Original investigation

Sensory Effects of Menthol and Nicotine in an E-Cigarette

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Abstract

Introduction: Despite the longstanding use and popularity of menthol as a flavorant in tobacco products, its sensory interactions with inhaled nicotine have never been measured independently of the other irritants in tobacco smoke. We therefore measured the perception of menthol in an E-cigarette with the primary goal of assessing its analgesic effect on the sensory irritation produced by inhaled nicotine.

Methods: Adult cigarette smokers sampled aerosolized E-liquids containing five different concentrations of nicotine with 0%, 0.5%, or 3.5% l-menthol, as well as two commercial menthol flavors with and without nicotine. For each of the E-liquids participants used a labeled magnitude scale to rate the Overall Sensation intensity, Coolness/Cold, and Irritation/Harshness they experienced, and a Labeled Hedonic Scale to indicate how much they liked/disliked the overall flavor.

Results: The main findings were that (1) perceived Irritation/Harshness was unaffected by a low (0.5%) menthol concentration, whereas a high menthol concentration (3.5%) led to higher perceived Irritation/Harshness at low nicotine concentrations but to lower Irritation/Harshness at the highest nicotine concentration (24 mg/ml); (2) a commercial Menthol–Mint flavor produced similar results; (3) nicotine tended to enhance rather than suppress sensations of Coolness/Cold; and (4) menthol tended to slightly increase liking independently of nicotine concentration.

Conclusion: In addition to adding a sensation of coolness, menthol can reduce perceived airway irritation and harshness produced by inhalation when nicotine concentration is high, and contributes to the sensory impact of E-liquids when nicotine concentration is low.

Implications: The evidence presented here indicates that menthol can potentially improve the appeal of E-cigarettes not only via its coolness and minty flavor, but also by reducing the harshness from high concentrations of nicotine. As the first direct demonstration of an analgesic effect of menthol on inhaled nicotine in humans, these data also have implications for the role of menthol flavors in other inhaled tobacco products.

Introduction

Menthol has long been an important flavoring agent in commercial tobacco products. The reasons most often cited for its use are the cooling sensations it produces and its perceived ability to mask the harshness and taste of inhaled tobacco smoke.^{1–6} Tobacco industry research has also shown that highly mentholated cigarettes can

appeal to smokers who desire a stronger sensory impact, while low menthol cigarettes can appeal to smokers who prefer a cool and minty taste.^{2,7,8} Given the popularity of menthol cigarettes, it is not surprising that menthol is also a common flavorant in E-cigarettes, where its familiar cooling flavor appeals to current and former smokers of menthol cigarettes.⁹ It is unclear, however, what the sensory

effects of menthol are in E-cigarettes. Although few of the chemical and particulate irritants in cigarette smoke are present in E-liquids, in sufficient concentrations nicotine, and possibly other flavor constituents,¹⁰⁻¹³ may produce sensory irritation.

The present study was therefore designed to take a first step toward understanding the sensory effects of, and interactions between, menthol and nicotine in E-cigarettes. Menthol has been shown to have an analgesic effect on nociception produced by heat and low pH in mice,¹⁴ and to attenuate the *in vitro* response of human nicotinic acetylcholine receptors (nAChRs)¹⁵ that play a role in the sensory irritation from nicotine.¹⁶⁻¹⁸ An analgesic effect of menthol on nicotine irritation in humans has been demonstrated in a desensitization paradigm¹⁹ in which menthol was applied repeatedly to the tongue before nicotine was applied 5 minutes later. However, evidence of a direct effect on the sensory irritation from inhaled nicotine has been less clear. The authors of a recent study of menthol cigarettes concluded there was no consistent effect of menthol on perceived harshness and overall impact,²⁰ whereas a previous study indicated that menthol could either increase or decrease the perceived harshness of a cigarette depending on nicotine concentration.⁸ Studies in mice^{21,22} indicate that menthol can reduce the sensory irritation from constituents of cigarette smoke, including nicotine, that stimulate the pain receptor TRPA1,^{23,24} which has been shown to play a role in cough.²⁵ However, the numerous other chemical and particulate irritants in tobacco smoke^{5,26} have precluded direct measurement of the effect of menthol on the perception of nicotine irritation alone. Here we took advantage of the atomizing technology of E-cigarettes, and the ability to deliver different flavor constituents alone and in mixture in E-liquids, to make the first direct measurements of the effect of menthol on the sensory irritation produced by nicotine in the airways.

Because menthol can itself produce sensations of burning and stinging,^{19,27-29} another goal of the study was to measure the sensory irritation, overall sensation, and liking of menthol inhaled via an E-cigarette. Finally, because menthol is a primary constituent of mint-flavored E-liquids,³⁰ a second experiment measured the sensory effects of a commercial Menthol-Mint flavor and a commercial menthol flavor, with and without nicotine.

Methods

Subjects

Thirty-two adult smokers (16F, 16M) 18–45 years of age were paid to participate in each experiment and were recruited by flyers posted around the Yale University campus and online advertisements in the New Haven, Connecticut area. The subject sample was racially diverse, including eight African Americans in experiment 1 and 10 in experiment 2, all of whom were self-reported menthol cigarette smokers, as were the majority of white and multiracial participants (75% and 81% in experiments 1 and 2, respectively). Three females and three males served in both experiments; the remaining 26 participants in the second experiment were new to the study. Potential subjects were screened by telephone and only individuals who reported to be current daily cigarette smokers for at least 1 year, in overall good health, not pregnant, and who spoke fluent English were invited to participate in the study. The experimental protocol was approved by the Yale School of Medicine Human Investigations Committee, and all subjects gave written consent before participation.

Subjects were asked to abstain from smoking for 10 hours before each session, which was verified by expired air carbon monoxide

levels < 10 ppm³¹ using the MC02 carbon monoxide monitor (Micro Direct, Inc, Lewiston, ME). If the subject's alveolar carbon monoxide was greater or equal to 10 ppm, the session was rescheduled for another day. Subjects also completed an information sheet indicating the time they last smoked, when and what they last ate or drank, preferred cigarette type (menthol, non-menthol or both), the estimated number of cigarettes smoked per day, and if they smoked E-cigarettes, the estimated frequency of use per day. The majority of subjects in both experiments were self-reported menthol cigarette smokers: 25 in experiment 1 and 26 in experiment 2. Five subjects in experiment 1 reported regular use of E-cigarettes and 12 subjects in experiment 2.

Equipment and Stimuli

All testing was done using the V2 Standard E-Cigarette (79 mm; VMR Products, LLC) and V2 blank cartridges. In experiment 1, the blank cartridges were filled with 15 different E-liquids that were prepared by Pace Engineering Concepts, LLC: five concentrations of nicotine (0, 6, 12, 18, 24 mg/ml) with 0.0%, 0.5% or 3.5% l-menthol in a 70% propylene glycol (PG)/30% vegetable glycerin (VG) base. The decision to use 3.5% menthol was based on preliminary tests which indicated this concentration produced cool/cold sensations approximately equal to those experienced from a mentholated commercial flavor E-liquid (Menthol, AmericanELiquidStore) when inhaled using the V2 E-cigarette.

In experiment 2, the blank cartridges were filled with six different E-liquids, also in a 70%PG/30%VG base: two Menthol and two Menthol-Mint commercial flavors (AmericanELiquidStore) with one of each flavor containing 0 or 24 mg/ml nicotine; and two Unflavored E-liquids (PG/VG base only) containing 0 or 24 mg/ml nicotine prepared by Pace Engineering Concepts, LLC.

For all stimuli 0.5 ml of the E-liquids was carefully pipetted into the blank cartridges. Leakage of the E-liquid onto the heating element inside each cartridge was prevented by inserting a cylindrical wooden toothpick into the core during pipetting. After filling, each cartridge was pre-tested using a syringe to create simulated puffs and was used with only one subject.

Experiment Design

In both experiments data were collected in two sessions on separate days. In experiment 1 each session contained 10 trials divided into two blocks, with each block having all five nicotine concentrations with one concentration of menthol. The inter-trial interval was 90 seconds, which appeared in preliminary testing to be sufficient to prevent noticeable nicotine desensitization between successive trials. Subjects were randomly assigned to either a menthol-first group, which received 0.5% or 3.5% menthol in the first block followed by 0.0% menthol in the second block, or a menthol-second group, which received 0.0% menthol in the first block. Within each group the order of menthol concentration (0.5% or 3.5%) was counterbalanced across sessions. Also to reduce the potential for nicotine desensitization,^{32,33} subjects sampled E-cigarettes in one of six pseudo-random orders in both sessions. A 10-minute break between blocks allowed lingering effects of menthol and nicotine to diminish.

In experiment 2, each of the two sessions contained just six trials comprised of three E-liquids (Flavorless base, Menthol, and Menthol-Mint) with 0 and 24 mg/ml nicotine. There were four different orders of flavor presentation, and the order of nicotine exposure within each flavor pair (0 or 24 mg/ml) was counterbalanced

across sessions. There was a 5-minute break between flavor pairs to allow lingering sensory effects to diminish.

Training Procedures

In the first session subjects were familiarized with the general Labeled Magnitude Scale³⁴ for rating sensation intensity and the Labeled Hedonic Scale^{35,36} for rating flavor liking/disliking. The general version of the LMS is a category ratio scale of perceived sensation intensity with seven semantic labels: “no sensation,” “barely detectable,” “weak,” “moderate,” “strong,” “very strong,” and “strongest imaginable sensation of any kind.” The five intermediate labels are positioned quasi-logarithmically on the scale according to their empirically determined semantic magnitudes. The Labeled Hedonic Scale is a bipolar scale of liking/disliking with “neutral” in the middle and five symmetrical semantic labels: “like/dislike slightly,” “like/dislike moderately,” “like/dislike very much,” “like/dislike extremely,” “most liked/dislike imaginable,” also positioned on the scale according to their semantic magnitudes. Both scales were displayed on a monitor and subjects used a computer mouse to register their ratings on the scale. Practice using the general Labeled Magnitude Scale and the Labeled Hedonic Scale was given by having subjects rate the intensities and liking/disliking of 15 remembered or imagined sensations.

Subjects were also given instructions and practice in using the V2 E-cigarette. Because the heating element in the V2 atomizer is activated by inhalation, the first puff generally produces less vapor than subsequent puffs. To avoid this as a variable in stimulus generation, subjects were instructed to take two “priming puffs” into the mouth only, then to fully inhale the third puff as they normally would when smoking a cigarette and to exhale through the mouth.

Testing Procedure

Subjects were given color coded E-cigarettes that gave no indication of flavor content or concentration and were instructed to use the same inhalation procedure as during training. After exhalation the subject was prompted to make sequential ratings of Overall Sensation, Coolness/Cold, Harshness/Irritancy, and Liking/Disliking. Following the harshness intensity rating subjects indicated on a pick-list where, if any, harsh sensations were perceived during inhalation (mouth, throat, or lungs/chest). Subjects were then prompted to rate and localize residual harshness, if any, 60 seconds after the initial rating.

Because in experiment 2 the cooling sensation from menthol occurred together with a peppermint flavor in the Menthol-Mint E-liquid, subjects were instructed that when making ratings of Coolness/Cold to discriminate as best they could the cool temperature sensation from the peppermint flavor.

Results

Experiment 1

Figure 1 displays the intensity ratings for Overall Sensation as a function of E-liquid nicotine concentration, with menthol concentration as the parameter. Without menthol, Overall Sensation increased monotonically with nicotine concentration from a baseline (no nicotine) rating of mean- $\log_{10}$ 0.78, to a maximum intensity rating of 1.42 (24 mg/ml nicotine). This change of +0.64 $\log_{10}$ reflects a 4.4-fold increase in perceived intensity, which corresponds to a change in rated sensation from “weak” to a little less than “strong.” The weak sensation reported when no nicotine (or menthol) was present

Figure 1. Log₁₀ mean ratings of Overall sensation intensity as a function of nicotine concentration (0, 6, 12, 18, 24 mg/ml) in E-liquids containing 3.5%, 0.5%, or 0% menthol. Letters on the right y-axis denote semantic labels of sensation intensity on the general Labeled Magnitude Scale (gLMS): BD = Barely Detectable; W = Weak; M = Moderate; S = Strong. Error bars represent the standard errors of the means (SEM).

resulted from the 70%VG/30%PG E-liquid base alone plus the oral and airway sensations that normally result from inhaling cool ambient air (see below).

Menthol contributed significantly to the intensity and quality of the E-cigarette flavor and influenced the perception of nicotine, both in a concentration dependent manner. As shown in Figure 1, 0.5% menthol increased Overall Sensation only when no nicotine was present, whereas 3.5% menthol significantly increased Overall Sensation at all but the highest nicotine concentration. These effects were confirmed by a two-factor (Menthol, Nicotine) repeated-measures analysis of variance with Gender as a between-groups factor. In addition to the main effects of Menthol ($F_{(2,60)} = 24.3$, $P < .00001$) and Nicotine ($F_{(4,120)} = 25.6$, $P < .00001$), the analysis revealed a significant Menthol $\times$ Nicotine interaction ($F_{(8,240)} = 6.3$, $P < .00001$), which reflected the strong effect 3.5% menthol had on Overall Sensation when nicotine concentration was low. The convergence of the nicotine and menthol plus nicotine functions at the highest nicotine concentration indicates that the sensations produced by menthol and nicotine were not additive. The analysis also shows there was a significant interaction among the factors Menthol, Nicotine, and Gender ($F_{(8,240)} = 2.72$, $P < .01$). However, the source of the interaction was significantly higher ratings of Overall Sensation by females only in the 0.5% menthol condition when no nicotine was present. Data from females was therefore the primary factor in producing the higher mean Overall Sensation ratings in that condition.

As expected, Figure 2 shows that both 0.5% and 3.5% menthol significantly increased perceptions of Coolness/Cold (main effect of

Figure 2. Log₁₀ mean ratings of Coolness/Cold sensations as a function of nicotine concentration (0, 6, 12, 18, 24 mg/ml) in E-liquids containing 3.5%, 0.5%, or 0% menthol. Letters on the right y-axis denote semantic labels of sensation intensity on the general Labeled Magnitude Scale (gLMS): BD = Barely Detectable; W = Weak; M = Moderate; S = Strong. Error bars represent the standard errors of the means (SEM).

menthol; $F_{(2,60)} = 57.6$, $P < .000001$). As alluded to above, the ratings of coolness when no menthol was present resulted from physical cooling of the mouth and airways caused by inhaling ambient air (24°C). The psychophysical functions for 0%, 0.5%, and 3.5% menthol were nearly parallel across nicotine concentrations, indicating that the increase in Coolness/Cold was proportionally similar whether 0.5% menthol was added to the E-liquid base or concentration was increased to 3.5%. More surprisingly, Figure 2 also shows that ratings of Coolness/Cold steadily increased with nicotine concentration in the 3.5% menthol condition, and also increased for all but the highest nicotine concentration in the 0% and 0.5% menthol conditions. These visible trends were confirmed by a significant main effect of Nicotine ($F_{(4,120)} = 4.87$, $P < .005$). Despite a downturn in Coolness/Cold at 24 mg/ml nicotine in both the 0% and 0.5% menthol conditions, there was not a significant Menthol $\times$ Nicotine interaction.

Ratings of Irritation/Harshness during inhalation are shown in Figure 3. Harshness increased monotonically with nicotine concentration in all conditions, but not at the same rate. The ratings followed nearly identical accelerating functions in the 0% and 0.5% menthol conditions, indicating no significant effect of 0.5% menthol on nicotine harshness, whereas the 3.5% menthol condition produced higher mean ratings when nicotine concentration was low, and lower mean ratings at the highest nicotine concentration. A significant Nicotine $\times$ Menthol interaction ($F_{(8,240)} = 4.33$, $P < .0001$) confirmed this differential effect across menthol concentrations.

Figure 3. Log₁₀ mean ratings of Irritation/Harshness perceived during inhalation of E-liquid aerosols as a function of nicotine concentration (0, 6, 12, 18, 24 mg/ml) in E-liquids containing 3.5%, 0.5%, or 0% menthol. Letters on the right y-axis denote semantic labels of sensation intensity on the general Labeled Magnitude Scale (gLMS): BD = Barely Detectable; W = Weak; M = Moderate; S = Strong. Error bars represent the standard errors of the means (SEM).

Including gender as a between groups factor revealed a Menthol $\times$ Nicotine $\times$ Gender interaction ($F_{(8,240)} = 3.32$, $P < .0005$), and perusal of the data showed that the higher Irritation/Harshness ratings for 3.5% menthol at low nicotine concentrations were driven more strongly by males, whereas the lower ratings at the highest nicotine concentration were driven primarily by females.

Analysis of liking/disliking ratings indicated that on average the E-liquids were only “slightly liked,” and the degree of liking did not vary significantly across nicotine or menthol concentrations. The only significant effect, a Gender $\times$ Nicotine interaction ($F_{(4,120)} = 3.76$, $P < .05$), was due to slightly higher mean ratings of liking for males compared to females for the three lowest nicotine concentrations, which disappeared at the two highest concentrations.

Sensations of Irritation/Harshness were experienced more frequently in the mouth and throat than in the lungs, and the response patterns differed across sites: The frequency of localization to the mouth increased with menthol concentration but not with nicotine concentration, indicating the oral cavity was effectively insensitive to nicotine delivered via the V2 E-cigarette (Supplementary Figure 1). In contrast, Irritation/Harshness was localized to the throat in a concentration-dependent manner: menthol drove localization to the throat at the low nicotine concentrations but showed signs of reducing localization at the two highest nicotine concentrations. Localization to the lungs was much less frequent than to the upper airway, with irritation from nicotine rarely sensed in the lungs/chest except at the highest concentration (24 mg/ml), where it

was reported on slightly more than half of trials (53.1%). However, 3.5% menthol significantly reduced localization to the lungs at that concentration (Cochran $Q = 6.63$, $P < .05$), with reports falling to only 25% of trials. Tendencies toward reduced frequency of localization to the throat visible for 18 and 24 mg/ml nicotine were not statistically significant (Cochran $Q = 2.0$, $P = .37$). No significant effect of gender was found, although in general agreement with intensity ratings, localization of Irritation/Harshness to the lungs for 3.5% menthol was reduced somewhat more for women (43.8% to 12.5% = -31.3%) than for men (62.2% to 37.5% = -24.8%).

Experiment 2

Figure 4 displays log-mean ratings of Overall Sensation, Coolness/Cold, and Irritation/Harshness from 0 and 24 mg/ml nicotine for the three E-liquids. Not surprisingly, the Overall Sensation intensity when nicotine was absent was dominated by the Menthol and Menthol-Mint flavors, whereas the Irritation/Harshness produced by 24 mg/ml nicotine dominated when no flavor was present. These trends were supported by a three-factor (Flavor $\times$ Nicotine $\times$ Replicate) repeated measures analysis of variance which identified main effects of Flavor ($F_{2,62} = 28.7$, $P < .00001$) and Nicotine ($F_{2,62} = 89.0$, $P < .00001$) on the perception of Overall Sensation, and an interaction between these two factors ($F_{2,62} = 28.1$, $P < .00001$) that can be attributed to a much greater effect of nicotine on perception of the unflavored E-liquid compared to the Menthol and Menthol-Mint flavors. Menthol and Menthol-Mint produced similarly intense sensations of Coolness/Cold, although these sensations tended to be stronger for the Menthol-Mint flavor. Consistent with this visible trend, an analysis of variance found a significant main effect of Nicotine on perceived Coolness/Cold ($F_{1,31} = 4.3$, $P < .05$). Although there was no significant interaction between the factors Nicotine and Flavor, the main effect appears to have been driven more strongly by the higher ratings of Coolness/Cold for the Unflavored E-liquid with 24 mg/ml nicotine. Finally, an analysis of variance conducted on the Irritation/Harshness ratings revealed only a main effect of Nicotine ($F_{1,32} = 90.4$, $P < .00001$) and a Nicotine $\times$ Flavor interaction ($F_{2,62} = 34.6$, $P < .00001$). This interaction resulted from a combination of higher ratings for the two flavored

E-liquids without nicotine and progressively lower ratings for the two flavored E-liquids with 24 mg/ml of nicotine. Tukey HSD tests ($P_s < .05$) confirmed that the Menthol-Mint flavor led to significantly lower Irritation/Harshness ratings than either of the other two E-liquids, whereas the lower log₁₀-mean ratings for the Menthol E-liquid compared to the Unflavored E-liquid did not reach statistical significance. Unlike experiment 1, no significant effects of gender were found for any ratings of perceived intensity.

As in experiment 1, average liking ratings of the E-liquid flavors did not exceed “like slightly” on the Labeled Hedonic Scale. A trend toward higher ratings for liking of the Menthol and Menthol-Mint flavors over the unflavored E-liquid was supported by a main effect of Flavor ($F_{2,60} = 8.11$, $P < .001$). However, a trend toward a greater effect of Flavor on liking when 24 mg/ml nicotine was present fell just below statistical significance ($F_{2,60} = 3.15$, $P = .05$), while there was a significant interaction between Flavor and Gender ($F_{2,60} = 4.62$, $P < .05$). The latter interaction resulted from males tending to give higher liking ratings to the unflavored and Menthol Flavor E-liquids than did females.

Localization of Irritation/Harshness sensations in the airway was generally consistent with the results of experiment 1: localization to the mouth was similar whether or not nicotine was present, whereas localization to the throat and lungs/chest was driven primarily by the harshness of 24 mg/ml nicotine (Supplementary Figure 2). Also consistent with the data of experiment 1 were trends toward reduced frequencies of localization to these two regions for the two E-liquids containing menthol. Statistical analysis of these trends showed that reductions in frequency of localization were significant for the throat (Cochran $Q = 9.19$, $P < .02$) but not for the lungs/chest ($Q = 3.14$, $P = .15$). In contrast, the same analysis also showed a significant localization effect for the mouth ($Q = 9.53$, $P < .01$), but in the opposite direction, with more frequent localization of the Menthol and Menthol-Mint flavors to the mouth.

Discussion

The present study provides the first quantitative data on the sensory effects of, and interactions between, menthol and nicotine when inhaled via an E-cigarette. The most expected and straightforward of

Figure 4. Shown are Log₁₀ mean ratings of Overall sensation, Coolness/Cold, and Irritation/harshness reported for two concentrations of nicotine (0 and 24 mg/ml) and three E-liquid flavors: No Menthol (light grey bars), Menthol (grey bars), and Menthol-Mint (black bars). For each sensation the bars having different letters (a–d) are significantly different from one another. Letters on the right y-axis denote semantic labels of sensation intensity on the general Labeled Magnitude Scale (gLMS): BD = Barely Detectable; W = Weak; M = Moderate; S = Strong. Error bars represent the standard errors of the means (SEM).

the sensory effects were the concentration-dependent sensations of both Coolness/Cold and Irritation/Harshness produced by menthol, and the monotonic increase in sensations of Irritation/Harshness produced by nicotine across concentrations. Menthol's perceptual effects of cooling and sensory irritation have been well studied on the skin and in the mouth,^{28,37–39} and a recent investigation showed that menthol sniffed into the nose also produced burning and stinging as well as cooling.⁴⁰ Whereas menthol's innocuous cold sensations have been shown to depend on stimulation of the cold-sensitive receptor TRPM8,^{41–43} its nociceptive sensations appear to arise at least in part from activation of the receptor TRPA1,^{44,45} which plays an important role in pain perception,⁴⁶ including the sensitivity to painfully cold temperatures.⁴⁷ The data from experiment 1 show that when inhaled using the V2 E-cigarette, the coolness produced by 3.5% menthol was the most prominent flavor quality until the highest concentration of nicotine alone produced equal ratings of Overall Sensation. The sensory irritation from menthol was also the primary driver of Irritation/Harshness at nicotine concentrations below 18 mg/ml. Although Overall Sensation intensity was highest when 24 mg/ml nicotine was present in the menthol flavored E-liquids, in both experiments mean ratings of Irritation/Harshness were lower compared to when nicotine was presented alone. Thus instead of the two sources of sensory irritation adding together to increase harshness, there was a downward trend in intensity ratings for the 24 mg/ml nicotine E-liquid, which is consistent with an analgesic effect of menthol on airway irritation from nicotine. In addition, both experiments showed that menthol reduced the frequency of localization of Irritation/Harshness in the throat and lungs for 24 mg/ml nicotine. Notably, the absence of a measurable analgesic effect of 3.5% menthol at lower nicotine concentrations was most likely due to a masking effect of the sensory irritation produced by menthol, which was stronger than the sensory irritation produced by nicotine below 18 mg/ml.

The suppression by menthol of nicotine's sensory irritation in the throat and lungs is consistent with the results from prior studies which have shown that in humans, pre-treatment with menthol can attenuate both the sensory irritation from nicotine applied to the tongue¹⁹ and the frequency of cough from inhaled capsaicin,⁴⁸ and that in mice, exposure to menthol reduces the irritant responses to cigarette smoke and some of its constituent irritants.^{21,22} Evidence from the studies in mice also indicate that menthol's inhibitory effects depend upon TRPM8, which has been shown to underlie the analgesic effects of cold on acute and inflammatory pain via an opioid-dependent central neural pathway.⁴⁹ An analgesic action of menthol via a central neural mechanism raises the possibility that menthol stimulation in both the upper and lower airways may contribute to reducing the sensory irritation of nicotine that is sensed most acutely in the throat and lungs. However, other evidence that menthol has an inhibitory effect on the TRPA1 receptor^{45,50} implies that menthol reaching the epithelia of the throat and lungs may also suppress the harshness of nicotine locally.

An unexpected finding was the evidence that nicotine tended to increase sensations of Coolness/Cold. Although as was noted above, TRPA1 appears to function exclusively as a pain receptor within the somatosensory system, there is evidence that in airway epithelia, TRPA1 receptors of the vagus nerve are sensitive to cooling to temperatures as mild as 24°C.⁵¹ The latter finding raises the possibility that stimulation of TRPA1 by nicotine²³ may contribute to the rise in Coolness/Cold that we observed up to a nicotine concentration of 18 mg/ml.

The dominance of menthol's sensations of Coolness/Cold and Irritation/Harshness at low nicotine concentrations, as well as the maintenance of a strong Overall sensation at high nicotine concentrations even though menthol attenuated the harshness of nicotine, may help to explain why smokers of menthol cigarettes have cited both higher impact and milder flavor as reasons for preferring menthol brands.^{2,7,8} However, the present data on liking/disliking, which were collected from cigarette smokers, most of whom did not report frequent use of E-cigarettes, gave little indication of the contribution of menthol to E-cigarette flavor preferences. Future studies directed specifically toward understanding the appeal of menthol in E-cigarettes will need to collect similar sensory and hedonic data from regular E-cigarette users who do or do not prefer menthol flavors. In addition, use here of only a single type of E-cigarette with a relatively low-powered atomizer that likely delivers less nicotine and flavor constituents than do some other types of E-cigarettes, places limits on the generalizability of the findings. The results nevertheless provide clear evidence that menthol is an important sensory additive in E-liquids that has the ability to contribute flavor qualities which add to sensory impact while also attenuating the perceived harshness of high concentrations of inhaled nicotine.

Supplementary Material

Supplementary Figures 1 and 2 can be found online at <http://www.ntr.oxfordjournals.org>

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Declaration of Interests

None declared.

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