Published in final edited form as:

Behav Brain Res. 2017 September 15; 334: 72–77. doi:10.1016/j.bbr.2017.07.027.

Menthol disrupts nicotine's psychostimulant properties in an age and sex-dependent manner in C57BL/6J mice

Benjamin W. Falt¹, David Thompson¹, Tenna Mose, MSc¹, Peter Jatlow, MD², Sven Jordt, PhD³, Marina R. Picciotto, PhD¹, and Yann S. Mineur, PhD^{1,4}

¹Department of Psychiatry, Yale University School of Medicine, 34 Park Street, 3rd floor research, New Haven, CT 06520

²Department of Laboratory Medicine, Yale University School of Medicine, 330 Cedar Street, 4th floor, New Haven, CT 06520

³Department of Cellular and Molecular Pharmacology University of California, San Francisco, California 94143-2140, USA

Abstract

Menthol is a commonly used flavorant in tobacco and e-cigarettes, and could contribute to nicotine sensitivity. To understand how menthol could contribute to nicotine intake and addiction, it is important to determine whether specific mechanisms related to sex and age could underlie behavioral changes induced by menthol-laced nicotinic products. Using a validated paradigm of nicotine-dependent locomotor stimulation, adolescent and adult C57BL/6J mice of both sexes were exposed to nicotine, or nicotine laced with menthol, as their sole source of fluid, and psychostimulant effects were evaluated by recording home cage locomotor activity for ten days. Nicotine and cotinine blood levels were measured following exposure. Results show an interaction between treatment, age, and sex on liquid consumption, indicating that mice responded differently to menthol and nicotine based on their age and sex. Adult male mice greatly increased their nicotine intake when given menthol. In female mice of both age groups, menthol did not have this effect. Despite an increase in nicotine intake promoted by menthol, adult male mice showed a significant decrease in locomotion, suggesting that menthol blunted nicotine-induced psychostimulation. This behavioral response to menthol was not detected in adolescent mice of either sex. These data confirm that menthol is more than a flavorant, and can influence both nicotine intake and its psychostimulant effects. These results suggest that age- and sex-dependent mechanisms could underlie menthol's influence on nicotine intake and that studies including adolescent and adult menthol smokers of both sexes are warranted.

⁴Corresponding Author, yann.mineur@yale.edu.

Postal Address: Department of Psychiatry, Yale University School of Medicine, 34 Park Street, 3rd floor research, New Haven, CT 06520

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Menthol; nicotine; age difference; sex difference; locomotion

1. INTRODUCTION

Cigarette smoking contributes to about 1 in 5 deaths in the United States alone [1]. Many smokers express the desire to quit, but relapse is observed in 70 to 80% of quit attempts, even with treatment to aid in smoking cessation [2]. Mentholated cigarettes are used more frequently by populations more vulnerable to tobacco addiction including adolescent and female smokers [3–5] but the underlying reasons remain unclear. Although nicotine is the primary psychoactive component of cigarettes, 90% of tobacco products also contain menthol – often at levels not detectable by taste [6].

A growing body of behavioral, epidemiological, and biochemical evidence suggests that menthol intensifies the addictive properties of nicotine. Smokers of mentholated cigarettes experience stronger cravings [7] and have lower quit rates [8, 9]. Menthol can act as an analgesic to alleviate the irritated lungs of first-time smokers [10] and it creates a “cooling” sensation via activation of TRPM8 receptors [11]. This sensation may also serve as a sensory cue that could strengthen the ability of nicotine to reinforce behavior [12]. Recently, data have emerged to implicate menthol more directly in the underlying neurobiology of nicotine dependence. Menthol binds to and modulates the function of nicotinic acetylcholine receptors (nAChRs) [13] and administration of menthol alone is sufficient to up-regulate midbrain nAChRs in mice, which has somewhat conflicting behavioral effects, either facilitating behaviors related to nicotine addiction [14] or preventing nicotine reward [15]. In human subjects, menthol slows nicotine metabolism [16, 17] and adolescent smokers with slow nicotine metabolism, in contrast to adults, are more likely to progress to addiction [18]. Studies have also uncovered genetic vulnerability to consume mentholated cigarettes in women [19], which can lead to greater nicotine exposure [20]. Thus, while several molecular mechanisms may explain how menthol can alter nicotine’s pharmacological properties, sex- and age-dependent specificities appear to lead to significant differences in regards to menthol-induced modulation of nicotine related behavior, including reward and addiction.

A common behavioral response to addictive drugs in mice, including chronic nicotine exposure, is hyperlocomotion, stemming from activity of the mesostriatal dopamine system [21, 22]. This behavioral model is also of interest because it requires the expression of nicotinic acetylcholine receptors (nAChRs) containing the $\beta 2$ subunit in mesostriatal neurons, which are essential for behaviors related to nicotine addiction [21, 23] and are upregulated by menthol after long term exposure [15]. Furthermore, maintenance of locomotor sensitization requires long-term exposure to nicotine, which is critical for the development of addictive behaviors. We hypothesized that menthol could modulate the addictive properties of nicotine by potentiating its psychostimulant effects, but that specific patterns might be observed between sexes and age-groups that could explain conflicting data about the effects of menthol on nicotine’s pharmacological properties. We therefore investigated whether menthol can alter nicotine-induced hyperlocomotion differentially in

adult and adolescent, male and female wild-type mice and mice lacking the $\beta 2$ subunit of the nAChR. Finally, we determined whether adding menthol could contribute to greater nicotine intake and changes in blood nicotine and/or cotinine levels, and whether this might explain any effects of menthol on nicotine-induced locomotor stimulation.

2. METHODS

2.1 Animals

All experiments were carried out in accord with guidelines from the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Yale University Committee on the Care and Use of Animals. Male and female C57BL/6J mice were purchased from Jackson Laboratory (Bar Harbor, ME) and acclimated for about a week in our animal facility. Adult mice were 77–91 days old when treatment was administered. Adolescent mice were 21–28 days old at the start of treatment. Mice were housed on a 12:12 light/dark cycle (7:00 am – 7:00 pm) at $22.1 \pm 1^\circ \text{C}$ with *ad libitum* access to food. $\beta 2$ subunit-knockout mice were generated as described previously [24] and backcrossed onto C57BL/6J background for more than 30 generations. Controls were wild-type littermates. Twelve animals were assigned to each pharmacological treatment. Mice were euthanized on the last day of the locomotor activity experiments between 9 and 11 am and tissues were taken for further biochemical analyses from 7 to 8 mice per group, chosen at random.

2.2 Drug solutions

Treatments groups were as follows: **1.** nicotine (as hydrogen bitartrate salt) 200 $\mu\text{g/ml}$ (Sigma) in 2% saccharin (Sigma); **2.** nicotine 200 $\mu\text{g/ml}$ + 10 $\mu\text{g/ml}$ L-menthol in 2% saccharin; **3.** 2 mM tartaric acid (vehicle; Sigma) in 2% saccharin; **4.** 2 mM tartaric acid + 10 $\mu\text{g/ml}$ L-menthol in 2% saccharin. The pH of all solutions was adjusted to 7. The concentration of nicotine was based on previous studies [21, 25, 26] and menthol concentration was based on pilot studies showing no baseline preference for this concentration in acute administration, with higher menthol concentrations becoming aversive [27]. This concentration corresponds to a menthol to nicotine ratio similar to what is found in un-mentholated cigarettes [28]. All solutions were administered in modified glass bottles to prevent menthol decomposition in plastic, and covered with foil tape to prevent nicotine decomposition in light.

2.3 Locomotor activity

Mice were single-housed ($19 \times 29 \times 13 \text{ cm}$) with free access to food. Nesting material was removed to prevent obstruction of infrared beams in the locomotor monitoring system. All groups were habituated to single-housing for 5–7 days, weighed, and then moved to the testing cages for at least 3 days. When mice were transferred to their testing cage, drinking water was also replaced by a solution composed of 2 mM tartaric acid in 2% saccharin to allow animals to habituate to the taste and testing conditions. This approach prevents behavioral effects that might be induced by changes in housing and drinking solution. After this period of habituation, mice were randomly assigned to one of the 4 treatment solutions as their sole source of liquid. Locomotor data was collected using an Accuscan Instruments (Columbus, Ohio) behavioral monitoring system and software. Locomotion was monitored

for 10 days using six photocells placed 4 cm apart. Locomotor counts were monitored in 10 min blocks to obtain an “ambulatory activity count” consisting of the number of beam breaks recorded during a period of ambulatory activity (linear motion rather than quick, repetitive beam breaks associated with behaviors such as scratching and grooming). Mice were not disturbed during the testing period. Bottles were weighed at the beginning and end of treatment to measure nicotine intake.

2.4 Nicotine and cotinine levels

After measurement of locomotor activity was completed, between 9 and 11 am, mice were rapidly decapitated, trunk blood samples were taken and immediately frozen at -80°C . Blood was centrifuged, serum removed and frozen at -80°C for later analysis. A separate group of wild-type male mice were analyzed for blood nicotine and cotinine levels. Serum nicotine and cotinine levels were measured. Plasma nicotine and cotinine were assayed by HPLC interfaced with tandem mass spectrometry (LC/MS/MS) with stable isotope (deuterated)-labeled internal standards as previously described [29].

2.5 Data analysis and statistics

Locomotor data were processed using R and Statview (SAS institute, Inc.). The 10-min activity counts for each mouse were assigned a numeric vector inside a data frame that included a time vector. Using this data structure, the number of “locomotor bursts” was tabulated by taking all of the mice in each control group and finding the 90th percentile. Then, the number of 10-min time-bins exceeding that cutoff value was determined for each mouse relative to its control group (code included in supplementary materials). Counts for day and night were calculated for each mouse. Because the data showed little overall differences in locomotion within groups over the 10 days of measurement, with no overall day effect when data were binned as 12-hour cells, the 5th night (midpoint) was chosen as a representation of activity cross the study. Statistical analysis was performed using both Microsoft Excel and the R Statistical computing language (<https://www.r-project.org/>) with the RStudio interface (<https://www.rstudio.com/>).

Data were analyzed by ANOVA with age (2 levels), sex (2 levels), and treatment (4 levels) as main factors. When interactions were found to be significant, posthoc analyses were conducted by t-tests against the null hypothesis. T-test post-hoc analyses were performed (with Bonferroni corrections) on the control group vs. nicotine, the control group vs. menthol, and nicotine alone vs. nicotine and menthol combined. Unless stated otherwise, p value was set at 5%.

3. RESULTS

3.1 Menthol significantly increases nicotine intake in male mice

ANOVA for liquid consumption indicated an overall treatment effect ($F(3, 155) = 269.7$; $P < 0.0001$) but fluid intake was sex and age specific (treatment*sex*age: ($F(3, 155) = 24.7$, $p < 0.0001$). Relative to the control group, adult male mice given nicotine consumed slightly less liquid (~10%; $t(21) = 2.71$; $p = 0.013$), while the groups given menthol alone consumed more liquid (~30%; $t(22) = -9.25$; $p < 0.001$). Adult male mice given nicotine and menthol

together consumed significantly more liquid than nicotine alone (~25%; $t(20) = 7.62$, $p < 0.001$). Adult female mice consumed less liquid when given nicotine alone (~20% $t(16) = 4.36$; $p < 0.001$), but there was no significant difference in fluid intake if menthol was added (Fig. 1a). This pattern was mirrored in the adolescent male group but not in the adolescent female group, and no significant differences were observed (Fig. 1b). Menthol did not cause a significant change in cotinine levels; however, the adult females had a significantly higher nicotine:cotinine ratio with menthol than without ($t(18) = 3.60$; $p = 0.002$) (Fig. 1c).

3.2 Locomotor activity during the active phase

ANOVA of locomotion average on the 5th night (19:00–07:00) showed a significant treatment effect ($F(3, 156) = 22.1$; $p < 0.0001$), but it was different depending on sex and age (treatment * age * sex ($F(3, 156) = 2.77$; $p = 0.044$; Fig. 2). Adult male mice drinking menthol significantly decreased their locomotion compared to mice drinking the control solution ($t(22) = 6.84$; $p < 0.0001$; Fig. 2a). In line with previous experiments [21], all treatment groups had higher locomotor counts during the night when given nicotine, although this only reached significance in the male mice (adolescents: $t(21) = 2.08$; $p = 0.0019$; adults: $t(22) = 1.7$, $p = 0.06$; Fig. 2b). There were no significant changes in locomotor counts in response to menthol drinking alone in adolescent mice of either sex.

Adding menthol to the nicotine solution decreased locomotor activity relative to the nicotine-only group, but to different degrees depending on age and sex. Adult male mice showed ~50% reduction in activity when given menthol+nicotine vs. nicotine alone ($t(20) = 3.57$; $p = 0.0019$), while adolescent males showed a modest (~ 10%) decrease in activity that did not reach significance. There was no significant difference in the effects of menthol +nicotine vs. nicotine alone in adolescent female mice; however, there was a significant reduction of locomotor activity in the menthol+nicotine group compared to menthol alone ($t(17) = 0.904$; $p = 0.033$).

3.3 Locomotion during the inactive (day) phase

Limited, but significant ($F(3, 156) = 2.83$; $p = 0.040$; Fig. 2c and d), treatment effects on overall activity counts were observed during the inactive phase (07:00–19:00) that were similar in direction to those observed during the dark phase, although smaller in magnitude. No significant effects were detected by ANOVA with sex and age as factors.

A focus on bursts of activity (which represent the 90th percentile of locomotor activity) during the inactive phase showed significant differences between treatment groups ($F(3, 156) = 11.8$; $p < 0.001$; Fig. 3). Adult male mice given nicotine alone had significantly more locomotor bursts than mice given menthol+nicotine ($t(20) = 2.86$; $p = 0.0098$). However, male mice given menthol alone had significantly fewer locomotor bursts relative to adult males drinking the control solution ($t(22) = 4.20$; $p < 0.001$; Fig. 3a). No other significant effects on locomotor bursts were observed.

3.4 Menthol effects in knockout mice lacking the $\beta 2$ nAChR subunit

Menthol increased fluid consumption significantly in $\beta 2$ WT female mice ($t(17) = -4.08$; $p < 0.01$), but not in $\beta 2$ KO female mice (Fig. 4a). Menthol also increased 5th night

locomotion in $\beta 2$ KO female mice ($t(19) = -3.15$; $p = 0.003$) but not $\beta 2$ WT females (Fig. 4). $\beta 2$ WT females given menthol had significantly higher 5th day locomotion averages during the day ($t(15) = -2.14$; $p = 0.05$; supFig. 4b). $\beta 2$ KO female mice also had a significantly higher number of locomotor bursts in response to menthol ($t(19) = -2.48$; $p < 0.02$), while $\beta 2$ WT females did not (supFig. 4c). Menthol also induced a decrease in locomotor bursts in adult male $\beta 2$ WT male mice ($t(19) = 1.73$; $p = 0.089$), but not in $\beta 2$ KO male mice.

4. DISCUSSION

Behavioral, epidemiological, and biochemical evidence suggest that menthol may enhance nicotine addiction through several mechanisms [12]. In addition to its cooling sensation and its role as an analgesic in cigarette smoke, menthol may also act directly on nAChRs in the mesolimbic dopamine system [15] and could affect the psychostimulant properties of nicotine. These mechanisms are not mutually exclusive and are sex and/or age-dependent, which could explain seemingly inconsistent outcomes between experimental groups and demographic factors in human studies. We therefore evaluated the effects of menthol in a model of chronic nicotine administration, combined with the evaluation of locomotor activity (as a measure of its psychostimulant effects), nicotine intake, and blood cotinine levels. Because age and sex are important determinants of smoking behavior and addiction, these experiments were carried out in male and female, adult and adolescent mice. In parallel, we measured menthol intake and its effects on locomotor activity in mice lacking $\beta 2$ nAChRs, a critical receptor subtype necessary for locomotor activation in response to nicotine [21, 24].

Epidemiological studies have emphasized that teenagers and women are more prone to smoking mentholated products. This study, however, did not demonstrate significant effects of menthol on nicotine intake or nicotine-induced locomotor activity in adolescent or female mice. It is therefore possible that the cooling effects of menthol are more important for increasing susceptibility to tobacco addiction in vulnerable populations, especially in first-time users and adolescents who would be expected to be more sensitive to the aversive taste of tobacco products. Instead, adult male mice appear to be particularly sensitive to locomotor effects of menthol, and its ability to modulate nicotine pharmacodynamics and intake.

A striking increase in fluid intake induced by menthol was observed in adult male mice. This increase was unexpected since 10 $\mu\text{g/ml}$ menthol had no effect on water intake in acute studies, and became aversive at higher concentrations [27]. Although menthol does not affect water intake following acute administration, it is possible that tolerance to some aspects of menthol response over time could lead to a preference for menthol following chronic administration. In the current study, the control solution contained tartaric acid in saccharin rather than water to mimic the bitter taste of the nicotinic salts used in the experiment [21, 30]. It is therefore possible that menthol masked the taste of the control solution that would otherwise be aversive; however, the finding that adult female mice and adolescents of both sexes did not show a change in mentholated water intake suggests that this is likely not the case. When normalized to body weight, adolescent mice drank about

twice as much fluid as their adult counterparts, suggesting the possibility that these mice may have reached a ceiling for fluid intake. Our results, however, are in line with previously published results in adolescent C57BL/6J mice showing similar fluid intake that could still be altered significantly by changing the concentration of nicotine. Thus, it is unlikely that the high fluid consumption at baseline would prevent us from observing changes in response to nicotine and/or menthol.

In addition to changes observed in fluid intake, the current data also suggest that menthol alters nicotine titration in adult male mice, leading to an increase in consumption and a greater exposure to nicotine. Previous studies have shown that mice can tightly titrate their nicotine intake, potentially within a range that is rewarding [31]. Adult male mice drank less fluid when presented with nicotine alone in the drinking water, but drank significantly more menthol or nicotine laced with menthol. Thus, menthol could increase tolerance to nicotine in adult male mice. Several anecdotal reports suggest that menthol can decrease appetite, and a recent study showed that menthol's aftertaste decreases food wanting and liking [32]. Thus, menthol-induced increase in fluid consumption is unlikely related to general hyperphagia that would underlie fluid intake. Female mice did not increase nicotine intake when menthol was added to the drinking solution, whereas women are more likely to consume mentholated cigarettes. Interestingly, genetic haplotypes related to bitter taste have been linked to a preference for mentholated cigarettes in humans [19], and this has not been investigated in mice. It is also difficult to extrapolate the current results to make a conclusion about menthol preference, because each mouse only had access to one solution. Thus, we cannot exclude that female mice may have preferred a mentholated solution if they had a choice of intake. More experiments will be necessary to determine the underlying mechanism for this sex difference in intake.

Interestingly, while the intake of nicotine and menthol+nicotine differed in adult male mice, this was not paralleled by differences in blood cotinine levels or nicotine:cotinine ratios, nor did it correlate with changes in locomotor activity. Although cotinine levels do not correlate strongly with the addictive and stimulant properties of ingested nicotine [30], cotinine results from nicotine metabolism and is related to the rate of nicotine clearance. Adolescent and adult female mice had blood cotinine levels that varied with nicotine intake. Adult male mice, however, had similar blood cotinine levels whether they drank nicotine alone or with menthol, despite drinking nearly twice as much nicotine when it was laced with menthol. In adult male mice, chronic menthol exposure may therefore increase nicotine clearance and tolerance and thereby lead to the observed increase in nicotine intake. As noted above, the increase in nicotine intake following menthol was not mirrored by an increase in locomotor activity, suggesting that in adult males, menthol decreases the psychostimulant response to nicotine. It is also possible that a menthol-induced increase in nicotine intake actually led to decreased locomotion, as nicotine can induce hyperlocomotion only within a narrow range of concentrations [30]. The ability of menthol to disrupt nicotine titration, an effect observed in previous studies [31] could also suggest a menthol-induced increase in nicotine clearance; however, previous studies suggest that menthol may inhibit oxidation of nicotine by human CYP2A6 [17], which would be expected to decrease nicotine clearance, although species differences cannot be excluded.

Adult male mice exposed to menthol+nicotine showed a decrease in locomotor activity when compared to nicotine alone, despite greater intake of nicotine and similar cotinine levels. This was not observed in adolescent mice or adult female mice. This effect was even more prominent when quantifying locomotor bursts (locomotor activity above the 90th percentile of the average locomotor activity during the inactive phase) because menthol greatly decreased the number of locomotor bursts in adult male mice on its own, or when combined with nicotine. Menthol therefore appears to limit the psychostimulant effect of nicotine in adult male mice.

Recent studies have demonstrated that menthol can change the expression and pharmacology of $\beta 2$ -containing nAChRs [15] and can up-regulate high affinity nAChRs *in vivo* [33]. In males, the effects of menthol on locomotor activity may not be due to actions at $\beta 2$ subunit-containing nAChRs, since male $\beta 2$ KO mice show similar locomotor responses to menthol as their wild type siblings. In this experiment with siblings bred in-house, however, a decrease in locomotion in male wild type mice treated with menthol was not observed. While this difference between wild-type animals of the different experiments was somewhat surprising, stress of transport and/or subtle strain effects (including flanking alleles, maternal effects or genetic drifts) could underlie a different dose-response to menthol, as has been seen in other studies controlling for genetic background (see for instance [34]). As a result, future studies could focus on additional behavioral responses to menthol or different doses. In female $\beta 2$ KO mice, however, there was greater locomotor activity in the menthol-treated group, which suggests that unlike in males, menthol may normally decrease behaviors related to nicotine-induced psychostimulation in female animals through an nAChR-related mechanism. In addition, it is not yet known whether menthol crosses the blood-brain barrier and results in brain concentrations sufficient to affect receptors on central neurons. It is therefore possible that menthol acts in the periphery to exert its locomotor effects. Menthol acts primarily through temperature-detecting TRPM8 receptors [35], but can also activate κ -opioid receptors [11]. Thus, the behavioral effects of menthol could result from activity at a number of potential molecular targets outside the central nervous system.

Taken together, these data indicate that menthol can alter responses to nicotine through multiple mechanisms, all of which may affect the efficacy and stimulant effects of nicotine-containing products including cigarettes. This study identifies sex differences in intake of menthol and nicotine and their psychostimulant effects. There are also significant differences in responses to menthol and nicotine in adolescent and adult mice, suggesting that mentholated nicotine products may be used by smokers for different reasons across the lifespan, and may contribute differentially to nicotine dependence in women and men. Our study demonstrates that menthol can increase nicotine intake even over many days of exposure. These effects are likely not due to a direct effect of menthol on $\beta 2$ subunit-containing nAChRs, and could partly be explained by age and sex specific menthol-induced changes in nicotine clearance and metabolism. In addition, menthol facilitated bursts of nicotine-induced activity in female $\beta 2$ KO mice, indicating a potential role for $\beta 2$ nAChRs in decreasing locomotor response to menthol in female mice. Human studies of the effects of menthol on both nicotine metabolism and arousal in women and men are warranted to determine how menthol alters tobacco dependence in young smokers and in adulthood.

Acknowledgments

Research reported in this manuscript was supported by Yale TCORS (P50DA036151) grant from NIDA and the FDA Center for Tobacco Products. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or the FDA. We thank Dr. Ralitz Gueorgieva for her help with statistical analyses and Haleh Nadim for cotinine and nicotine quantification.

References

1. SD Alberg, AJ., Cummings, KM. The Health Consequences of Smoking-50 Years of Progress: A Report of the Surgeon General. United States Center for Disease Control and Prevention; Atlanta (GA): 2014.
2. Fiore MC, Smith SS, Jorenby DE, Baker TB. The effectiveness of the nicotine patch for smoking cessation. A meta-analysis. *Jama*. 1994; 271(24):1940–7. [PubMed: 8201739]
3. Rosenbloom J, Rees VW, Reid K, Wong J, Kinnunen T. A cross-sectional study on tobacco use and dependence among women: Does menthol matter? *Tobacco induced diseases*. 2012; 10(1):19. [PubMed: 23181980]
4. Collins CC, Moolchan ET. Shorter time to first cigarette of the day in menthol adolescent cigarette smokers. *Addictive behaviors*. 2006; 31(8):1460–4. [PubMed: 16303257]
5. Moolchan ET. Adolescent menthol smokers: will they be a harder target for cessation? *Nicotine Tob Res*. 2004; 6(Suppl 1):S93–5. [PubMed: 14982712]
6. Giovino GA, Sidney S, Gfroerer JC, O'Malley PM, Allen JA, Richter PA, Cummings KM. Epidemiology of menthol cigarette use. *Nicotine Tob Res*. 2004; 6(Suppl 1):S67–81. [PubMed: 14982710]
7. Wackowski O, Delnevo CD. Menthol cigarettes and indicators of tobacco dependence among adolescents. *Addict Behav*. 2007; 32(9):1964–9. [PubMed: 17229528]
8. Levy DT, Blackman K, Tauras J, Chaloupka FJ, Villanti AC, Niaura RS, Vallone DM, Abrams DB. Quit attempts and quit rates among menthol and nonmenthol smokers in the United States. *Am J Public Health*. 2011; 101(7):1241–7. [PubMed: 21566032]
9. Smith SS, Fiore MC, Baker TB. Smoking cessation in smokers who smoke menthol and non-menthol cigarettes. *Addiction*. 2014; 109(12):2107–17. [PubMed: 24938369]
10. Willis DN, Liu B, Ha MA, Jordt SE, Morris JB. Menthol attenuates respiratory irritation responses to multiple cigarette smoke irritants. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology*. 2011; 25(12):4434–44. [PubMed: 21903934]
11. Galeotti N, Di Cesare Mannelli L, Mazzanti G, Bartolini A, Ghelardini C. Menthol: a natural analgesic compound. *Neurosci Lett*. 2002; 322(3):145–8. [PubMed: 11897159]
12. Wickham RJ. How Menthol Alters Tobacco-Smoking Behavior: A Biological Perspective. *The Yale journal of biology and medicine*. 2015; 88(3):279–87. [PubMed: 26339211]
13. Ashoor A, Nordman JC, Veltri D, Yang KH, Al Kury L, Shuba Y, Mahgoub M, Howarth FC, Sadek B, Shehu A, Kabbani N, Oz M. Menthol binding and inhibition of alpha7-nicotinic acetylcholine receptors. *PLoS One*. 2013; 8(7):e67674. [PubMed: 23935840]
14. Henderson BJ, Wall TR, Henley BM, Kim CH, McKinney S, Lester HA. Menthol Enhances Nicotine Reward-Related Behavior by Potentiating Nicotine-Induced Changes in nAChR Function, nAChR Upregulation, and DA Neuron Excitability. *Neuropsychopharmacology*. 2017
15. Henderson BJ, Wall TR, Henley BM, Kim CH, Nichols WA, Moaddel R, Xiao C, Lester HA. Menthol Alone Upregulates Midbrain nAChRs, Alters nAChR Subtype Stoichiometry, Alters Dopamine Neuron Firing Frequency, and Prevents Nicotine Reward. *J Neurosci*. 2016; 36(10):2957–74. [PubMed: 26961950]
16. Fagan P, Pokhrel P, Herzog TA, Pagano IS, Franke AA, Clanton MS, Alexander LA, Trinidad DR, Sakuma KL, Johnson CA, Moolchan ET. Nicotine Metabolism in Young Adult Daily Menthol and Nonmenthol Smokers. *Nicotine Tob Res*. 2015
17. Benowitz NL, Herrera B, Jacob P 3rd. Mentholated cigarette smoking inhibits nicotine metabolism. *The Journal of pharmacology and experimental therapeutics*. 2004; 310(3):1208–15. [PubMed: 15084646]

18. Rubinstein ML, Shiffman S, Moscicki AB, Rait MA, Sen S, Benowitz NL. Nicotine metabolism and addiction among adolescent smokers. *Addiction*. 2013; 108(2):406–12. [PubMed: 22823143]
19. Oncken C, Feinn R, Covault J, Duffy V, Dornelas E, Kranzler HR, Sankey HZ. Genetic Vulnerability to Menthol Cigarette Preference in Women. *Nicotine Tob Res*. 2015; 17(12):1416–20. [PubMed: 25832883]
20. Women who smoke menthol cigarettes have greater nicotine exposure. *Oncology (Williston Park)*. 1999; 13(7):915. [PubMed: 10442338]
21. Mineur YS, Brunzell DH, Grady SR, Lindstrom JM, McIntosh JM, Marks MJ, King SL, Picciotto MR. Localized low-level re-expression of high-affinity mesolimbic nicotinic acetylcholine receptors restores nicotine-induced locomotion but not place conditioning. *Genes, brain, and behavior*. 2009; 8(3):257–66.
22. Nestler EJ. Common molecular and cellular substrates of addiction and memory. *Neurobiol Learn Mem*. 2002; 78(3):637–47. [PubMed: 12559841]
23. Maskos U, Molles BE, Pons S, Besson M, Guiard BP, Guilloux JP, Evrard A, Cazala P, Cormier A, Mameli-Engvall M, Dufour N, Cloez-Tayarani I, Bemelmans AP, Mallet J, Gardier AM, David V, Faure P, Granon S, Changeux JP. Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. *Nature*. 2005; 436(7047):103–7. [PubMed: 16001069]
24. Picciotto MR, Zoli M, Lena C, Bessis A, Lallemand Y, Le Novère N, Vincent P, Pich EM, Brulet P, Changeux JP. Abnormal avoidance learning in mice lacking functional high-affinity nicotine receptor in the brain. *Nature*. 1995; 374(6517):65–7. [PubMed: 7870173]
25. King SL, Caldarone BJ, Picciotto MR. Beta2-subunit-containing nicotinic acetylcholine receptors are critical for dopamine-dependent locomotor activation following repeated nicotine administration. *Neuropharmacology*. 2004; 47(Suppl 1):132–9. [PubMed: 15464132]
26. Sparks JA, Pauly JR. Effects of continuous oral nicotine administration on brain nicotinic receptors and responsiveness to nicotine in C57Bl/6 mice. *Psychopharmacology*. 1999; 141(2):145–53. [PubMed: 9952038]
27. Fan L, Balakrishna S, Jabba SV, Bonner PE, Taylor SR, Picciotto MR, Jordt SE. Menthol decreases oral nicotine aversion in C57BL/6 mice through a TRPM8-dependent mechanism. *Tob Control*. 2016
28. Ai J, Taylor KM, Lisko JG, Tran H, Watson CH, Holman MR. Menthol Content in US Marketed Cigarettes. *Nicotine Tob Res*. 2016; 18(7):1575–80. [PubMed: 26259988]
29. Sofuoglu M, Herman AI, Nadim H, Jatlow P. Rapid nicotine clearance is associated with greater reward and heart rate increases from intravenous nicotine. *Neuropsychopharmacology*. 2012; 37(6):1509–16. [PubMed: 22334123]
30. Matta SG, Balfour DJ, Benowitz NL, Boyd RT, Buccafusco JJ, Caggiula AR, Craig CR, Collins AC, Damaj MI, Donny EC, Gardiner PS, Grady SR, Heberlein U, Leonard SS, Levin ED, Lukas RJ, Markou A, Marks MJ, McCallum SE, Parameswaran N, Perkins KA, Picciotto MR, Quik M, Rose JE, Rothenfluh A, Schafer WR, Stolerman IP, Tyndale RF, Wehner JM, Zirger JM. Guidelines on nicotine dose selection for in vivo research. *Psychopharmacology*. 2007; 190(3):269–319. [PubMed: 16896961]
31. Vieyra-Reyes P, Picciotto MR, Mineur YS. Voluntary oral nicotine intake in mice down-regulates GluR2 but does not modulate depression-like behaviors. *Neurosci Lett*. 2008; 434(1):18–22. [PubMed: 18261852]
32. Hutchings SC, Horner KM, Dible VA, Grigor JM, O’Riordan D. Modification of aftertaste with a menthol mouthwash reduces food wanting, liking, and ad libitum intake of potato crisps. *Appetite*. 2017; 108:57–67. [PubMed: 27663531]
33. Alsharari SD, King JR, Nordman JC, Muldoon PP, Jackson A, Zhu AZ, Tyndale RF, Kabbani N, Damaj MI. Effects of Menthol on Nicotine Pharmacokinetic, Pharmacology and Dependence in Mice. *PLoS One*. 2015; 10(9):e0137070. [PubMed: 26355604]
34. Crusio WE, Goldowitz D, Holmes A, Wolfer D. Standards for the publication of mouse mutant studies. *Genes, brain, and behavior*. 2009; 8(1):1–4.
35. Bautista DM, Siemens J, Glazer JM, Tsuruda PR, Basbaum AI, Stucky CL, Jordt SE, Julius D. The menthol receptor TRPM8 is the principal detector of environmental cold. *Nature*. 2007; 448(7150):204–8. [PubMed: 17538622]

Fig. 1.

Menthol and nicotine consumption. Liquid consumed by adult (PND: 77–91 days; a) and adolescent (PND 21–28 days; b), male and female, C57BL/6J mice adjusted for body mass. (c) Average blood serum nicotine: cotinine ratios in C57BL/6J adult and adolescent mice of both sexes administered nicotine without menthol ($n = 8–9$) and with menthol ($n = 10–11$). Data are expressed as mean $\pm$ SEM ($n = 9–12$). Mice were provided with a saccharine vehicle mixture, nicotine, menthol, and a menthol-nicotine mixture as the sole source of fluid ($n = 9–12$ /group). Significance was determined using post-hoc Bonferroni-corrected t-tests. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, compared to Vehicle group unless brackets state otherwise.

Fig. 2.

Analysis of locomotor activity in adult (PND: 77–91 days; a) and adolescent (PND: 21–28 days; b) C57BL/6J male and female mice ($n = 9–12$) during the 5th night (active phase) and 5th day (c, d; inactive phase). Locomotion was defined as more than one beam break that was not highly-repetitive (indicating stereotypic behavior such as scratching). Significant differences between groups was determined using post-hoc Bonferroni-corrected t-tests. **, $p < 0.01$; ***, $p < .001$, compared to Vehicle group unless brackets state otherwise.

Fig. 3.

Locomotor Bursts of activity. A “burst” of locomotor activity was defined as a 10-min time period in which a mouse exhibited greater than the 90th percentile of locomotor counts compared to its respective control group. Data represent locomotor bursts on Night 5 in adult (PND: 77–91 days; a) and adolescent (PND: 21–28 days; b), male and female, mice ($n = 9–12$) given vehicle, nicotine, menthol, or a mixture of nicotine and menthol. Significant differences between treatment groups were determined using post-hoc Bonferroni-corrected t-tests. **, $p < 0.01$, compared to Vehicle group unless brackets state otherwise.

Fig. 4.

Liquid consumed by adult $\beta 2$ subunit nAChR knockout ($\beta 2$ KO) mice and their wild-type littermate controls ($\beta 2$ WT) adjusted for body mass (a). Average locomotor activity of menthol in $\beta 2$ subunit knockout ($\beta 2$ KO) mice and their wild-type littermate controls ($\beta 2$ WT) during night (b) and day (c). Locomotor burst totals (d), where one “burst” was classified as a 10-min period of activity greater than the 90th percentile of activity in the respective control group. The mice were given saccharine vehicle or menthol as the sole source of fluid ($n = 10-12$). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, compared to Vehicle group unless brackets state otherwise.