OPEN

Neuropsychopharmacology (2017) 42, 2285–2291
Official journal of the American College of Neuropsychopharmacology

www.neuropsychopharmacology.org

Menthol Enhances Nicotine Reward-Related Behavior by Potentiating Nicotine-Induced Changes in nAChR Function, nAChR Upregulation, and DA Neuron Excitability

Brandon J Henderson[1,2], Teagan R Wall[1], Beverley M Henley[1], Charlene H Kim[1], Sheri McKinney[1] and Henry A Lester*[,1]

[1]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, USA; [2]Department of Biomedical Sciences, Marshall University, Joan C Edwards School of Medicine, Huntington, WV, USA

Understanding why the quit rate among smokers of menthol cigarettes is lower than non-menthol smokers requires identifying the neurons that are altered by nicotine, menthol, and acetylcholine. Dopaminergic (DA) neurons in the ventral tegmental area (VTA) mediate the positive reinforcing effects of nicotine. Using mouse models, we show that menthol enhances nicotine-induced changes in nicotinic acetylcholine receptors (nAChRs) expressed on midbrain DA neurons. Menthol plus nicotine upregulates nAChR number and function on midbrain DA neurons more than nicotine alone. Menthol also enhances nicotine-induced changes in DA neuron excitability. In a conditioned place preference (CPP) assay, we observed that menthol plus nicotine produces greater reward-related behavior than nicotine alone. Our results connect changes in midbrain DA neurons to menthol-induced enhancements of nicotine reward-related behavior and may help explain how smokers of menthol cigarettes exhibit reduced cessation rates.
Neuropsychopharmacology (2017) **42,** 2285–2291; doi:10.1038/npp.2017.72; published online 3 May 2017

INTRODUCTION

Menthol cigarettes are used by 1 out of 3 smokers and >85% of African–American smokers (McCarthy *et al*, 1995). Smokers of menthol cigarettes are less likely to quit compared to smokers of non-menthol cigarettes (Ahijevych and Garrett, 2010). Youth smokers of menthol cigarettes are twice as likely to become lifelong smokers compared to youth smokers of non-menthol cigarettes (D'Silva *et al*, 2012). With e-cigarettes, consumption rates of flavored products (including menthol) are rising, especially among young smokers (Singh *et al*, 2016). Thus, it is important that we understand how menthol and other tobacco flavorants alter nicotine reward.

VTA neurons containing α4, α6, and β2 nAChR subunits ($\alpha4\beta2$, $\alpha4\alpha6\beta2$, $\alpha6\beta2\beta3$) mediate aspects of nicotine addiction (Tapper *et al*, 2004; Pons *et al*, 2008). Upregulation of these nAChRs is considered a biomarker for addiction. Smokers of menthol cigarettes exhibit greater upregulation of brain nAChRs compared to smokers of non-menthol cigarettes (Brody *et al*, 2013). Menthol enhances nicotine withdrawal (Alsharari *et al*, 2015) and nicotine intravenous self-administration (IVSA) in rats (Wang *et al*, 2014; Biswas *et al*, 2016). We have previously reported that menthol alone upregulates α4-containing ($\alpha4^*$) and $\alpha6^*$ nAChRs in the VTA and substantia nigra pars compacta (SNc) (Henderson *et al*, 2016). Also, menthol alone alters nAChR function on midbrain DA neurons (Henderson *et al*, 2016). This suggests that menthol directly alters midbrain neurons, in addition to any sensory and metabolic actions that may increase nicotine exposure in the brain.

Here we demonstrate that menthol plus nicotine enhances nicotine reward-related behavior and nicotine's actions on midbrain neurons. We suggest that menthol's ability to enhance nicotine-induced changes in nAChR function, nAChR number, and DA neuron excitability are important for menthol's enhancement of nicotine reward-related behavior.

MATERIALS AND METHODS

Menthol Dose Selection

We estimated the pharmacologically relevant dose of menthol by analogy with nicotine doses used in mouse studies. Typical menthol cigarettes contain 1–5 mg of menthol (Ai *et al*, 2015) and ~1 mg of nicotine (Rodgman and Perfetti, 2009). Therefore, menthol is 1–5 times that of nicotine. Steady state and peak concentrations of nicotine in human smokers are replicated in mice using 0.4 and 2.0 mg/kg/h doses of nicotine, respectively (Matta *et al*, 2007). CPP

*Correspondence: Professor HA Lester, Division of Biology and Biological Engineering, California Institute of Technology, 1200 East California Boulevard MC 156-29, Pasadena, CA 91125-2900, USA, Tel: +626 395 4946, Fax: +626 564 8709, E-mail: Lester@Caltech.edu
Received 20 December 2016; revised 28 March 2017; accepted 1 April 2017; accepted article preview online 12 April 2017

assays use 0.5 mg/kg nicotine (Tapper *et al*, 2004). Assays for *in vivo* upregulation use 2.0 mg/kg/h nicotine (Henderson *et al*, 2014). We selected 1 mg/kg and 2 mg/kg/h menthol for CPP and *in vivo* upregulation assays, respectively. Both dose selections fall within the 1–5 menthol-to-nicotine ratio of menthol cigarettes.

We previously discussed our menthol dose selection for cultured cells and neurons (Henderson *et al*, 2016). In preliminary assays to determine the concentration of menthol in a mouse brain, our chronic dosing methods (2 mg/kg/h, osmotic pump) produced concentrations of menthol at 0.5–2.5 μM. Thus, 500 nM menthol is appropriate in studying cultured neurons and cells and is consistent with previous investigations (Henderson *et al*, 2016).

All material and methods are described in detail in the Supplementary Material.

RESULTS

Menthol Enhances Nicotine Reward-Related Behavior

Mice administered with saline or menthol alone (1.0–10 mg/kg) did not exhibit CPP (Figure 1a). Nicotine and menthol plus nicotine produced a significant effect on CPP (Figure 1b, one-way ANOVA, $F_{(4,\ 83)} = 14.3$, $p < 0.001$). 0.5 mg/kg nicotine produced CPP ($p < 0.05$) (Figure 1b), similar to previous observations (Tapper *et al*, 2004; Henderson *et al*, 2016). Menthol (1.0 mg/kg) was administered with either 0.25 mg/kg or 0.5 mg/kg nicotine (Figure 1b). In both pairs, menthol plus nicotine produced a significant increase in CPP compared to respective nicotine-only doses ($p < 0.05$, Figure 1b). This suggests that menthol enhances nicotine reward-related behavior and agrees with previous nicotine IVSA assays (Wang *et al*, 2014; Biswas *et al*, 2016).

Menthol Plus Nicotine Alters Baseline Midbrain Neuron Firing Frequency

Using midbrain cultures from tyrosine hydroxylase (TH)-eGFP mice, we identified TH+/DA and TH − /non-DA (putative GABA) neurons. Cultured midbrain neurons were treated with control medium (control), 200 nM nicotine, or 500 nM menthol plus nicotine for 10 days. Control TH+/DA neurons exhibited a firing frequency of 4.1 ± 0.7 Hz (Figure 2a1–a3). Drug treatments produced a significant change in firing frequency for TH+/DA (one-way ANOVA, $F_{(2,61)} = 13.07$, $p < 0.001$) and TH − /non-DA neurons (one-way ANOVA, $F_{(2,29)} = 8.69$, $p = 0.002$). Nicotine treatment significantly decreased TH+/DA neuron firing frequency (1.5 ± 0.3 Hz, $p < 0.005$). Menthol plus nicotine decreased TH +/DA neuron firing frequency significantly more than nicotine treatment alone (1.2 ± 0.2 Hz, $p < 0.05$). TH − /non-DA neurons exhibited a baseline firing frequency of 8.1 ± 1.2 Hz (Figure 2b1–b3). Nicotine treatment increased firing frequency (13.9 ± 1.0 Hz, not significant) but menthol plus nicotine increased firing frequency more than nicotine alone (22.4 ± 2.1 Hz, $p < 0.005$).

Menthol Enhances nAChR-Stimulated TH+/DA Neuron Excitability

Midbrain TH+/DA neurons exhibit transient increases in firing frequency following nAChR activation, and nicotine's ability to alter tonic and phasic firing of these TH+/DA neurons is necessary for nicotine reward (Mansvelder *et al*, 2002). Thus, we examined menthol's effect on TH+/DA neuron transient increases in firing frequency following nAChR activation.

Neurons were treated for 10 days with control, 200 nM nicotine, or 500 nM menthol plus 200 nM nicotine and were recorded in current clamp mode before and after a brief application of ACh (Figure 2c1–c3). Drug treatments produced a significant effect on TH+/DA neuron excitability (one-way ANOVA, $F_{(2,29)} = 9.01$, $p = 0.001$). After ACh application, control TH+/DA neurons exhibited a transient, ~2-fold increase in firing frequency to 8.0 ± 1.1 Hz (Figure 2c1–c3). Nicotine-treated TH+/DA neurons exhibited a ~5-fold increase in firing frequency, with a maximum of 13.6 ± 2.5 Hz (Figure 2c1–c3). TH+/DA neurons treated with menthol plus nicotine exhibited an ~8-fold increase in firing frequency with a maximum of 19.6 ± 3.1 Hz (Figure 2c1–c3). Thus, we observed that menthol plus nicotine increased firing frequency more than nicotine alone ($p < 0.05$).

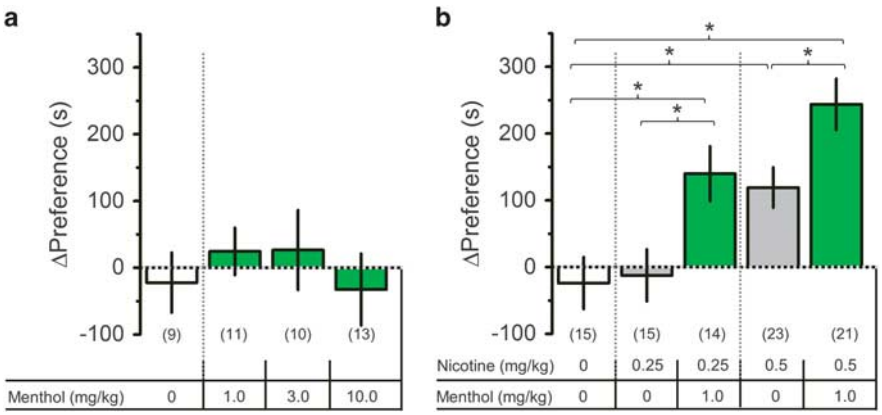

Figure 1 Menthol alone is not rewarding but enhances nicotine reward-related behavior. (a and b) Mice were administered saline, menthol, nicotine, or menthol plus nicotine at doses indicated. The data are mean ± SEM. *$p < 0.05$, one-way ANOVA, post *hoc* Tukey. Numbers in parentheses represent number of mice per group.

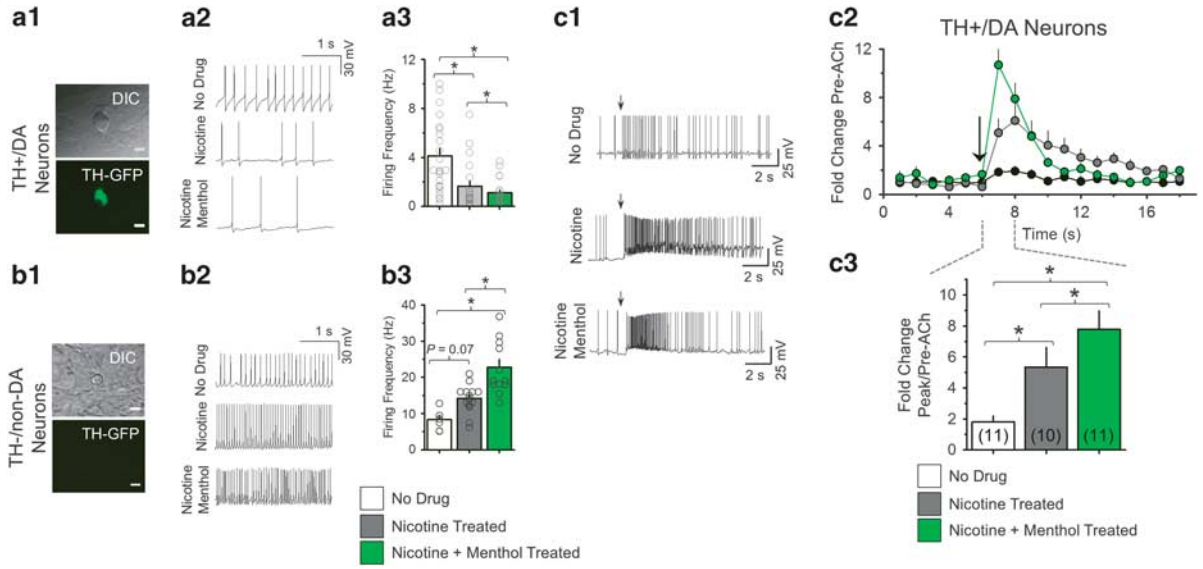

Figure 2 Menthol enhances nicotine-induced changes midbrain neuron firing frequency. (a1, b1) Neurons were identified as TH+/DA or TH-/non-DA using TH-eGFP fluorescence. Bars, 20 μm. (a2–3, b2–3, c1–3) Neurons were treated 10 days with control, 200 nM nicotine, or 500 nM menthol plus 200 nM nicotine and firing frequencies were recorded in current clamp mode. (a3, b3) Mean firing frequency of neurons ($n = 20$–22 for TH+/DA neurons and 9–13 for TH − /non-DA neurons). (c1) Current-clamp recordings of TH+/DA neurons before and after ACh puff (100 ms, 300 μM at arrows). (c2) Mean firing frequency over time before and after ACh application. (c3) Mean 'peak' firing frequency during 2 s post-ACh puff. For (c1–3), number of neurons recorded is indicated in parenthesis of (c3). The data are mean ± SEM: *, $p < 0.05$, one-way ANOVA, post *hoc* Tukey.

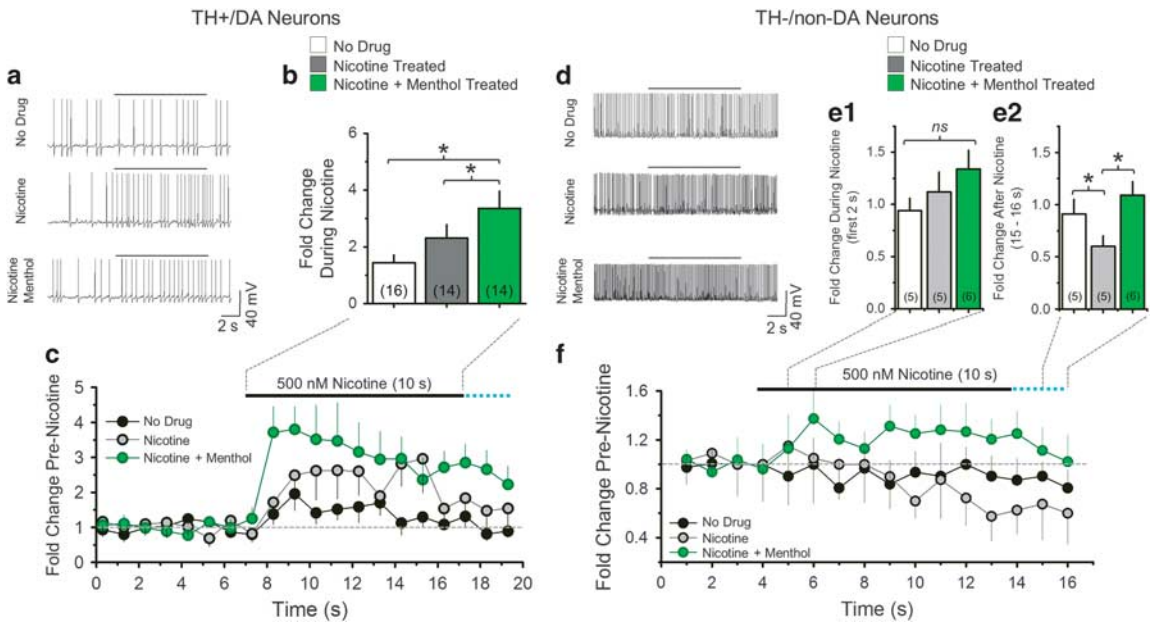

Figure 3 Menthol plus nicotine alters neuron excitability during acute exposure to smoking-relevant nicotine concentrations. Neurons were treated 10 days with control, nicotine, or menthol plus nicotine. (a and d) Representative current-clamp traces from TH+/DA and TH − /non-DA neurons puffed with nicotine. (b) Mean change in TH+/DA neuron firing frequency during 10 s nicotine applications. (c) and (f), mean firing frequency over time for TH+/DA and TH − /non-DA neurons, respectively. (e1–2) Mean fold-change in TH − /non-DA firing frequency following the first 2 s of nicotine puff (e1) and 2 s following end of nicotine puff (e2). For (c) and (b), number of individual neurons recorded is indicated in parenthesis in (b). For (f) and (e1–2), number of individual neurons recorded is indicated in parenthesis in (e1-2). The data are mean ± SEM. *, $p < 0.05$, one-way ANOVA, post *hoc* Tukey. Bars indicate 10 s, 500 nM nicotine application and (c, f) dotted blue lines indicate nicotine remains briefly after the end of puff due to perfusion rate.

In humans, brain nicotine concentrations peak at ~ 500 nM seconds after a puff on a cigarette (Matta *et al*, 2007). Accordingly, we investigated how TH+/DA neuron firing frequency changed during a 10 s exposure to 500 nM nicotine after 10-day treatment with control, nicotine, or menthol plus nicotine (Figure 3). Drug treatment produced a significant effect (one-way ANOVA, $F_{(2,41)} = 7.29$, $p = 0.002$). Control TH+/DA neurons exhibited a transient, 1.4-fold increase in firing frequency during nicotine application. Nicotine-treated TH+/DA neurons exhibited a transient,

2.3-fold increase in firing frequency during nicotine application. TH+/DA neurons treated with menthol plus nicotine exhibited a larger, slowly decaying increase in firing frequency during nicotine application that peaked at 4.0-fold over baseline and was significantly greater than control-treated ($p<0.005$) or nicotine-treated ($p<0.05$, Figure 3) TH+/DA neurons.

We completed similar assays on TH − /non-DA neurons (Figure 3d–f). Control-treated TH-/non-DA neurons exposed to acute nicotine (10 s, 500 nM) exhibited no change in firing frequency. Nicotine-treated TH − /non-DA neurons exposed to acute nicotine exhibited an initial increase in firing frequency followed by a significant decrease as the nicotine puff continued (Figure 3e1–e2 and f) (one-way ANOVA, $F_{(2,27)}=9.21$, $p<0.005$, Figure 3e2). TH − /non-DA neurons treated with menthol plus nicotine exposed to acute nicotine exhibited a non-significant increase in firing frequency that returned to baseline. We have previously reported that acute applications of menthol (up to 1 μM) do not activate nAChRs or potentiate nAChR currents (Henderson *et al*, 2016). We also stress that 500 nM menthol failed to alter firing frequency or stimulate inward currents in TH+/DA or TH − /non-DA neurons (Supplementary Figure S1). Thus, the effects we observed result from chronic and not acute actions of menthol.

Menthol Enhances Nicotine-Induced Upregulation of α4* But Not α6(non-α4)* nAChRs

Midbrain DA neurons contain α4β2(non-α6)*, α6β2(non-α4)*, and α4α6β2* nAChRs, while midbrain GABA neurons contain α4β2(non-α6)* nAChRs (Champtiaux *et al*, 2003). These nAChR subtypes are most sensitive to nicotine and are vital for nicotine reward (Pons *et al*, 2008). To study α4* and α6* nAChR upregulation, we used α4-mCherryα6-GFP mice that were treated for 10 days with vehicle, nicotine (2 mg/kg/h), or menthol plus nicotine (2 mg/kg/h, each). Similar to previous studies (Henderson *et al*, 2014, 2016), increases in integrated density of GFP or mCherry fluorescence was used to determine change in nAChR number. In midbrain, α6* nAChRs are selectively expressed in DA neurons (Mackey *et al*, 2012), so DA neurons of the SNc and VTA were identified by the presence of α6-GFP fluorescence (Figure 4a and b1–b2).

We observed a significant effect of drug treatment on α4-mCherry and α6-GFP fluorescence intensity in the VTA (one-way ANOVA: $F_{(2,12)}=24.1$, $p<0.001$ and $F_{(2,12)}=5.43$, $p=0.017$, α4-mCherry and α6-GFP, respectively) and SNc (one-way ANOVA: $F_{(2,12)}=4.14$, $p=0.043$ and $F_{(2,12)}=7.87$, $p=0.007$, α4-mCherry and α6-GFP respectively) and substantia nigra pars reticulata (SNr) (one-way ANOVA: $F_{(2,12)}=8.68$, $p=0.005$, α4-mCherry). Nicotine treatment robustly increased α4* nAChR number in VTA DA neurons and SNr GABA neurons (Figure 4c1–c3). Menthol plus nicotine produced an increase in α4* nAChRs in these same regions that was significantly greater than nicotine alone ($p<0.05$). In SNc DA neurons, α4* nAChR number was increased by nicotine ($p<0.05$) and the addition of menthol yielded no difference. As observed previously (Henderson *et al*, 2014), nicotine increased α6* nAChR number in VTA and SNc DA neurons (Figure 4c1–c2). In VTA neurons, menthol plus nicotine increased α6* nAChR number but was not different from nicotine treatment alone. In SNc DA neurons, menthol plus nicotine did not increase α6* nAChR number. These data suggest that menthol enhances only α4* nAChR upregulation in VTA DA and SNr GABA neurons, and does not enhance α6* nAChR upregulation.

We observed a similar trend in functional upregulation of nAChRs: nicotine alone upregulates α4* and α6* nAChR function, but menthol plus nicotine provides a further increase in only α4* nAChR function (Supplementary Figure S2). *In vitro,* we observed that menthol enhanced nicotine-induced upregulation of α4* and not α6* nAChRs (Supplementary Figure S3) and the upregulated α4* nAChRs were of the high-sensitivity stoichiometry (Supplementary Figure S4).These data further suggest that menthol may selectively enhance upregulation of α4* and not α6* nAChRs. Using RNA-seq, we found that nAChR upregulation by menthol plus nicotine was not accompanied by any changes in mRNA (Supplementary Figure S3G). Full descriptions of these results can be found in the Supplementary Results section of the Supplementary Material.

Menthol Enhances Nicotine-Induced Upregulation of α4α6* nAChRs

Using pixel-based FRET methods (Henderson *et al*, 2014), we identified regions where α4-mCherry and α6-GFP nAChR subunits co-assembled to form α4α6* nAChRs (Figure 4b1–b2). Drug treatment produced a significant effect on α4α6* nAChR number in the VTA (one-way ANOVA: $F_{(2,12)}=14.4$, $p<0.001$) and SNc (one-way ANOVA: $F_{(2,12)}=8.23$, $p=0.006$). In the VTA, α4α6* nAChR number did not significantly increase following nicotine treatment, but menthol plus nicotine significantly increased α4α6* nAChR number (Figure 4c1). In the SNc, nicotine and menthol plus nicotine increased α4α6* nAChR number to a similar extent (Figure 4c2).

Next, we examined whether menthol potentiated nicotine-induced nAChR upregulation on VTA DA neurons under the same drug exposure conditions as our CPP assays. α4-mCherryα6-GFP mice were given injections of saline, nicotine, or menthol plus nicotine, with dosing identical to CPP assays (presented in Figure 1). Because VTA DA neurons are critical for nicotine reward (Pons *et al*, 2008), we examined only VTA DA neurons (Figure 4d1–d3). We observed a significant effect on α4* nAChRs ($F_{(2,15)}=3.90$, $p=0.04$, Figure 4d1). Nicotine produced 20.1% increase in α4* nAChR number (not significant), while menthol plus nicotine produced a 41.3% increase ($p<0.05$). We observed no change in α6* nAChR number (Figure 4d2). These less-robust observations with alternating daily injections, compared to osmotic pumps, agreed with previous observations of nAChR upregulation.

We observed a significant effect on α4α6* nAChR number ($F_{(2,15)}=15.7$, $p<0.001$, Figure 4d3). Nicotine produced a 54.7% increase ($p<0.05$), while menthol plus nicotine produced a 117.6% increase ($p<0.005$). The increased α4α6* nAChR number in VTA neurons is accounted for by an increased number of FRET pixels, rather than by increased fluorescence intensity. This was consistent in both osmotic pump and injected preparations.

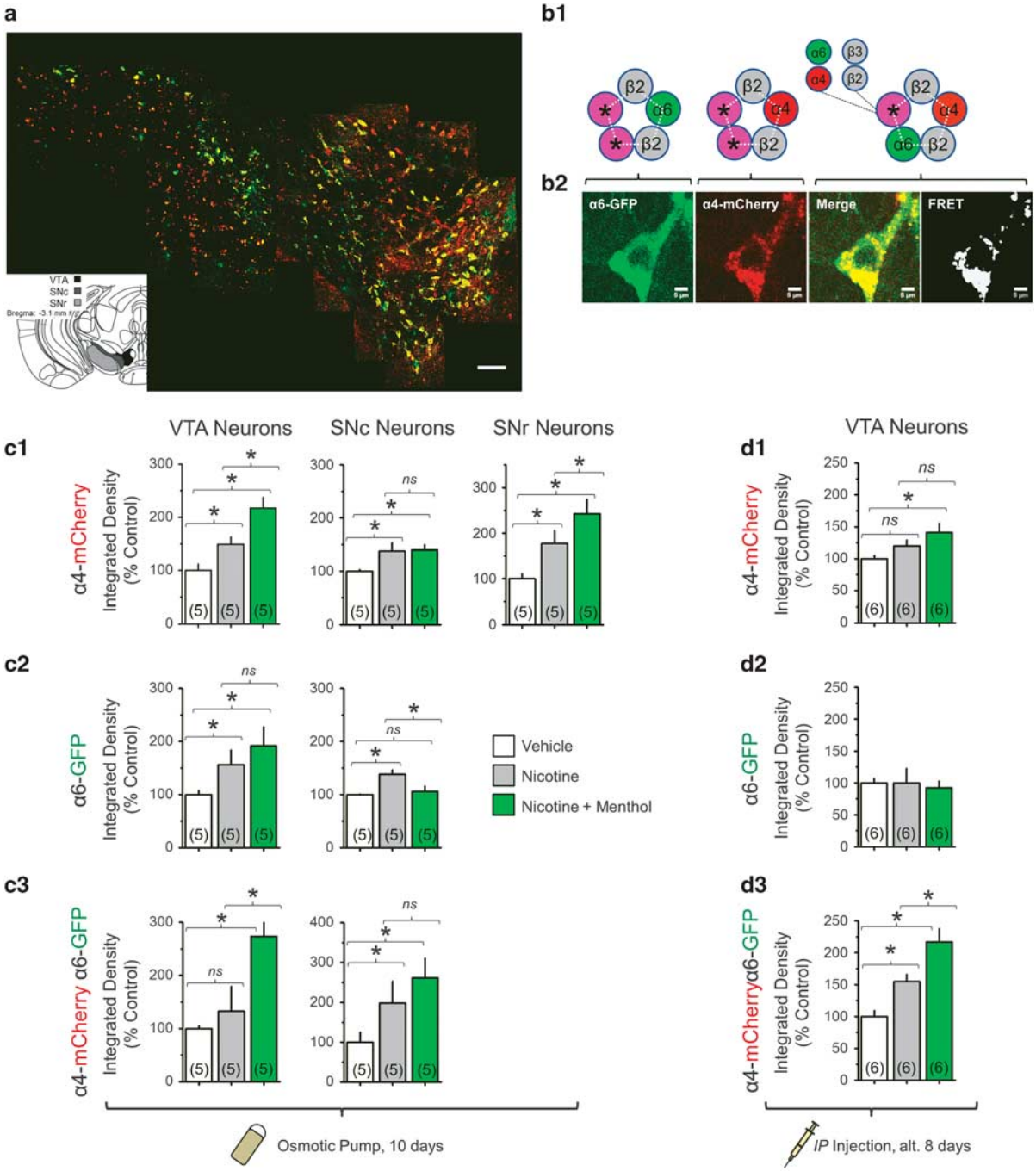

Figure 4 Menthol enhances nicotine-induced upregulation. (a) Representative coronal slice (bregma − 3.1) with α4-mCherryα6-GFP-labeled neurons in the VTA, SNc, and SNr. Bar, 100 μm. (b1) Potential assemblies of midbrain nAChRs revealed by GFP and mCherry fluorescence. The subunits indicated by * are uncertain (α4, α6, β2, or β3). (b2) Representative α4-mCherryα6-GFP* neurons. (c1–3) α4-mCherry*, α6-GFP*, or α4α6* nAChR-integrated density. The data are mean ± SEM, normalized to vehicle. (c1–3) Chronic treatments were 10 days: vehicle, 2 mg/kg/h nicotine, or 2 mg/kg/h nicotine with 2 mg/kg/h menthol. (d1–3) Mice were given drug dosing identical to CPP assays. Number of mice for each treatment is indicated in parenthesis. *$p < 0.05$, one-way ANOVA, post *hoc* Tukey.

DISCUSSION

Individual deletions of $\alpha4$, $\alpha6$, or $\beta2$ nAChR subunits prevent self-administration of nicotine in mice; and selective re-expression of these deleted subunits in the VTA (not SNc or SNr) re-instates self-administration (Pons *et al*, 2008). Selective activation of $\alpha6\beta2^*$ or $\alpha4\beta2^*$ nAChRs by smoking-relevant concentrations of nicotine stimulates depolarization and elevates firing frequency of VTA DA neurons (Liu *et al*, 2012; Engle *et al*, 2013). Both $\alpha4$ and $\alpha6$ nAChR subunits are necessary for this as preparations lacking either $\alpha4$ or $\alpha6$ nAChR subunits failed to stimulate depolarization or elevate VTA DA neuron firing in response to nicotine. Together, these data highlight VTA $\alpha4\alpha6\beta2^*$ nAChRs as the primary targets of smoking-relevant concentrations of nicotine.

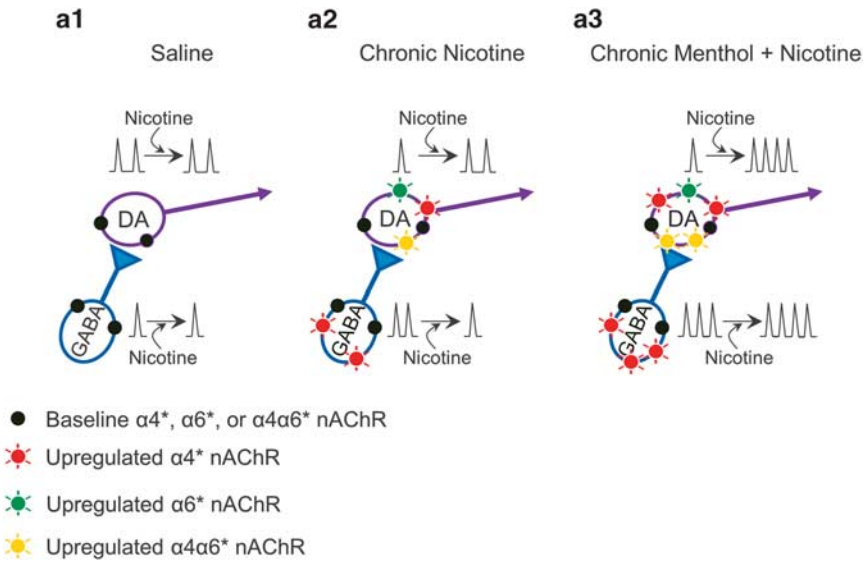

Figure 5 Summary. Simplified circuit diagram showing GABAergic neurons (blue) projecting to VTA DA neurons (purple). Arrows indicate acute applications of nicotine and its effect on firing frequency. See discussion for complete description.

We observed a significant increase in $\alpha4\alpha6\beta2^*$ nAChR number following menthol plus nicotine treatment, greater than the increase observed with nicotine alone (Figure 4c3 and d3). In assays using alternating daily injections, we observed little or no increase in total $\alpha4^*$ or $\alpha6^*$ nAChRs (Figure 4d1–d2). Considering the increase in $\alpha4\alpha6^*$ nAChRs, detected primarily by an increase in FRET pixels, this suggests that nicotine and menthol plus nicotine selectively upregulate the rather small, highly nicotine-sensitive subpopulation of nAChRs that contain both $\alpha4$ and $\alpha6$ subunits. Note that our brain slice microscopy method measures both intracellular and plasma membrane nAChRs and is not a direct measurement of functional nAChRs.

If $\alpha4\alpha6\beta2^*$ nAChRs are the primary targets of smoking-relevant concentrations of nicotine, their enhanced upregulation by menthol plus nicotine may underlie the enhancement in reward-related behavior (Figure 1b). The enhancement in TH+/DA-neuron firing frequency that we observed with menthol plus nicotine (Figure 3c) also supports this. Liu *et al* (2012) found that 300 nM nicotine applications elevated VTA DA neuron firing in a biphasic manner where $\alpha4(\text{non-}\alpha6)\beta2^*$ nAChRs mediated a rapid, desensitizing response and $\alpha4\alpha6\beta2^*$ mediated a sustained elevation in firing. We did observe robust, sustained increases in TH+/DA-neuron firing with similar nicotine applications. This may be attributed to the upregulated $\alpha4\alpha6\beta2^*$ nAChRs. GABAergic inputs on VTA neurons also play an important role in nicotine's actions. Following chronic exposure to nicotine, $\alpha4^*$ nAChRs are upregulated on SNr GABAergic neurons (Figure 4)(Nashmi *et al*, 2007). Acute nicotine exposure enhances GABAergic activity transiently, followed by sustained depression due to the upregulated $\alpha4^*$ nAChRs' rapid desensitization, leading to increased DA neuron excitability (Mansvelder *et al*, 2002). In TH − /non-DA neurons, we did measure transient increases in firing, followed by a maintained depression in activity (Figure 3f) in agreement with Mansvelder *et al*. TH-/non-DA neurons treated with menthol plus nicotine did not decrease in firing frequency during nicotine applications. We have previously observed that menthol (alone) reduces $\alpha4\beta2^*$ nAChR desensitization (Henderson *et al*, 2016). This may explain how TH-/non-DA neurons may fail to desensitize during nicotine applications as they have only $\alpha4(\text{non-}\alpha6)\beta2^*$ nAChRs. These observations are summarized in Figure 5.

Comparing menthol alone (Henderson *et al*, 2016) to menthol plus nicotine (studied here), we observe a distinct difference. Menthol alone upregulates low-sensitivity $\alpha4^*$ and $\alpha6^*$ nAChRs, suppresses DA neuron excitability, and suppresses nicotine reward-related behavior. Menthol plus nicotine enhances DA neuron excitability and nicotine reward-related behavior (Figures 1,2,3). The likely molecular basis is that menthol plus nicotine increases the number of high-sensitivity $\alpha4^*$ and $\alpha6^*$ nAChRs, but menthol alone upregulates low-sensitivity $\alpha4^*$ and $\alpha6^*$ nAChRs (Supplementary Figure S4 and Henderson *et al*, 2016).

We note that smokers experience odorant and tastant effects of menthol. It was shown that menthol decreases oral nicotine aversion in mice through a TRPM8-dependent mechanism (Fan *et al*, 2016). We stress that our results presented here use a drug exposure paradigm that avoids tastant effects and the neurons in our preparations (slices or cultures) do not contain TRPM8 receptors (Henderson *et al*, 2016). We suggest that tastant and sensory effects act in addition to the direct, neuron-mediated effects we observed.

We provide a cellular basis for how menthol may enhance nicotine reward-related behavior. Our findings, combined with the knowledge that menthol may enhance nicotine withdrawal (Alsharari *et al*, 2015) and nicotine IVSA (Wang *et al*, 2014; Biswas *et al*, 2016), may provide insight into why smokers of menthol cigarettes report lower quit rates.

FUNDING AND DISCLOSURE

This work was supported by fundings from the National Institutes of Health (NIH) (DA040047, DA033721, DA036061, DA037161, and DA037743).The authors declare no conflict of interest.

REFERENCES

Ahijevych K, Garrett BE (2010). The role of menthol in cigarettes as a reinforcer of smoking behavior. *Nicotine Tob Res* **12**(Suppl 2): S110–S116.

Ai J, Taylor KM, Lisko JG, Tran H, Watson CH, Holman MR (2015). Menthol content in US marketed cigarettes. *Nicotine Tob Res* **18**: 1575–1580.

Alsharari SD, King JR, Nordman JC, Muldoon PP, Jackson A, Zhu AZX *et al* (2015). Effects of menthol on nicotine pharmacokinetic, pharmacology and dependence in mice. *PloS ONE* **10**: e0137070.

Biswas L, Harrison E, Gong Y, Avusula R, Lee J, Zhang M *et al* (2016). Enhancing effect of menthol on nicotine self-administration in rats. *Psychopharmacology* **233**: 3417–3427.

Brody A, Mukhin A, La Charite J, Ta K, Farahi J, Sugar C *et al* (2013). Up-regulation of nicotinic acetylcholine receptors in menthol cigarette smokers. *Int J Neuropsychopharmacol* **16**: 957–966.

CDC (2016). Tobacco use among middle and high school students - United States, 2011 - 2015. *MMWR Morb Mortal Wkly Rep* **65**: 361–367.

Champtiaux N, Gotti C, Cordero-Erausquin M, David DJ, Przybylski C, Lena C *et al* (2003). Subunit composition of functional nicotinic receptors in dopaminergic neurons investigated with knock-out mice. *J Neurosci* **23**: 7820–7829.

D'Silva J, Boyle RG, Lien R, Rode P, Okuyemi KS (2012). Cessation outcomes among treatment-seeking menthol and nonmenthol smokers. *Am J Prev Med* **43**: S242–S248.

Engle SE, Shih PY, McIntosh JM, Drenan RM (2013). Alpha4alpha6beta2* nicotinic acetylcholine receptor activation on ventral tegmental area dopamine neurons is sufficient to stimulate a depolarizing conductance and enhance surface AMPA receptor function. *Mol Pharmacol* **84**: 393–406.

Fan L, Balakrishna S, Jabba SV, Bonner PE, Taylor SR, Picciotto MR *et al* (2016). Menthol decreases oral nicotine aversion in C57BL/6 mice through a TRPM8-dependent mechanism. *Tob Control* **0**: 1–5.

Henderson BJ, Srinivasan R, Nichols WA, Dilworth CN, Gutierrez DF, Mackey ED *et al* (2014). Nicotine exploits a COPI-mediated process for chaperone-mediated up-regulation of its receptors. *J Gen Physiol* **143**: 51–66.

Henderson BJ, Wall T, Henley BM, Kim CH, Nichols WA, Moaddel R *et al* (2016). Menthol alone upregulates midbrain nAChRs, alters nAChR sybtype stoichiometry, alters dopamine neuron firing frequency, and prevents nicotine reward. *J Neurosci* **36**: 2957–2974.

Liu L, Zhao-Shea R, McIntosh JM, Gardner PD, Tapper AR (2012). Nicotine persistently activates ventral tegmental area dopaminergic neurons via nicotinic acetylcholine receptors containing alpha4 and alpha6 subunits. *Mol Pharmacol* **81**: 541–548.

Mackey ED, Engle SE, Kim MR, O'Neill HC, Wageman CR, Patzlaff NE *et al* (2012). α6* nicotinic acetylcholine receptor expression and function in a visual salience circuit. *J Neurosci* **32**: 10226–10237.

Mansvelder HD, Keath JR, McGehee DS (2002). Synaptic mechanisms underlie nicotine-induced excitability of brain reward areas. *Neuron* **33**: 905–919.

Matta SG, Balfour DJ, Benowitz NL, Boyd RT, Buccafusco JJ, Caggiula AR *et al* (2007). Guidelines on nicotine dose selection for *in vivo* research. *Psychopharm* **190**: 269–319.

McCarthy WJ, Caskey NH, Jarvik ME, Gross TM, Rosenblatt MR, Carpenter C (1995). Menthol vs nonmenthol cigarettes: effects on smoking behavior. *Am J Public Health* **85**: 67–72.

Nashmi R, Xiao C, Deshpande P, McKinney S, Grady SR, Whiteaker P *et al* (2007). Chronic nicotine cell specifically upregulates functional α4* nicotinic receptors: basis for both tolerance in midbrain and enhanced long-term potentiation in perforant path. *J Neurosci* **27**: 8202–8218.

Pons S, Fattore L, Cossu G, Tolu S, Porcu E, McIntosh JM *et al* (2008). Crucial role of α4 and α6 nicotinic acetylcholine receptor subunits from ventral tegmental area in systemic nicotine self-administration. *J Neurosci* **28**: 12318–12327.

Rodgman A, Perfetti TA (2009). *The Chemical Components of Tobacco and Tobacco Smoke*. CRC Press: Boca Raton, FL, USA.

Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C *et al* (2004). Nicotine activation of α4* receptors: sufficient for reward, tolerance and sensitization. *Science* **306**: 1029–1032.

Wang T, Wang B, Chen H (2014). Menthol facilitates the intravenous self-administration of nicotine in rats. *Front Behav Neurosci* **8**: 437.

Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)