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Nicotine as a Conditioned Stimulus: Impact of Attention-Deficit/Hyperactivity Disorder Medications

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Abstract: People diagnosed with attention-deficit/hyperactivity disorder (ADHD) are at an increased risk to start smoking and have greater difficulty quitting. Nicotine, one of the principal addictive components of tobacco smoke, functioned as a conditioned stimulus (CS) for intermittent sucrose delivery in a Pavlovian drug discrimination task with rats. This study compared the ability of commonly prescribed ADHD medications (i.e., methylphenidate, atomoxetine, and bupropion) and additional dopamine reuptake inhibitors (i.e., cocaine and GBR 12909) to substitute for the CS effects of nicotine. Atomoxetine was also used to antagonize these CS effects. Rats acquired the discrimination as evidenced by increased dipper entries in nicotine (0.2 mg base/kg) sessions as compared with saline sessions. Nicotine generalization was dose dependent. Bupropion (10 and 20 mg/kg), methylphenidate (10 mg/kg), and cocaine (5 and 10 mg/kg) partially substituted for the 0.2 mg/kg nicotine CS. Atomoxetine did not substitute for the nicotine CS; however, atomoxetine (1 to 10 mg/kg) partially blocked nicotine's CS effects. These results suggest that atomoxetine, bupropion, and/or methylphenidate may be effective treatments for people diagnosed with ADHD and addicted to nicotine.

Keywords: dopamine, drug discrimination, interoceptive Pavlovian conditioning, nicotinic acetylcholine receptors, norepinephrine

Nicotine is the primary psychoactive and addictive component of tobacco smoke. It is interesting to note that higher incidences of tobacco dependence and difficulty quitting are reported in clinical populations (Morris, Giese, Turnbull, Dickinson, & Johnson-Nagel, 2006; Williams & Ziedonis, 2004). For example, tobacco dependence commonly co-occurs in schizophrenic populations (de Leon & Diaz, 2005) and is highly correlated with mood disorders (Glassman et al., 1990; Pomerleau, Marks, & Pomerleau, 2000). Recent reports show that people diagnosed with attention-deficit/hyperactivity disorder (ADHD) are twice as likely to become smokers than their peers (Milberger, Biederman, Faraone, Chen, & Jones, 1997; A. S. Potter & Newhouse, 2004) and show more severe withdrawal symptoms when trying to quit tobacco use (Pomerleau et al., 2003). Thus, the abuse liability of nicotine appears exacerbated in ADHD patients. Therefore, pharmacotherapies simultaneously targeting ADHD symptoms and nicotine addiction may have some treatment utility for this clinical subpopulation.

Three drugs commonly prescribed to treat ADHD are methylphenidate, bupropion, and atomoxetine (Ritalin, Wellbutrin, and Strattera, respectively). Of these drugs, bupropion and methylphenidate increase dopamine release in brain reward

pathways (Ascher et al., 1995; Bymaster et al., 2002; Kuczenski & Segal, 1997; Nomikos, Damsma, Wenkstern, & Fibiger, 1992). Bupropion has lower affinity for the dopamine transporter than methylphenidate and also blocks norepinephrine reuptake (Ascher et al., 1995; Katz, Izenwasser, & Terry, 2000). Atomoxetine, on the other hand, acts primarily on the noradrenergic system by blocking the reuptake of synaptic norepinephrine (Bymaster et al., 2002). Drugs targeting the noradrenergic system may benefit patients with ADHD who are also smokers given that atomoxetine has impacted nicotine-induced responding in prior research. Specifically, Gould, Rukstalis, and Lewis (2005) demonstrated that an acute administration of nicotine (0.125 mg/kg) or atomoxetine (2 mg/kg) enhanced prepulse inhibition of the acoustic startle response in mice. However, this enhancement was blocked when atomoxetine was administered before nicotine, probably owing to overactivation of the noradrenergic system (Gould et al., 2005). Atomoxetine also alleviates attention deficits associated with nicotine withdrawal. For example, mice undergoing nicotine withdrawal show impaired contextual fear conditioning in comparison to mice in a control condition (Davis & Gould, 2007; Davis, James, Siegel, & Gould, 2005). This impairment was reversed by an acute injection of atomoxetine (Davis & Gould, 2007).

Current theories of tobacco abuse attribute associative learning processes as having a prominent role in the acquisition, maintenance, and/or relapse of drug use and abuse (e.g., Bevins & Palmatier, 2004; Di Chiara, 1999; Geier, Mucha, & Pauli, 2000; Lazev, Herzog, & Brandon, 1999; Pritchard,

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Robinson, Guy, Davis, & Stiles, 1996; Rose & Levin, 1991). In the case of nicotine conditioning, environmental cues (conditioned stimulus, CS) presented in close temporal proximity with the physiological effects of the drug (unconditioned stimulus, US) come to evoke behavioral and cognitive responses even in a non-drug state. From this framework, the interoceptive effects of nicotine are functioning as a US. Indeed, this framework is the most commonly used to study the potential conditioning processes contributing to nicotine use (see Bevins & Palmatier, 2004, for a review). Research from our laboratory and others has extended this conceptualization to include nicotine as an interoceptive CS (Besheer, Palmatier, Metschke, & Bevins, 2004; Bevins, Penrod, & Reichel, 2007; Clements, Glautier, Stolerman, White, & Taylor, 1996; Murray & Bevins, 2007; Troisi, 2006; Wilkinson et al., 2006). In our research, to train nicotine as a CS, rats had intermittent access to sucrose on nicotine sessions; on intermixed saline sessions no sucrose was present. Conditioned responding to the nicotine CS was evidenced by more head entries into the dipper receptacle before the first sucrose delivery on nicotine sessions (often termed *goal tracking*; Boakes, 1977; Farwell & Ayres, 1979) than in a comparable time period on saline sessions (Besheer et al., 2004; Bevins et al., 2007; Murray & Bevins, 2007; Wilkinson et al., 2006).

To date, bupropion is the only drug approved for the treatment of ADHD that has been tested for substitution of the interoceptive CS effects of nicotine. In brief, when 0.4 mg/kg nicotine was trained as a CS, 20 mg/kg bupropion increased conditioned responding above saline levels, but not quite equivalent to nicotine CS levels, suggesting that bupropion produces an interoceptive cue somewhat similar to nicotine (Besheer et al., 2004). Therefore, one purpose of the present study was to extend this research by determining whether the widely prescribed ADHD medications bupropion, methylphenidate, and atomoxetine substituted for a more moderate dose of nicotine (i.e., 0.2 mg/kg) functioning as a CS. Additionally, we tested cocaine and GBR 12909 (highly specific dopamine reuptake inhibitor) to determine whether dopamine reuptake inhibitors with varying affinity for the transporter would substitute for the nicotine CS (Katz et al., 2000). Finally, prompted by the research of Gould and colleagues described earlier, we tested whether atomoxetine would antagonize the nicotine-evoked conditioned response (CR).

Method

Subjects

Sixteen male Sprague-Dawley rats (weighing 300 ± 20 g at the start of the study) were obtained from Harlan (Indianapolis, IN). The rats had been used in a previous study and had experienced 15 consecutive daily exposures to the nicotine (0.2 mg/kg) and sucrose (see Bevins et al., 2007, Experiment 2). Animals were housed individually in clear polycarbonate tubs lined with wood shavings in a temperature- and humidity-controlled colony room. Water was continually pro-

vided in the home cage. Food access was restricted such that rats' body weights were maintained at 85% of their free-feeding weight. Approximately every 30 days, this target weight was increased by 2 g. All sessions were conducted during the light portion of a 12-hr light-dark cycle. Experimental protocols were approved by the University of Nebraska—Lincoln Institutional Animal Care and Use Committee and followed the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996).

Apparatus

Eight standard conditioning chambers (Med Associates, Georgia, VT) were used in this study. Each chamber was enclosed in a light- and sound-attenuating polyvinyl chloride cubicle fitted with a fan to provide airflow and mask noise. The conditioning chambers measured $30.5 \times 24.1 \times 21$ cm ($l \times w \times h$). The sidewalls were made of aluminum; the ceiling, front, and back walls were clear polycarbonate. Each chamber contained a recessed dipper receptacle ($5.2 \times 5.2 \times 3.8$ cm; $l \times w \times d$) in one of the aluminum sidewalls. When the dipper arm was raised, it allowed access to 0.1 ml of 26% sucrose solution (wt/vol) in the receptacle. An infrared emitter-detector unit located 1.2 cm inside the receptacle and 3 cm from the floor recorded head entries. A second infrared emitter-detector unit bisected the chamber 14.5 cm from the sidewall containing the receptacle and was mounted 4 cm above the rod floor. This unit provided a measure of activity in the chamber. A personal computer with Med Associates interface and software (Med-PC for Windows, Version 4) controlled sucrose deliveries and recorded dipper entries and chamber activity.

Drugs

(S)-nicotine hydrogen tartrate, bupropion hydrochloride, cocaine hydrochloride, GBR 12909, and methylphenidate hydrochloride were purchased from Sigma (St. Louis, MO). Atomoxetine hydrochloride was purchased from Tocris Cookson (Ellisville, MO). Nicotine, cocaine, bupropion, and methylphenidate were dissolved in 0.9% saline. All drug doses except nicotine are reported as salt form; nicotine is reported as base. Nicotine was adjusted to a pH of 7.0 ± 0.2 with a dilute NaOH solution. GBR 12909 and atomoxetine were dissolved in sterile water. All drugs were injected at a volume of 1 ml/kg. For substitution drugs, injection route, injection-to-placement intervals, and doses were based on previous studies (Besheer et al., 2004; Desai, Barber, & Terry, 2003; Gould et al., 2005; Mansbach, Rovetti, & Freedland, 1998; Mattiuz et al., 2003).

Acquisition

Rats were injected with nicotine or 0.9% saline 5 min before placement in the conditioning chamber for 20 min. On nicotine sessions, rats had 4 s access to sucrose on 36 separate occasions. On intermixed saline sessions, sucrose deliveries were withheld. To decrease the possibility of the rats learn-

ing to time when a sucrose delivery may occur, we used four different computer programs controlling sucrose deliveries. Comparable programs were written for saline sessions, with 4-s "empty" intervals occurring in place of sucrose to ensure identical session length. The average time before the first sucrose delivery (or equivalent time in saline sessions) across programs was 137 s, with a range of 124–152 s. Nicotine and saline sessions were randomly assigned to each rat so that there were no more than two of the same session types in a row. This acquisition phase lasted 24 days and consisted of 12 nicotine and 12 saline sessions.

Testing

Nicotine generalization. Following acquisition training, rats entered the nicotine generalization phase. On the first 4 days of a 5-day cycle, rats received two nicotine and two saline training sessions that were randomly intermixed. These sessions were identical to those described for acquisition and were used to ensure maintenance of the discrimination. On Day 5, a 4-min test session was conducted if discrimination criteria were met (discussed later). This 5-day testing cycle was used for the remainder of the experiment. On test days, rats were injected subcutaneously with saline or nicotine (0.025, 0.1, 0.2, or 0.3 mg/kg) 5 min before chamber placement. Rats were tested on all doses, and the order was randomly assigned for each rat.

Substitution. Upon completion of the nicotine generalization phase, rats started the substitution testing phase. On a given test day, rats were injected intraperitoneally with bupropion (5, 10, or 20 mg/kg), cocaine (1, 5, 10, or 20 mg/kg), GBR 12909 (1, 5, 10, 20, or 30 mg/kg), methylphenidate (1, 5, or 10 mg/kg), or atomoxetine (0.3, 1, 3, or 10 mg/kg) 15 min before placement in the chamber. Testing of these drugs was intermixed, and the order of drug dose was randomly assigned for each rat. Methylphenidate was tested after completion of all of the other test compounds.

Atomoxetine antagonism. Following substitution testing, we assessed whether pretreatment with atomoxetine would block conditioned responding evoked by the nicotine CS. On test days, rats were injected with atomoxetine (0.1, 0.3, 1, 3, or 10 mg/kg ip) 25 min before nicotine (cf. Gould et al., 2005, for comparable injection-to-place interval for antagonism). As in training, nicotine was injected 5 min before placement in the conditioning chamber. The order of drug dose was randomly assigned for each rat.

Dependent Measures and Testing Criteria

For acquisition and testing, the rate of infrared beam breaks in the dipper receptacle per second (i.e., dipper entry rate) before the first sucrose delivery in the nicotine sessions (or equivalent time in the saline and tests sessions) served as the main dependent measure. For a rat to qualify to test, during the substitution and antagonism phases, the dipper entry rate on each nicotine session had to be at least 0.01 dipper entries per second higher than on both saline sessions within the four

training sessions of a 5-day testing cycle (cf. Murray & Bevins, 2007). General chamber activity, defined as the number of beam breaks per second during the same time period as dipper entries, was also analyzed during acquisition and testing.

Data Analyses

For acquisition training, dipper entries and chamber activity were analyzed with two-way (Drug [nicotine or saline] \times Session) repeated measures analyses of variance (ANOVAs); Fisher's protected least significant difference (LSD) tests were used for post hoc comparisons. For generalization, substitution, and antagonism testing phases, dipper entries and chamber activity were analyzed with separate one-way repeated measures ANOVAs for each compound. One rat began having adverse reactions to the training dose of nicotine and was removed from the study before completion of GBR 12909 and methylphenidate substitution and atomoxetine antagonism tests. During substitution testing, bupropion, cocaine, GBR 12909, and atomoxetine were randomly intermixed. Accordingly, the same baseline values for nicotine and saline sessions were used for comparison purposes. For these comparisons, the baseline values were extracted from the nicotine and saline training sessions before testing with 3 mg/kg atomoxetine. This procedure allows for a random sample of baseline values because all drugs and drug doses were initially assigned in a random order. Methylphenidate substitution was added to the experiment after completion of all other test drugs. Accordingly, separate baseline values for nicotine and saline sessions were extracted before testing with 5 mg/kg methylphenidate. Fisher's protected LSD tests were used for post hoc comparisons. Of note, to reduce the Type I error rate, post hoc comparisons were conditional on a significant omnibus ANOVA. Additionally, these comparisons were limited to contrasting each test value with saline. Test doses that were significantly different from saline were then compared with the nicotine training dose. Full substitution or antagonism was declared when doses of the test compound significantly differed from saline but not from the nicotine training dose. Partial substitution or antagonism was declared when a dose differed statistically from both saline and the nicotine training dose. Statistical significance was set at $p < .05$ (two tailed) for all tests. The median effective dose (ED_{50}) was calculated using linear regression on doses from the ascending limb of the dose-effect function.

Results

Acquisition

Figure 1 illustrates that rats readily acquire the discrimination between nicotine and saline sessions (left graph). There was a main effect of drug, $F(1, 15) = 23.61, p < .001$, and session, $F(11, 165) = 5.63, p < .001$, and a significant Drug \times Session interaction, $F(11, 165) = 8.63, p < .001$. Relative to saline sessions, dipper entries were higher on Nicotine Sessions 6 to 12 (Fisher's LSD = .038). For chamber activity

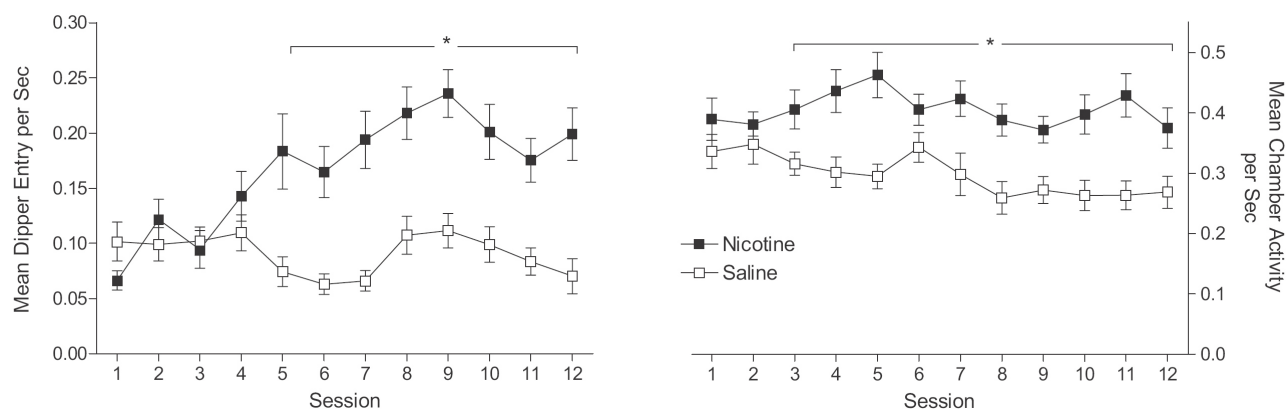


Figure 1. Mean (\pm SEM) dipper entries (left graph) and chamber activity (right graph) for rats ($n = 16$) trained with 0.2 mg/kg nicotine during acquisition. * $p < .05$ for difference from corresponding saline session.

(Figure 1, right graph), there was a significant main effect of drug, $F(1, 15) = 41.97$, $p < .001$, but not session, $F(11, 165) = 1.44$, $p = .16$. The Drug \times Session interaction was significant, $F(11, 165) = 1.88$, $p < .05$. Chamber activity was higher on Nicotine Sessions 3 to 12 in comparison to saline sessions (Fisher's LSD = .062).

Generalization and Substitution Testing

Nicotine dose effect. Conditioned responding evoked by nicotine was sensitive to test dose (Figure 2, left graph), yielding a dose main effect, $F(4, 60) = 17.70$, $p < .001$. Specifically, rats had higher levels of dipper entries when tested with 0.1, 0.2, and 0.3 mg/kg nicotine as compared with saline (Fisher's LSD = .045). Further, dipper entries at the training dose (0.2 mg/kg) were higher than at 0.025 and 0.3 mg/kg nicotine. The ED_{50} for nicotine was 0.075 mg/kg. Chamber activity (Figure 2, right graph) increased with nicotine dose, yielding a dose

main effect, $F(4, 60) = 9.72$, $p < .001$; activity on 0.1, 0.2, and 0.3 mg/kg nicotine was above saline levels (Fisher's LSD = .075).

Bupropion substitution. Bupropion partially substituted for the nicotine CS. Dipper entries (Figure 3A, left graph) increased with bupropion dose, yielding a dose main effect, $F(3, 45) = 9.50$, $p < .001$. Specifically, conditioned responding was higher at 10 and 20 mg/kg bupropion as compared with saline but remained significantly lower as compared with the training dose of nicotine (Fisher's LSD = .039). Chamber activity (Figure 3A, right graph) also increased with bupropion dose, yielding a dose main effect, $F(3, 45) = 10.46$, $p < .001$; 10 and 20 mg/kg increased activity to a level comparable with the nicotine training dose (Fisher's LSD = .105).

Cocaine substitution. Cocaine partially substituted for the nicotine CS, as dipper entries (Figure 3A, left graph) increased with cocaine dose, yielding a dose main effect, $F(4, 60) = 7.68$, $p < .001$. Specifically, conditioned responding

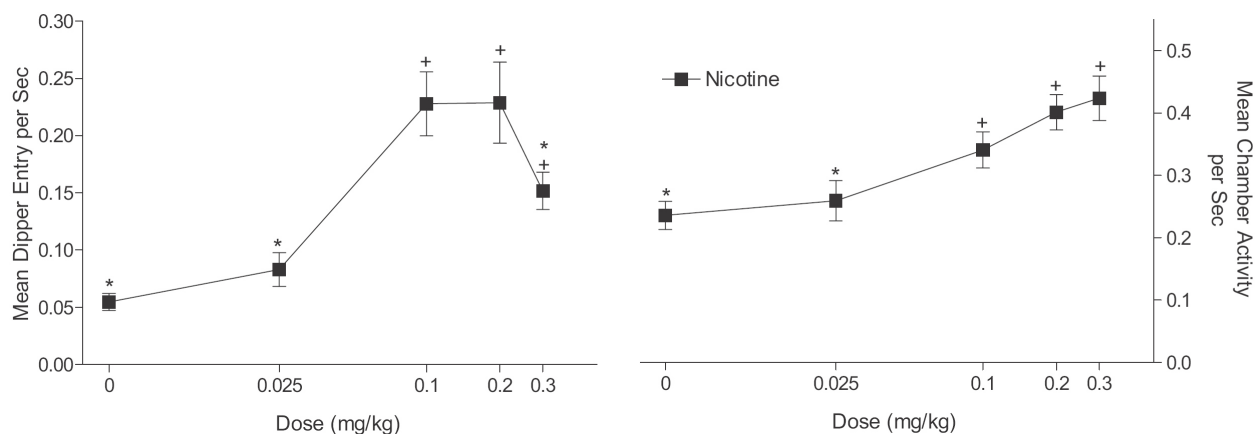


Figure 2. Mean (\pm SEM) dipper entries (left graph) and chamber activity (right graph) for the nicotine dose effect ($n = 16$). *Significantly different from 0.2 mg/kg nicotine, $p < .05$. +Significantly different from saline, $p < .05$.

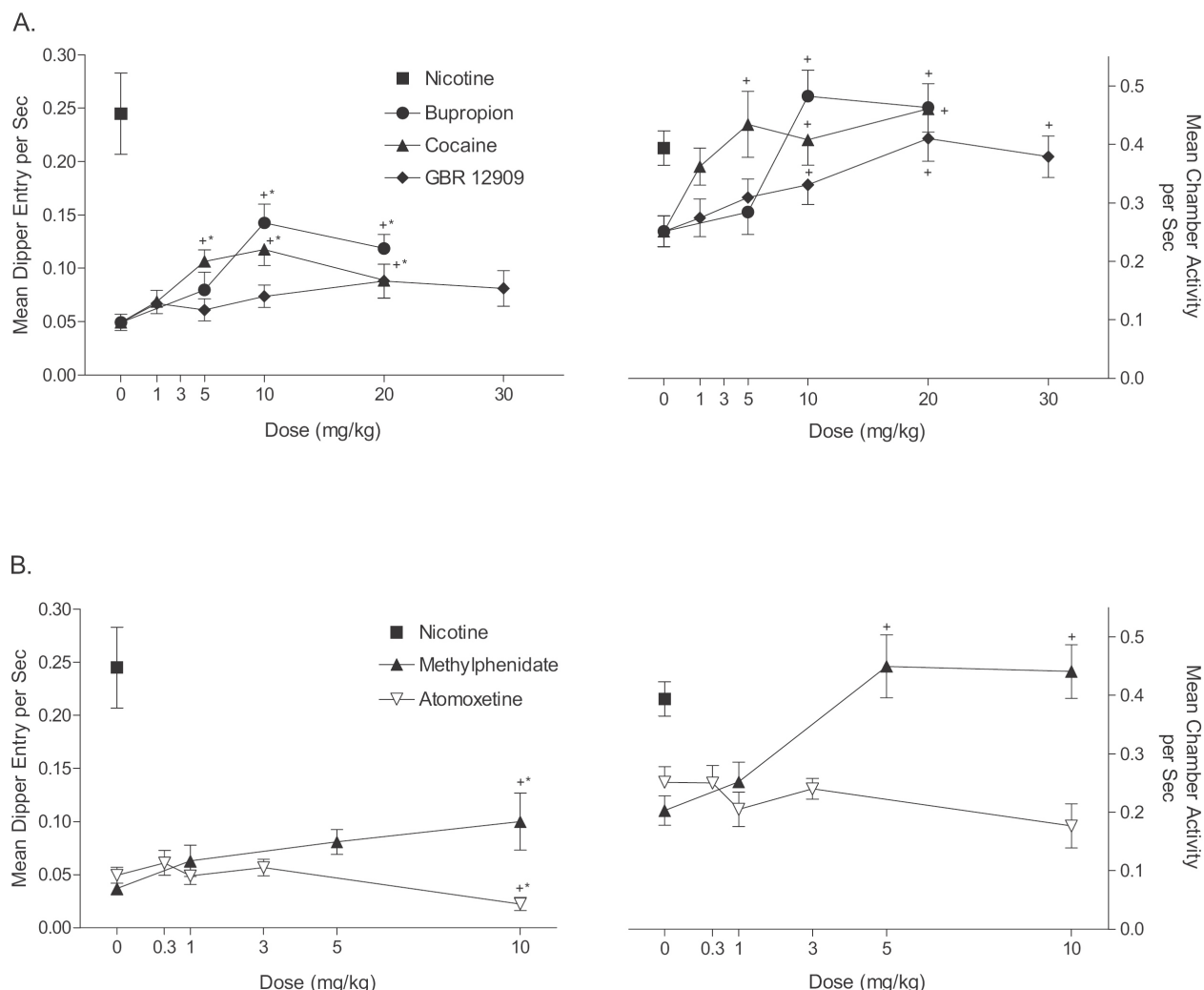


Figure 3. Mean (\pm SEM) dipper entries (left graphs) and chamber activity (right graphs) during substitution testing for bupropion ($n = 16$), cocaine ($n = 16$), and GBR 12909 ($n = 15$) are shown in Panel A. Panel B shows the dipper entries (\pm SEM) and activity scores (\pm SEM) for methylphenidate ($n = 15$) and atomoxetine ($n = 16$). *Significantly different from 0.2 mg/kg nicotine, $p < .05$. +Significantly different from saline, $p < .05$.

was increased at 5, 10, and 20 mg/kg cocaine in comparison to saline but remained significantly lower in comparison to the training dose of nicotine (Fisher's LSD = .031). Chamber activity (Figure 3A, right graph) also increased with cocaine dose, yielding a dose main effect, $F(4, 60) = 4.63$, $p < .003$; 5, 10, and 20 mg/kg increased activity to a level comparable with the nicotine training dose (Fisher's LSD = .110).

GBR 12909 substitution. GBR 12909 did not alter dipper entry rate (Figure 3A, left graph), $F(5, 70) = 1.45$, $p = .22$. However, GBR 12909 increased chamber activity (Figure 3A, right graph), yielding a dose main effect, $F(5, 70) = 4.48$, $p < .001$, with 10, 20, and 30 mg/kg increasing activity to a level comparable with the nicotine training dose (Fisher's LSD = .086).

Atomoxetine substitution. Dipper entries (Figure 3B, left graph) decreased with atomoxetine dose, $F(4, 60) = 3.66$, $p < .01$. The 10 mg/kg atomoxetine reduced dipper entries in comparison to saline and the training dose of nicotine (Fisher's LSD = .022). Chamber activity (Figure 3B, right graph) was unaffected by atomoxetine dose, $F(4, 60) = 2.03$, $p = .10$.

Methylphenidate substitution. Methylphenidate partially substituted for the nicotine CS (Figure 3B, left graph), yielding a dose main effect, $F(3, 42) = 2.83$, $p = .05$. Specifically, conditioned responding was increased at 10 mg/kg methylphenidate in comparison to saline but remained significantly lower in comparison to the training dose of nicotine (Fisher's LSD = .046). Chamber activity (Figure 3B, right graph) also increased with methylphenidate dose, yielding a dose main ef-

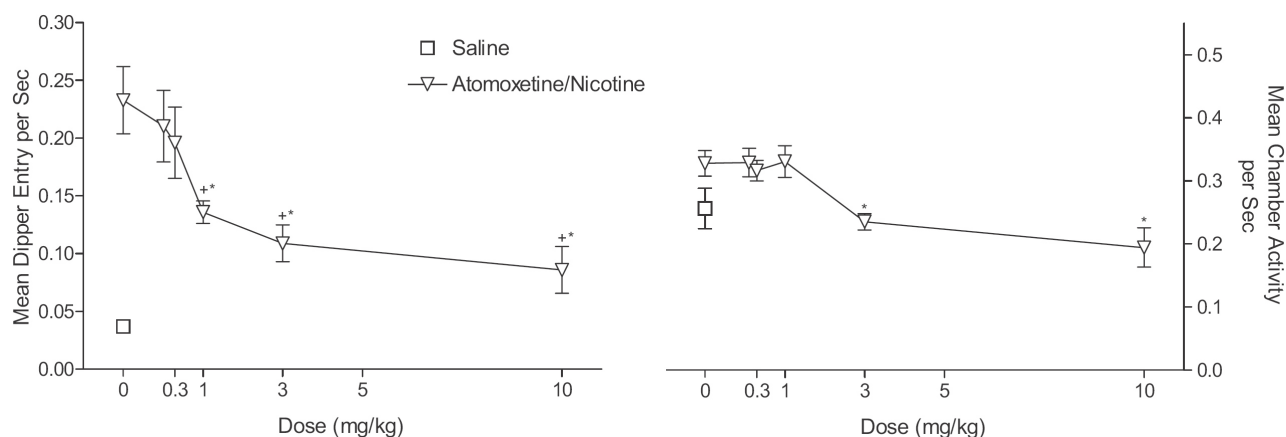


Figure 4. Mean (\pm SEM) dipper entries (left graph) and chamber activity (right graph) during antagonism testing with atomoxetine ($n = 15$). *Significantly different from 0.2 mg/kg nicotine, $p < .05$. +Significantly different from saline, $p < .05$.

fect, $F(4, 60) = 4.63$, $p < .003$, with 5 and 10 mg/kg increasing activity to a level comparable with the nicotine training dose (Fisher's LSD = .101).

Atomoxetine antagonism. Atomoxetine pretreatment partially blocked conditioned responding evoked by the nicotine CS (Figure 4, left graph), yielding a dose main effect, $F(5, 70) = 11.88$, $p < .001$. Specifically, the nicotine-evoked CR was decreased with pretreatment of 1, 3, and 10 mg/kg atomoxetine in comparison to vehicle pretreatment (i.e., nicotine training dose) but remained significantly higher in comparison to the saline baseline (Fisher's LSD = .046). Chamber activity (Figure 3B, right graph) decreased with atomoxetine pretreatment dose, $F(5, 70) = 8.51$, $p < .001$. Specifically, 3 and 10 mg/kg atomoxetine pretreatment suppressed chamber activity to a level consistent with the saline baseline (Fisher's LSD = .056). Together, these results indicate that pretreatment with 1 mg/kg atomoxetine partially blocks the cuing effects of nicotine (0.2 mg/kg) without blocking nicotine-induced hyperactivity.

Discussion

The present report replicated past research from our laboratory demonstrating that the interoceptive effects of nicotine can serve as a CS in a Pavlovian appetitive discrimination task (Besheer et al., 2004; Bevins & Palmatier, 2004; Bevins et al., 2007; Murray & Bevins, 2007; Wilkinson et al., 2006). The current research extends these previous findings by (a) demonstrating that cocaine and methylphenidate partially substitute for nicotine as a CS, (b) showing that bupropion partially substitutes for a lower dose of nicotine than previously studied, and (c) finding that atomoxetine does not substitute for nicotine but blocks the CR evoked by the nicotine CS, as well as nicotine-induced motor activity at the higher doses. Of note, two commonly prescribed ADHD medications (i.e., methylphenidate and bupropion) were able to evoke nicotine-

appropriate responding. Given the propensity for people diagnosed with ADHD to abuse tobacco products, this research has important implications for pharmacotherapy development focused on nicotine dependence in this clinical subpopulation (addressed later).

Of the drugs used to treat ADHD, bupropion and methylphenidate produced some nicotine-appropriate responding, whereas atomoxetine did not. This result is not surprising given the shared neuropharmacological mechanisms between bupropion, methylphenidate, and nicotine. In general, each of these drugs increases dopaminergic neurotransmission in brain reward pathways. More specifically, bupropion elevates dopamine concentrations in the nucleus accumbens and striatum by inhibiting dopamine and norepinephrine reuptake (Ascher et al., 1995; Miller, Sumithran, & Dwoskin, 2002; Nomikos et al., 1992). Methylphenidate increases dopamine and norepinephrine in the prefrontal cortex, nucleus accumbens, and striatum by blocking reuptake mechanisms (Bymaster et al., 2002; Kuczenski, & Segal, 1997). Nicotine indirectly increases dopaminergic neurotransmission by activating nicotinic acetylcholine receptors located on dopamine projections from the ventral tegmental area (Corrigall, Coen, & Adamson, 1994; Stoleran & Shoaib, 1991). On the other hand, atomoxetine does not increase synaptic dopamine in brain regions associated with reward. More specifically, atomoxetine increases extracellular norepinephrine and dopamine in the prefrontal cortex but not in the nucleus accumbens or striatum by inhibiting norepinephrine reuptake (Bymaster et al., 2002). Differences between cortical and subcortical dopaminergic neurotransmission suggest that atomoxetine lacks the abuse liability associated with psychostimulant drugs. Indeed, atomoxetine is not self-administered in animal models of drug taking that are predictive of human drug abuse liability (Gasior, Bergman, Kallman, & Paronis, 2005; Wee & Woolverton, 2004). In contrast, bupropion, methylphenidate, and nicotine are self-administered in laboratory animals (Cor-

rigall et al., 1994; Gasior et al., 2005; Lamb & Griffiths, 1990; Rauhut, Mullins, Dwoskin, & Bardo, 2002; Tella, Ladenheim, & Cadet, 1997).

We also tested whether the dopamine reuptake inhibitors cocaine and GBR 12909 would substitute for nicotine as a CS to assess drugs with varying affinity for the dopamine transporter. Cocaine partially substituted for the CS effects of nicotine, whereas GBR 12909 did not produce a statistically significant increase in conditioned responding. These drugs, as well as bupropion and methylphenidate, block dopamine reuptake with varying affinity. More specifically, in rank order from the weakest to strongest, affinity for the dopamine transporter is bupropion < cocaine < methylphenidate < GBR 12909 (Katz et al., 2000). It is interesting to note that this rank ordering matches the substitution pattern of these drugs for the CS properties of nicotine in the current study. That is, the drugs with the weakest affinity for the dopamine transporter appeared to substitute for the nicotine CS more readily than the drugs with stronger binding affinities. This finding could indicate that specific blockade of the dopamine transporter does not result in an interoceptive cue similar to the one experienced when nicotine is present in the central nervous system. More likely, a mixture of dopamine reuptake inhibition in combination with actions at norepinephrine, nicotinic acetylcholine, and/or serotonin receptors may better simulate the interoceptive CS effects of nicotine.

General chamber activity was measured throughout the entire experiment. The most notable observation from these data is that a difference in dipper entries was not necessarily paralleled in the general chamber activity. For example, during acquisition, dipper entries and chamber activity were substantially higher during nicotine than during saline sessions. In contrast, during substitution testing, drugs that increased motor activity (e.g., GBR 12909 at 10, 20, and 30 mg/kg) did not always increase dipper entries. This dissociation is methodologically important because it indicates that our measure of conditioned responding (i.e., goal tracking) is not attributable to nonspecific motor alterations of the test compounds.

The 1 mg/kg atomoxetine dose partially blocked the cuing effects of nicotine without altering the locomotor enhancing effect of nicotine. Additionally, higher atomoxetine doses (i.e., 3 and 10 mg/kg) blocked nicotine-induced motor activity as well as the nicotine-evoked CR. Thus, atomoxetine blocked both the CS and motor-activating effects of nicotine, with the CS effects being more sensitive to antagonism. The exact mechanisms by which atomoxetine antagonized these nicotine-related behaviors will require further investigation. However, research suggests a possible role of cortical norepinephrine, dopamine, and/or acetylcholine efflux (Tzavara et al., 2006). One suggestion for the procholinergic property is that converging dopamine and norepinephrine neurons modulate cortical acetylcholine release at the nucleus basalis (Tzavara et al., 2006). Thus, atomoxetine indirectly increases acetylcholine levels by activating $\alpha - 1$ norepinephrine receptors and/or dopamine D₁ receptors (Tzavara et al., 2006). Of note, reboxetine, another selective norepinephrine reuptake inhib-

itor, produced the same cortical acetylcholine efflux (Tzavara et al., 2006) and also inhibits nicotinic acetylcholine receptors (nAChRs; Miller, Wong, Chesnut, & Dwoskin, 2002). Albeit speculative, if atomoxetine shares the ability to inhibit nAChRs with reboxetine, one possible explanation for atomoxetine's impact on nicotine-mediated behaviors in the present experiment may be its antagonism of nAChRs.

An alternative explanation for blockade of the CS effects of nicotine is that nicotine and atomoxetine administered together produce an interoceptive cue that is distinct from that produced by nicotine alone. Both nicotine and atomoxetine separately increase noradrenergic neurotransmission in the hippocampus that, when combined, may result in overstimulation of norepinephrine neurons (Fu, Matta, James, & Sharp, 1998; Gould et al., 2005; P. E. Potter, Thorne, & Gaughan, 1997; Swanson et al., 2006). Thus, conditioned responding to nicotine would decrease if the nicotine cue was progressively changed with increases in atomoxetine dose. In previous drug discrimination studies atomoxetine substituted for several different drugs, which lends feasibility to this suggestion. For instance, atomoxetine partially substituted (i.e., 33% to 50% responding) for methylphenidate in a human drug discrimination study (Lile, Stoops, Durell, Glaser, & Rush, 2006). In studies with rats, atomoxetine fully substituted for a low dose of cocaine and partially substituted for the potassium channel blocker 4-aminopyridine (Brandsgaard, Barrett, & Rosenzweig-Lipson, 2000; Terry, Witkin, & Katz, 1994). However, our enthusiasm for this account is somewhat diminished because changes in the nicotine cue would not necessarily account for the concurrent blockade of nicotine-induced chamber activity. Further, conditioned responding was only partially blocked, whereas chamber activity was fully antagonized to saline levels.

Methylphenidate, bupropion, and atomoxetine are prescribed for the treatment of ADHD. Of these drugs, atomoxetine is the only drug that does not increase dopamine release in the mesolimbic dopamine system. In fact, volunteers with a history of light drug use, including exposure to various psychostimulant drugs, reported that the subjective effects of atomoxetine were "bad" or "sick" rather than "good" or "stimulating," indicating little or no abuse potential (Heil et al., 2002). Thus, atomoxetine provides a nonstimulant pharmacological treatment for ADHD. As mentioned previously, ADHD is a risk factor for initiation of cigarette smoking (Milberger et al., 1997). Of the ADHD drugs tested in the present study, atomoxetine was the only drug that did not at least partially substitute for the interoceptive stimulus effects of nicotine. However, atomoxetine did block the interoceptive stimulus properties and locomotor activating effects of nicotine. For these reasons, we suggest atomoxetine may have additional benefits for patients with ADHD who are also addicted to nicotine. Indeed, this suggestion was recently posited by Gould and colleagues (see Davis & Gould, 2007; Gould et al., 2005). In further support of this notion, a norepinephrine reuptake inhibitor (i.e., reboxetine) was reported to decrease nicotine self-administration in rats (Rauhut, Mullins, Dwoskin, &

Bardo, 2002). Thus, there may be clinical utility for atomoxetine as a smoking prevention and/or intervention aid for patients diagnosed with ADHD. The current findings merit further preclinical investigations of atomoxetine in the treatment of nicotine addiction.

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