional subject was removed from analysis due to insufficient data collection. All experiments were conducted according to the Guide for the Care and Use of Laboratory Animals and were approved by the Yale University Institutional Animal Care and Use Committee.

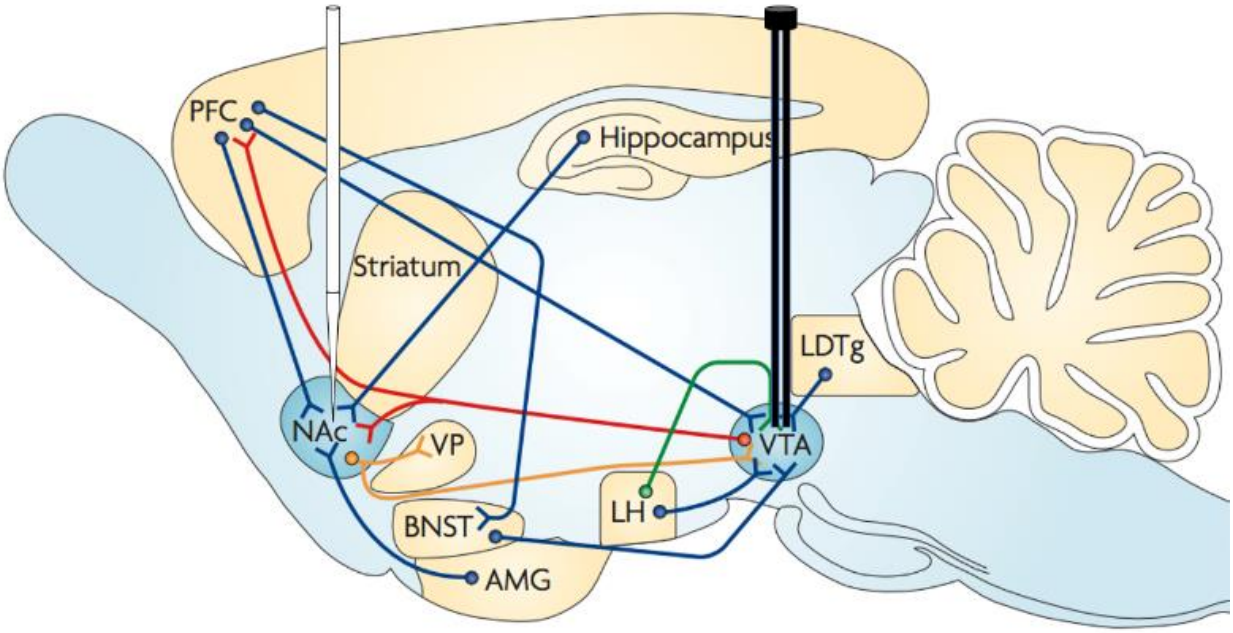
Surgery

Four small holes were drilled into the skulls of anesthetized rats for the insertion of a bipolar stimulator retrofitted with a cannula, a carbon microfiber electrode, a reference wire, and a screw to secure the reference electrode. Anteroposterior (AP), mediolateral (ML), and dorsoventral (DV) coordinates were referenced from bregma. A bipolar, stainless steel stimulating electrode was inserted into the VTA (AP −5.2 mm, ML 0.5-1.5 mm, DV from 7.4 to

8.1 mm below dura). An Ag/AgCl reference electrode was inserted into the contralateral cortex and was held with a screw attached to the skull to minimize the number of manipulators used. The pia matter was punctured and removed, and a carbon-fiber microelectrode was implanted vertically in the NAc core (AP +1.2 mm, ML −1.4 mm, DV from 6.2 to 6.9 mm) (see **Figure 2**).

Figure 2

Placement of Stimulating and Recording Electrodes in Rat Brains



Note. The stimulating electrode (black) was placed in the VTA to stimulate the release of DA in anesthetized rats and the recording electrode (white) was placed in the NAc to record DA release (Kauer & Malenka, 2007).

Fast-Scan Cyclic Voltammetry (FSCV)

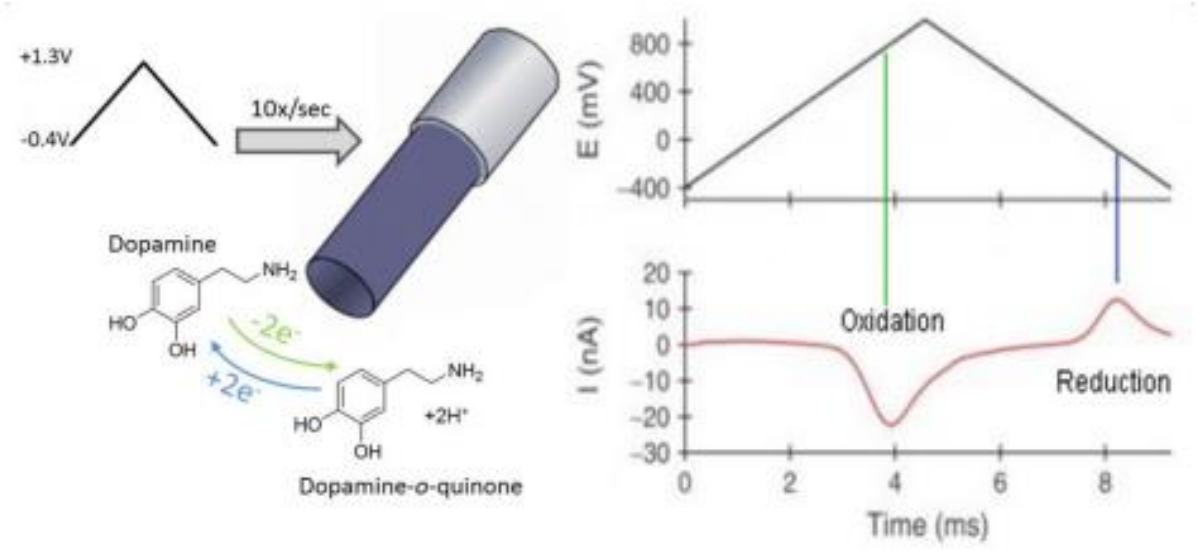
Electrical stimulation (300µA, 60Hz, 24 pulses) was applied to the VTA using a bipolar electrode to evoke phasic DA release in the NAc core of the anesthetized subjects. Each

stimulation was applied every 3 minutes so DA releasable stores could return to their original levels.

FSCV is a method of examining neurotransmitter signaling within the brain that provides high chemical selectivity and temporal resolution (Wickham et al., 2014). During FSCV current is generated at different potentials and the redox reactions for different neurotransmitters are recorded and displayed with distinct peaks for oxidation and reduction (Wickham et al., 2014). Dopamine is absorbed onto the FSCV carbon microfiber electrode and the electrode records the oxidation of DA into DA-orthoquinone and the subsequent reduction of DA-orthoquinone into DA (see **Figure 3**).

Figure 3

The Methodology of Fast-Scan Cyclic Voltammetry



Note. DA is absorbed onto the carbon fiber microelectrode, at 0.6V DA is oxidized into DA-orthoquinone and at -0.2V reduced back into DA, voltage is cycled every 100ms (Foster, 2014).

Experimental Design and Data Analysis

Six baseline recordings of DA release were recorded from each subject. Intraperitoneal (IP) injections of nicotine (n = 8, 0.35mg/kg in 0.09% saline) or menthol (n = 7, 400mg/kg in 5% DMSO, 45% Tween, and 50% vehicle) were then administered and DA measurements continued for 30 minutes. In five rats that received IP nicotine injections, IP menthol injections were administered 30 minutes later, and DA measurements were collected for an additional 30 minutes.

All FSCV recordings were converted to percent baseline for analysis. A one-way Analysis of Variance (ANOVA) was conducted to analyze the effect of time on each drug condition. A two-way within subjects ANOVA was conducted to analyze the effects of menthol when nicotine was already in the system, and a two-way mixed-model ANOVA was conducted to analyze the effects of nicotine versus menthol over time.

Results

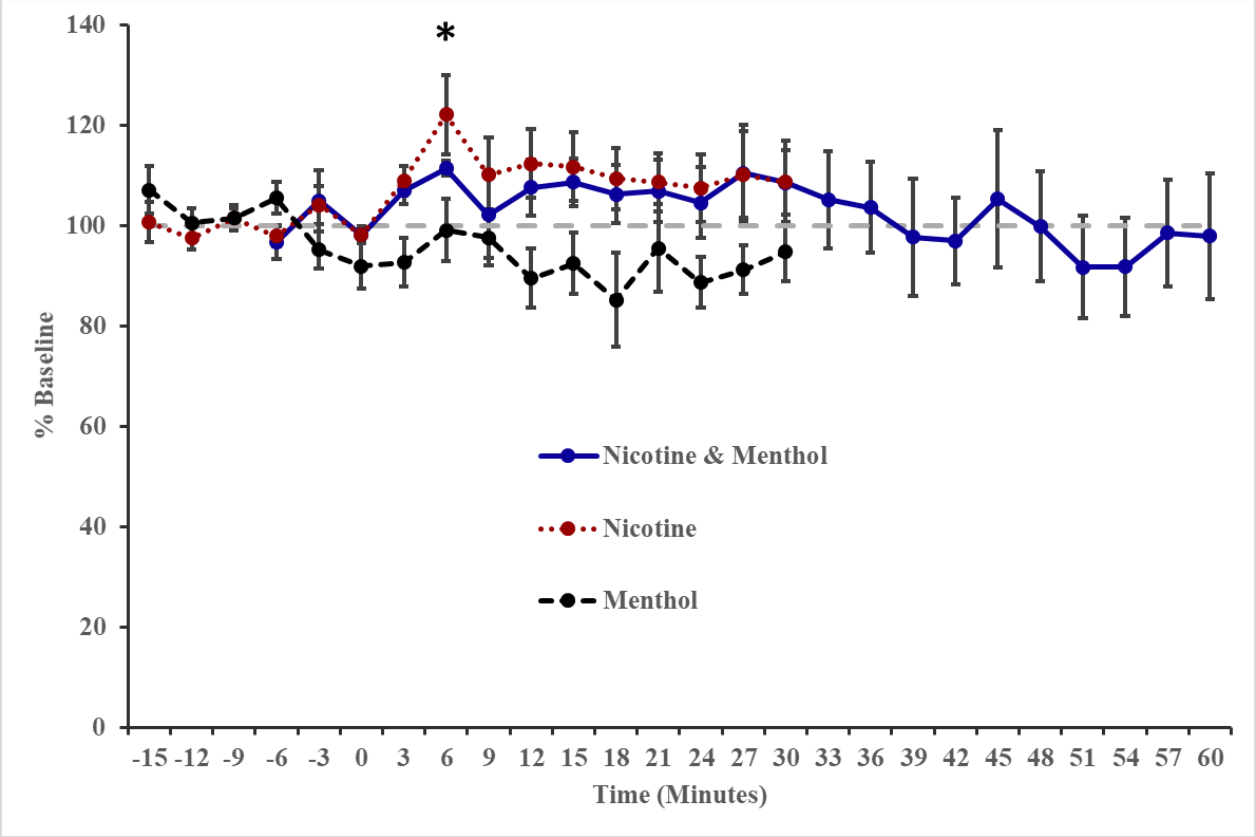
A one-way ANOVA revealed that there was a significant effect of time on nicotine exposure alone, $F(15, 105) = 2.056$, $p = 0.018$. Bonferroni's post-hoc test showed significance from baseline to six minutes post-injection, $p = 0.0034$ (see **Figure 4**). A one-way ANOVA revealed that there was no significant effect of time on menthol exposure alone $F(15, 90) = 1.581$, $p = 0.095$ or nicotine and menthol exposure combined $F(12, 48) = 1.270$, $p = 0.267$.

A two-way within subjects ANOVA on nicotine and menthol in combination revealed that there was no significant effect of time $F(12, 48) = 0.547$, $p = 0.872$, drug $F(1, 4) = 0.611$, $p = 0.478$, or time x drug $F(12, 48) = 1.304$, $p = 0.248$. A two-way between subjects ANOVA on nicotine alone and menthol alone revealed that there was no significant effect of time $F(15, 195)$

= 1.061, p = 0.396, however, there was a significant effect of drug $F(1, 13) = 5.946$, p = 0.030, and there was a significant effect of time x drug $F(15, 195) = 2.544$, p = 0.002.

Figure 4

Effects of Nicotine and Menthol Exposure on Phasic Dopamine Release in the Nucleus Accumbens



Note. Baseline recordings were taken before the administration of each drug condition: nicotine (n = 8), menthol (n = 7), and nicotine and menthol (n = 5). DA levels were recorded for 30 minutes post drug exposure. After 30 minutes, menthol was administered to a subset of 5 subjects from the nicotine condition and the subsequent DA levels were recorded for an additional 30 minutes and were compared to the last 3 DA recordings in the nicotine condition to

examine any additive effects of menthol when nicotine is already present in the system. Data are presented as the mean ± SEM. * = $p < 0.05$

Discussion

Study 1 explored whether acute menthol exposure could influence phasic DA release in the NAc. The results of this study confirm that nicotine enhances phasic DA release in the NAc, however, menthol alone does not increase DA release, nor does menthol have any additive effects on DA release in conjunction with nicotine.

In the current literature, systemic changes in nAChR expression are found after exposure to chronic menthol (Henderson et al., 2016; Henderson et al., 2017; Ton et al., 2015). While the results did not indicate significant changes in DA levels in this study, this could be because acute menthol exposure was utilized rather than chronic menthol exposure. It is possible that acute menthol has no significant systemic reactions, but rather is more important for the acquisition of nicotine consumption because of its ability to mask the aversive taste of nicotine and to coat the lungs so smoke inhalation is more tolerable (Wickham, 2019).

Nicotine is the primary psychoactive ingredient in tobacco cigarettes, it produces euphoric effects and is the driving force behind tobacco addiction (Wickham, 2015). Menthol alone has no euphoric effects but is able to facilitate tobacco addiction through enhancing the appeal of nicotine products and making them available to larger populations (Wickham, 2015). It is possible that acute menthol exposure does not affect the rewarding properties of nicotine and tobacco consumption and acute menthol exposure is instead more important for the initial acquisition of smoking behavior.

These data speak to acute effects of menthol and nicotine, not chronic effects of menthol where many nAChR effects are observed. Keeping this in mind, one major limitation to Study 1 is the small sample size, most notably that only five rats received nicotine and then menthol to examine potential additive effects. Future studies should examine menthol's influence on DA release in the NAc using at least ten rats per experimental condition and more conditions should be examined to gain a better understanding for how menthol interacts with DA release alone and in combination with nicotine. These conditions should include acute menthol exposure, acute nicotine exposure, acute menthol exposure after acute nicotine exposure to examine any additive effects, acute menthol and nicotine exposure simultaneously to examine combined effects, as well as chronic effects of menthol and nicotine exposure with each condition.

These results imply that the role of menthol in nicotine and tobacco addiction is more complex than simply increasing DA release, leading to a greater feeling of pleasure. Nicotine produces rewarding behaviors through increased DA release, which implies that nicotine is the driving force behind tobacco addiction. The role of menthol in tobacco addiction appears to be more complex and requires further research to be fully understood.

Study 2

Study 2 compared smoking behavior and motivations between menthol and nonmenthol preferring smokers. It was hypothesized that menthol smokers would have stronger urges to smoke associated with environmental cues, more difficulty quitting, and be more likely to use a smoking cessation tool than nonmenthol preferring smokers.

Methods

Participants

One hundred participants, 42 menthol smokers and 58 nonmenthol smokers, completed a five-minute online survey about their smoking preference and behavior. Data from three respondents were removed from analysis because when asked "at what age did you begin using nicotine products" they responded with "never." Of the 100 respondents that were included in analysis, 22 were 18-24, 15 were 25-39, 46 were 40-59, and 17 were 60+; 93 were White, 3 were Black/African American, 1 was Latino/Hispanic, and 3 classified themselves as "other"; 75 were female, 22 were male, and 3 were nonbinary; 2 had less than a high school degree, 20 had a high school degree or equivalent, 5 had a trade school degree, 26 had some college but no degree, 9 had an Associate Degree, 23 had a Bachelor's Degree, 13 had a Master's Degree, and 2 had a PhD or higher; 10 lived in an urban area, 31 lived in a suburban area, and 59 lived in a rural area; 58 grew up in a smoking household and 42 grew up in a nonsmoking household; and 7 believed menthol cigarettes are less dangerous than nonmenthol cigarettes.

Participants were recruited from different social media platforms utilizing the snowball effect method. One initial request was sent out, to take the survey if eligible or to share the survey link, and participants were recruited through subsequent reposts of the initial request. This study was approved by the Elizabethtown College Institutional Review Board in February 2021.

Design

Participants were asked to respond to yes/no, fill in the blank, and multiple-choice questions to understand each individual's background and smoking preference. These questions included "have you smoked in the last 30 days?" (yes/no), "I prefer to smoke _____(menthol/nonmenthol) cigarettes", "Have you ever smoked mentholated cigarettes?" (yes/no), "do you currently smoke tobacco on a daily basis, less than daily, or not at

all?", "have you smoked tobacco daily in the past?" (yes/no), "in the past, have you smoked tobacco on a daily basis, less than daily, or not at all?", "have you ever wanted to quit smoking?" (yes/no), "have you ever attempted to quit smoking?" (yes/no), "have you ever successfully quit smoking?" (yes/no), "how many times have you attempted to quit smoking?" (1-6+), "how many times have you begun smoking again after attempting to quit?" (1-6+), "have you ever used a smoking cessation tool (i.e. Chantix [Varenicline] or Zyban [Bupropion]) or nicotine replacement therapy (i.e. nicotine patch, gum, spray, lozenge, inhaler, etc.)?" (smoking cessation tool, nicotine replacement therapy, both, neither), and "during the past 12 months, have you tried to stop smoking?" (yes/no).

Participants were then asked to respond to statements using a five-point Likert scale, where 1 indicated *strongly disagree,* 2 indicated *disagree,* 3 indicated *neutral,* 4 indicated *agree,* and 5 indicated *strongly agree,* in order to understand how environmental cues may impact the smoking behaviors of each individual. Likert scale statements included: "when I am in a specific environment, I feel a stronger urge to smoke", "I feel the urge to smoke in inappropriate places", "I often feel the urge to smoke when I see a cigarette", "I smoke to feel better", "I smoke to feel good", "I smoke because I am bored", "I smoke to relieve cravings", "I smoke to relieve withdrawal symptoms", "I often feel the urge to smoke when I see an ashtray", "I smoke without thinking about it", "Smoking feels like a habit", "I often feel the urge to smoke when I see a lighter", and "I often smoke in the same location"; some of these questions were taken from a scale used by Newton and colleagues (2009) to examine theories of addiction (Cronbach's α = 898).

Demographics collected included current age, the age participants began using nicotine products, race, gender, education level, community type, if the participant grew up in a smoking

or nonsmoking household, and if the participant believes menthol cigarettes are less dangerous than nonmentholated cigarettes.

An independent samples t-test was run to examine the relationship between menthol status and environmental cues and to examine the relationship between menthol status and age of acquisition, and chi-square test of association tests were run to examine the relationships between menthol status and the frequency of nicotine consumption, successful quit rate, utilization of smoking cessation tools, and demographics.

Results

An exploratory factor analysis revealed three main categories when examining the relationship between menthol status and the reasons behind smoking behavior: emotion, urges, and environment (see **Table 1**). An independent samples t-test revealed no significant difference in emotion driving smoking behavior between menthol (M = 3.17, SD = 1.02) and nonmenthol smokers (M = 2.94, SD = 1.24), $t(98) = 0.994$, $p = 0.323$, $d = 0.201$; no significant difference in urges driving smoking behavior between menthol (M = 2.83, SD = 1.19) and nonmenthol smokers (M = 3.05, SD = 1.33), $t(98) = -0.868$, $p = 0.388$, $d = -0.176$; and no significant difference in environment driving smoking behavior between menthol (M = 1.68, SD = 0.999) and nonmenthol smokers (M = 1.94, SD = 1.11), $t(98) = -1.21$, $p = 0.230$, $d = -0.245$ (see **Figure 5**). An independent samples t-test revealed no difference age of acquisition between menthol (M = 16.6, SD = 3.72) and nonmenthol smokers (M = 16.6, SD = 5.48), $t(97) = -0.0307$, $p = 0.976$, $d = -0.00625$ (see **Figure 6**).

Table 1

Exploratory Factor Analysis of the Variables Related to Smoking Behavior

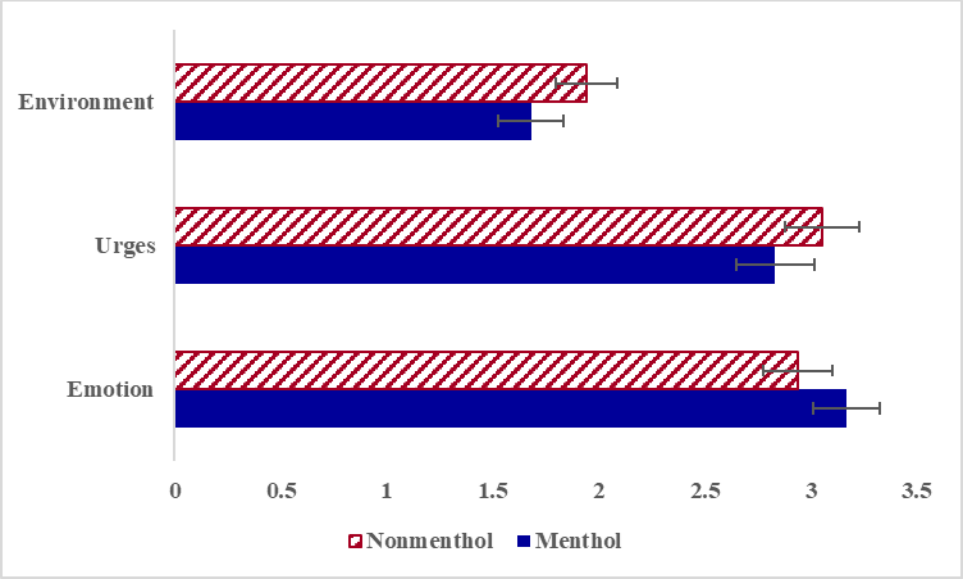
Factor Loadings

	Factor			
	1	**2**	**3**	**Uniqueness**
When I am in a specific environment, I feel a stronger urge to smoke.	0.568			0.6148
I feel the urge to smoke in inappropriate places.				0.6869
I often feel the urge to smoke when I see a cigarette.				0.4799
I smoke to feel better.	0.718			0.3612
I smoke to feel good.	0.895			0.2198
I smoke because I am bored.	0.616			0.3841
I smoke to relieve cravings.		0.607		0.4166
I smoke to relieve withdrawal symptoms.		0.619		0.4801
I often feel the urge to smoke when I see an ashtray.			0.719	0.3448
I smoke without thinking about it.		0.778		0.4323
Smoking feels like a habit.		0.641		0.4219
I often feel the urge to smoke when I see a lighter.			0.971	0.0479
I often smoke in the same location.				0.5945

Note. 'Minimum residual' extraction method was used in combination with an 'oblimin' rotation. Factor loadings below 0.5 were excluded from analysis. Factor 1 indicates variables in the Emotion category, Factor 2 indicates variables in the Urges category, and Factor 3 indicates variables in the Environment category.

Figure 5

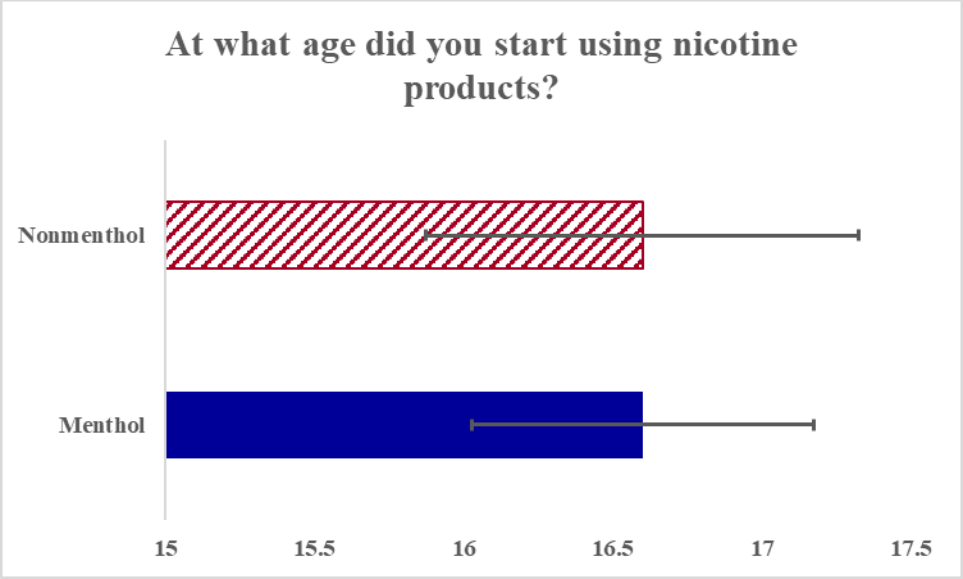
Independent Samples T-Test Examining the Motivation Behind Smoking Behavior



Note. Higher numbers are indicative of stronger motivations to smoke. Data is displayed as mean ± SEM.

Figure 6

The Relationship of Menthol Status with Age of Acquisition

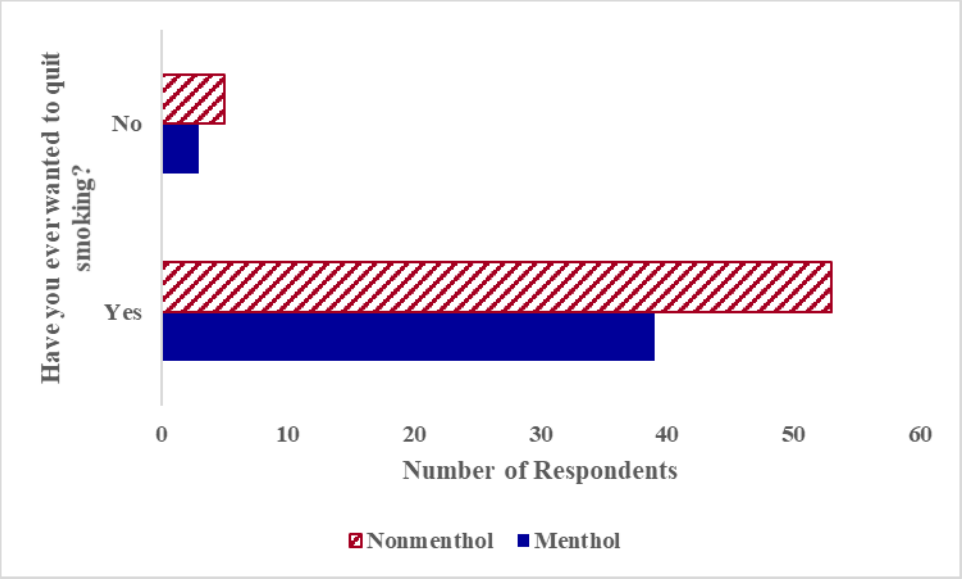


Note. Data is displayed at mean ± SEM.

Chi-square test of association were run to examine the relationship between menthol status and smoking behavior. There was no relationship between menthol status and desire to quit smoking, χ^2 (1, N = 100) = 0.072, p = 0.788, Cramer's V = 0.027 (see **Figure 7**). There was no relationship between menthol status and successful quit rate, χ^2 (1, N = 100) = 0.493, p = 0.483, Cramer's V = 0.070 (see **Figure 8**). There was no relationship between menthol status and number of attempts to quit smoking, χ^2 (6, N = 100) = 4.15, p = 0.656, Cramer's V = 0.204 (see **Figure 9a**), or number of times relapsing after attempting to quit, χ^2 (6, N = 100) = 2.43, p = 0.877, Cramer's V = 0.156 (see **Figure 9b**). There was no relationship between menthol status and utilization of a smoking cessation tool, χ^2 (3, N = 100) = 1.50, p = 0.682, Cramer's V = 0.123 (see **Figure 10**). There was no relationship between menthol status and smoking daily, χ^2 (1, N = 100), p = 0.116, Cramer's V = 0.157 (see **Figure 11**). There was no relationship between menthol status and if participants believed menthol cigarettes are less dangerous than nonmentholated cigarettes, χ^2 (1, N = 100) = 0.00227, p = 0.962, Cramer's V = 0.00476 (see **Figure 12**).

Figure 7

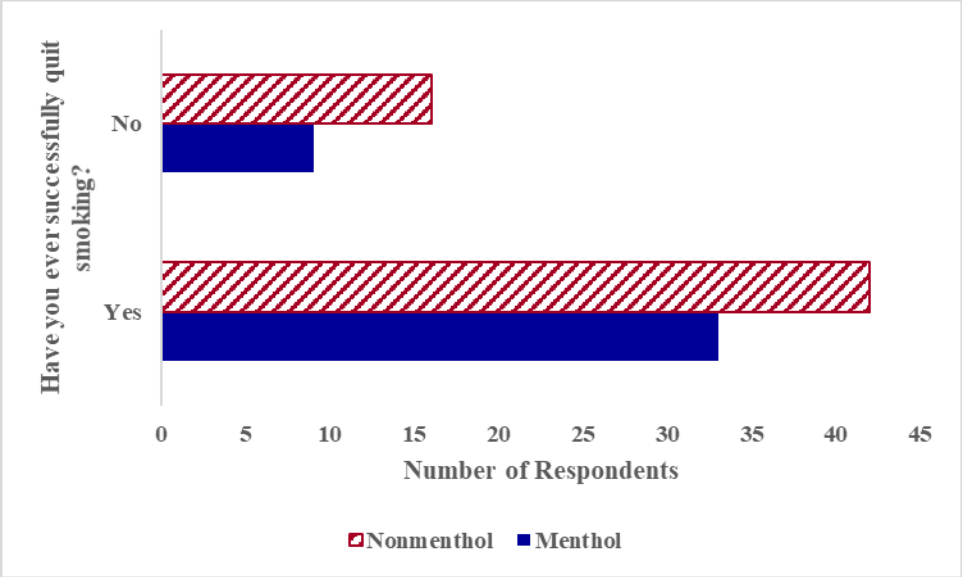
Menthol Status Differences in Desire to Quit Smoking



Note. Data is displayed as the mean of each group.

Figure 8

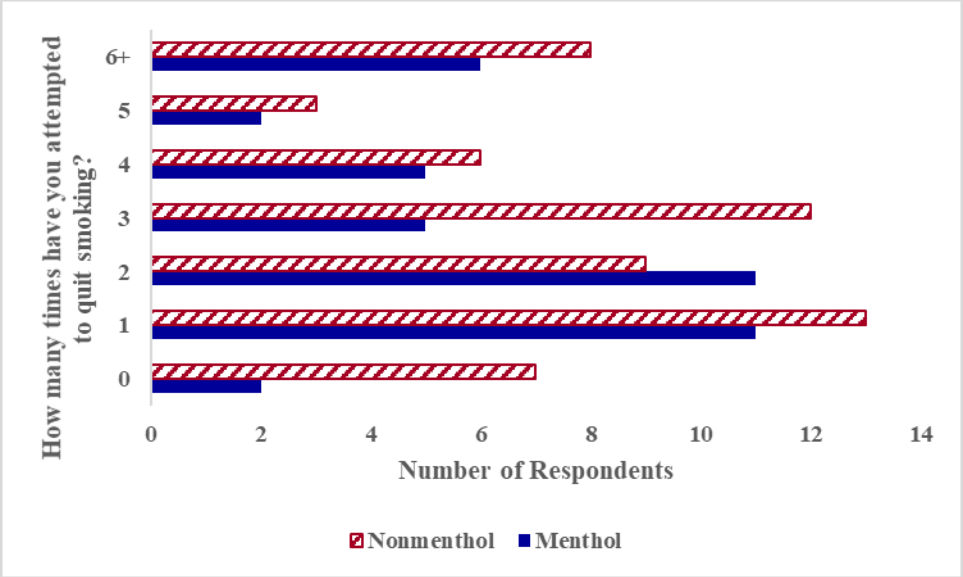
Menthol Status Differences in Successful Smoking Cessation



Note. Data is displayed as the mean of each group.

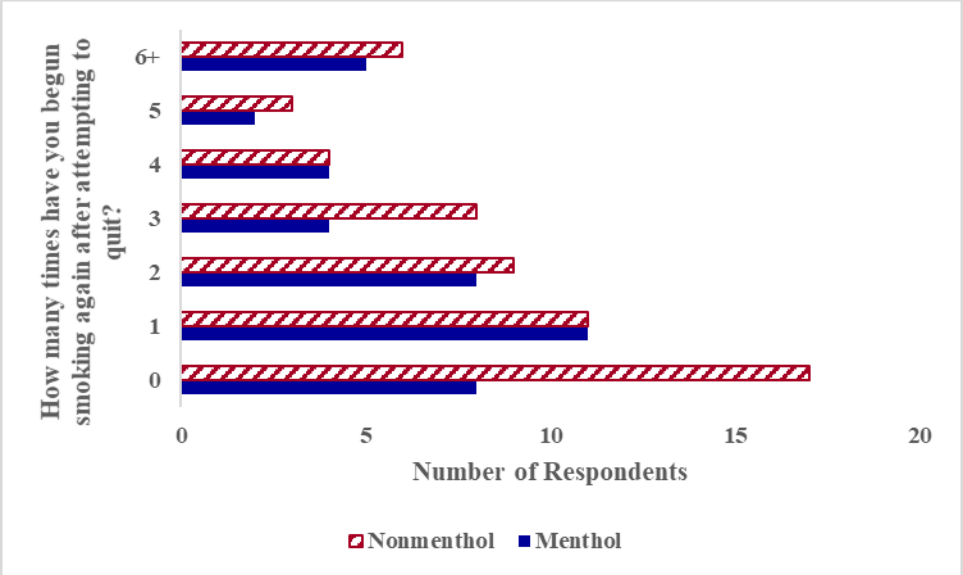
Figure 9

Menthol Status Differences in Number of Attempts to Quit



Note. Figure 9a: Data is displayed as the mean of each group.

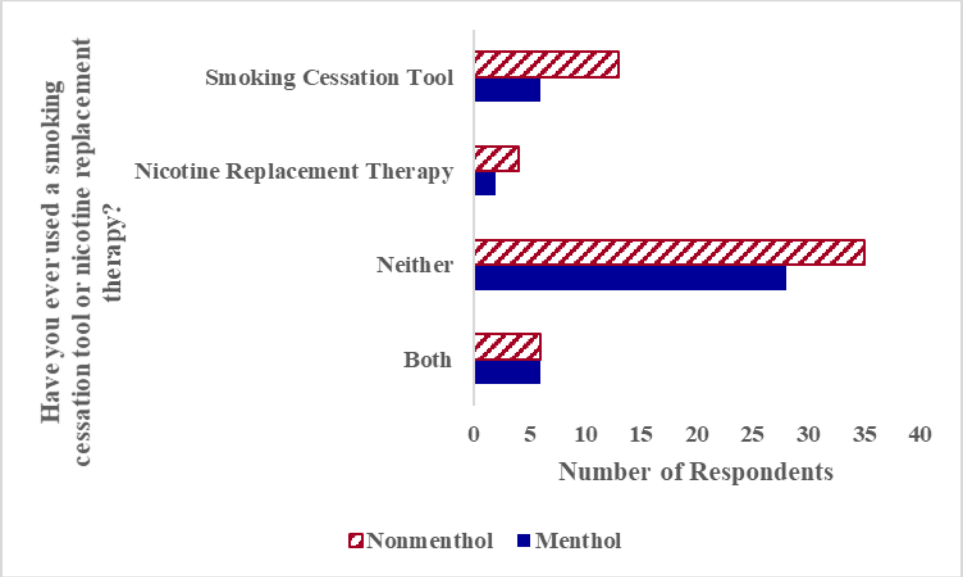
Menthol Status Differences in Number of Relapses



Note. Figure 9b: Data is displayed as the mean of each group.

Figure 10

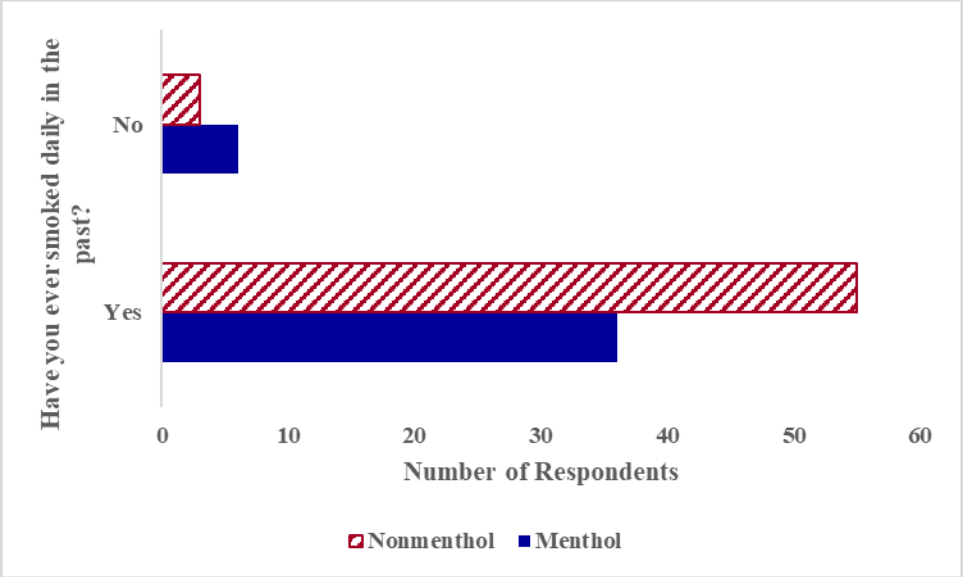
Menthol Status Differences in Use of a Smoking Cessation Tool



Note. Data is displayed as the mean of each group.

Figure 11

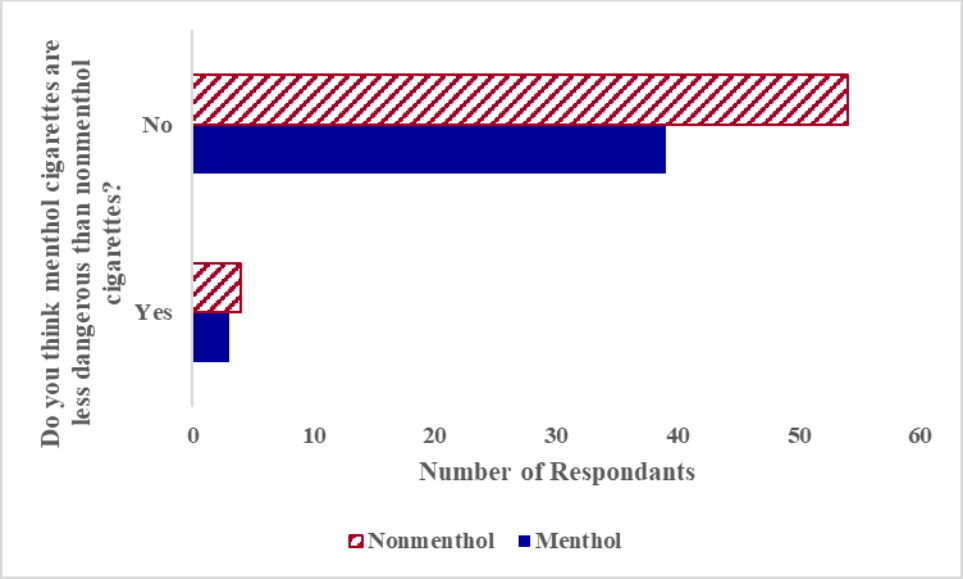
Menthol Status Differences in Smoking Daily



Note. Data is displayed as the mean of each group.

Figure 12

Menthol Status Differences in Opinion on if Menthol Cigarettes are Less Dangerous than Nonmenthol Cigarettes



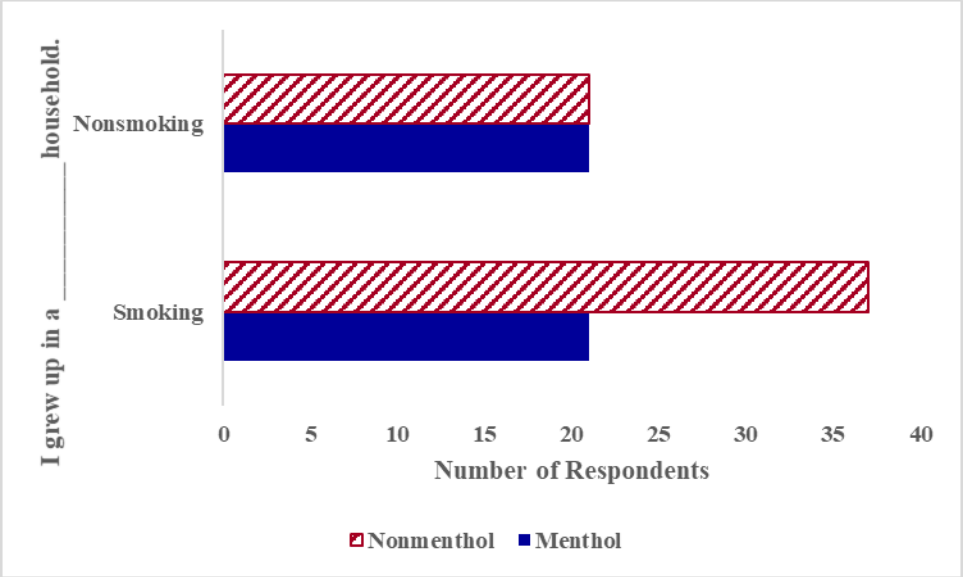
Note. Data is displayed as the mean of each group.

Chi-square test of association were also run to compare menthol status within each demographic collected. There was no relationship between menthol status and growing up in a smoking or nonsmoking household, χ^2 (1, N = 100) = 1.90, p = 0.168, Cramer's V = 0.138 (see **Figure 13**). There was no relationship between menthol status and type of area participants lived in, χ^2 (2, N = 100) = 0.610, p = 0.737, Cramer's V = 0.078 (see **Figure 14**). There was no relationship between menthol status and the highest level of education participants had completed, χ^2 (7, N = 100) = 13.9, p = 0.054, Cramer's V = 0.372 (see **Figure 15**). There was no relationship between menthol status and gender, χ^2 (2, N = 100) = 2.72, p = 0.256, Cramer's V = 0.165 (see **Figure 16**). There was no relationship between menthol status and race, χ^2 (5, N =

100) = 5.01, $p = 0.415$, Cramer's V = 0.224 (see **Figure 17**). There was a relationship between menthol status and age, χ^2 (3, $N = 100$) = 21.7, $p < .001$, Cramer's V = 0.466 (see **Figure 18**).

Figure 13

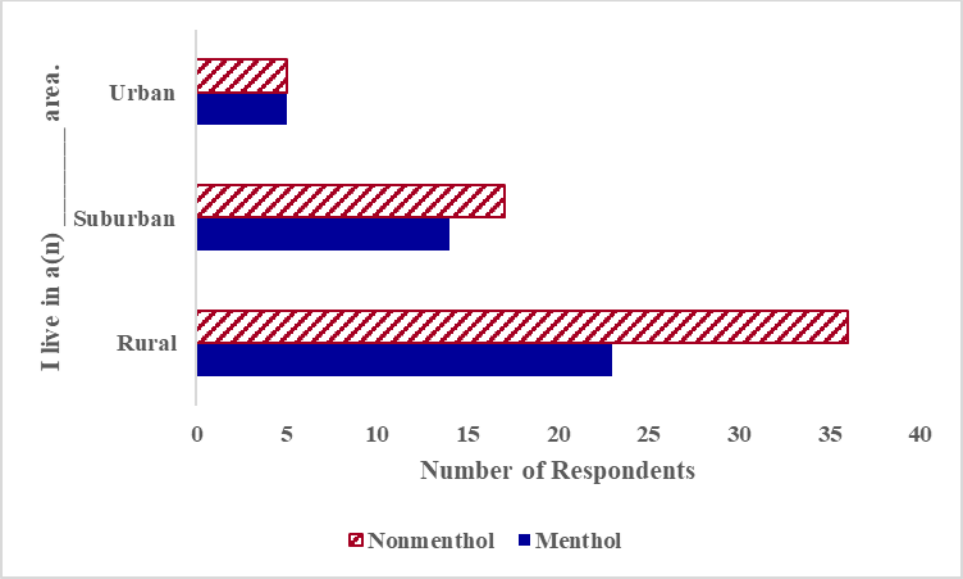
Menthol Status Differences in Household Environment Growing Up



Note. Data is displayed as the mean of each group.

Figure 14

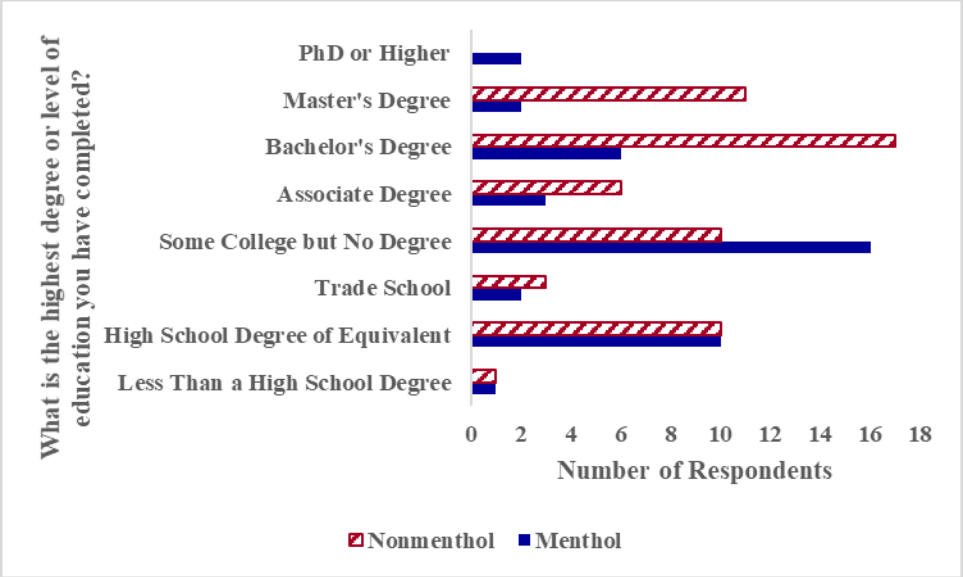
Menthol Status Differences in Participant Living Location



Note. Data is displayed as the mean of each group.

Figure 15

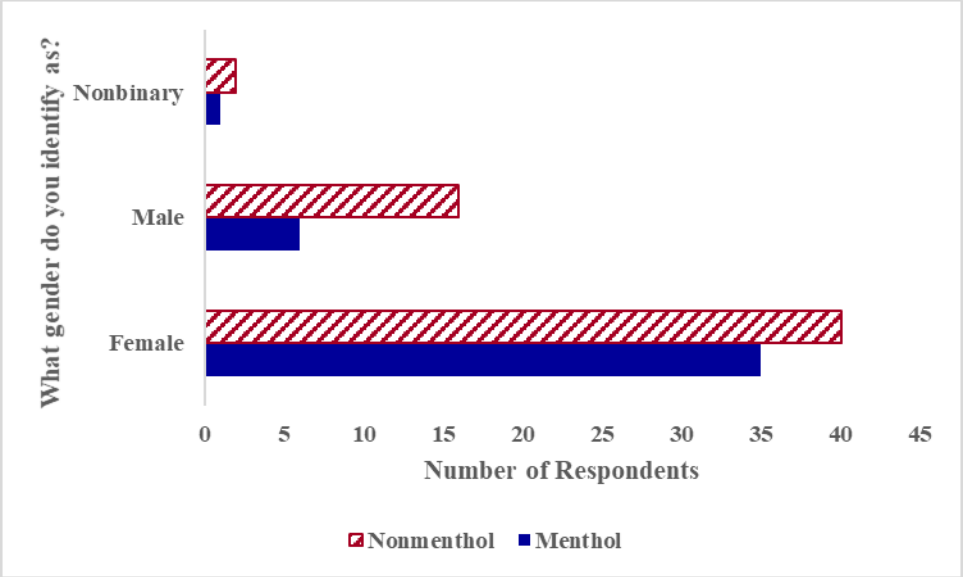
Menthol Status Differences in Level of Education



Note. Data is displayed as the mean of each group.

Figure 16

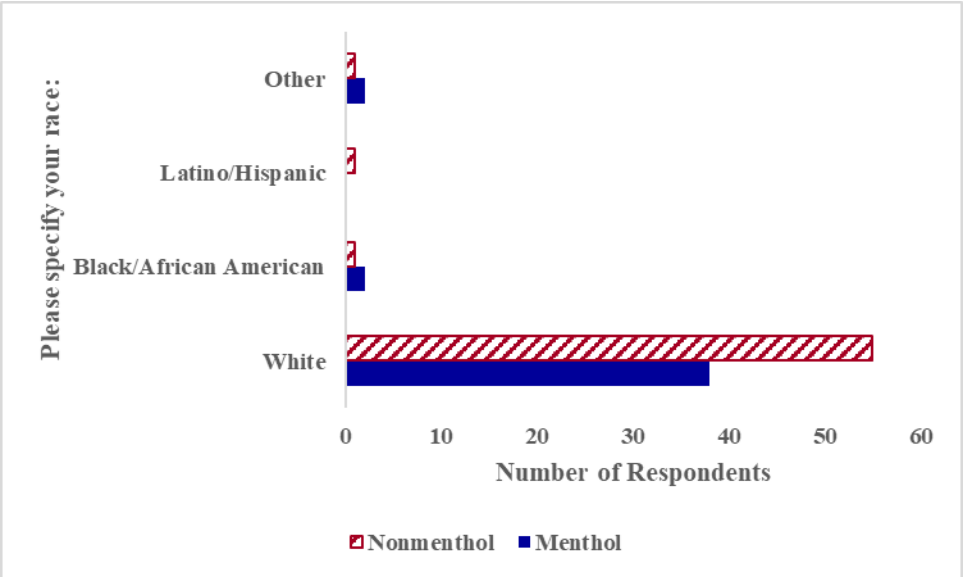
Menthol Status Differences in Gender



Note. Data is displayed as the mean of each group.

Figure 17

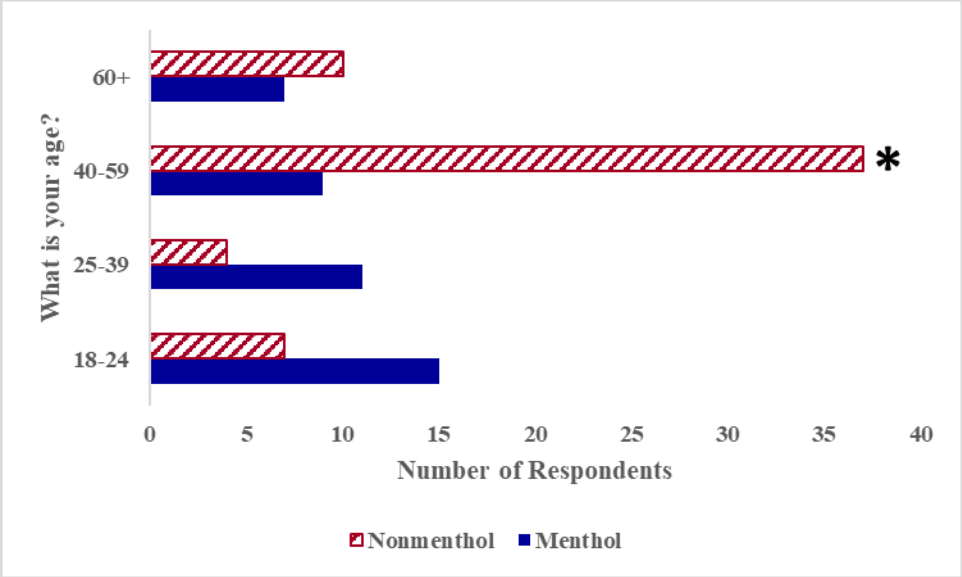
Menthol Status Differences in Race



Note. Data is displayed as the mean of each group.

Figure 18

Menthol Status Differences in Age



Note. There were significantly more nonmenthol smokers aged 40-59 than menthol smokers. Data is displayed as the mean of each group. * = $p < .001$

Discussion

While the results from Study 2 do not support the hypothesis that menthol smokers would have stronger associations with environmental cues and would have a more difficult time with smoking cessation, these results do have interesting implications. Abstinence outcomes are independent of menthol status which implies that menthol status does not affect the rate of successful smoking cessation, although a recent meta-analysis from Smith and colleagues (2020) indicates this may only be in Black populations (Jao et al., 2017). Pre-existing differences, including biological differences, between menthol preferring and non-preferring smokers may influence their perception of nicotine as well as their response to short term abstinence programs (DeVito et al., 2016). This suggests that individual differences contribute more to successful

smoking cessation than menthol status itself, and furthermore implies that there may be biological reasons behind why an individual prefers mentholated cigarettes to nonmentholated cigarettes and it may be those reasons, not the menthol itself, that leads to less success with smoking cessation that is seen in established literature (DeVito et al., 2016; Fait et al., 2017; Henderson et al., 2017; Wickham, 2019). It is possible that future research on this could lead to enhancements in smoking cessation interventions.

Results did indicate that individuals who are 40-59 years old are more likely to smoke nonmenthol cigarettes than menthol cigarettes. This makes sense within the current literature because menthol cigarettes are marketed toward younger populations (Lee & Glantz, 2011).

Nicotine is reinforcing on its own. Menthol may not necessarily increase how reinforcing nicotine is, but it can blunt the positive responses to nicotine and is correlated with less severe nicotine withdrawal after overnight nicotine deprivation (Valentine et al., 2018). Lifetime smokers exhibit no acute effects of menthol, which indicates that menthols' effects appear to be more long term in which the plasticity of the brain is altered via changes in nAChRs (Valentine et al., 2018; Wickham, 2019). It is possible that there is a ceiling effect wherein the effects of nicotine are so potent that menthol does not have the ability to create additive effects in established smokers, similar to how there is a decline in cue reactivity of established smokers due to a ceiling effect (Karelitz, 2020).

Keeping this in mind, it is important to address the limitations of this study. There are limitations in the design of the online study. Most questions were not taken from a questionnaire that has a known reliability, instead they were made by the author. Questions that target more specific behavior of menthol versus nonmenthol preferring smokers could have been asked and participants could have been directed to more specific questions depending on their responses to

questions about their menthol status. Specifics should have been asked about environmental cues as well, for example after each Likert scale question was answered there could have been a follow up question asking participants to explain their responses, for example what specifically makes them feel a stronger urge to smoke. The questions that were ultimately asked were general in nature and it would have been interesting to see if there were any commonalities between menthol status if participants were asked to elaborate for themselves.

Future studies should examine smoking behavior throughout the duration of an individual's time smoking cigarettes. Questions should examine motivation behind beginning to smoke, current smoking behaviors, attitudes toward menthol and nicotine in general, and intentions toward smoking cessation. I would also be interesting to conduct personal interviews with participants to gather more information from participants in an open-ended manner. It is possible that environmental cues related to smoking behavior may operate nonconsciously which would mean participants could not report their reactions to these cues themselves. Instead, a behavioral measure could be used to examine if this is the case. For a follow-up like this, menthol and nonmenthol smokers would both experience the same cue, for example watching a video of someone lighting a cigarette, and each participant would be asked to rate their craving for a cigarette before and after the cue is administered. Overall, it is important to note that while there was no difference in menthol status on efficacy of environmental cues or smoking cessation, there could be underlying differences in menthol status that were not addressed with this survey.

Conclusion

The results of Study 1 confirm that nicotine elevates phasic dopamine release in the mesolimbic pathway, which aligns with the first hypothesis of the study. The second hypothesis,

that menthol alone would have no significant effect on dopamine release, was also supported by data from Study 1. The third hypothesis, that the addition of menthol would have an additive effect on dopamine release elicited by nicotine, was not supported. Menthol had no additive effect on dopamine release when nicotine was already present in the system. This is likely due to the use of acute menthol exposure for Study 1, or due to the delay in administration of menthol after nicotine. It is possible that there are effects that are dependent on when nicotine and menthol are administered in relation to each other.

Results of Study 2 did not support the hypothesis that menthol smokers would have stronger urges to smoke associated with environmental cues, a more difficult time quitting, and be more likely to use a smoking cessation tool than nonmenthol smokers. The present results indicate that there is no difference in menthol status on the salience of environmental cues acting as motivation behind smoking behavior.

Taken together the results of Study 1 and Study 2 have interesting implications. Nicotine is the addictive substance in tobacco cigarettes, not menthol, however, menthol can help facilitate the acquisition of smoking because it makes the consumption of nicotine less aversive by providing relief from smoke inhalation and masking the aversive taste associated with nicotine (Wickham, 2019). The mechanisms behind the interaction between menthol and nicotine are more complex than initially believed and future research should examine menthol independently from nicotine, as well as combined, in several different conditions to examine how DA release and nAChR expression are affected. Results from these preclinical studies can be applied in human research to better understand how menthol status can affect nicotine acquisition, continued use, and smoking cessation. The use of more specific survey questions

geared toward understanding the motivations behind smoking behavior may yield a deeper understanding of menthol's role in the acquisition and continuations of nicotine use.

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