ORIGINAL INVESTIGATION

Effects of Nicotine on Olfactogustatory Incentives: Preference, Palatability, and Operant Choice Tests

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ABSTRACT

Introduction: The use of additives in tobacco may capitalize on the incentive motivational properties of tastes and scents such as mint (menthol), vanilla, and strawberry. These incentives are intended to increase tobacco experimentation, but their salience may also be enhanced by the incentive amplifying effects of nicotine (NIC). The goal of the present studies was to investigate the potential interaction between the incentive amplifying effects of NIC and gustatory incentives.

Methods: One of two discriminable tastes (grape or cherry Kool-Aid®; 0.05% wt/vol; unsweetened) was paired with sucrose (20% wt/vol; conditioned stimulus [CS+]) in deionized water, whereas the other taste (CS-) was presented in deionized water. Experiment 1 investigated the effects of NIC pretreatment on preference for the CS+ versus CS- in 2-bottle choice tests. Experiment 2 investigated the effects of NIC on palatability of the CS+ and CS- using orofacial taste reactions. Experiment 3 investigated the effects of NIC on reinforcement by the CS+ and CS- using a concurrent choice operant task.

Results: NIC pretreatment robustly increased operant responding for the CS+ but did not alter responding for the CS- in the operant choice task (Experiment 3). However, NIC pretreatment did not alter intake or palatability of the CS+ or CS- (Experiments 1 and 2).

Conclusions: NIC increases the reinforcing effects of gustatory incentive stimuli, even though these stimuli were not paired with NIC administration. The findings suggest that adding taste incentives to tobacco products may increase the attractiveness of these products to consumers and the probability of repeated use.

INTRODUCTION

Nicotine (NIC) is a weak primary reinforcer (Le Foll & Goldberg, 2009). Despite these weak primary reinforcing effects, NIC potently increases responding for nondrug rewards such as visual stimuli (Palmatier, Liu, Caggiula, Donny, & Sved, 2007a) and sucrose (Palmatier, O'Brien, & Hall, 2012a). This latter "incentive amplifying" effect probably depends on NIC-induced increases in incentive motivation (Palmatier et al. 2012a, 2012b). For example, we recently found that NIC administration preferentially increased approach to a conditioned stimulus (CS) paired with sucrose (sign tracking) but did not change approach to the location of the sucrose reward (goal tracking; Palmatier et al., 2012b). These findings converge with findings from other paradigms showing that NIC increases other behaviors motivated by appetitive CSs (Chaudhri et al., 2006; Olausson, Jentsch, & Taylor, 2003, 2004; Thiel, Sanabria, & Neisewander, 2009).

Incentive motivation traditionally refers to behavior that is energized by stimuli associated with a primary reward; as such, a stimulus described as an incentive usually energizes

behavior based on acquired rather than inherent appetitive properties (Spence, 1956). The hypothesis that NIC amplifies the effects of incentive stimuli on behavior makes specific predictions about the role of associative and motivational processes in NIC self-administration and tobacco use (Bevins & Palmatier, 2004; Caggiula et al., 2009). For example, initiation of tobacco use may be facilitated if the effect of NIC coincides with stimuli that have acquired incentive properties. Tobacco products that contain scent and flavor additives, such as basil, bay leaf, cinnamon, coconut, and vanilla, may exploit the incentive salience that these stimuli have acquired by their association with other rewards such as sweet tastes. Indeed, some evidence from the Legacy Tobacco Documents Library (UCSF, 1972) suggests that these additives may be intended to attract smokers from younger demographics who have not previously experimented with tobacco products. Based on previous research, we hypothesized that NIC administration in conjunction with these stimuli could increase their ability to evoke approach and consumption (i.e., self-administration).

Gustatory additives to tobacco may have important sensory effects on self-administration of NIC; however, the current

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preclinical literature is limited. One study has investigated the effect of NIC on palatability of bitter and sweet tastes (Parker & Doucet, 1995)—acute but not chronic NIC pretreatment weakly increased the palatability of sucrose. Oral self-administration of NIC increased the palatability of NIC-containing solutions (Flynn, Webster, & Ksir, 1989). Preclinical models have been underused as a strategy for investigating the role of gustatory additive to tobacco, especially as their salience may be altered by NIC. The goal of the present studies was to investigate the incentive amplifying effects of NIC on reward-associated taste stimuli. The basic paradigm involved access to discriminable unsweetened Kool-Aid® taste stimuli (e.g., cherry and grape, 0.05% wt/vol); one taste was paired with sucrose (20% wt/vol; CS+), whereas the other taste was “unpaired” (CS−). Experiment 1 investigated whether NIC pretreatment increased intake of the CS+ relative to the CS−. Experiment 2 investigated whether NIC altered the palatability of the CS+ and CS−. Experiment 3 investigated whether NIC altered operant responding for the CS+ and CS− in a concurrent choice paradigm. These studies begin to evaluate the effects of adding gustatory incentives to tobacco products.

METHODS

Subjects

Male Sprague-Dawley rats (275–325 g on arrival, $N = 53$) were purchased from Charles River Laboratories and housed individually in solid-bottom cages with contact bedding. The cages were housed in a temperature- and humidity-controlled colony room on a 12-hr light:dark cycle. For Experiments 1 and 3, the light:dark cycle of the colony room was reversed (lights off from 07:00 a.m. to 07:00 p.m.), and all procedures were performed out in the dark portion of the cycle to maintain consistency with our previous investigations of the incentive amplifying effects of NIC. In Experiment 2, facial reactions were recorded by a video camera for later scoring. To facilitate recording and scoring facial reactions, the light:dark cycle was standard (lights off from 07:00 p.m. to 07:00 a.m.), and all procedures were carried out in the light portion of the cycle. After 7 days of habituation to the colony room, feed was restricted for all rats such that they maintained 100% of their initial free-feeding body weight throughout the studies. Free access to water was provided throughout the experiments. There were 16 rats in Experiments 1 and 2—eight in each drug pretreatment condition (NIC or saline [SAL]). For Experiment 3, 22 rats were used with 11 in each drug pretreatment condition. However, one rat in the NIC pretreatment condition received the wrong taste solution on a paired test day (cherry instead of grape) and was dropped from the experiment. All experiments were carried out at Kansas State University, and all procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the institutional animal welfare oversight committee (Animal Welfare Assurance: A3609-01).

Drugs and Solutions

NIC hydrogen tartrate salt (Sigma) was dissolved in sterile physiological SAL (0.9% wt/vol), and the pH was adjusted to 7.0 (± 0.2) with a dilute NaOH solution. The NIC dose (0.4 mg/

kg) was calculated as base and selected from previous studies showing robust increases in approach behavior (Palmatier et al., 2012a, 2012b). Grape and cherry Kool-Aid® (unsweetened) were purchased from a local convenience store and dissolved in deionized water (0.05% wt/vol). Deionized water contains no ionic or mineral content, and purification of the water was used to ensure that no adventitious additives altered the flavor of the test solutions. Selection of taste and concentration was based on previous studies showing that these concentrations are discriminable and can acquire conditional appetitive properties (Myers, Ferris, & Sclafani, 2005; Myers & Sclafani, 2001a, 2001b, 2006). Also, both tastes are derived from citric acid and therefore unlikely to have any inherent differences in palatability. Sucrose (20% wt/vol) was dissolved in deionized water with the cherry and grape taste stimuli.

Apparatus: Taste Reaction Test Cage

The taste reactivity test was conducted in a clear plexiglass cylinder measuring 25 cm in diameter. The cylinder was placed on a glass base above a mirror, which allowed videotaping of the mouth and tongue movements from the ventral side of the rat. The cylinder was fitted with a lid that could be lowered to approximately 7.5 cm above the glass base in order to restrict vertical movements (i.e., rearing). A small hole was punched in the lid, and the infusion tubing was threaded through this hole.

Apparatus: Operant Test Cages

Operant conditioning tests were conducted in eight chambers measuring 30.5×25.4×30.5 (w × d × h). Operant test cages were interfaced to a personal computer with Med Associates hardware and software. Each chamber was fitted with two liquid dipper receptacles located on the left and right sides of one wall, approximately 2 cm from the floor and 2.5 cm from the rear (left) and front (right) side. Liquid dippers with 0.1-ml cups were attached to the outside of the chamber on each side; activation of a motor raised the cup to the bottom of the receptacle. Fluids delivered in the liquid dipper could be accessed through an aperture in the bottom of the receptacle (approximately 0.5 cm diameter). Two fixed levers were mounted above each liquid dipper. Meeting the reinforcement schedule (see later) on a lever mounted above one of the dippers activated the dipper arm and provided access to the fluid for 5 s.

Acquisition

Naïve rats were used for each experiment, and each test began in subjects with identical histories of NIC exposure and flavor–sucrose pairings. Only acquisition data from Experiment 1 are presented to conserve space. Prior to each experiment, rats were randomly assigned to NIC- or SAL-exposure groups. Within each drug-exposure group, one taste was randomly assigned as paired (CS+) with the constraint that the number of rats receiving cherry and grape as the paired taste was balanced in each drug-exposure condition. Conditioning sessions were conducted on alternating days. On paired sessions, two 50-ml drinking tubes containing the assigned paired taste and sucrose were placed on the home cage for 20 min. On unpaired sessions (CS−), the same procedure was used, but drink tubes contained the other taste stimulus dissolved in water. This alternating procedure was carried out for 30 test sessions, so that each rat

received 15 exposures to the paired and unpaired taste stimuli. In each experiment, half of the rats received cherry as the CS+ and grape as the CS- ($n = 4-6$ per drug condition); the remaining rats received the converse treatment.

In order to reduce the impact of initial motor suppressant/aversive effects of NIC on tests, all rats received subcutaneous (sc) injections of their assigned drug solution approximately 1 h after each acquisition session. Rats received their daily food ration approximately 1 h after injections. This procedure was employed to limit the strength of associations among conditioning sessions, injections, and feeding and reduce the impact of these associations in the principal dependent measures (Palmatier et al., 2007a).

Experiment 1: Intake Tests

Following conditioning, rats were provided access to both tastes in two-bottle choice tests (paired vs. unpaired) over four consecutive days. On test sessions, rats were injected with their assigned drug solution 30 min before access to the taste stimuli. During the first 2 days, rats had access to the paired and unpaired tastes at the same time, but both tastes were presented "unsweetened." Bottle position (left vs. right, paired vs. unpaired) was balanced on the first test session and reversed on the second test session. The following two tests were comparable, but both taste solutions were dissolved in 20% sucrose.

Experiment 2: Palatability Tests

Experiment 2 was similar to Experiment 1, with the following exceptions. After 24 conditioning tests, rats were anesthetized with a mixture of ketamine (80 mg/kg) and diazepam (5 mg/kg) and implanted with intraoral fistulae. The fistulae were constructed from polyethylene tubing (PE-160; 1.14 mm internal diameter) with one #0 nylon washer affixed to the intraoral end to hold the fistula in place in the cheek. The fistulae were inserted by threading the tubing onto a curved 18-gauge spinal needle. The needle was inserted into the left cheek next to the first maxillary molar and threaded under the skin up to the top of the scalp, allowing the tubing to emerge just medial and anterior to the left ear. A second washer was fitted over the tubing during surgery and glued to the scalp to hold the fistula in place and a small piece (approximately 2 cm) of 18 gauge (1.27 mm outer diameter) hypodermic tubing was glued into the top of the PE tubing in order to couple the fistula to the infusion line.

After 4 days of recovery, conditioning resumed for the remaining six sessions. Throughout the rest of conditioning, fistulae were flushed daily with 1 ml deionized water followed by 5 ml of air. Fistulae were flushed 1 h after conditioning sessions, immediately before NIC/SAL injections. On the final 3 days of conditioning, rats were habituated to the test apparatus for 30 min (10 min per day) 30 min after their sc NIC/SAL injections. On the third habituation test, deionized water was infused into the intraoral fistula with a syringe pump and facial reactions were recorded. The habituation procedures were included to familiarize the rats to the handling/infusion procedures, reduce exploratory behavior, and facilitate recording of facial reactions.

Facial reaction tests were conducted by infusing approximately 1 ml of solution through the intraoral fistula. To equate for total volume and to account for dead space in the tubing, the

syringe pump was set to an infusion rate of 1 ml/min and the pump remained activated for 1 min after an initial facial reaction (mouth movement) was observed by the experimenter (Bice & Kiefer, 1990). At the end of 1 min, the syringe pump was turned off, and facial reactions were recorded for one additional minute. The palatability tests were conducted over 2 days and were comparable to the intake tests. All rats were injected with their assigned drug solution 30 min prior to the palatability tests. On the first day, rats received unsweetened infusions of the paired and unpaired tastes in balanced order; on the second day, they received sweetened infusions of these tastes in the reverse order. After the first infusion of each day, the rat was removed from the test apparatus, and the fistula was flushed with deionized water to clear the taste solution and then with air to clear the water from the fistula. The rat was then replaced into the chamber and tested with the second solution. This procedure was employed in order to test both solutions while equating the pharmacological effects of NIC as much as possible.

Experiment 3: Operant Tests

Following acquisition, the rats were shaped to respond for 20% sucrose from each dipper in the operant box. Initially, the right lever was removed and responses on the left lever resulted in delivery of sucrose in the left dipper. Subsequently, the left lever was removed and responses on the right lever resulted in delivery of sucrose in the right dipper. A brief preference test with both levers installed but dippers inactivated was then used to detect unconditional side biases. Data from this test were used to ensure that side preferences were equated as much as possible when paired and unpaired solutions were assigned to each dipper location.

All operant tests were 60 min in duration. For the first five tests, rats were injected with their assigned drug solution (NIC or SAL) 15 min before placement in the operant box. Taste solutions were presented in each dipper (unsweetened). Because this test amounted to a test of conditioned reinforcement, the schedule of reinforcement was comparable to other conditioned reinforcement tests (see Chaudhri et al., 2006; Olausson et al., 2004; Palmatier et al., 2008 for further details). Because both the CS+ and CS- tastes were concurrently available, the schedule shifted for both reinforcers at the same time, after a total of three reinforcers earned, regardless of combination. This procedure was employed to discourage changeover behavior based on differences in reinforcement schedule between levers. The sixth and final session was identical to the previous five tests; however, all rats were injected with SAL prior to this test. There was no time-out period after a reinforcer was earned.

Data Analysis

Intake of each solution was used as the principal dependent measure in Experiment 1. In Experiment 2, the primary dependent measure was facial reactions, which were categorized as either ingestive or aversive (Grill & Norgren, 1978). Ingestive responses are associated with fluid consumption because they move the fluid to the rear of the mouth where the fluid can be swallowed. The responses identified as ingestive are low-amplitude mouth movements, tongue protrusions, and lateral tongue protrusions. Aversive responses are associated with expulsion of fluid from the oral cavity. The aversive responses were

gape, drip, headshake, forelimb flail, chin rub, and paw pushing (Grill & Norgren, 1978). Responses were scored by a research assistant who was blind to experimental condition. Reliability tests were performed by a second research assistant who was also blind to experimental condition. Interrater reliability for individual measures (tongue protrusions, gapes, etc.) was high ($rs \geq 0.84$). Analyses were performed on data summed within the ingestive and aversive categories, as well as within the individual reactions. In Experiment 3, the principal dependent measures were lever presses and reinforcers earned (paired vs. unpaired). Because all three experiments were designed as taste discrimination tasks, taste (paired vs. unpaired) was always a within-subjects factor and drug (NIC vs. SAL) was a between-subjects factor. Acquisition data from Experiment 1 and test data from Experiment 3 (see Results section) included session as a repeated measure. For all three experiments, analyses of unconditional taste preferences (cherry vs. grape) were conducted for intake during acquisition (all experiments) and for each test measure (intake, palatability, operant responding) from Experiments 1–3. There were no significant differences on any of the intake or palatability measures ($F_s < 1$). There was a slight trend for increased responding for cherry, relative to grape, over operant tests (Test $\times$ Taste interaction, $F(4,76) = 1.68$, $p = .1$). However, there was no main effect of taste, and this may have resulted from one more rat receiving cherry as the CS+ ($n = 11$), relative to grape ($n = 10$) in that experiment.

RESULTS

Experiment 1: Acquisition

Rats rapidly acquired a discrimination between the paired taste (CS+) and the unpaired taste (CS–), and intake during these sessions did not vary as a function of drug exposure (Figure 1A). This was confirmed by repeated measures analysis of variance (ANOVA) with significant main effects of CS ($F(1,196) = 207.1$, $p < .001$) and session ($F(14,196) = 8.56$, $p < .001$) and a significant CS $\times$ Session interaction ($F(14,196) = 2.7$, $p < .01$). None of the main effects or interactions involving drug were significant ($F_s < 1$).

Experiment 1: Intake Tests

During the first intake test (CSs unsweetened), rats drank more of the CS+ than the CS–, but NIC pretreatment did not alter intake of either taste (Figure 1B). This was confirmed by two-way ANOVA with a significant main effect of CS ($F(1,14) = 11.2$, $p < .01$); no other main effects of interactions were significant ($F_s < 1$). Because of the large reduction in intake of the CS+ (see Figure 1A vs. B), an additional test was conducted in which both taste solutions were presented in conjunction with the unconditioned stimulus. As illustrated in Figure 1C, there was only a slight trend for increased intake of the CS+, and NIC did not alter intake. Two-way ANOVA confirmed that there were no significant main effects or interactions ($ps \geq .18$).

Experiment 2: Taste Reactivity Tests

A preference for the CS+ was confirmed in the palatability tests as increased ingestive reactions to the CS+ relative to the

CS– when each CS was presented in unsweetened solution (Myers & Sclafani, 2001a). However, NIC pretreatment did not alter expression of this preference (Figure 2A). The finding was confirmed by separate two-way ANOVAs conducted on aversive and ingestive reactions. For aversive reactions, there were no significant main effects or interactions ($ps \geq .25$). For ingestive reactions, there was only a significant main effect of CS ($F(1,14) = 9.37$, $p < .01$). Additional analyses of the individual ingestive (Figure 2A) and aversive (Figure 2B) responses showed that NIC did not alter any specific taste reaction. Also, many of the ingestive taste reactions to the unsweetened CS+ and CS– were mouth movements rather than tongue protrusions (Figure 2B). The two-way ANOVAs on each ingestive reaction showed a significant main effect of CS ($F(1,14) = 15$, $p < .01$) on the mouth movement measure; for the other measures, the main effect of CS only approached statistical significance ($F_s \geq 3.8$, $ps \leq .07$). Individual aversive reactions were highly variable, with no significant main effects or interactions.

No preference was observed when the CS+ and CS– were presented in conjunction with the sucrose unsweetened stimuli (Figure 3A and B); however, tongue protrusions and lateral tongue protrusions contributed to a higher proportion of the ingestive reactions (Figure 3B). This was confirmed by ANOVAs with no significant main effects or interactions ($F_s < 1$). Independent analyses of aversive reactions were not performed because no responses were recorded for many of the reactions.

Experiment 3: Operant Choice Tests

In the operant tests, the discrimination between the CS+ and CS– was observed as more CS+ dippers earned (Figure 4A) and more responses on the CS+ lever (Figure 4B), relative to CS– dippers earned and lever responses. NIC pretreatment potentiated the increase in responding for the CS+. This finding was confirmed by two-way ANOVAs on each measure with significant main effects of drug ($F_s(1,19) \geq 21.9$, $ps < .001$) and CS ($F_s(1,76) \geq 21.9$, $ps < .001$), as well as significant Drug $\times$ CS ($F_s(1,76) \geq 14.5$, $ps < .01$), Session $\times$ Drug ($F_s(4,76) \geq 2.6$, $ps < .05$), and Session $\times$ CS $\times$ Drug ($F_s(4,76) \geq 3.5$, $ps \leq .01$) interactions. Follow-up analyses (t tests with Bonferroni correction) confirmed that rats pretreated with NIC responded more on the CS+ lever and earned more CS+ dippers, relative to the CS–, on Tests 4 and 5 ($ps < .05$). When NIC pretreatments were replaced with SAL (Session 6), responding for the CS+ declined to basal levels, although responding for the CS– was still greater than responding for the CS+. This was confirmed by two-way ANOVA on responses (Figure 4B) with significant main effects of CS and drug ($F_s(1,19) \geq 6.5$, $ps \leq .02$). For dippers earned, there were no significant main effects or interactions ($ps \geq .17$, Figure 4B).

DISCUSSION

NIC pretreatment selectively increased operant responding for a CS+ but did not alter responding for a comparable stimulus that was not paired with a reward (CS–). This effect of NIC was selective to approach behavior; NIC pretreatment did not alter consumption or palatability of the tastes. These experiments confirm that the incentive amplifying effects of NIC can be observed with olfactogustatory incentive stimuli, even those that have acquired their incentive properties outside of

Figure 1. This figure illustrates mean (± 1 SEM) intake of the CS+ and CS- during acquisition of the taste discrimination (Panel A) and two-bottle tests in which both taste stimuli were presented unsweetened (0% sucrose, Panel B) or when both tastes were sweetened with 20% sucrose (Panel C). Filled symbols and bars represent intake of the CS+ and open symbols represent intake of the CS-. During acquisition, nicotine (NIC; 0.4 mg/kg calculated as base, $n = 8$) or placebo (SAL; 0.9% saline, $n = 8$) was administered 1 h after testing (e.g., CS+ // NIC). During the discrimination tests (Panels B and C), injections were administered 30 min prior to the 20 min drink. *Indicates intake of CS+ significantly greater than intake of CS-, $p < .05$.

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Figure 2. This figure illustrates mean (± 1 SEM) facial reactions to the CS+ and CS- during the initial test with both solutions unsweetened. Panel A illustrates average ingestive and aversive responses summed across all reactions; filled bars represent reactions to the CS+ and open bars represent reactions to the CS-. Panels B and C represent the individual ingestive and aversive reactions, respectively; filled bars represent rats tested 30 min after NIC (0.4 mg/kg nicotine, $n = 8$) injections and open bars represent rats tested 30 min after 0.9% saline (SAL) (0.9% wt/vol, $n = 8$) injections. All rats in this experiment received acquisition training identical to rats in Experiment 1 (Figure 1A) prior to taste reaction tests. *Indicates reactions to CS+ significantly greater than reactions to CS- (main effect of CS), $p < .05$.

Figure 3. This figure illustrates mean (± 1 SEM) facial reactions to the CS+ and CS- during the second test with both solutions sweetened. Panel A illustrates average ingestive and aversive responses summed across all reactions; filled bars represent reactions to the CS+ and open bars represent reactions to the CS-. Panel B represents the individual ingestive reactions; filled bars represent rats tested 30 min after NIC (0.4 mg/kg nicotine) injections and open bars represent rats tested 30 min after 0.9% saline (SAL) (0.9% wt/vol) injections. These data represent data from the same subjects as Figure 2, but sweetened taste palatability tests were conducted 1 day after the tests with unsweetened tastes (Figure 2, see Methods section).

the context of NIC administration. Thus, the present studies provide a preclinical model in which self-administered NIC in tobacco products may be facilitated by combining NIC with incentive motivational flavor additives that acquire their incentive properties in other contexts (e.g., basil, vanilla, menthol, etc.).

Previous studies have established that chronic NIC treatment does not reliably increase the palatability (Parker & Doucet, 1995) or intake (Palmatier et al., 2012a) of sweet tastes such

as sucrose. The present studies confirm that NIC pretreatments do not increase palatability or consumption of flavor incentives that have been associated with sweet tastes. Rather, the incentive amplifying effects of NIC may be limited to approach and search behaviors that may depend on neuropharmacological underpinnings that diverge from those mediating palatability (Cagniard & Murphy, 2012; Robinson & Berridge, 1993). However, an important caveat to this conclusion is that we did not explicitly attempt to establish the CS+ as an incentive in

Figure 4. This figure illustrates mean (± 1 SEM) dippers earned (Panel A) and lever presses (Panel B) for the CS+ and CS- during the operant conditioning tests (both tastes presented unsweetened). Filled symbols represent CS+ dippers earned or lever presses on the CS+ associated lever; open symbols represent CS- dippers earned and lever presses for the CS-. Circles represent rats tested 30 min after NIC (0.4 mg/kg nicotine) injections and squares represent rats tested 15 min after 0.9% saline (SAL) (0.9% wt/vol) injections. All rats in this experiment received acquisition training identical to rats in Experiment 1 (Figure 1A) prior to operant tests. *Indicates that NIC pretreatment increased responding for the CS+ relative to the CS-, $p < .05$.

the presence of NIC effects. Nor was the CS+ established as an incentive as a result of pairing with NIC administration. Thus, we cannot rule out the hypothesis that NIC might alter palatability of a CS if it were associated with NIC or if it were paired with sucrose in the context of NIC effects. For example, access to NIC in drinking solutions for 16 days increased ingestive taste reactions elicited by solutions containing 1 or 5 $\mu\text{g/ml}$ NIC (Flynn et al., 1989). The authors suggest that pairings between the taste of NIC and its pharmacological effect established the taste as a CS and increased the palatability of this stimulus.

A recent study by Chen and colleagues (2011) attempted to build on this finding by pairing a bitter flavor solution (quinine mixed with grape Kool-Aid®) and intravenous NIC infusions. The coadministration of NIC and gustatory cues did not result in increased self-administration.

The strong brand preferences exhibited by menthol smokers suggest that overlap between NIC administration and exposure to gustatory incentives may alter the palatability of these stimuli (Hymowitz et al., 1995; Richter, Beistle, Pederson, & O'Hegarty, 2008). Although the primary reinforcing effects

of NIC are weak, they can establish nondrug stimuli as conditioned reinforcers (Palmatier et al., 2008; Palmatier et al., 2007b). Once established, the incentive amplifying effects of NIC can increase responding for these conditioned reinforcers (Palmatier et al., 2007b). The findings of Flynn and colleagues (1989) that the palatability of NIC-associated tastes may be enhanced by the pharmacological effects of NIC lend support to the hypothesis that both the primary reinforcing and incentive amplifying effects of NIC may contribute to taste and brand preferences in tobacco users. An important question for future research will be to investigate the relative roles of these effects of NIC in promoting palatability and taste preferences in preclinical models. Preclinical models of palatability and associative learning represent a vital source of information about the interaction between NIC and tobacco flavor additives.

The goal of the present studies was to model the addition of familiar, appetitive taste stimuli to tobacco products, which may represent a strategy for promoting tobacco use in nonusers. For example, adding menthol flavorings to cigarettes produces a smoke that is perceived to be less harsh than nonmenthol cigarette smoke (Hymowitz et al., 1995). Focus group interviews suggest that “taste” is the most common reason for smoking menthol cigarettes (Ahijevych & Garrett, 2010). However, these studies also suggest that the appeal of menthol cigarettes is also based on a prior familiarity of menthol as a taste with incentive salience—“tasting like a peppermint patty” (Richter et al., 2008). The present studies suggest that NIC administration may increase the salience of such appetitive tastes, even though it does not appear to alter their palatability. This finding may be important to the regulation of tobacco products because the addition of familiar flavor incentives (fruit, vanilla, etc.) is increasingly being added to products that are currently not regulated by the Food and Drug Administration (cigars, cigarillos). Based on the present findings, we predict that the incentive amplifying effects of NIC would increase the frequency or probability of tobacco use (i.e., number of cigarettes smoked) in products with additives that have familiar, appetitive flavor additives. Notably, this increased use might occur without any changes in measures of palatability or satisfaction.

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DECLARATION OF INTERESTS

None.

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