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Review of the Role of Environmental Cues in Various Aspects of Nicotine Addiction

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Abstract

Nicotine in cigarettes is a highly addictive substance but can also alter the incentive value of cues associated with smoking as well as other natural reinforcers, such as food. While previous work has shown nicotine to enhance the saliency of and reactivity to sucrose-paired cues by serving as an occasion-setting stimulus while also enhancing reward-learning, much of this work has been done in male rats. In the present study, we explore whether nicotine can enhance sucrose self-administration and sucrose-seeking in female rats. For ten days female Sprague-Dawley rats were either given subcutaneous saline (ST, saline trained) or nicotine (NT, nicotine trained, 0.4 mg/kg) prior to sucrose self-administration. Then, rats were given two separate tests for sucrose-seeking in which they received either a saline or nicotine challenge. We show that ST and NT animals have mostly comparable acquisition of sucrose self-administration, with the exception of NT animals lever pressing at a higher rate when the sucrose-paired audiovisual cue was on. We also show that NT trained animals elevate responding during sucrose-seeking tests when given a nicotine challenge compared to a saline challenge while ST animals have comparable responding. Thus, we show that nicotine is an occasion-setting stimulus for sucrose-seeking in female rats, as is in male rats, but unlike in male rats where the effects of nicotine on reward-learning are profound, we observed subtler effects on acquisition of sucrose-self administration. Consideration of nicotine and cue-saliency leads to implications for treatment in those with a nicotine addiction, targeting various aspects of the addiction cycle. Menthol has been shown to effect various aspects of nicotine's mechanism of action and would be worth further investigation into its effect nicotine's relationship with cue-seeking.

Introduction

Addiction to nicotine-containing products is one of the leading causes of preventable death in the world (World Health, 2011). Individuals who use these products typically have a difficult time quitting and have a high rate of relapse (Hughes et al., 2004). Individuals who smoke often gain weight and body fat when trying to quit, which can be partially attributable to a variety of factors such as insulin-resistance (Chiolero et al. 2008). An additional possibility is that food may have enhanced reinforcing value due to its association with nicotine in these products. Indeed, nicotine's interoceptive properties are well-known to serve as a discriminative stimulus, conditioned stimulus for sucrose, or an occasion-setting stimulus for cue-induced sucrose-seeking (Shoaib et al. 1997, Chaudhri et al. 2006, Palmatier and Bevins 2008, Grimm et al. 2012, Stringfield et al. 2019).

Sex differences in nicotine reinforcement is relatively well-known, but whether there are differences in nicotine's nonreinforcing (e.g., aside from its primary reinforcing effects) effects are less studied. Recent meta-analyses reveal that while male and female rats tend to self-administer nicotine at roughly the same rates (e.g., no differences in reinforcing effects), female rats may be more sensitive to the nonreinforcing effects of nicotine such as enhancing cue saliency or serving as a more robust discriminative stimulus (Pogun et al. 2017, Flores et al. 2019). One study has shown that in male rats nicotine can enhance both sucrose self-administration and cue-induced sucrose-seeking (Grimm et al. 2012, Grimm et al. 2013) , sometimes called just sucrose-seeking (Addy et al., 2015). However, whether the effects of nicotine on sucrose self-administration and sucrose-seeking generalizes to female rats is unknown. The goal of this study is to assess whether nicotine, when paired with sucrose availability, will enhance sucrose self-administration or sucrose-seeking in female rats.

In this study, female adult rats were trained to press a lever for a sucrose reward paired with an audiovisual cue and administered either nicotine or saline prior to each training session. This phase allows for studying the effects of nicotine on the primary reinforcing effects of sucrose. The second phase of the study included a sucrose-seeking test where rats were responding for only the sucrose-paired environmental cues (extinction training), thus assessing the secondary reinforcement of sucrose.

General Nicotine Overview

Pharmacokinetics

Nicotine can be administrated in a variety of different ways, such as through smoking, chewing, or patches. In cigarette smokers, nicotine from the smoke of the cigarette is inhaled directly into the lungs, where it is immediately absorbed into the bloodstream. Nicotine enters the bloodstream in those who use chewing tobacco through the mucosa of the mouth into the bloodstream. With nicotine patches, nicotine is absorbed through the skin into the bloodstream. Once in the bloodstream, nicotine makes its way to the heart, where it is pumped out throughout the body, including the brain. The liver is then able to extract 70% of nicotine in liver blood flow (Benowitz et al., 2009) In order to be expelled from the body, nicotine is metabolized mainly by the enzyme CYP2A6, which is found in the liver (Benowitz, 2009). Through CYP2A6 and aldehyde oxidase, 70-80% of nicotine is converted into cotinine (Benowitz et al., 2009). However, 85-90% of that cotinine is broken down into other various metabolites. 3’-Hydroxycotinine is the main metabolite of cotinine, which is metabolized by CYP2A6 alone (Benowitz et al., 2009). The liver then excretes nicotine and its metabolites through urine.

Since CYP2A6 is the primary enzyme in the metabolism of nicotine, the nicotine-metabolite ratio is an indicator of the ability for the body to expel nicotine from the system. The NMR is the ratio between the concentrations of 3'-hydroxycotinine to cotinine, a reaction exclusively mediated by CYP2A6. A higher NMR indicates a faster nicotine metabolism and vice versa. Usually, those with a faster metabolism for nicotine have a stronger dependence and more difficulty with cessation. A smoker's NMR can be affected by a variety of factors, including age, sex, race, and use of menthol. For example, generally white smokers have been found to metabolize nicotine faster than black smokers (Fagan et al., 2016). Even though white smokers metabolize nicotine faster, black smokers have lower successful cessation rates, despite black smokers have more quitting attempts (Babb et al., 2017). Therefore, a lower NMR does not necessarily lead to less dependence.

Pharmacodynamics

Nicotine directly affects areas of the brain involved in reward learning, a key pathway defining nicotine's addictive properties. One pathway critical to reward learning starts in the ventral tegmental area and travels to the prefrontal cortex and nucleus accumbens (Biasi & Dani, 2011). The VTA, made up of mostly dopamine neurons, is known for its important role in reward, motivation, cognition, and aversion (Bouarab et al., 2019). When these dopamine neurons in the VTA are depolarized, dopamine is released, and signals are sent through interneurons in this pathway to primarily the prefrontal cortex and nucleus accumbens (Biasi & Dani, 2011). Nicotine is relevant to this pathway because it binds to nicotinic acetylcholine receptors (nAChRs) on neurons throughout the central nervous system, including this pathway. When bound, nAChRs open, allowing cations to flow into the neuron, causing local depolarization, leading to an action potential, eliciting dopamine release by the neuron. Therefore, nicotine's presence leads to

increased firing rate and phasic burst rate in the VTA and increases dopamine levels in the prefrontal cortex and nucleus accumbens (Biasi & Dani, 2011). Phasic firing occurs in response to reward, such as nicotine, while tonic firing relates to relatively sustained single-spike firing (Biasi & Dani, 2011). Nicotine reinforces its use by increasing dopamine levels in a critical reward learning pathway. However, this pathway gets more complicated.

Not all nAChRs are the same when it comes to affecting dopamine neurons in the VTA. NAChRs consist of five subunits, which can be made up from a combination of nine different α subunits and three different β subunits (Nair & Liu, 2019). The two most common forms of nAChRs contain α4β2 (along with a combination of α and β subunits) and α7 (with other α subunits only) subunits (Nair & Liu, 2019). α4β2 nAChRs rapidly desensitize with prolonged nicotine exposure, while α7 subunits desensitize more slowly (Paradiso & Steinbach, 2003; Nair & Liu, 2019). Therefore, nicotine acts as a full agonist on α4β2 nAChRs initially, but then acts as an antagonist once α4β2 nAChRs are desensitized by prolonged nicotine exposure.

Desensitization of α4β2 receptors cause an increase in cue saliency. Dopamine neurons receive excitatory and inhibitory inputs affecting its firing rate. Glutamate neurons release glutamate onto dopamine neurons, causing an excitatory response. α7-containing nAChRs are found in the presynaptic terminals of these GLU-DA synapses (Picciotto et al., 2008). GABA neurons inhibit dopamine neurons through GABA release. β2-containing nAChRs are found in the presynaptic terminals of these GABA-DA synapses (Picciotto et al., 2008). β2-containing nAChRs are also found directly on dopamine neurons themselves (Picciotto et al., 2008). Normally, nicotine activates nAChRs on glutamate and dopamine neurons, causing increased dopamine release, while nAChR activation on GABA neurons inhibits dopamine release (Biasi & Dani, 2011). Baseline tonic firing is determined from sustained single-spike firing resulting from nicotine interaction on

these three important locations of nAChRs. However, with prolonged nicotine exposure, β2-containing nAChRs become desensitized, while α7 nAChRs are more resistant (Paradiso & Steinbach, 2003; Nair & Liu, 2019). Therefore, dopamine neurons are no longer being modulated by GABA release nor by the β2 receptors directly on the dopamine neuron. Tonic firing decreases as a result of the inhibition of these neurons. As a result, the glutamate neuron then becomes the primary excitatory neuron to the dopamine neuron, because α7 nAChRs are still nicotine sensitive. The prefrontal cortex and pedunculopontine release glutamate into the VTA in response to cues (Geisler & Wise, 2008). Since the phasic:tonic dopamine release ratio is higher after β2 receptor desensitization, the glutamate released due to cues is more influential, leading to an increase of cue saliency in relation to nicotine use. As one learns the association between the cue and the reward from nicotine, the craving for nicotine due to that cue increases.

Materials and Methods

Subjects

36 female Sprague-Dawley rats (aged 9-12 weeks) were bred at Elizabethtown College. Rats were housed in a room on a reverse light-dark cycle. Rats were given at least one week to adjust to the reverse light-dark cycle, singly housed, and handled daily five days before training. Rats were weighed daily. All procedures were approved by the Elizabethtown College Institutional Animal Care and Use Committee.

Apparatus

Sucrose self-administration training and sucrose-seeking test days took place within two operant conditioning chambers (21.6 x 21.6 x 27.9 cm; Model 80003NS, Lafayette Instrument). The chamber contained two stationary levers, a tone generator, a stimulus light above each lever,

and a liquid hopper between the levers. A dispenser dropped the sucrose reward into this hopper. The operant chambers were contained within a soundproof box with a ventilation fan. The far corner of the box contained a white house light on the ceiling opposite from the levers.

Drugs

(-)-Nicotine hydrogen tartrate salt (Glentham Life Sciences, Corsham, UK) was dissolved in saline to 0.4 mg/mL (free base) for SC injection at 1 mL/kg (0.4 mg/kg). The pH was balanced to 7.0 ± 0.3 with NaOH. This nicotine dosage has been previously shown to be able to enhance sucrose self-administration in male rats (Grimm et al., 2012; Murray, Penrod, & Bevins, 2009).

Procedure

Training Period

Rats were split between two drug treatment groups: nicotine-trained (NT) and saline-trained (ST). Rats were administered either nicotine (0.4 mg/kg, s.c.) or saline (s.c.) three days prior to sucrose self-administration to familiarize them with the effects of injection and the drug in their home cages. Rats were then trained to self-administer sucrose for 1 hour per day for 10 days and given their respective drug 5 minutes prior to each session. Pressing the active lever produced an audiovisual cue, which consisted of a 6 second light located above the active lever and a tone, as well as delivery of 0.3 mL of 10% sucrose into the hopper. A 25 second timeout period in which the rats could not earn another reward followed the termination of the audiovisual cue. Lever presses for both the active and inactive lever were recorded for the number of times pressed before the cue onset (e.g., initiating the cue and reward), during the cue, and during the timeout period.

Prior to the first day of training, rats were water deprived for 21 hours, and 22 hours thereafter to increase motivation for the sucrose reward. Hand-shaping typically occurred during the first 2-3 days of training and was no longer needed after six days at the latest. Successful acquisition of sucrose self-administration was defined as receiving at least 20 rewards for two consecutive days of training by the 6th day of training. Upon successful acquisition, rats were given *ad libitum* water access. Rats that failed to acquire (9/36) were excluded from the study and subsequent analyses.

Sucrose-seeking Test Days

After the 10 training days, both NT and ST groups completed two subsequent 30-minute sucrose-seeking tests (Test Day 1) where the liquid sucrose reward was omitted, but the audiovisual cue would be triggered upon lever pressing. Rats were given a saline challenge prior to the first 30-minute sucrose-seeking test. Then, they were removed from the operant chamber and were given a nicotine challenge. Five minutes later, they completed a second 30-minute sucrose-seeking test. After two days of re-training with sucrose available, a second set of sucrose-seeking tests (Test Day 2) were completed, with the nicotine challenge occurring first and the saline challenge occurring second. This second test was performed to compare saline and nicotine challenges in the absence of an earlier extinction phase.

Statistical Analyses

Training Period

Total rewards earned, total active lever presses, total inactive lever presses, active and inactive lever presses during the timeout period, and active and inactive lever presses during the cue were all separately analyzed between the saline-trained and nicotine-trained treatment groups across the training period using two-way repeated measures ANOVA.

Sucrose-seeking Test Days

Total active lever presses and total inactive lever presses were separately analyzed between training groups and drug pretreatment on both test days using a two-way repeated measures ANOVA. Planned comparisons (paired t-test) examining the effects of saline versus nicotine challenge on NT and ST animals were employed since it has been shown that a nicotine challenge enhances sucrose-seeking in NT, and not ST, male rats (Grimm et al. 2012). Outliers were identified using the IQR method, in which animals that exhibited lever pressing 1.5 IQR below the first quartile or 1.5 IQR above the third quartile were considered outliers. Only one rat (NT) met this criterion due to dramatically reduced lever pressing from Test Day 1 to Test Day 2 (70% reduction).

Results

Training Period

For total rewards earned (**Fig. 1A)**, there was no main effect of drug treatment, $F(1, 24) = 0.839$, $p = 0.369$, a main effect of time, $F(9, 216) = 65.9$, $p < 0.0001$, and no interaction between drug treatment and time, $F(9, 216) = 0.336$, $p = 0.962$. For total active lever presses, there was no main effect of drug treatment, $F(1, 24) = 0.0097$, $p = 0.923$, a main effect of time, $F(9, 216) = 23.8$, $p < 0.0001$, and no interaction between drug treatment and time, $F(9, 216) = 0.652$, $p = 0.752$. For total inactive lever presses, there was no main effect of drug treatment, $F(1, 24) = 0.397$, $p = 0.936$, a main effect of time, $F(9, 216) = 2.33$, $p = 0.016$, and no interaction between drug treatment and time, $F(9, 216) = 0.397$, $p = 0.936$.

For active lever presses during the timeout period (**Fig. 1B)**, there was no main effect of drug treatment, $F(1, 24) = 0.881$, $p = 0.357$, but a main effect of time, $F(9, 216) = 5.51$, $p < 0.0001$, and no interaction between drug treatment and time, $F(9, 216) = 0.837$, $p = 0.583$. For active lever presses during the cue, there was a main effect of drug treatment, $F(1, 24) = 2.73$, $p = 0.0049$, a main effect of time, $F(9, 216) = 2.48$, $p = 0.010$, and an interaction between drug treatment and time, $F(9, 216) = 2.73$, $p = 0.0049$. Post hoc analyses using Sidak's multiple comparisons test revealed that nicotine-trained animals pressed the active lever during the cue more than saline-trained animals on session5, $p = 0.0010$, and on session 6, $p = 0.0086$.

Sucrose-seeking Test Days

As outlined in the procedure, saline was injected first into both treatment groups, followed by nicotine in the second half hour, for Test Day 1, while the order was reversed for Test Day 2. For Test Day 1 active lever presses, two-way repeated-measures ANOVA revealed no main effect of training (NT or ST), $F(1, 23) = 2.055$, $p = 0.165$, a main effect of drug injection (nicotine challenge or saline challenge), $F(1, 23) = 47.61$, $p < .0001$, and an interaction between training and drug injection, $F(1, 23) = 8.178$, $p = 0.009$ (**Fig. 2A)**. Post hoc analyses using Sidak's multiple comparisons test revealed that nicotine-trained animals had more total active lever presses than saline-trained animals when given nicotine for the second 30 minute session for Test Day 1, $p = 0.0181$. For Test Day 2 active lever presses, two-way ANOVA revealed no main effect of training, $F(1, 23) = 0.597$, $p = 0.448$, a main effect of drug injection, $F(1, 23) = 84.06$, $p < .0001$, and no interaction between training and drug injection, $F(1, 23) = 0.834$, $p = 0.370$. For Test Day 1 inactive lever presses, two-way ANOVA revealed a main effect of training, $F(1, 23) = 9.355$, $p = 0.006$, a main effect of drug injection, $F(1, 23) = 16.85$, $p = 0.0004$, and no interaction between training and drug injection, $F(1, 23) = 0.373$, $p = 0.547$ (**Fig. 2B**). For Test Day 2 inactive lever

presses, two-way ANOVA revealed a main effect of training, $F(1, 23) = 18.18$, $p = 0.0003$, no main effect of drug injection, $F(1, 23) = 3.028$, $p = 0.095$, and an interaction between training and drug injection, $F(1, 23) = 4.35$, $p = 0.048$.

Drug Effects on Sucrose-seeking

To directly compare the effects of saline and nicotine challenge without prior extinction influencing behavior, we compared the sucrose-seeking data for the first injection on Test Day 1 (saline) to the first injection of Test Day 2 (nicotine). ST rats did not increase active lever responding when given a nicotine challenge ($M = 53.42$, $SEM = 4.182$) compared to saline ($M = 43.67$, $SEM = 4.038$), $t(11) = 2.894$, $p = 0.956$ (**Fig. 2C)**, but NT rats did (nicotine: $M = 53.42$, $SEM = 3.369$; saline: $M = 43.67$, $SEM = 4.31$; $t(12) = 2.894$, $p = 0.015$, **Fig. 2D)**. Inactive lever presses did not differ with pretreatment in saline-trained animals, $t(12) = 1.86$, $p = 0.09$, nor in nicotine-trained animals, $t(11) = 0.71$, $p = 0.50$ (data not shown).

Between-Test Training Period

The average lever presses for the last three days of acquisition was compared to the average of the two-day retraining period between Test Day 1 and Test Day 2 in order to evaluate behavioral changes between the sucrose-seeking tests. For total active lever presses, a two-way ANOVA revealed no main effect of training, $F(1, 23) = 0.52$, $p = 0.48$, no main effect of time (acquisition versus retraining) , $F(1, 23) = 1.16$, $p = 0.29$, and no interaction between training and time, $F(1, 23) = 0.59$, $p = 0.45$. For total inactive lever presses, two-way ANOVA revealed a main effect of training, $F(1, 23) = 9.30$, $p = 0.006$, no main effect of time, $F(1, 23) = 2.85$, $p = 0.10$, and an interaction between training and time, $F(1, 23) = 11.95$, $p = 0.002$. Sidak's multiple comparisons test revealed that ST animals had significantly lower inactive lever presses than nicotine-trained

animals between test days, $p = 0.0002$, and that saline-trained animals lowered their inactive lever pressing between the two trials, $p = 0.003$ (data not shown).

Discussion of Research

There are three key findings from this study. First, nicotine pretreatment did not significantly alter the primary reinforcing effects of sucrose, as evidenced by similar responding during acquisition (**Fig. 1A).** Second, both NT and ST animals had comparable levels of sucrose-seeking when given a saline or nicotine challenge (**Fig. 2A)**. Third, and most importantly, a nicotine challenge relative to a saline challenge does increase sucrose-seeking only in NT animals (**Fig. 2D)**—those who have associated nicotine with sucrose availability and sucrose cues. Thus, we interpret these findings to mean that nicotine is serving as an occasion-setting discriminative stimulus which can promote sucrose-seeking.

A study using male rats and examining nicotine's effects on sucrose self-administration and sucrose-seeking had similar conclusions about nicotine's occasion-setting properties, but with some interesting differences (Grimm et al. 2012). Reward-learning effects in male rats were much more pronounced and may likely be due to a strong presence of locomotor sensitization in their study. Indeed, both active and inactive lever presses were elevated in this study, indicating a general locomotor effect. In the present study, we did not measure locomotor activity, but we did not observe any changes in inactive lever responding hinting that nicotine did not produce hyperlocomotor responses (**Fig. 1A**). The lack of locomotor sensitization in our study is in line with previous work showing that female rodents have substantially lower locomotor responses to chronic nicotine exposure (Caldarone et al. 2008). However, it is important to note that other studies show either similar (Elliott et al. 2004) or higher responses to nicotine (Kanýt et al. 1999) in female rats relative to male rats. Surprisingly, we also saw elevated lever press responding

during the cue-on period during training in the nicotine pre-treated animals (**Fig. 1B)**. We interpret this to mean that nicotine may be invigorating responding in the presence of a sucrose-paired cue, and fits with the idea of nicotine producing higher cue-induced sucrose-seeking.

In the present study in female rats, a nicotine challenge significantly elevated sucrose-seeking but only in rats previously trained with nicotine (**Fig 2C and D)**, suggesting an occasion-setting effect (Grimm et al. 2012, Stringfield et al. 2019). In other words, animals administered with nicotine prior to conditioning (NT group) may identify the nicotine itself as a discriminative stimulus that goes part and parcel with the reward-predictive stimuli (sucrose cue). In the absence of this discriminative stimulus, sucrose-seeking is reduced relative to when nicotine is present (**Fig 2A,** compare Test Day 1 SAL to Test Day 2 NIC in the NT group**)**. This is not the case for animals administered saline prior to conditioning (ST group), as responding is identical whether given a saline or nicotine challenge (**Fig 2A,** compare Test Day 1 SAL to Test Day 2 NIC in the ST group**).** There is also evidence of nicotine serving as an occasion-setter based on comparing sucrose-seeking on Test Day 1 where all rats were given a saline challenge followed by a nicotine challenge. Responding was lower for the nicotine challenge on this test day, likely due to extinction occurring from the saline challenge. However, responding during the nicotine challenge was much higher in NT animals relative to ST animals. We interpret this to mean that the during the saline challenge, the extinction experienced by the NT animals was somewhat discounted relative to ST animals, since contextually NT animals are used to have nicotine during sucrose self-administration. Again, this suggests that nicotine may be creating a contextual context for sucrose-seeking. Interestingly, NT and ST animals had comparable sucrose-seeking overall when given a nicotine challenge (**Fig. 2A, Test 2)**, suggesting nicotine is not simply making animals press more for the lever. In male rats, NT animals had elevated sucrose-seeking relative to ST animals when

given a nicotine challenge (Grimm et al. 2012). The differences in the present study in females and the Grimm et. al., study in male rats may be due to differences in locomotor responses produced by nicotine.

One limitation of our study is that we did not directly assess differences between males and females. We elected to omit a comparison male group since a comparable study was performed already in males (Grimm et al., 2012), and this kind of study asks a slightly different question than our main inquiry which is whether or not nicotine enhances sucrose self-administration and sucrose-seeking specifically in female rats. Thus, our study affirms that indeed, nicotine produces similar occasion-setting effects in female rats as in male rats, but we cannot directly compare the size of or differences between these effects between the sexes. A recent study has compared male and female rats in a Pavlovian approach task, and showed that female rats find cues more salient induces greater Pavlovian approach (Stringfield et al., 2019), thus a future study may find that nicotine pretreated rats might respond more vigorously during sucrose-seeking tests.

Treatments for Nicotine Addiction

As demonstrated by the discussed research, cues play an important role in nicotine's addictive cycle. In order to help treat nicotine addiction, there are a variety of therapeutic treatments on the market to target various aspects of the addiction cycle. The two main categories of treatments for nicotine cessation are nicotine replacement therapy, consisting of tobaccoless nicotine alternatives, and drug therapy, utilizing drugs such as mecamylamine and varenicline.

Nicotine replacement therapy is a common treatment for nicotine addiction. When nicotine levels are high in the brain, nAChRs become desensitized and upregulated. As nicotine levels fall, nAChRs resensitize, and withdrawal effects emerge (Flowers, 2016). Withdrawal effects are

unpleasant; they consist of depressed mood, dysphoria, anxiety, irritability, craving, gastrointestinal discomfort, increased appetite, etc. (Jackson et al., 2015). Withdrawal is a major driver in cessation difficulty and relapse, and nicotine replacement therapy can help alleviate this withdrawal. Nicotine replacement therapy can be found in the forms of gum, inhalers, lozenges, nasal sprays, and transdermal patches. The chances of quitting increases by 50-80% when a nicotine replacement therapy is used (Flowers, 2016). Transdermal patches slowly release nicotine through the skin at a slow rate throughout the day. Slow nicotine delivery, such as through transdermal patches, can reduce nicotine's reinforcing effects, because the nicotine being metabolized is constantly being replaced, and therefore nAChRs are not able to resensitize as quickly (Flowers, 2016). The lessening of withdrawal symptoms then reduces the craving to use tobacco products. From a binge-intoxication standpoint, transdermal patches maintain nicotine levels in the body, so when one tries to use a tobacco product, the additional nicotine consumption is not as rewarding and potentially aversive. This reduction in added euphoria from the use of tobacco products makes their use less salient. The goal would be to use transdermal patches with smaller and smaller nicotine doses over time until none are needed. This method can also be applied with other nicotine replacement therapies. Acute dosing nicotine products have the benefit of allowing the timing and amount of nicotine dosage to be titrated in order to meet one's needs (Wadgave & Nagesh, 2016). During intense acute craving periods, a nicotine replacement (such as gum or lozenge) can be used to alleviate this craving and therefore helps prevent relapse (Wadgave & Nagesh, 2016). Nicotine replacement therapies, such as transdermal patches and gum, slow nicotine absorption into the brain, helping to reduce withdrawal, to reduce reinforcing euphoria, and to reduce craving.

Mecamylamine can be used as a drug therapy for nicotine addiction. Mecamylamine is a nonselective nAChR noncompetitive antagonist. (Nickell et al., 2013). When mecamylamine is taken, it binds allosterically to nAChRs, modulating the receptor into an inactive state (Nickell et al., 2013). Therefore, nAChRs are not able to open with nicotine binding, including rapidly desensitizing α4β2 nAChRs. With mecamylamine, the α4β2 nAChR is transitioned directly into a state mimicking a desensitized nAChR without the channel ever opening (Nickell et al., 2013). Therefore, nicotine intake while on mecamylamine will reduce the euphoric reinforcing effect of nicotine, decreasing the urge to smoke. Functional desensitization of α4β2 nAChRs by mecamylamine reduces withdrawal by preventing resensitization when nicotine leaves the body. Combination therapy, the use of both mecamylamine and nicotine replacement therapy in tandem, has shown to be more successful in helping smoking cessation than either treatment alone (Stead, 2011). With combination therapy, it has been suggested that more nAChRs are blocked than either alone, reducing the reward of additional tobacco use (Stead, 2011).

Varenicline is another drug therapy that can be used for nicotine addiction, but works differently from mecamylamine. Varenicline is a partial agonist at α4β2 nAChRs (Garcia-Rivas et al., 2019). Varenicline allows nAChRs to open, causing increased dopamine firing, but not as much as nicotine, a full agonist (Benowitz, 2009). Partial agonism reduces euphoria from additional nicotine intake and withdrawal effects. NAChRs bound to varenicline are also not as susceptible to desensitization as those bound to nicotine (Lotfipour et al., 2012). This reduction in desensitization leads to a decrease in the phasic:tonic ratio in dopamine neurons. With a reduced phasic:tonic ratio, cues associated with nicotine are less salient when on varenicline (Garcia-Rivas et al., 2019). Those that experience craving from cues associated with nicotine benefit the most from varenicline treatment.

Menthol as an Area of Further Research

A further area of research to examine is that of menthol's affects on nicotine and cue-seeking. Menthol is the only flavor of cigarette legal under U.S. law. However, menthol is more than just a flavor, it has the potential to alter the pharmacokinetic and pharmacodynamic properties of nicotine itself, as well as increasing the difficulty for cessation. Menthol cigarette marketing has been traditionally targeted towards the African-American community, so menthol's effects on nicotine's properties, such as in cue-seeking, is worth further study.

Menthol negatively effects the pharmacokinetics of nicotine in the body. In smokers, menthol's cooling sensation helps to mask the irritation caused by smoke, making the experience of smoking less adverse (Kabbani, 2013). Menthol interacts with TRPM8 receptors found on cold-responsive somatosensory neurons, helping to mask the irritation and bitter flavor from smoke (Willis et al., 2011). With the harshness of smoking reduced due to menthol, it is easier for first-time smokers to try smoking again, putting them at greater risk for increased use and dependence. Fagan et al. found that the nicotine metabolite ratio in menthol-preferred smokers is significantly lower than those who use nonmentholated products (2016). The lower NMR in mentholated-preferred smokers indicate that they metabolize nicotine more slowly than their nonmentholated-preferred counterparts. As mentioned previously, a lower NMR usually corresponds to lower dependence rates and easier cessation. However, studies have shown that those that prefer mentholated tobacco products have a harder time with nicotine cessation, especially in minorities (Foulds et al., 2010). Therefore, when it comes to the interaction between menthol and nicotine, a slower metabolism does not lead to lower dependence and higher cessation rates, but rather the opposite surprisingly. The link between menthol's effect on nicotine metabolism and dependence rates needs more research to solve this discrepancy. Perhaps the link between slower metabolism

and more difficulty quitting lies in increased bioavailability of nicotine. Those with slower nicotine metabolism, such as with mentholated-preferred smokers, are exposed to nicotine in the brain for a longer period of time. This greater bioavailability of nicotine, due to menthol's inhibition of CYP2A6, may complexly influence nicotinic acetylcholine receptor (nAChR) expression in a way that increases dependence (Wickham, 2020). Overall, more research is needed to evaluate how menthol's effect on CYP2A6 is linked to observed increased dependence rates in mentholated-preferred smokers.

Menthol enhances the pharmacodynamic properties of nicotine through nAChR upregulation, changes in prevalent nAChR subunit expression, and less nAChR desensitization. In mentholated-preferred smokers, α4β2 nAChRs are upregulated to a further extent than nonmentholated-preferred smokers, meaning that more nAChRs are found in the brain when mentholated cigarettes are used (Brody et al., 2012). Greater nAChR upregulation may cause mentholated smokers to have a harder time quitting smoking, because more nAChRs would increase the effect of nicotine on neurons found in the VTA, as in the mechanism described above. (Brody et al., 2012). In addition, menthol also stabilizes and increases the number of lower-sensitivity α4 and α6 containing nAChRs, which leads to less desensitization in those that use mentholated tobacco products (Henderson et al., 2016). The increase of nAChRs overall and lower sensitivity nAChRs helps enhance nicotine's reinforcement in the reward learning pathway. Menthol's enhancement of nicotine's pharmacodynamics suggest mentholated tobacco products are more dangerous than their nonmentholated counterparts.

Menthol may impact one's resistance to smoking cessation. As discussed earlier, menthol's cooling sensation helps to mask the irritation caused by smoking through TRPM8 receptors (Kabbani, 2013; Willis et al., 2011). Since the experience of smoking is not as irritable in those

that use mentholated cigarettes, the motivation for one to quit may not be as strong. In addition, the increased upregulation of nAChRs and expression of less desensitizing nAChRs in the reward learning pathway due to menthol may enhance nicotine's reinforcement. The resulting stronger reinforcement of nicotine due to menthol would make it harder to quit. The ability of menthol to make smoking less adverse and its ability to reinforce nicotine use indicates that mentholated cigarettes may reduce one's willingness to quit smoking.

Despite the various therapeutic treatment available, black smokers have a lower success rate in smoking cessation than their white counterparts, despite attempting to quit more frequently than white smokers (Babb et al., 2017). One can attribute this discrepancy with the higher likelihood of black smokers using mentholated cigarettes (76.8%) than white smokers (24.6%) (U.S. HHS, 2017). Newer studies suggest that those that prefer mentholated cigarettes have a harder time with smoking cessation than those that use nonmentholated cigarettes, especially in minorities, but more research is needed to confirm this trend (Foulds et al., 2010).

Conclusion

Our study shows in female rats that nicotine serves as an occasion-setting stimulus in the context of operant conditioning for natural rewards and is involved in serving as an interoceptive stimulus which sets the level of sucrose-seeking for sucrose-paired cues. In the context of the broader literature, our findings support a picture that nicotine's nonreinforcing effects, such as occasion-setting or serving as an interoceptive discriminative stimulus, may generalize to both male and females. Given that both male and female individuals who smoke gain weight after smoking cessation and may find food paired-cues more salient (Parker and Doucet 1995, Perkins et al. 1995, Chiolero et al. 2008), our findings that nicotine can enhance sucrose-seeking by serving as an occasion-setter implies comparable behavioral mechanisms between male and female

smokers. These findings contribute to the theory behind various treatments of nicotine addiction. Menthol would be valuable to evaluate in the future in a cue-seeking paradigm due to its modulation of nicotine's mechanism of action.

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Figures

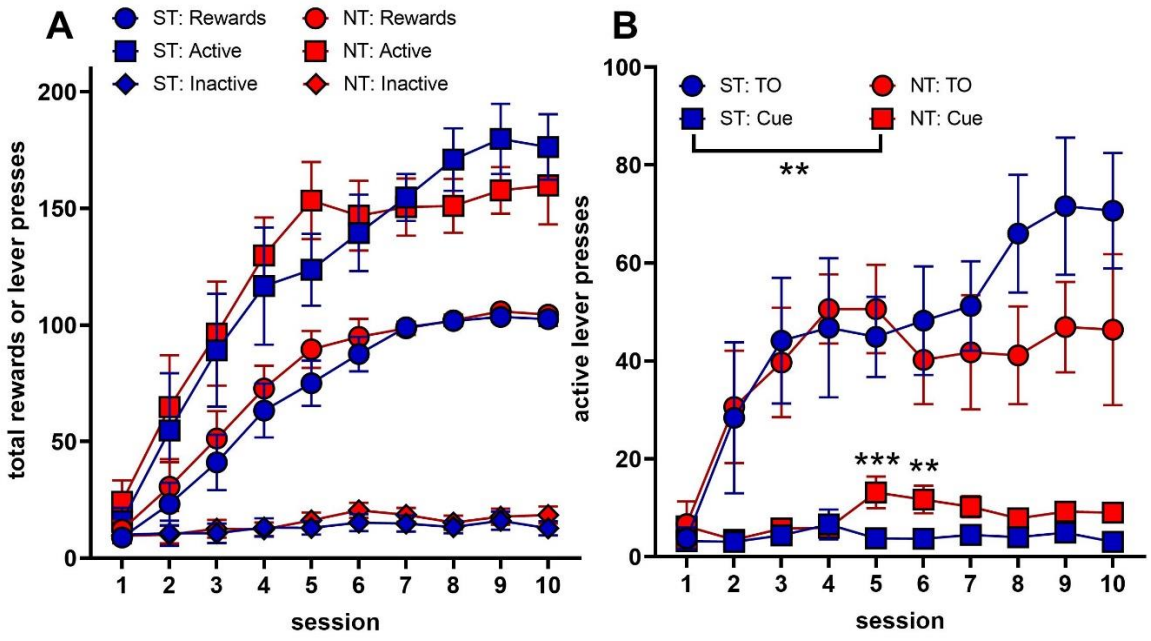

Figure 1: Comparison of acquisition of operant conditioning for sucrose reward between nicotine trained (NT) and saline trained (ST) rats. (A) Total rewards, total active lever presses, and total inactive lever presses did not differ between groups. (B) When examining active lever presses alone, NT rats pressed the active lever more during the cue period compared to ST animals. Statistical significance is indicated as ** = $p < 0.01$; *** = $p < 0.001$. Error bars are standard error of the mean.

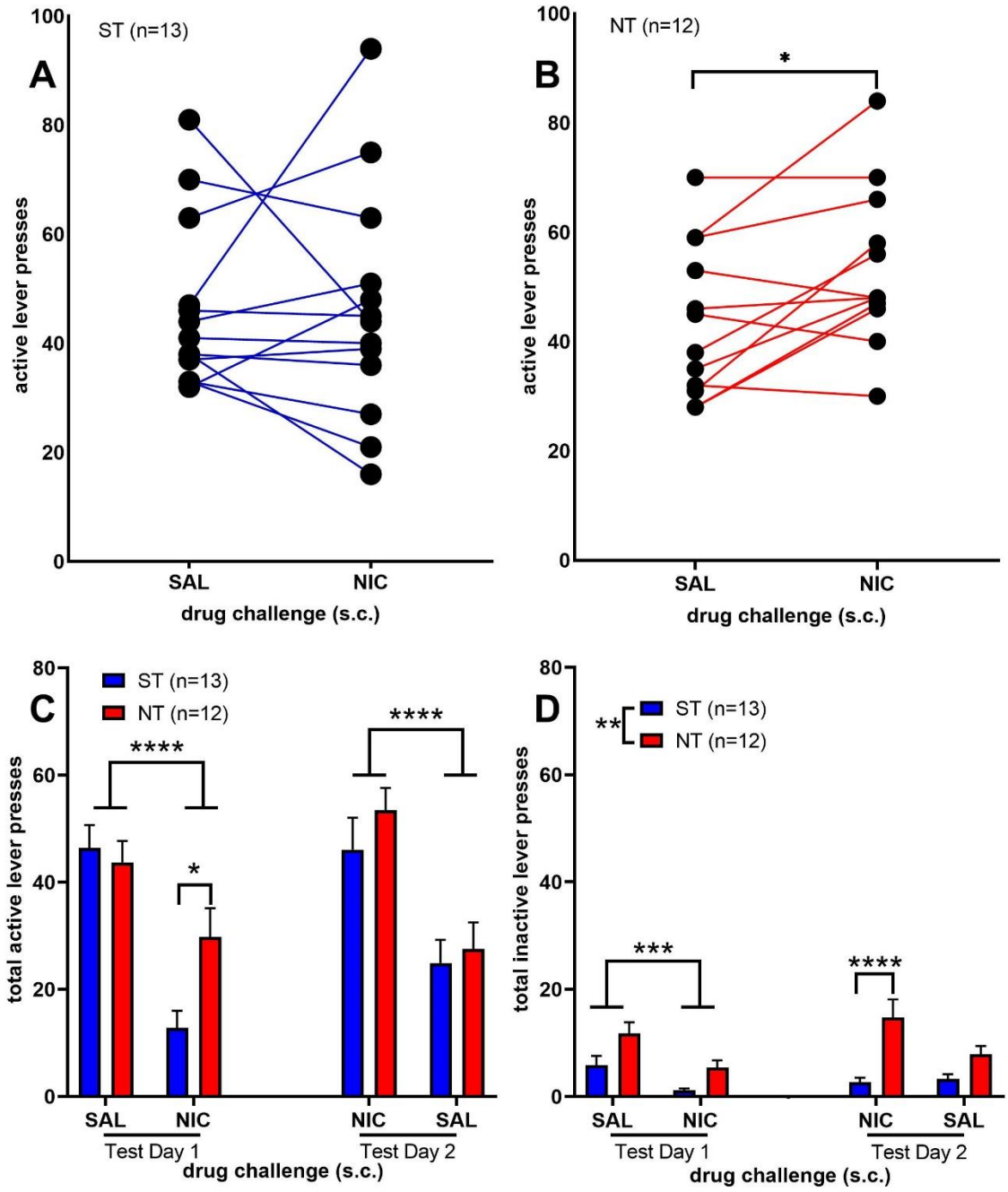

Figure 2: Sucrose-seeking Test Day 1 and Test Day 2. (A) Active lever presses decreased from the first to second challenge, indicative of extinction. However, NT rats had more active lever presses on Test Day 1 when administered nicotine. (B) Inactive lever presses similarly decreased from the first to second drug challenge, but nicotine animals had greater inactive lever presses on both test

days. (C/D) Since there was significant extinction between the first and second challenge on each test day, the first challenge for each test day was compared. ST animals showed similar responding with saline and nicotine challenges, but NT trained animals had more active lever responding. Statistical significance is indicated as ** = $p < 0.01$; *** = $p < 0.001$, **** = $p < 0.0001$. Error bars are standard error of the mean.