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Menthol facilitates dopamine-releasing effect of nicotine in rat nucleus accumbens

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Abstract

Menthol is a significant flavoring additive in tobacco products. Accumulating clinical evidence suggests that menthol may promote tobacco smoking and nicotine dependence. Our previous studies demonstrated that menthol enhanced nicotine reinforcement in rats. However, it is unclear whether menthol interacts with nicotine at the neurochemical level. The present study used intracranial microdialysis to examine whether and the ways in which menthol affects nicotine-induced dopamine release in rats in the nucleus accumbens core (NAc), a terminal field of brain reward circuitry. To make comparisons with our previous work that showed an enhancing effect of menthol on nicotine self-administration behavior, male Sprague-Dawley rats were first trained in 20 daily 1-h sessions to press a lever for intravenous nicotine self-administration (15 µg/kg/infusion). Dopamine levels were then measured in the right NAc using intracranial microdialysis coupled with high-performance liquid chromatography. Five minutes before microdialysis, the rats received an intraperitoneal injection of menthol (0, 1, 2.5, and 5 mg/kg), a subcutaneous injection of nicotine (0.2 mg/kg or its vehicle), or both. Menthol alone did not affect dopamine levels in dialysates, whereas nicotine alone elevated dopamine levels. Combined nicotine and menthol administration significantly increased dopamine levels compared with nicotine alone. These data indicate a facilitating effect of menthol on nicotine-induced dopamine release in the NAc. These findings shed light on our understanding of the neurobiological mechanisms that underlie the menthol-induced enhancement of nicotine reinforcement.

Keywords

Dopamine; menthol; microdialysis; nicotine; reinforcement

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Conflict of Interest
None.

1. Introduction

In the United States, approximately 40 million adults are current smokers (CDC, 2015), and approximately one-third of these smokers use menthol cigarettes (Caraballo and Asman, 2011; Curtin et al., 2014; Pearson et al., 2012; SAMHSA, 2009). Relative to the overall significant progress in reducing tobacco smoking over the past half century, the use of mentholated cigarettes, particularly among the younger population, has become a growing concern (Giovino et al., 2015; SAMHSA, 2009; USDHHS, 2014).

Accumulating clinical and epidemiological studies have reported the significant impact of menthol on tobacco smoking and addiction. Such an impact manifests as higher rates of smoking experimentation and progression to regular smoking and lower rates of smoking cessation success (Anderson, 2011a; b; Benowitz and Samet, 2011; Delnevo et al., 2011; Delnevo et al., 2015; Fagan et al., 2015; Giovino et al., 2015; Tobacco, 2011; TPSAC, 2011). For example, evidence indicates that menthol smokers have a shorter latency to smoke their first cigarette in the morning after waking up, inhale more deeply, and have higher levels of nicotine addiction (Ahijevych and Parsley, 1999; Hoffman and Simmons, 2011; Muscat et al., 2009; Okuyemi et al., 2003; Richter et al., 2008). Moreover, menthol cigarette smokers have more difficulty quitting smoking and higher rates of relapse compared with their counterparts who smoke non-menthol tobacco (Ahijevych and Garrett, 2010; Besaratinia and Tommasi, 2015; Gardiner and Clark, 2010; Levy et al., 2011; Reitzel et al., 2013; Reitzel et al., 2011; Rojewski et al., 2014).

Our recent research, using the drug self-administration and reinstatement procedures that have showed good validities and translational value for our understanding of drug addictive behavior (Bossert et al., 2013; Henningfield et al., 2016 for exemplary reviews), demonstrated the impact of the interaction between menthol and nicotine on nicotine-addictive behavior. Specifically, menthol was found to enhance the reinforcing action of nicotine in a self-administration procedure (Biswas et al., 2016) and sustained and reinstated nicotine-seeking responses in an extinction-reinstatement paradigm (Harrison et al., 2017). However, the neurobiological mechanisms that underlie the observed behavioral effects of menthol and its interaction with nicotine remain unknown. The present study examined whether and the ways in which menthol interacts with nicotine to alter dopamine release in the brain reward system.

To make comparisons with the behavioral history of nicotine self-administration that was reported in our previous studies, the present study first allowed male Sprague-Dawley rats to intravenously self-administer nicotine (15 µg/kg/infusion) in daily 1-h sessions. Intracranial microdialysis coupled with high-performance liquid chromatography (HPLC) were then performed to measure dopamine levels in the right nucleus accumbens core (NAc). The NAc is an important terminal region of the brain mesocorticolimbic circuitry (Berridge and Robinson, 1998; Di Chiara and Bassareo, 2007; Ikemoto and Panksepp, 1999; Koob, 1992), which also plays a critical role in mediating the reinforcing actions of nicotine (Balfour et al., 1998; Clarke, 1990; Corrigall et al., 1992; Di Chiara, 2000; Picciotto and Corrigall, 2002; Watkins et al., 2000). We hypothesized that menthol would enhance the dopamine-

releasing action of nicotine, but that menthol administration alone might not alter dopamine levels.

2. Materials and Methods

2.1. Subjects

Male Sprague-Dawley rats were housed in a humidity- and temperature-controlled colony room on a reverse 12 h/12 h light/dark cycle. To facilitate nicotine self-administration learning and to be consistent with our previously reported behavioral data (Biswas et al., 2016), a food-restriction regimen (20 g chow/day) was employed with unlimited access to water throughout the experiments. The feeding regimen allowed the rats to have consistent but low weight gain (i.e., approximately 85% of their free-feeding weight). All of the experimental tests were conducted during the dark phase at the same time each day (9:00 AM–3:00 PM). The experimental protocol was approved by the University of Mississippi Medical Center Institutional Animal Care and Use Committee.

2.2. Surgical procedures

The rats were anesthetized with isoflurane (1–3% in 95% O₂ and 5% CO₂) and then underwent catheterization surgery. They were implanted with an indwelling intravenous catheter in the right jugular vein after lever-press training with food reward as previously reported (Biswas et al., 2016). These rats were also implanted with a unilateral guide cannula immediately after intravenous catheterization without the interruption of anesthesia. The rats were placed on a stereotaxic frame, and a stainless-steel guide cannula (CMA/Microdialysis) was implanted in the right NAc (1.7 mm anterior to bregma, 1.2 mm lateral to bregma, and 7.0 mm below the surface of the skull; (Paxinos and Watson, 2007). The concentric microdialysis probe (membrane length, 2 mm; outer diameter, 0.5 mm) was used to collect dialysate samples from the entire NAc region.

The rats were allowed to recover from surgery for 7–10 days. The catheters were flushed daily with 0.1 ml of sterile saline that contained heparin (30 U/ml) and gentamicin (20 mg/ml) to maintain catheter patency and prevent infection. Thereafter, the catheters were flushed with heparinized saline before and after the experimental sessions.

After performing microdialysis, probe placements were confirmed histologically. A representative microphotograph of a Cresyl violet stained brain section is shown in Fig. 1. Rats were excluded from the data analysis if more than half of the active portion of the dialysis membrane was outside the border of the NAc.

2.3. Nicotine self-administration

The rats were allowed to intravenously self-administer nicotine (15 µg/kg/infusion, free base) in standard operant conditioning chambers (Med Associates, St. Albans, VT, USA) in 20 daily 1-h sessions. (–)-Nicotine hydrogen tartrate (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in physiological saline, and the pH was adjusted to 7.0 ± 0.4 with 1N sodium hydroxide solution. The rats were placed in the operant conditioning chambers and connected to the intravenous drug infusion system. The sessions began with extension of the

two levers into the chamber and illumination of a red house light. Once the rats reached the fixed-ratio (FR) requirement of responses on the active lever, an infusion of nicotine was delivered in a volume of 0.1 ml over approximately 1 s, depending on the rats' body weight. Each nicotine infusion was signaled by the presentation of an auditory/visual stimulus that consisted of 5-s tone and 20-s illumination of the light above the active lever, which also indicated a 20-s timeout period that followed each nicotine infusion. An FR1 schedule of reinforcement was used for sessions 1–5. An FR2 schedule was used for sessions 6–8. An FR5 schedule was used for sessions 9–20. Responses on the inactive lever were recorded but had no programmed consequences. The sessions were conducted 5 days/week with weekend off.

2.4. Microdialysis and dopamine assay

On the evening of the 20th day of nicotine self-administration, a microdialysis probe was inserted into the right NAc and perfused at 0.2 μ l/min overnight. The next day (12–14 h after probe insertion), the rats were placed in the operant chambers, and the perfusion rate was increased to 2.0 μ l/min. After 1-h equilibrium, dialysate sampling in 10-min fractions began and continued until the test was completed. Baseline sampling lasted 30 min, and then drug treatments were given at 60-min intervals. The dialysate samples were immediately frozen on dry ice and stored at -80°C until the dopamine assay.

Dopamine concentrations in the dialysate samples were measured by reverse-phase HPLC coupled with electrochemical detection. The mobile phase was 0.54 nM monobasic sodium phosphate buffer that was pumped through the column at a rate of 20 μ l/min. Dialysate samples were injected into an ODS-2 C-18 microbore column; 100 mm $\times$ 1.0 mm) in a volume of 1 μ l using an electrically actuated valve (Model ECI4W1). Analytes were detected amperometrically (Model MP 1304) with a dual glassy carbon working electrode (Model RE1). The applied potential was +700 mV (vs. Ag/AgCl). The detection limit for dopamine was 0.2 nM, defined by a signal/noise ratio > 3 .

2.5. Dose-response effects of menthol and nicotine on dopamine levels in the NAc

Eighteen rats were used for microdialysis test 1. After 20 daily nicotine self-administration sessions, these rats were divided into two groups ($n = 9/\text{group}$) with similar levels of nicotine intake. Although all of the animals received menthol administration, half of the rats immediately received nicotine, and the other half received saline (i.e., the vehicle for nicotine). After 30-min baseline dialysate sampling, the rats in the menthol+nicotine group received an intraperitoneal injection of menthol, immediately followed by a subcutaneous injection of nicotine (0.2 mg/kg in a volume of 1 ml/kg). The 0.2 mg/kg nicotine was used because it was the amount that rats self-administered in the daily 1-h sessions so that the microdialysis results could be readily interpreted as a neurochemical substrate for our behavioral observations reported earlier (Biswas et al., 2016). The menthol+saline group received an intraperitoneal injection of menthol, immediately followed by a subcutaneous injection of saline (1 ml/kg). Four doses of menthol (0, 1, 2.5, and 5 mg/kg) were given to the rats in a counterbalanced order so that every rat received each dose once. As reported previously (Biswas et al., 2016; Harrison et al., 2017), a 50% DMSO in deionized water served as the vehicle (0) of menthol. These menthol doses were used based on our previous

behavioral studies showing a threshold dose of 2.5 mg/kg to enhance nicotine reinforcement (Biswas et al., 2016). Since menthol content varies from 1.8 µg/cigarette in nonmenthol-labeled to 19.6 mg/cigarette in menthol-labeled cigarettes (e.g., Ai et al., 2016; FTC, 2016), these doses of menthol should be comparable to the amount smokers take. The four menthol +nicotine or menthol+saline treatments were arranged one hour apart in order to eliminate possible carryover effects.

2.6. Trajectory of dopamine levels in the NAc following menthol and/or nicotine administration

Twenty-four rats were used for microdialysis test 2. After 20 daily nicotine self-administration sessions, these rats were divided into three groups ($n = 8/\text{group}$) with similar levels of nicotine intake. After 30-min baseline dialysate sampling, the first group received an intraperitoneal injection of menthol (2.5 mg/kg), the threshold dose in both previous behavioral tests (Biswas et al., 2016; Harrison et al., 2017) and the above described microdialysis test 1. The second group received a subcutaneous injection of nicotine (0.2 mg/kg). The third group received an intraperitoneal injection of menthol, immediately followed by a subcutaneous injection of nicotine. Dialysate sampling lasted 2 h after drug administration.

2.7. Statistical analyses

The nicotine self-administration data are expressed as the mean $\pm$ SEM number of lever presses and corresponding nicotine infusions pooled across the final three sessions. These data were analyzed using one-way analysis of variance (ANOVA), followed by Bonferroni's post hoc test. The data from microdialysis test 1 are expressed as the mean $\pm$ SEM of raw dopamine concentrations in the 30-min baseline period and 30-min period following the administration each of the four menthol doses in both the nicotine and saline groups. These data were first analyzed using two-way ANOVA and then using one-way ANOVA for nicotine or its vehicle separately. The data from microdialysis test 2 are expressed as the mean $\pm$ SEM of the percentage changes in 10-min-bin dopamine concentrations in the three groups (menthol, nicotine, and menthol+nicotine). These data were analyzed using two-way repeated-measures ANOVA, followed by one-way ANOVAs for each group and within each group. Finally, Bonferroni's post hoc tests were used to confirm significant differences among individual means.

3. Results

3.1. Nicotine self-administration

In the 20 daily 1-h self-administration sessions, the rats acquired lever-press responding to earn intravenous nicotine infusions (15 µg/kg/infusion). Averaged across the final three sessions (18, 19, and 20), these rats emitted a mean $\pm$ SEM of 67.1 ± 13.3 responses on the active lever and 8.1 ± 3.2 responses on the inactive lever, consequently self-administering 12.8 ± 4.2 infusions of nicotine.

These animals were randomly divided into two groups for microdialysis test 1 ($n = 9/\text{group}$) and three groups for microdialysis test 2 ($n = 8/\text{group}$). These groups had self-administered

similar numbers of nicotine infusions (test 1: $F_{1,22} = 0.36$, $p > 0.05$; test 2: $F_{2,21} = 0.56$, $p > 0.05$; Table 1).

3.2. Dose-response effects of menthol and nicotine on dopamine levels in the NAc

Fig. 2 shows the results of the microdialysis assay in rats with the aforementioned history of nicotine self-administration. The two-way repeated-measures ANOVA revealed a significant main effect of nicotine ($F_{1,16} = 6.74$, $p < 0.05$) and a significant nicotine $\times$ menthol interaction ($F_{3,48} = 3.68$, $p < 0.05$), with no main effect of menthol ($F_{3,48} = 3.68$, $p = 0.096$). A t -test showed a significant difference between nicotine (in the top panel) and saline (in the below panel) at 0 menthol condition, indicating a dopamine-releasing action of nicotine.

The top panel in Fig. 2 shows that pretreatment with menthol significantly elevated dopamine levels in the NAc in rats that subsequently received nicotine (0.2 mg/kg). The one-way repeated-measures ANOVA revealed a significant main effect of menthol dose ($F_{3,24} = 4.42$, $p < 0.05$). Bonferroni's *post hoc* test confirmed a significant ($p < 0.05$) difference between 2.5 and 5 mg/kg menthol vs. vehicle and 1 mg/kg menthol. As shown in the bottom panel in Fig. 2, in rats that received saline instead of nicotine immediately following menthol treatment, dopamine levels in the NAc remained unchanged. The one-way repeated-measures ANOVA did not indicate a significant effect of menthol dose ($F_{3,24} = 0.61$, $p > 0.05$).

3.3. Trajectory of dopamine levels in the NAc following menthol and/or nicotine administration

As shown in Fig. 3, pretreatment with menthol significantly enhanced the dopamine-releasing effect of nicotine. Menthol alone did not alter dopamine levels in the NAc. The two-way repeated-measures ANOVA of dopamine levels in dialysate samples revealed significant effects of group ($F_{2,21} = 7.41$, $p < 0.01$) and time ($F_{8,168} = 13.25$, $p < 0.0001$) and a significant group $\times$ time interaction ($F_{16,168} = 3.43$, $p < 0.0001$). Further one-way ANOVAs and subsequent Bonferroni's *post hoc* tests within each individual group (repeated measures) and at each time point across different groups confirmed significantly higher dopamine levels after the administration of nicotine and menthol+nicotine compared with baseline and menthol administration alone. In particular, there was a significant difference between menthol+nicotine and nicotine alone groups in the first 3 dialysate samples following drug administration. Detailed statistical results are shown in Fig. 3.

4. Discussion

The present study demonstrated that menthol enhanced the effect of nicotine on the release of dopamine in the NAc, an important terminal region in the brain dopaminergic reward system. These findings shed light on our understanding of the neurochemical mechanisms that underlie the menthol-induced enhancement of nicotine reinforcement that was reported recently by our laboratory (Biswas et al., 2016; Harrison et al., 2017). Menthol facilitated nicotine-induced dopamine release in the brain reward system and thus enhanced nicotine reinforcement, providing important empirical evidence that supports the U.S. Food and Drug Administration's initiative to regulate the addition of menthol to tobacco products.

Menthol alone did not alter dopamine levels in the NAc in rats that received menthol without subsequent nicotine administration. The doses of menthol (up to 5 mg/kg) that were used in the present study were shown to be effective in enhancing nicotine-taking and -seeking behavior in our previous studies (Biswas et al., 2016; Harrison et al., 2017), thus precluding possible concerns about the doses of menthol being too low to produce an effect. The lack of effect of menthol in this microdialysis assay is consistent with a recent report that showed that the intraoral administration of menthol, in contrast to sucrose or saccharin, produced no effect on phasic dopamine release, measured by fast-scan cyclic voltammetry, in the NAc (Wickham et al., 2018). A previous study found that menthol reduced the frequency of dopamine neuron firing in mouse brain slices and cultured neuron preparations (Henderson et al., 2016). The inability of menthol alone to increase dopamine release is consistent with behavioral observations that showed that menthol did not produce conditioned place preference in mice (Henderson et al., 2017). An earlier mouse study found that the menthol-induced increase in ambulation was prevented by pretreatment with dopamine receptor antagonists (Umezu and Morita, 2003), indicating the involvement of dopamine neurotransmission in the effects of menthol. However, the extraordinarily high doses of menthol (400–800 mg/kg, subcutaneous) in this previous study made the findings irrelevant in terms of menthol's actions in mentholated cigarette smokers. Altogether, these data suggested that menthol may not significantly influence dopaminergic signaling in brain reward circuitry in smokers who use mentholated tobacco products.

In the present study, nicotine administration increased dopamine concentrations in dialysate samples. These results are consistent with numerous studies that used intracranial microdialysis to examine the effects of nicotine on dopamine release in mesocorticolimbic rewarding circuits (Cadoni and Di Chiara, 2000; D'Souza et al., 2011; Fu et al., 2000; Johnson et al., 2000; Lecca et al., 2006; Mifsud et al., 1989; Rahman et al., 2004; Zocchi et al., 2003). Nicotine, like other drugs of abuse and natural rewards, facilitates dopamine release to produce reinforcing actions. The trajectory of nicotine to increase dopamine release in the NAc (i.e., a rapid onset that lasted approximately 10 min) was similar to our previous report (Liu et al., 2001). In our previous study, the facilitating effect of nicotine that was locally applied via the microdialysis probe elevated norepinephrine release in the rat supraoptic nucleus up to 20 min after application. These microdialysis observations corroborate many electrophysiological studies that showed that nicotine facilitated the firing rate of dopamine neurons in the NAc (Bahk et al., 2002; Dani et al., 2011; Erhardt et al., 2002; Kleijn et al., 2011; Morud et al., 2016). However, no correlation of the acute nicotine-elevated dopamine levels with previous nicotine intake during self-administration phase was observed, and neither is the case for the menthol+nicotine-elevated dopamine levels discussed below. Notwithstanding, the issue of whether and how the previous history of nicotine self-administration impacts the dopamine-releasing effect of either nicotine alone or menthol+nicotine deserves investigation in future.

The present study demonstrated that menthol interacted with nicotine to elevate dopamine levels in the NAc. Menthol pretreatment prior to nicotine administration resulted in a greater elevation of dopamine levels compared with the nicotine-alone condition. Moreover, with menthol pretreatment, the dopamine-releasing effect of nicotine lasted up to 30 min compared with 10 min in the no-menthol condition. The enhancing effect of menthol on

nicotine-induced dopamine release is consistent with a recent electrophysiological study that showed that menthol+nicotine increased the frequency of dopamine neuron firing more than nicotine alone (Henderson et al., 2017). In adolescent rats, functional magnetic resonance imaging indicated greater functional connectivity in reward circuitry (i.e., the ventral tegmental area and its dopaminergic projection terminal regions) with combined treatment with menthol (5.38 mg/kg) and nicotine (0.4 mg/kg) compared with nicotine alone (Thompson et al., 2017). Additionally, menthol was shown to alter dopamine neurotransmission in rodents by directly interacting with dopamine uptake (Umezaki and Morita, 2003). Thus, converging evidence suggests that menthol may strengthen dopaminergic neuron signaling that is activated by nicotine. In the present study, the rats had a history of nicotine self-administration that was exactly the same as in our previous studies of nicotine-taking and -seeking (Biswas et al., 2016; Harrison et al., 2017). A reasonable interpretation of the present data is that dopamine is a neurochemical substrate that is involved in the enhancing effect of menthol on nicotine reinforcement.

In addition to the enhancing effect of menthol on nicotine-induced dopaminergic signaling, menthol may also influence other neurotransmitters and their receptors to facilitate nicotine reinforcement. Menthol was shown to act on nicotinic acetylcholine receptors (nAChRs) and γ -aminobutyric acid (GABA) receptors. Recent studies showed that menthol regulated the function of $\alpha 4\beta 2$, $\alpha 3\beta 4$, and $\alpha 7$ nAChRs (Ashoor et al., 2013; Hans et al., 2012; Henderson et al., 2017; Henderson et al., 2016; Ton et al., 2015). In rodents, menthol and nicotine interacted to produce an additive effect on $\alpha 4$, $\alpha 6$, and $\beta 2$ nAChR-expressing neurons in the mesocorticolimbic reward circuit (Alsharari et al., 2015; Henderson et al., 2017). Such actions of menthol reconcile well with a human study that found that menthol cigarette smokers had a higher density of $\alpha 4\beta 2$ nAChRs in several brain regions, including the prefrontal cortex, corpus callosum, cerebellum, and brainstem, compared with nonmenthol smokers (Brody et al., 2013). An effect of menthol on GABA receptor activity has been reported (Henderson et al., 2016; Lau et al., 2014; Watt et al., 2008; Zhang et al., 2008). Interestingly, however, activation of the classic menthol receptors (i.e., transient receptor potential melastatin 8 [TRPM8] ion channels; (Peier et al., 2002) does not appear to be required for the observed effects of menthol. TRPM8 receptors are located at peripheral sensory nerve terminals, and the intraperitoneal route of menthol administration eliminates the topical and sensory effects of menthol. In our recent study (Harrison et al., 2017), the selective TRPM8 receptor antagonist RQ-00203078 did not block the enhancing effect of menthol on nicotine-seeking behavior. In cultured dopamine neurons from the rodent midbrain and in Neuro-2a cells with genetic deletion of TRPM8 receptors, menthol altered the electrophysiological activity of nAChRs (Henderson et al., 2016). Thus, it is reasonable to conclude that the direct interaction of menthol with central neurotransmitter receptors rather than menthol's sensory actions underlies the observed behavioral and neurochemical effects of menthol in these preparations.

In conclusion, the present study demonstrated an enhancing effect of menthol on nicotine-induced dopamine release in the NAc. The present results provide further insights into the neurochemical mechanisms that underlie observations in clinical and animal research, showing that menthol interacts with nicotine to increase nicotine reinforcement (Ahijevych

and Garrett, 2004; 2010; Alsharari et al., 2015; Biswas et al., 2016; Harrison et al., 2017; Hoffman and Simmons, 2011; Wang et al., 2014; Wickham, 2015).

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Highlights

- Dopamine levels in the nucleus accumbens core (NAc) were measured using intracranial microdialysis in rats with a history of nicotine self-administration.
- Nicotine elevated dopamine concentrations in the dialysates from the NAc.
- Menthol alone did not alter dopamine concentrations.
- Menthol enhanced the dopamine-releasing effect of nicotine.

Figure 1.

A representative microphotograph of the brain section showing the placement of microdialysis probe in the nucleus accumbens core region.

Figure 2.

Effect of menthol on dopamine concentrations in microdialysis samples from the right NAc in rats with a history of nicotine self-administration. (Top) Results from rats that received an intraperitoneal injection of menthol, immediately followed by subcutaneous nicotine administration (0.2 mg/kg). (Bottom) Results from rats that received an intraperitoneal injection of menthol, immediately followed by subcutaneous saline administration. * $p < 0.05$, significant difference from the 0 or 1 mg/kg condition; + $p < 0.05$, significant difference from the 0 condition of the saline rats showed in the below panel.

Figure 3.

Trajectory of changes in dopamine concentrations after the administration of menthol, nicotine, or menthol+nictotine in rats with a history of nicotine self-administration. The data are expressed as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, significant difference from time point 0 (baseline); + $p < 0.05$, ++ $p < 0.01$, significant difference from nicotine alone group at respective time points.

Table 1.

Similar profiles of nicotine self-administration in different groups of rats prior to the intracranial microdialysis assay. The number of nicotine infusions was averaged across the final three nicotine self-administration sessions and is expressed as the mean $\pm$ SEM.

Microdialysis tests and groups	Nicotine Infusions
Test 1 ($n = 9/\text{group}$)	
Saline	14.2 ± 3.6
Nicotine	13.4 ± 4.1
Test 2 ($n = 8/\text{group}$)	
Menthol	13.2 ± 3.5
Nicotine	12.9 ± 2.9
Menthol+Nicotine	14.1 ± 4.2