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Brief report

Nicotine Enhances Footshock- and Lithium Chloride-Conditioned Place Avoidance in Male Rats

Deanne M. Buffalari PhD¹, Julianna K. Mollica BS¹, Tracy T. Smith PhD², Rachel L. Schassburger MS¹, Linda Rinaman PhD¹, Edda Thiels PhD³, Eric C. Donny PhD², Alan F. Sved PhD¹

¹Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA; ²Department of Psychology, University of Pittsburgh, Pittsburgh, PA; ³Department of Neurobiology, University of Pittsburgh, Pittsburgh, PA

Corresponding Author: Deanne M. Buffalari, PhD, Department of Psychology, Westminster College, New Wilmington, PA 16147, USA. Telephone: 724-946-7358; Fax: 724-946-7725; E-mail: buffald@westminster.edu

Abstract

Introduction: Numerous studies have shown that nicotine (NIC) can enhance the reinforcing effects of non-NIC stimuli through a nonassociative mechanism. To date, it is unclear whether NIC reinforcement enhancement serves to increase behaviors motivated by rewarding stimuli only, or whether NIC potentiates behavior motivated by all stimuli, regardless of valence.

Methods: The current study used a place conditioning procedure to examine whether acute NIC injection modulates avoidance of an environment previously associated with an aversive stimulus. Separate groups of rats underwent place conditioning using either lithium chloride (125 mg/kg/ml, i.p.) or footshock (0.75 mA) as the aversive stimulus. Other rats served as nonconditioned controls. The magnitude of place avoidance was assessed after acute NIC (0.1 or 0.4 mg/kg/ml, s.c.) or saline.

Results: Rats avoided chambers previously paired with either lithium chloride or footshock, and conditioned place avoidance was significantly enhanced by NIC pre-treatment.

Conclusions: These results demonstrate that the ability of NIC to enhance motivated behavior extends to behaviors elicited by aversive stimuli, evidence that NIC affects behavior motivated by a broader range of stimuli than previously appreciated.

Implications: The current study examined whether the reinforcement enhancement properties of NIC apply to aversive stimuli by testing NIC enhancement of conditioned place avoidance in rats. The results demonstrate that NIC enhances the motivational impact of these distinct aversive stimuli, providing novel evidence that NIC affects behavior motivated by a broader range of stimuli than has previously been demonstrated.

Introduction

The prevalence of tobacco use has been partially attributed to the primary reinforcing effects of nicotine (NIC) and its interactions with non-NIC stimuli.^{1–3} NIC establishes non-reinforcing stimuli as conditioned reinforcers via associative mechanisms.^{4,5} NIC can also enhance the existing reinforcing properties of non-NIC stimuli via nonassociative mechanisms,^{4,6–8} a property referred to as reinforcement enhancement. These complex interactions between NIC and

stimuli, along with the primary reinforcing actions of NIC, likely contribute to the persistence of tobacco use as a pervasive epidemic.

Studies examining the ability of NIC to enhance stimulus control of behavior have utilized stimuli that function as primary reinforcers.^{9–12} In some cases, NIC enhances responding for a change in stimulus conditions (eg, in rats, NIC enhances lever-pressing behavior that results in the turning off of the chamber light).¹³ However, it is unclear whether this enhancement reflects an increase in the value

of positive reinforcers (eg, an “off” house light has greater value in the presence of NIC) as demonstrated with other stimuli,^{9–12} or an increase in the value of aversive stimuli (eg, an “on” house light is more aversive in the presence of NIC, and therefore, rats increase lever pressing that results in its removal). Experiments designed to discriminate between these possibilities will improve our understanding of the mechanisms by which NIC exerts its enhancement effects.

Reinforcement enhancement is most commonly studied using operant procedures,^{14,15} which are less useful for examining whether NIC modulates the ability of aversive stimuli to regulate behavior. We recently demonstrated that a single injection of NIC enhances the expression of a conditioned place preference for sucrose solution or cocaine.¹⁶ Place conditioning may also provide a suitable approach for understanding how NIC alters avoidance behavior. This study examined whether a single, acute, noncontingent NIC injection can enhance avoidance of a testing chamber previously paired with either systemic lithium chloride (LiCl) treatment or footshock (FS), both of which support conditioned place avoidance (CPA) in rats.^{17,18} Avoidance behavior in rats previously exposed to aversive conditioning was compared to behavior in nonconditioned controls to ensure that the actions of acute NIC were specific in modulating behavior guided by prior stimulus-context pairing.

Methods

Subjects

Male, Sprague-Dawley rats ($n = 36$, Harlan Farms, Indianapolis, IN), 200–225 g, were singly housed in a colony room with a 12-hour reverse light-dark cycle (lights off at 7 AM) with *ad libitum* access to water and rat chow. After 6 days of housing acclimation, animals were weighed and restricted to 20 g of food daily for 7–10 days prior to the start of the study (to approximately 85%–90% free feeding weight). All behavioral procedures occurred during the dark phase of the cycle. Experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals under local IACUC oversight.

Place Conditioning Procedure

Rats were conditioned and tested in modified three-compartment place conditioning chambers (MED-CPP-RS, Med Associates, Inc, St Albans, VT) encased in sound-attenuating cabinets. Compartment A had horizontally striped wall coverings; compartment B had vertically striped wall coverings; A and B had comparable illumination. Compartment C was a smaller middle compartment with gray walls, and was more brightly illuminated in order to reduce baseline preference for the smaller chamber. Chambers A and B had floors that could deliver FS. Chambers were separated by manual guillotine-style doors. Time spent in each chamber was automatically detected via infrared photobeam sensors connected to a data collection system (Med PC, Med Associates, Inc).

Initial Avoidance Assessment

On Day 1 of behavior, all animals ($n = 36$) underwent a 20-minute initial assessment to establish time spent in each chamber at baseline. Animals were placed into the center compartment and allowed to freely explore all compartments for 20 minutes (all doors open). Animals were then returned to their home cage.

FS Conditioning

One group of animals ($n = 27$) received either FS ($n = 15$) or control ($n = 12$) conditioning. FS and control conditioning occurred over

2 days (one FS and one control pairing for FS conditioned rats, or one control pairing in each compartment for control conditioned rats, with the order and FS compartment pairing counterbalanced in an unbiased design). Doors were closed during conditioning to restrict access to the FS or control compartment only. Rats were placed into the conditioning compartment for 10 minutes total. During FS conditioning, 3 minutes after placement into the compartment, 10 single FSs (0.75 mA, 0.5 seconds duration) were given via electrified flooring over 6 minutes (variable time, ITI of 70 seconds, range of 10–110 seconds). FS parameters were chosen based on pilot testing prior that determined an appropriate intensity, duration, and number of FS that would produce aversion without complete avoidance. Rats remained in the compartment for 1 additional minute after delivery of the last FS, and were then returned to their home cage. During control conditioning, rats remained undisturbed in the control compartment for 10 minutes, and then were returned to their home cage.

LiCl Conditioning

A separate group of rats underwent LiCl conditioning ($n = 9$). LiCl conditioning occurred over 4 days (two saline pairings, two LiCl pairings, with injection order and compartment pairing counterbalanced; the compartment paired with LiCl was randomly assigned in an unbiased design). Pilot studies confirmed that 2 days of LiCl conditioning were necessary to support significant CPA. Doors were closed during conditioning to restrict access only to the conditioning compartment. Rats were placed into the conditioning compartment immediately after injection of either saline or LiCl (125 mg/kg/ml, i.p.). Rats remained in the compartment for 60 minutes, and then were returned to their home cage.

Avoidance Testing

Avoidance Tests 1, 2, and 3 were conducted on the days immediately following conditioning and were identical to the initial assessment (ie, free access to all compartments for 20 minutes). Testing was done using an ABA design, where A represented two saline tests separated by a single NIC test (B). For saline tests, rats received a single injection of saline (1 ml/kg, s.c.). For NIC tests, rats in the FS group were split into two groups and given either 0.1 mg/kg/ml or 0.4 mg/kg/ml NIC, s.c., while all LiCl-conditioned rats received the 0.4 mg/kg/ml NIC dose. Five minutes after injection, rats were removed from their home cage and placed into the center compartment of the chamber for testing (20 minutes). Rats in the LiCl experiment were given a second saline injection immediately prior to placement in the chamber in order to mimic the LiCl and saline injections given during conditioning.

Drugs

NIC solutions were made by dissolving (–)NIC hydrogen tartrate (Sigma, St Louis, MO) in 0.9% NaCl. NIC doses (0.1 and 0.4 mg/kg/ml, s.c., free base concentration, pH adjusted to 7.0 ± 0.2 and filtered for sterility) were based on previous reinforcement-enhancement studies of NIC.^{1,7,19} LiCl was dissolved in distilled water, and given at a dose (125 mg/kg/ml, i.p.) previously shown to produce CPA.²⁰

Data Analysis

Two-way ANOVA was used to reveal interactions and main effects of prior FS (vs. no FS) and acute NIC (0, 0.1, or 0.4 mg/kg/ml) on total time spent in the FS-paired chamber. One-way ANOVA was used to compare total time in the LiCl-paired chamber across the

three tests (SAL1, NIC, SAL2) to confirm a significant CPA to LiCl, and to reveal potential enhancement of CPA by NIC (0.4mg/kg). Bonferroni post hoc tests were used to examine specific, pair-wise differences. For all statistical tests, an alpha criterion of 0.05 was set to indicate significant effects.

Results

FS-Induced CPA

FS supported the development of strong CPA. Rats that received FS conditioning spent significantly less time in the FS-paired chamber compared to nonconditioned controls (significant interaction, $F_{(9,69)} = 9.69$, post hoc $P < .0001$, Figure 1). Time in the FS-paired chamber during the initial preference test significantly decreased after FS-conditioning ($P < .01$), confirmation of this strong CPA. In FS-exposed rats, but not in controls, NIC significantly enhanced avoidance of the FS-paired chamber compared to saline (NIC TEST vs. SAL1 and SAL2 $P < .01$, Figure 1). There was no effect of NIC dose on FS-induced CPA magnitude, with no significant differences in avoidance across dose (0.1 mg/kg vs. 0.4 mg/kg, $P > .05$).

LiCl-Induced CPA

LiCl supported the development of CPA, as revealed by a significant main effect of test ($F_{3,23} = 12.18$, $P < .0001$, Figure 2), and post hoc comparisons that revealed a significant difference between post-conditioning saline test 1 and initial baseline preference ($P < .01$). Further, NIC enhanced the magnitude of LiCl-induced CPA; CPA after NIC treatment was significantly greater than in all other tests ($P < .001$, Figure 2).

Discussion

The present study is the first to examine the ability of NIC to enhance behavior that is driven by aversive stimuli. NIC enhanced the avoidance of a context previously paired with either FS or LiCl. Therefore, the reinforcement-enhancing effect of NIC appears to impact both

negative and positive reinforcement processes, significantly expanding the potential range of behaviors affected by NIC.

The reinforcement-enhancing effect of NIC on aversive conditioned stimuli is similar in several regards to the effect of NIC on appetitive stimuli. First, the effect does not depend on associative conditioning, as NIC was never directly paired with the chamber, FS, or LiCl prior to testing. Further, the effect is not modality-specific, nor does it depend on a specific number of pairings or dose of NIC. Avoidance of contexts associated with LiCl, a drug that causes nausea and altered interoceptive state, and FS, a nociceptive exteroceptive stimulus, were both enhanced by NIC. Disparate appetitive stimuli also have been shown to be subject to NIC enhancement, including gustatory stimuli, sucrose-paired stimuli, drug-paired stimuli, and social interactions.^{9,12,16,21} Moreover, although the number of stimulus-context pairings differed between FS and LiCl conditioning, NIC enhanced avoidance behavior supported by both aversive stimuli. Finally, the reinforcement-enhancement effect of NIC was present at both NIC doses, consistent with previous results.^{14,21,22}

NIC has previously been reported to enhance aversive learning. However, the current results are unlikely to reflect NIC enhancement of recall of the learned association between the unconditioned and conditioned stimuli. In previous reports, contextual fear conditioning was enhanced only when NIC was administered prior to conditioning and on test day,²³ whereas enhancement of CPA in the present study was present after a single injection prior to expression. Indeed, the previously reported enhancement of contextual fear conditioning could be partially explained by reinforcement enhancement, that is, NIC-induced enhancement of the ability of the shock-paired cue to drive freezing behavior. It is also unlikely that the current results reflect NIC-induced disruption of extinction of CPA; avoidance of a LiCl¹⁸ or FS-paired chamber²⁴ does not significantly extinguish in 3 days. In the present study, NIC enhanced avoidance above baseline levels, while NIC given during fear extinction training does not enhance freezing above baseline.²⁵ Thus, the NIC-driven disruption of extinction of contextual fear²⁵ could instead be interpreted as evidence that NIC enhances the ability of an aversively-paired context to support freezing behavior.

The reinforcement-enhancement properties of NIC have been suggested to enhance the ability of positive stimuli to drive

Figure 1. Footshock supported the development of conditioned place avoidance. Nicotine (NIC; 0.1 or 0.4 mg/kg/ml) enhanced the expression of footshock (FS)-conditioned place avoidance ($n = 15$) compared to control conditioned rats ($n = 12$) or saline. Data are represented as total time in FS-paired chamber $\pm$ standard error of the mean (SEM). *Significantly different from CTRL rats SAL1 and significantly different from FS rats pretest, +significantly different from FS rats non-NIC test days (SAL1 and SAL2).

Figure 2. Lithium chloride (LiCl) supported the development of conditioned place avoidance. Nicotine (NIC; 0.4 mg/kg/ml) enhanced the expression of LiCl-conditioned place avoidance ($n = 9$). Data are represented as total time in LiCl-paired chamber (s) $\pm$ standard error of the mean (SEM). *Significantly different from saline test 1, +significantly different from all other tests.

behavior.⁹⁻¹² Alternatively, NIC might promote behavior that results in the removal of a potentially aversive stimulus.¹³ We attempted to resolve this issue by utilizing two distinct stimuli known to be aversive. NIC enhanced CPA, which suggests that NIC exerts a stimulus-enhancing effect regardless of the valence of the stimulus. To reduce the possibility that NIC enhanced a preference for the control chamber that emerged when pairing the alternate chamber with either FS or LiCl, we utilized a three-compartment chamber²⁶ in which the properties of the neutral center compartment do not change during conditioning. We confirmed that rats distributed their time approximately equally between the control and neutral chambers during testing, evidence that the NIC enhancement effect is unlikely to reflect enhancement of a preference for the control chamber.

In summary, the current demonstration of NIC's ability to enhance negative reinforcement processes indicates that NIC, a modest primary reinforcer, may affect a wide range of motivated behaviors. Continued research to reveal neural mechanisms through which NIC enhances behavior driven by aversive stimuli or negative affective states may lead to improved understanding of how NIC reinforces smoking behavior, and thereby lead to more successful treatments for NIC addiction.

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Declaration of Interests

None declared.

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References

- Caggiula AR, Donny EC, Palmatier MI, Liu X, Chaudhri N, Sved AF. The role of nicotine in smoking: a dual-reinforcement model. *Nebr Symp Motiv*. 2009;55:91-109. www.ncbi.nlm.nih.gov/pmc/articles/PMC2694565/. Accessed October 16, 2015.
- Rose JE, Behm FM, Westman EC, Johnson M. Dissociating nicotine and nonnicotine components of cigarette smoking. *Pharmacol Biochem Behav*. 2000;67(1):71-81. doi:10.1016/S0091-3057(00)-00301-4.
- Perkins KA, Karelitz JL. Reinforcement enhancing effects of nicotine via smoking. *Psychopharmacology (Berl)*. 2013;228(3):479-486. doi:10.1007/S00213-013-3054-4.
- Chaudhri N, Caggiula AR, Donny EC, Palmatier MI, Liu X, Sved AF. Complex interactions between nicotine and nonpharmacological stimuli reveal multiple roles for nicotine in reinforcement. *Psychopharmacology (Berl)*. 2006;184(3-4):353-366. doi:10.1007/s00213-005-0178-1.
- Palmatier MI, Coddington SB, Liu X, Donny EC, Caggiula AR, Sved AF. The motivation to obtain nicotine-conditioned reinforcers depends on nicotine dose. *Neuropharmacology*. 2008;55(8):1425-1430. doi:10.1016/j.neuropharm.2008.09.002.
- Palmatier MI, O'Brien LC, Hall MJ. The role of conditioning history and reinforcer strength in the reinforcement enhancing effects of nicotine in rats. *Psychopharmacology (Berl)*. 2012;219(4):1119-1131. doi:10.1007/s00213-011-2439-5.
- Barret ST, Bevins RA. Nicotine enhances operant responding for qualitatively distinct reinforcers under maintenance and extinction conditions. *Pharmacol Biochem Behav*. 2013;114-115:9-15.
- Rupprecht LE, Smith TT, Schassburger RL, Buffalari DM, Sved AF, Donny EC. Behavioral mechanisms underlying nicotine reinforcement. *Curr Top Behav Neurosci*. 2015;24:19-53. doi:10.1007/978-3-319-13482-6_2.
- Palmatier MI, Lantz JE, O'Brien LC, Metz SP. Effects of nicotine on olfactory gustatory incentives: preference, palatability, and operant choice tests. *Nicotine Tob Res*. 2013;15(9):1545-1554. doi:10.1093/ntr/ntt016.
- Olausson P, Jentsch JD, Taylor JR. Nicotine enhances responding with conditioned reinforcement. *Psychopharmacology (Berl)*. 2004;171(2):173-178. doi:10.1007/s00213-003-1575-y.
- Perkins KA, Karelitz JL. Sensory reinforcement-enhancing effects of nicotine via smoking. *Exp Clin Psychopharmacol*. 2014;22(6):511-516. doi:10.1037/a0037823.
- Chaudhri N, Caggiula AR, Donny EC, et al. Operant responding for conditioned and unconditioned reinforcers in rats is differentially enhanced by the primary reinforcing and reinforcement-enhancing effects of nicotine. *Psychopharmacology (Berl)*. 2006;189(1):27-36. doi:10.1007/s00213-006-0522-0.
- Caggiula AR, Donny EC, White AR, et al. Environmental stimuli promote the acquisition of nicotine self-administration in rats. *Psychopharmacology (Berl)*. 2002;163(2):230-237. doi:10.1007/s213-002-1156-5.
- Chaudhri N, Caggiula AR, Donny EC, et al. Self-administered and non-contingent nicotine enhance reinforced operant responding in rats: impact of nicotine dose and reinforcement schedule. *Psychopharmacology (Berl)*. 2007;190(3):353-362. doi:10.1007/s00213-006-0454-8.
- Palmatier MI, Evans-Martin FF, Hoffman A, Caggiula AR, Chaudhri N, Donny EC. Dissociating the primary reinforcing and reinforcement-enhancing effects of nicotine using a rat self-administration paradigm with concurrently available drug and environmental reinforcers. *Psychopharmacology (Berl)*. 2006;184(3-4):391-400. doi:10.1007/s00213-005-0183-4.
- Buffalari DM, Marfo NY, Smith TT, et al. Nicotine enhances the expression of a sucrose or cocaine conditioned place preference in adult male rats. *Pharmacol Biochem Behav*. 2014;124:320-325. doi:10.1016/j.pbb.2014.06.013.
- Holahan MR, White NM. Intra-amygdala muscimol injections impair freezing and place avoidance in aversive contextual conditioning. *Learn Mem*. 2004;11(4):436-446. doi:10.1101/lm.64704.
- Tenk CM, Kavaliers M, Ossenkopp KP. Dose response effects of lithium chloride on conditioned place aversions and locomotor activity in rats. *Eur J Pharmacol*. 2005;515(1-3):117-127. doi:10.1016/j.ejphar.2005.04.007.
- Palmatier MI, Matteson GL, Black JJ, et al. The reinforcement enhancing effects of nicotine depend on the incentive value of non-drug reinforcers and increase with repeated drug injections. *Drug Alcohol Depend*. 2007;89(1):52-59. doi:10.1016/j.drualdep.2006.11.020.
- Carvalho AF, Van Bockstaele EJ. Direct intra-accumbal infusion of a beta-adrenergic receptor antagonist abolishes WIN 55,212-2-induced aversion. *Neurosci Lett*. 2011;500(1):82-85. doi:10.1016/j.neulet.2011.06.008.
- Thiel KJ, Sanabria F, Neisewander JL. Synergistic interaction between nicotine and social rewards in adolescent male rats. *Psychopharmacology (Berl)*. 2009;204(3):391-402. doi:10.1007/s00213-009-1470-2.
- Liu X, Palmatier MI, Caggiula AR, Donny EC, Sved AF. Reinforcement enhancing effect of nicotine and its attenuation by nicotinic antagonists in rats. *Psychopharmacology (Berl)*. 2007;194(4):463-473. doi:10.1037/a0012807.
- Kenney JW, Gould TJ. Nicotine enhances context learning but not context-shock associative learning. *Behav Neurosci*. 2008;122(5):1158-1165. doi:10.1016/j.bbr.2012.09.024.
- Huang AC, Shyu BC, Hsiao S, Chen TC, He AB. Neural substrates of fear conditioning, extinction, and spontaneous recovery in passive avoidance learning: a c-fos study in rats. *Behav Brain Res*. 2013;237:23-31. doi:10.1016/j.bbr.2014.01.031.
- Kutlu MG, Gould TJ. Acute nicotine delays extinction of contextual fear in mice. *Behav Brain Res*. 2014;263:133-137.
- Prus AJ, James JR, Rosecrans JA. Conditioned Place Preference. In: Buccafusco JJ, ed. *Methods of Behavior Analysis in Neuroscience*. 2nd ed. Boca Raton, FL: CRC Press; 2015.