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Nicotine and cocaine self-administration using a multiple schedule of intravenous drug and sucrose reinforcement in rats

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Abstract

There appears to be a relatively narrow range of contingencies in which intravenous (i.v) infusions of nicotine will maintain responding in rats. The schedule of reinforcement typically used when investigating i.v. nicotine self-administration is a simple fixed-ratio (FR) schedule. The current study determined if responding in rats could be established using a multiple (Mult) schedule of either i.v. cocaine or nicotine and sucrose reinforcement. Following training of individual components with each reinforcer, rats were placed on an FR15 60-sec timeout Mult schedule of cocaine (0.3 mg/kg/infusion) and sucrose (45 mg pellets) reinforcement or an FR5 60-sec timeout Mult schedule of nicotine (0.03 mg/kg/infusion) and sucrose (45 mg pellets) reinforcement. Both cocaine and nicotine maintained significant levels of responding under the Mult schedule. Pretreatment with the dopamine D_1 antagonist SCH 23390 increased cocaine-maintained responding, but not sucrose responding. Acute pretreatment with the nicotinic antagonist mecamylamine or SCH 23390 specifically decreased nicotine self-administration. Extinction of the individual nicotine and sucrose components resulted in decreases in responding in each component under extinction. These results indicate that i.v. nicotine maintains responding under a Mult schedule. This procedure may be useful when studying the specificity of drug pretreatments on nicotine self-administration.

Keywords

Nicotine; cocaine; self-administration; multiple schedule; mecamylamine; SCH 23390; rats

Introduction

Since the findings of Corrigall and Coen (1989) that intravenous (i.v.) infusions of nicotine can maintain responding in rats, the number of publications using the i.v. rodent nicotine self-administration model has been increasing. The use of the i.v. rodent nicotine self-administration procedure has led to a better understanding of the reinforcing properties of nicotine (Donny et al., 1998; Stolerman & Jarvis, 1995), the neural mechanisms underlying nicotine self-administration (Corrigall, Coen, & Adamson, 1994; Corrigall, Franklin, Coen, & Clarke, 1992), and the ability of nicotine to enhance the reinforcing effects of non-drug reinforcers (Chaudhri, Caggiula, Donny, Booth et al., 2006; Chaudhri, Caggiula, Donny, Palmatier et al., 2006). However, there has been relatively little work examining behaviors reinforced by both nicotine and non-drug stimuli within the same animal and same experimental session, which more closely models human tobacco use.

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The schedule of reinforcement used by Corrigall and Coen (1989) was a fixed-ratio (FR) 5, with each i.v infusion of nicotine followed by a 60-sec light-signaled timeout (TO) period during which another nicotine infusion could not be earned. Rats responded under this schedule of reinforcement during 60-min daily sessions. The majority of experiments investigating nicotine reinforcement using the i.v. rodent self-administration paradigm have used slightly modified versions of the FR5 limited access schedule (Corrigall, Coen, Adamson, Chow, & Zhang, 2000; Donny, Caggiula, Knopf, & Brown, 1995; Rauhut, Neugebauer, Dwoskin, & Bardo, 2003; Shoaib & Stolerman, 1999); however, there are some exceptions. For example, it has been found that nicotine can maintain responding under a progressive-ratio (PR) schedule of reinforcement (Donny et al., 1999), under unlimited access schedules (23hr/day sessions; LeSage, Keyler, Collins, & Pentel, 2003; LeSage et al., 2002).

Another schedule variation from the typical FR5 limited access schedule of nicotine self-administration has been a series of studies conducted to investigate the ability of nicotine infusions to maintain responding under a concurrent schedule of i.v. nicotine and light stimuli (Palmatier et al., 2006; Palmatier, Liu, Caggiula, Donny, & Sved, 2007). Using this model, the results from these studies illustrate that nicotine has two distinct reinforcing effects. First, nicotine was found to have weak primary reinforcing effects in the absence of any paired visual stimuli. Secondly, nicotine was found to enhance the reinforcing effects of a visual stimulus which was available on a concurrent lever, indicating that nicotine also has reinforcement-enhancing effects (Palmatier et al., 2006). When the reinforcing-visual stimuli were paired with an i.v. infusion of nicotine, the two “synergistically” increased rates of responding (Palmatier et al., 2006). Thus, in the typical rodent model of nicotine self-administration, an i.v. nicotine infusion paired with a light cue results in a powerful compound-reinforcing stimulus which can aid in our preclinical understanding of tobacco addition.

In an attempt to further improve the validity of the rodent i.v. nicotine self-administration paradigm as a preclinical tool, the current study attempted to assess both nicotine- and food-maintained responding using a multiple (Mult) schedule of reinforcement. With a Mult schedule, two or more reinforcement schedules are typically presented one at a time in an alternating sequence each day, and each schedule component is signaled by a different discriminative stimulus (Ferster & Skinner, 1957). Although a number of studies in monkeys have used a Mult schedule to assess cocaine self-administration and food-maintained responding (Caine & Koob, 1995; Claytor, Lile, & Nader, 2006; Glowa & Wojnicki, 1996; Kleven & Woolverton, 1993; Lile, Morgan, Birmingham, Davies, & Nader, 2004; Nader, Sinnott, Mach, & Morgan, 2002; Woolverton & Virus, 1989, relatively few studies have used a multiple schedule in rats (Caine & Koob, 1994). In one study, Caine and Koob (1994) used a Mult schedule of cocaine- and food-maintained responding to show that a low dose of the D_1 dopamine antagonists SCH 23390 or SCH 39166 (5 or 10 μg/kg, respectively) decreased cocaine-maintained responding, while having no effect on food-maintained responding; however, higher doses of SCH 23390 decreased responding non-specifically during both the cocaine- and food-maintained schedule components. These results indicate that rodents can be trained under a Mult schedule of cocaine and food reinforcement and that behavior maintained under this schedule is sensitive to pharmacological manipulations. More recently, LeSage (2009) used a Mult schedule in which rats were trained daily with a single 60-min nicotine-maintained component, followed by a single 30-min sucrose-maintained component. However, the purpose of training rats on this atypical Mult schedule was to subsequently assess contingency management effectiveness, rather than to assess any pharmacological manipulations.

The main purpose of the current study was to determine if responding could be maintained by i.v. nicotine infusions (+ visual stimuli) using a daily alternating-component Mult schedule procedure in rats, as has been shown with cocaine. The second purpose of the study was to determine whether this Mult schedule could be used to assess the ability of SCH 23390 and the noncompetitive nicotinic receptor antagonist mecamylamine to specifically decrease nicotine self-administration. In order to demonstrate drug self-administration under a multiple schedule in our laboratory, cocaine self-administration under a Mult schedule of cocaine and sucrose was first established. The sensitivity of this procedure to pharmacological manipulation was then illustrated with pretreatments of SCH 23390.

Methods

Subjects

Twenty-four male Sprague-Dawley rats, weighing 230–300 g at the start of the experiments, were obtained from Harlan (Indianapolis, IN). Rats were acclimated to the colony for 7 days and subsequently were handled daily for 3–5 days before the start of each experiment. Animals had unlimited access to food and water in their home cage, except where noted, and were maintained on a light/dark cycle in which the lights were on from 06:00–20:00 h. All procedures were conducted during the animals light cycle. All procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee and conformed to the 1996 NIH Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources (U.S.), 1996).

Apparatus

Rat operant conditioning chambers (ENV-001; MED Associates, St. Albans, VT), located inside sound-attenuating chambers, were used. The front and back walls of the operant chambers were made of aluminum, while the side walls were made of Plexiglas. There was a recessed food tray (5 × 4.2 cm) located in the bottom-center of the front wall. One retractable response lever was located on each side of the recessed food tray on the front wall. A 28-V white cue light was located 6 cm above each response lever. A house light with diffuser (ENV-227M; MED Associates, St. Albans, VT) was located on the top-middle of the back wall. All responses and scheduled consequences were recorded and controlled by a computer interface.

Procedure

Cocaine Self-Administration – Initial Training—Eighteen rats were given restricted access to food in order to reduce body weight to 85% of free feeding weight. Rats were then placed individually into the operant conditioning chamber for 60-min daily sessions with one lever (the position was counterbalanced across subjects) extended into the chamber and the house light turned on. Each response on the lever resulted in the delivery of a sucrose pellet (45 mg; BioServ, Frenchtown, NJ); this lever was designated the sucrose lever. The schedule was increased from an FR1 to an FR15 over 10 daily sessions. Once the FR15 ratio was reached, a 60-sec TO was implemented following the delivery of each reinforcer. The TO following each reinforcer was signaled by illumination of the cue light above the sucrose lever. Rats were trained for five 60-min daily sessions on the FR15 60-sec TO schedule of reinforcement with only the sucrose lever extended.

Following acquisition of the sucrose lever press, rats received unlimited access to standard rat chow in their home cage in order to regain their free-feeding body weights. Once rats regained their free-feeding weights (~5 days), they were anesthetized with an i.p. injection of ketamine (80 mg/kg) and diazepam (5 mg/kg). A silastic catheter was inserted into the right jugular vein and threaded subcutaneously with the open end of the catheter exiting the

skin and secured to an acrylic head mount, which allowed for the catheter to be connected to an infusion pump. Following surgery, rats received daily i.v. infusions of heparinized saline (Pharmacia, Columbus Ohio; 250,000 IU, 2 mg/ml heparinized saline, 0.1 ml/rat/day).

Following 7 days of recovery from surgery, food was again removed from the home cage and for the remainder of the experiment rats were given 20 g of rat chow at the end of each daily operant conditioning session in order to maintain their body weights at 85% of free feeding weight for the remainder of the experiment. Rats were placed back into the operant conditioning chamber and the lever that was retracted previously was extended into the chamber; this lever was designated the cocaine lever. During this time, only the cocaine lever was extended into the chamber with the house light remaining off. Each press on the cocaine lever resulted in an i.v. infusion of cocaine (0.3 mg/kg/infusion) on an FR1, TO 60-sec schedule of reinforcement during 60-min daily sessions. The dose of 0.3 mg/kg/infusion of cocaine was chosen because this dose has been shown to be near the apex of the dose-effect curve of cocaine self-administration (Caine & Koob, 1994). Under this schedule, each infusion of 0.06 ml was delivered over 3.33 sec and coincided with a 60-sec TO during which the cue light above the cocaine lever was illuminated. The ratio was then increased from FR1 to FR15 across daily sessions in the following manner: 3 sessions FR1, 3 sessions FR3, 2 sessions FR5, 2 sessions FR10 and 3 sessions FR15.

Mult Schedule of Cocaine and Sucrose Reinforcement—Following the last session of cocaine alone responding, behavior was maintained under a *mult* FR15, TO 60-sec schedule of cocaine and sucrose reinforcement. Both levers were extended into the operant conditioning chamber. Each daily session was 75 min, consisting of four 15-min components that were separated by 5-min TO periods. During the first and third 15-min components (cocaine components) the house light remained off and each FR15 completed on the cocaine lever resulted in delivery of an i.v. infusion of cocaine; a 60-sec TO followed each infusion and was signaled by illumination of the cue light above the cocaine lever for the duration of the TO. During the cocaine components, each response on the cocaine lever resulted in a brief 1-sec flash of the cue light above the cocaine lever. Responses on the sucrose lever during cocaine components were recorded, but had no scheduled consequence. During the second and fourth 15-min components (sucrose components), the house light was illuminated and completion of an FR15 on the sucrose lever resulted in delivery of a sucrose pellet. A 60-sec TO followed each pellet delivery and was signaled by illumination of the cue light above the sucrose lever for the duration of the TO. During the sucrose components, each response on the sucrose lever resulted in a brief 1-sec flash of the cue light above the sucrose lever. Responses on the cocaine lever during sucrose components were recorded, but had no scheduled consequence. Inserted between each 15-min component was a 5-min TO in which only the cue lights above each lever were illuminated; both the sucrose and the cocaine levers remained extended into the chambers. During the 5-min TO, responses on the sucrose or cocaine levers were recorded but had no scheduled consequence.

Training continued until stable responding was achieved across daily sessions. Stable responding was defined as three consecutive sessions with less than 25% variation in the total number of responses during the cocaine components and at least a 2:1 ratio in the number of responses on the cocaine lever compared to the sucrose lever during the cocaine components. Using these criterion, which our laboratory have use previously with a simple FR schedule (Stairs et al., 2007), 12 animals acquired stable cocaine self-administration after 12–14 sessions; 3 other rats did not reach criteria and 3 had a catheter malfunction that prevented inclusion of the data. Once responding stabilized, varying doses of SCH 23390 (0, 2.5, 5, and 10 μg/kg, s.c.) were administered in a latin square design 25 min prior to the session. Following each pretreatment session, rats received at least two sessions of maintenance training (no pretreatment) in order to maintain stable responding on the Mult

schedule. Following completion of the experiment, catheter patency was verified by observing a rapid cataleptic response following i.v. administration of morphine (15 mg/kg).

Nicotine Self-Administration – Initial Training—Initial training to press the sucrose lever was as described previously in the cocaine self-administration experiment, except that only six experimentally naïve rats were used and the ratio value was increased from an FR1 to a terminal FR5 schedule over 6 sessions. Once the FR5 was reached, a 60-sec TO was included in the session. Following three sessions on the terminal FR5 60-sec TO schedule, rats remained in their home cage for two days and food-restriction was continued.

Since nicotine engenders less robust responding compared to cocaine, rats were then trained briefly to press the nicotine lever using sucrose reinforcement as described previously (Corrigall & Coen, 1989; Donny et al., 1995). Rats were placed into the chamber and the lever that was retracted previously was extended into the chamber; this lever was designated the nicotine lever. During this time, only the nicotine lever was extended into the chamber with the house light remaining off. Each press on the nicotine lever resulted in delivery of a sucrose pellet. During these session there were no cue lights paired with sucrose pellet or a time out following the delivery of the reinforcer. The ratio requirement was increased from FR1 to FR5 over five daily 60-min sessions. Once rats completed two FR5 sessions, they received unlimited access to standard rat chow in order to regain their free-feeding body weights and then underwent catheterization surgery as described previously.

Following a seven day recovery period, rats were placed back on restricted access to food (20 g per day, given immediately following each session) for the remainder of the experiment. Two days after the start of food restriction, animals were placed into the chamber with only the nicotine lever extended into the chamber and the house light remaining off. Each press on the nicotine lever resulted in an infusion of nicotine (0.03 mg/kg/infusion) on an FR1 60-sec signaled TO schedule of reinforcement in 60-min daily sessions. This unit dose was selected because it has been shown previously to yield the maximal rate of self-infusions (Donny et al., 1998; Rose & Corrigall, 1997). Each infusion was delivered in a volume of 0.06 ml over 3.33 sec, which coincided with the beginning of the 60-sec TO signaled by the illumination of the cue light above the nicotine lever. The ratio was increased from an FR1 to FR5 in the following manner: 5 sessions FR1, 3 sessions FR2, 3 sessions FR3 and 3 sessions FR5.

Mult Schedule of Nicotine and Sucrose Reinforcement Procedure—Following the last day of nicotine alone responding, rats were placed under a *mult* FR5, TO 60-sec schedule of nicotine and sucrose reinforcement for 75-min daily sessions with both levers extended into the chamber. Procedures for the Mult schedule were similar to those described in the cocaine self-administration experiment, except that responding in the drug and sucrose components were maintained on an FR5 using nicotine infusions as the reinforcer in the drug components. Among the six rats used, 4 reached stable responding using the same criteria described previously, 1 did not reach stable criteria, and 1 had a catheter malfunction that prevented inclusion of the data.

When responding stabilized, varying doses of mecamylamine (0, 1, 2, and 4 mg/kg, s.c.) were administered in a latin square design 15 min prior to the session. Following completion of the mecamylamine acute dose-effect curve, acute doses of SCH 23390 (0, 2.5, 5, and 10 μg/kg, s.c.) were also administered in a latin square design 25 min prior to the session. Following each pretreatment session, rats received at least two sessions of maintenance training (no pretreatment) in order to maintain stable responding on the Mult schedule.

Following completion of the acute mecamylamine and SCH 23390 dose-effect curves, responding in the sucrose and nicotine components underwent extinction. In this phase, the sucrose pellet dispenser was first inactivated, but all other aspects of the schedule remained the same. Following six days in which responding was under extinction during the sucrose components, the pellet dispenser was reactivated and responding on the sucrose lever was allowed to return to baseline levels. Responding on the nicotine lever during the nicotine components then underwent extinction by replacing nicotine with saline; all other aspects of the schedule remained the same. Extinction of responding on the nicotine lever during the nicotine components lasted for 10 sessions, after which contingent nicotine infusions were reinstituted. Following completion of the experiment, catheter patency was verified as described previously.

Drugs

Cocaine HCl was obtained from NIDA (Bethesda, MD) and was dissolved in 0.9% w/v NaCl (saline). S(−)-Nicotine bitartrate was purchased from Sigma/RBI (Natick, MA) and was dissolved in saline; the solution was adjusted to pH 7 using sodium hydroxide (1 M). SCH 23390 HCl and mecamylamine HCl were purchased from Sigma/RBI, dissolved in saline and injected in a volume of 1 ml/kg body weight. Morphine HCl was obtained from NIDA (Bethesda, MD), prepared in saline, and administered i.v. in a volume of 1 ml/kg body weight. Nicotine doses are expressed as free base and all other drug doses are expressed as the salt weight.

Data Analysis

The level and pattern of responding in the first and second drug components of the multiple schedules were not significantly different and therefore responses during the drug components were combined into a single value. The level and pattern of responding in the first and second sucrose components were also not significantly different so the responses during the sucrose components were combined into a single value. Due to significant differences in the rates of responding between drug-maintained and sucrose-maintained behavior, data were analyzed statistically as a percent of control using a repeated measures analysis of variance (ANOVA) with pretreatment dose and reinforcer type as within-subject factors. Percent of control was calculated as the percent change in number of responses on the active (reinforced) lever following a pretreatment compared to the baseline control day (session prior to pretreatment). To determine if there was a time-dependent change across the session due to drug pretreatment, the number of responses from the first and second components and the number of responses from the third and fourth components were also analyzed separately as a percent of control using a repeated measures ANOVA with pretreatment dose and reinforcer type as within-subject factors. The number of responses during the extinction phase was analyzed using a repeated measures ANOVA, with session and lever (active versus inactive) as within-subject factors; a supplemental linear contrast analysis was conducted. Post hoc comparisons of interest for both experiments were conducted using Tukey's HSD test in order to control for multiple comparisons. All results were deemed significant at $p<0.05$. When reporting significant results for the repeated measures ANOVA the Huynh-Feldt statistics was used in order to control for potential violations of sphericity. Representative cumulative records from individual rats were used to assess the overall pattern of behavior within a session; the behavior of the animal that most closely matched the group mean in active responses was used as the representative cumulative record. Two cumulative response records were created from one experimental session. This was done for both the cocaine and nicotine experiments. One record displays responding on either the cocaine or nicotine lever throughout the entire session (Figures 1B and 3B, respectively) and the second record displays responding on the sucrose levers throughout the entire session (Figures 1C and 3C).

Results

Cocaine Self-Administration Experiment

Figure 1A illustrates that rats learned to respond on the appropriate lever during both the cocaine and sucrose components of the Mult schedule. A repeated measures ANOVA revealed a significant within-subject effect of lever, ($F_{3, 24} = 160.51$, $p < 0.001$). Post-hoc comparisons found that responding on the active (reinforced) lever was significantly higher than responding on the inactive (nonreinforced) lever in each component. Figure 1B is a representative cumulative record of responding on the cocaine lever from a rat that reached the stable response criteria on a maintenance session under the Mult schedule of cocaine and sucrose. This figure indicates that the rate of responding on the cocaine lever during the cocaine components was high; there was some responding on the cocaine lever during the first sucrose component with a burst of responding at the beginning of the sucrose component. While there was some responding on the cocaine lever during the first sucrose component, there was no responding on the cocaine lever during the second sucrose component. The figure also indicates that there was no responding during the timeouts between cocaine and sucrose components. Figure 1C is the a cumulative record of responding in the same session as illustrated in Figure 1B, except that it illustrates responding on the sucrose lever. There was a high rate of responding on the sucrose lever during the sucrose components. Also, there were greater levels of responding on the sucrose lever during the cocaine components than the levels of responding seen on the cocaine lever during the sucrose components.

Figure 2 illustrates that SCH 23390 pretreatment produced a biphasic dose effect on cocaine self-administration, with a low dose increasing, and a high dose decreasing cocaine self-administration. In contrast, SCH 23390 pretreatment produced a monophasic decrease on sucrose-maintained responding. A repeated measures ANOVA revealed a main effect of SCH 23390, ($F_{7, 49} = 5.58$, $p < 0.001$). Post hoc comparisons revealed that, relative to saline pretreatment, responding during cocaine components was increased following the administration of 2.5 μg/kg of SCH 23390. Post hoc comparisons also revealed that 10 μg/kg of SCH 23390 decreased responding nonspecifically during both the cocaine and sucrose components.

Nicotine Self-Administration Experiment

Figure 3A illustrates that rats learned to respond on the appropriate lever during both the nicotine and sucrose components of the Mult schedule. A repeated measures ANOVA revealed a significant within-subject effect of lever, ($F_{3, 9} = 151.10$, $p < 0.001$). Post-hoc comparisons found that responding on the active (reinforced) lever was significantly higher than responding on the inactive (nonreinforced) lever in each component. Figure 3B is a representative cumulative record of responding on the nicotine lever from a rat that reached the stable response criteria on a maintenance session under the Mult schedule of cocaine and sucrose. The cumulative record shows steady FR responding on the nicotine lever during both the first and second nicotine components, with little responding on the nicotine lever during the sucrose components. Figure 3C is the a cumulative record of responding in the same session as illustrated in Figure 3B, except that it illustrates responding on the sucrose lever. As seen in the cocaine experiment, sucrose maintained a high rate of responding on the sucrose lever during the sucrose components. Also, there were a greater number of responses emitted on the sucrose lever during the first nicotine component than during the second nicotine component. Figures 3B and C indicate that within a Mult schedule of intravenous nicotine and sucrose reinforcement, nicotine maintained a steady rate of behavior in both the first and second nicotine components, while corresponding high rates of behavior were maintained within the sucrose components.

Figure 4 illustrates that mecamylamine pretreatment produced a dose-dependent decrease in nicotine self-administration, but not sucrose-maintained responding. As shown in Figure 4A, repeated measures ANOVA revealed a significant within-subjects effect of mecamylamine when components 1 and 3 (nicotine components) were combined and components 2 and 4 (sucrose components) were combined ($F_{7, 21} = 4.03$, $p < 0.01$). Post hoc comparisons revealed that, relative to saline pretreatment, the highest dose of mecamylamine (4 mg/kg) decreased nicotine-maintained responding specifically, while not altering sucrose-maintained responding. Mecamylamine had similar effects on behavior in both the first half of the session (Figure 4B; components 1 and 2) and the second half of the session (Figure 4C; components 3 and 4).

Figure 5 illustrates the dose effects of SCH 23390 pretreatment on nicotine- and sucrose-maintained responding under the Mult schedule. In contrast to the results from the cocaine self-administration experiment, when the data were combined across components, there was no significant alteration in responding on the active nicotine lever at any dose of SCH 23390 tested (Figure 5A). However, separate analyses of the data from first and second halves of the session (components 1 and 2 vs components 3 and 4, Figures 5B and 5C respectively) revealed a time-dependent effect of SCH 23390 pretreatment. SCH 23390 decreased nicotine-maintained responding, but not sucrose-maintained responding, during only the second half of the session ($F_{7, 21} = 7.00$, $p < 0.01$). Post-hoc comparisons revealed that, relative to saline pretreatment, 2.5 and 10 μg/kg of SCH 23390 decreased nicotine-maintained responding during the second nicotine component.

Figure 6 illustrates the effects of omitting the sucrose reinforcer on responding in each schedule component. When the sucrose reinforcer was omitted, a repeated measures ANOVA on responding in the nicotine component revealed a significant main effect of lever ($F_{1, 2} = 23.14$, $p < 0.05$), but no significant main effect of session or interaction between session and lever. Thus, extinction of responding during the sucrose component did not significantly affect responding during the nicotine component (Figure 6A). A repeated measures ANOVA on responding in the sucrose component found significant main effect of session ($F_{6, 12} = 17.07$, $p < 0.001$), as well as an interaction of session × lever ($F_{6, 12} = 11.13$, $p < 0.001$), indicating that responding on the sucrose lever extinguished to a level similar to that recorded on the inactive (nicotine) lever across extinction sessions (Figure 6B).

Figure 7 illustrates the effect of omitting the nicotine reinforcer on responding in each schedule component. When the nicotine reinforcer was omitted, there was a clear trend for responding on the nicotine lever to decrease across blocks of nicotine extinction sessions; however, a repeated measures ANOVA revealed no significant main effect of session, although there was a significant interaction of session × lever. A subsequent linear contrast confirmed the significant interaction of session × lever, ($F_{1, 3} = 21.12$, $p < 0.05$), indicating that responding decreased across extinction sessions on the nicotine lever to levels equal to those on the sucrose lever by the final extinction sessions. Post-hoc analysis revealed that animals responded more on the nicotine lever than on the sucrose lever early during extinction, but by the final three sessions of extinction, animals responded similarly on both levers (Figure 7A). In contrast, responding during the sucrose components remained unchanged during nicotine extinction (Figure 7B). A repeated measures ANOVA of these latter results revealed a main effect of lever ($F_{1, 3} = 113.48$, $p < 0.01$), with no effect of session or significant interactions.

Discussion

The current results demonstrate that both cocaine and nicotine maintain responding under a Mult schedule of alternating i.v. drug and sucrose reinforcement. With cocaine self-administration, SCH 23390 pretreatment resulted in a biphasic effect on cocaine self-administration, but not on sucrose-maintained responding. Of greater interest, both nicotine and sucrose responding was maintained under the Mult schedule. With nicotine self-administration, pretreatment with the highest dose of mecamylamine tested (4 mg/kg) decreased nicotine self-administration, but not sucrose-maintained responding. SCH 23390 pretreatment also specifically decreased nicotine self-administration, but only during the second component of the Mult schedule. These results confirm that both nicotinic acetylcholine receptors and D_1 dopamine receptors play a role in maintaining nicotine self-administration (Corrigall & Coen, 1991; Watkins, Epping-Jordan, Koob, & Markou, 1999).

In the cocaine self-administration experiment, performance of rats at stable criteria under the Mult schedule in terms of the pattern and amount of cocaine intake was generally consistent with results reported previously using similar procedures (Caine & Koob, 1994; Weissenborn, Yackey, Koob, & Weiss, 1995). Although the component duration used in the current report (15 min) was shorter than the 30-min duration used by others (Caine & Koob, 1994; Weissenborn et al., 1995), the amount of cocaine intake using a similar unit dose (0.3 mg/kg/infusion) was comparable (13 infusions versus 15 infusions). However, the dose-dependent effects of SCH 23390 on cocaine- and sucrose-maintained responding under the Mult schedule contrast with those reported previously (Caine & Koob, 1994). Caine and Koob (1994) found that 5 μg/kg of SCH 23390 decreased cocaine self-administration specifically, whereas the current study found that 2.5 μg/kg of SCH 23390 increased cocaine self-administration specifically. Despite these discrepant findings, the SCH 23390-induced increase in cocaine self-administration observed in the current report is consistent with other studies using simple FR schedules (Caine & Koob, 1994; Koob, Le, & Creese, 1987). The discrepant findings between Caine and Koob (1994) and the current report may be due to a difference in session length (120 min versus 75 min). In any case, the current results suggest that low doses of SCH 23390 may attenuate the reinforcing effects of cocaine, thus resulting in a compensatory increase in responding in an attempt to surmount the antagonistic effect (Hubner & Moreton, 1991; Koob et al., 1987).

The decrease in nicotine self-administration following mecamylamine pretreatment observed in the current study is congruent with results from previous reports using simple FR schedules with similar doses of mecamylamine (Rauhut, Mullins, Dwoskin, & Bardo, 2002; Shoaib, Schindler, & Goldberg, 1997; Watkins et al., 1999). The absence of an antagonist-induced increase in nicotine self-administration following pretreatment with mecamylamine is also consistent with the previous studies. The absence of the antagonist-induced increase in nicotine intake is unique to nicotine self-administration relative to other drugs of abuse, for a review of this issue see, Rose & Corrigall (1997). Moreover, the specificity of mecamylamine to decrease nicotine self-administration is a novel finding, since previous studies have not examined the effect of mecamylamine on food or sucrose-maintained responding in rats (Corrigall & Coen, 1989; Shoaib et al., 1997; Watkins et al., 1999). Thus, these results indicate that current Mult schedule of nicotine self-administration is sensitive to pharmacological manipulations using a nicotinic antagonist.

The decrease in nicotine self-administration found during the second nicotine component following pretreatment with SCH 23390 is also congruent with previous reports (Corrigall & Coen, 1991). Corrigall and Coen (1991) found that 10 μg/kg of SCH 23390 decreased nicotine self-administration, and that this decrease was most apparent after the first 15 min of the session had elapsed. A pharmacokinetic explanation of this time-dependent effect

seems unlikely, since this dose of SCH23390 (10 μg/kg) decreased cocaine self-administration during the first cocaine component in the current study. An alternate explanation is that, because nicotine pretreatment decreases nicotine self-administration (Green, Phillips, Crooks, Dwoskin, & Bardo, 2000), SCH 23390 may not have affected nicotine self-administration until a threshold level of nicotine intake was reached, thus resulting in an additive effect of SCH 23390 and nicotine. Regardless of the explanation, however, the specific SCH 23390-induced decrease in nicotine self-administration observed in the current report contrasts with a previous report showing that SCH 23390 (10 μg/kg) decreased both nicotine and food-reinforced responding (Corrigall and Coen, 1991). The discrepancy between studies may reflect procedural differences, as Corrigall and Coen (1991) tested the effect of SCH 23390 in a separate group of rats using a simple FR schedule, whereas the current study used a within-subject Mult schedule.

Behavior maintained under the current procedure was not only sensitive to pharmacological manipulations, but was also sensitive to omission of the reinforcers maintaining behavior. When sucrose was omitted, sucrose-maintained behavior was extinguished within 6 sessions, while nicotine self-administration was not affected. Once sucrose was reinstated, sucrose responding returned to baseline levels. Likewise, when nicotine was omitted, responding on the nicotine lever extinguished, while sucrose responding was unaffected.

While the results of these studies are promising, there are some limitations with the multiple schedule of drug and sucrose reinforcement used in the current experiments. First, the level of responding maintained by drug was lower than that maintained by sucrose, this effect has been reported previously in studies using a multiple schedule of cocaine and food (Woolverton & Virus, 1989). Thus, some of the changes in responding could be due to rate-dependent effects (Dews, 1958). Also, differences in rates of responding maintained by drug and sucrose could indicate differences in reinforcer efficacy between drug and sucrose. The potential differences in reinforcer efficacy between drug and sucrose could result in the behavior maintained by the two reinforcers to be differentially sensitive to manipulation.

Despite these limitations the current Mult schedule procedure may be a useful preclinical screen for the acute effects of drugs with potential utility as pharmacotherapies for nicotine addiction. Further work should determine if it is this model is also suitable for assessing the effects of repeated pretreatments. When developing novel pharmacotherapies for nicotine addiction, researchers often determine the ability of a compound to selectivity decrease the reinforcing effects of nicotine by testing the effects of the compound in one group of animals self-administering nicotine and a second group of animals self-administering food. However, using the current multiple schedule procedure, the selectivity of a compound to decrease nicotine self-administration can be tested within one group of animals. By testing the selectivity of a potential pharmacotherapy within the same group of animals the validity of the rodent drug self-administration model as a preclinical screen for pharmacotherapy is increased. The preclinical use of a mult schedule of nicotine and sucrose or food may aid in the development of a more effective pharmaceutical adjunct in combating nicotine addiction.

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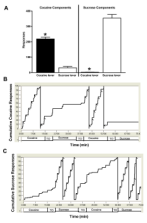

Figure 1. Responding under a Mult schedule of cocaine and sucrose reinforcement

In Panel A, data are expressed as mean (± SEM; N=9) total number of responses on the cocaine and sucrose levers during both of the cocaine and sucrose components, respectively. * denotes a significant difference from responding on the sucrose lever, $p<.05$. # denotes a significant difference from responding on the cocaine lever during the cocaine components, $p<.05$. In Panels B and C, data are cumulative records of a representative rat responding on the cocaine lever (Panel B) and sucrose lever (Panel C) under the Mult schedule of cocaine and sucrose reinforcement. Responses on the cocaine or sucrose lever resulted in the vertical movement of the pen. Upon completion of the FR15, the pen was diagonally displaced, indicating the delivery of either the cocaine infusion or sucrose pellet. The components and 5-min TO between components are indicated on event line.

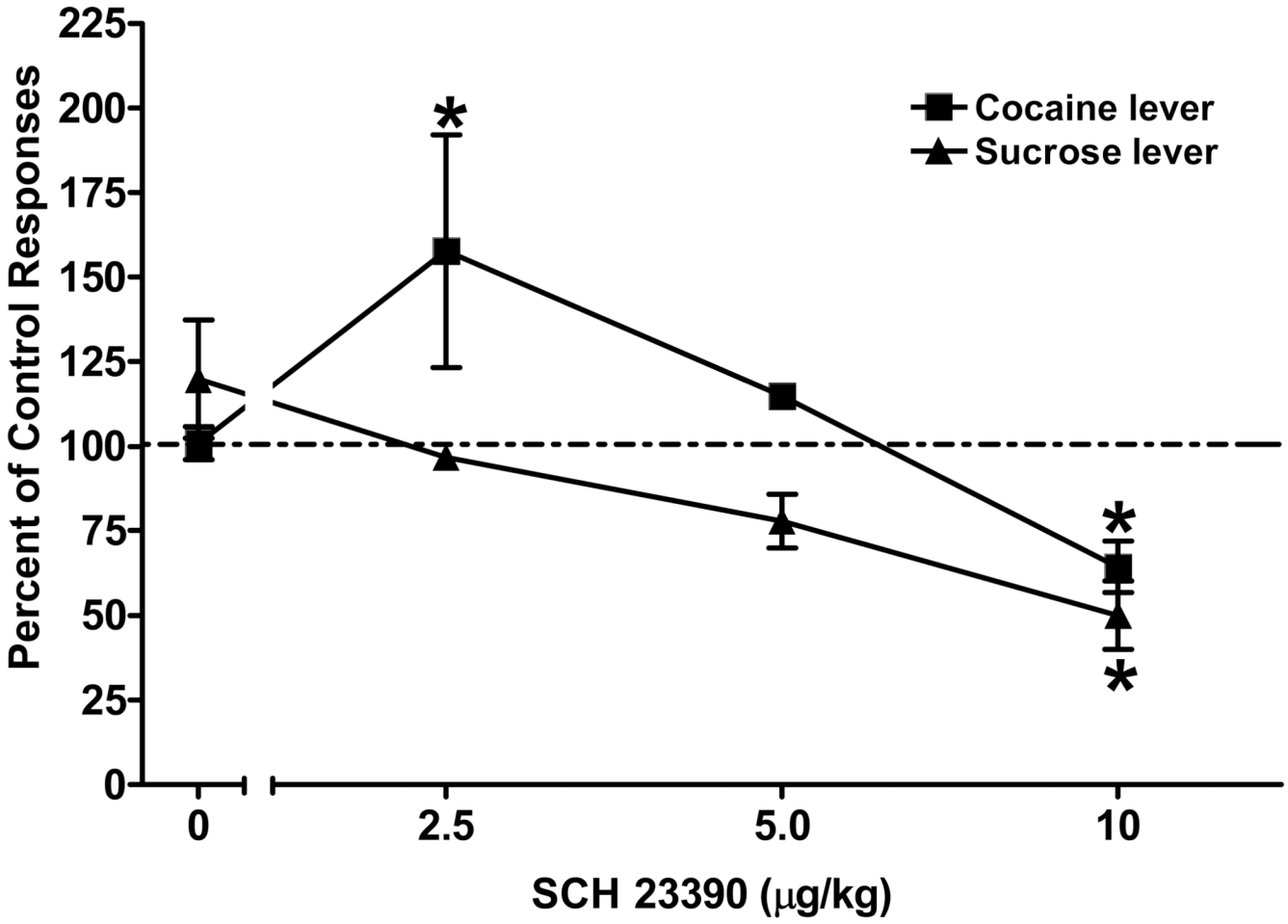

Figure 2. Effect of SCH23390 on responding under a Mult schedule of cocaine and sucrose reinforcement

Data are expressed as mean (± SEM; N=9) total number of responses as a percent of control responding on the cocaine lever during the cocaine component and on the sucrose lever during the sucrose components. * denotes a significant difference compared to saline control, $p < .05$.

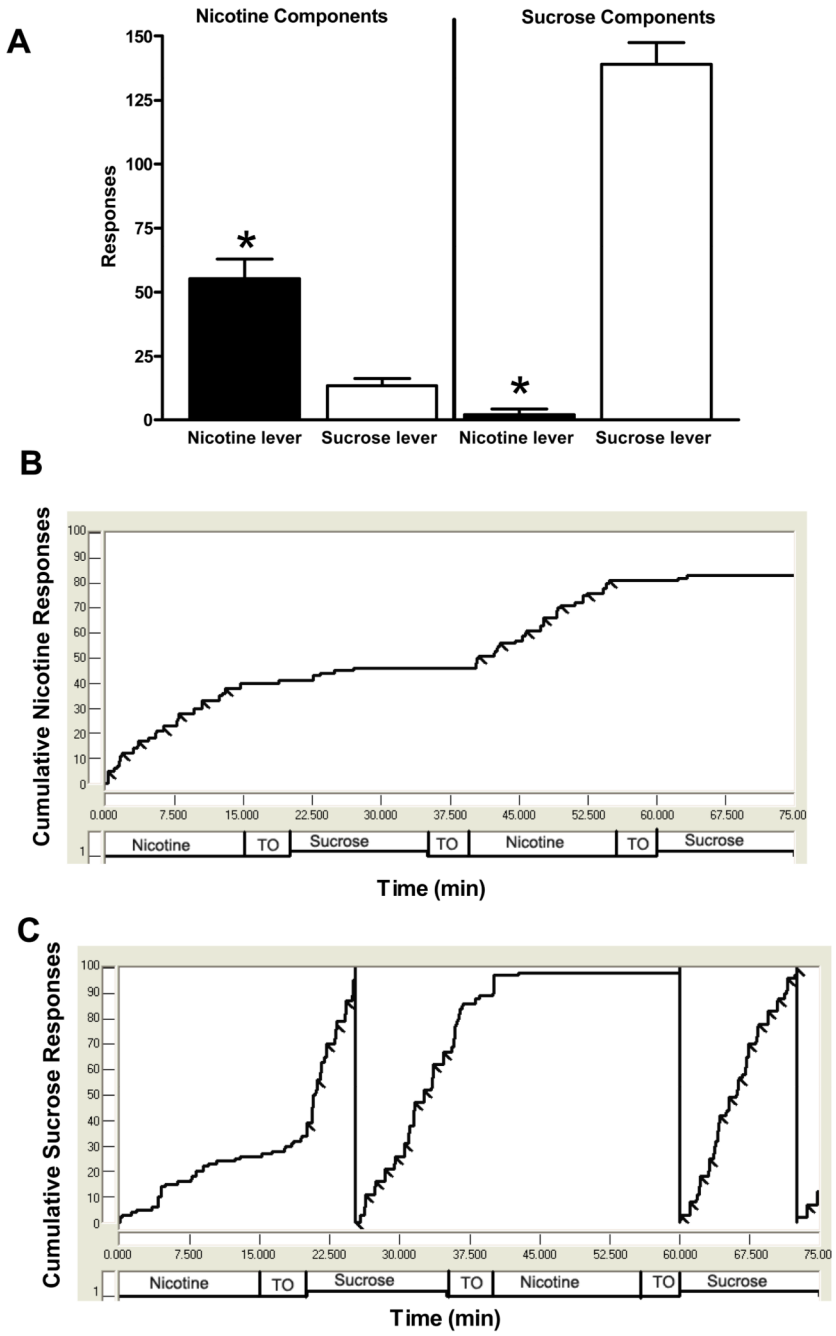

Figure 3. Responding under a Mult schedule of nicotine and sucrose reinforcement

In Panel A, data are expressed as mean (± SEM; N=4) total number of responses on the nicotine and sucrose levers during the nicotine and sucrose components, respectively. * denotes a significant difference from responding on the sucrose lever, $p<.05$. # denotes a significant difference from responding on the nicotine lever during the nicotine components, $p<.05$. In Panels B and C, data are cumulative records of responding on the nicotine lever (Panel B) and sucrose lever (Panel C) under the Mult schedule of nicotine and sucrose reinforcement for a representative subject. Responses on the nicotine or sucrose lever resulted in the vertical movement of the pen. Upon completion of the FR5, the pen was

diagonally displaced, indicating the delivery of either the nicotine infusion or sucrose pellet. The components and 5-min TO between components are indicated on event line.

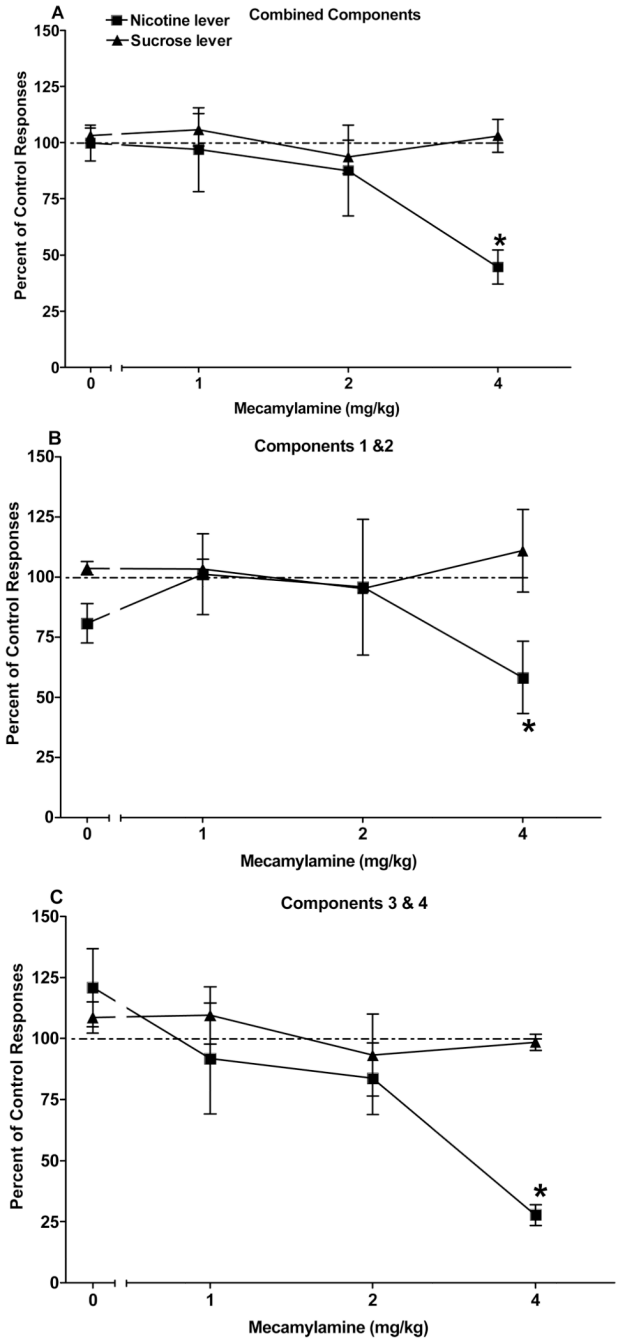

Figure 4. Effect of mecamylamine on responding under a Mult schedule of nicotine and sucrose reinforcement

Panel A shows the dose-dependent effects of mecamylamine on responding during the nicotine and sucrose components combined. In Panels B and C, data are graphed separately for the first (Panel B) and second (Panel C) set of nicotine and sucrose components. Data are expressed as mean (± SEM) number of responses as a percent change from baseline responding. * denotes a significant difference compared to saline, $p<.05$

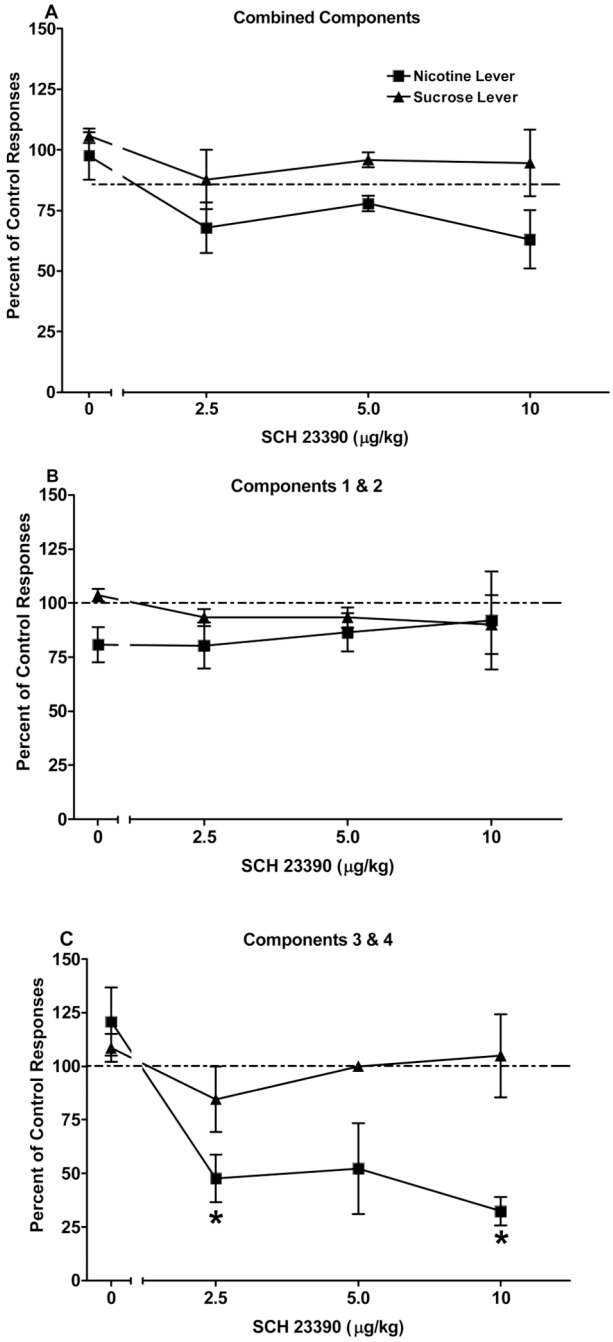

Figure 5. Effect of SCH23390 on responding under a Mult schedule of nicotine and sucrose reinforcement

In Panel A, data are expressed as mean (± SEM) total number of responses on the nicotine and sucrose levers during the nicotine and sucrose components, respectively. In Panels B and C, data are graphed separately for the first (Panel B) and second (Panel C) set of nicotine and sucrose components. * denotes a significant difference compared to saline, p<. 05.

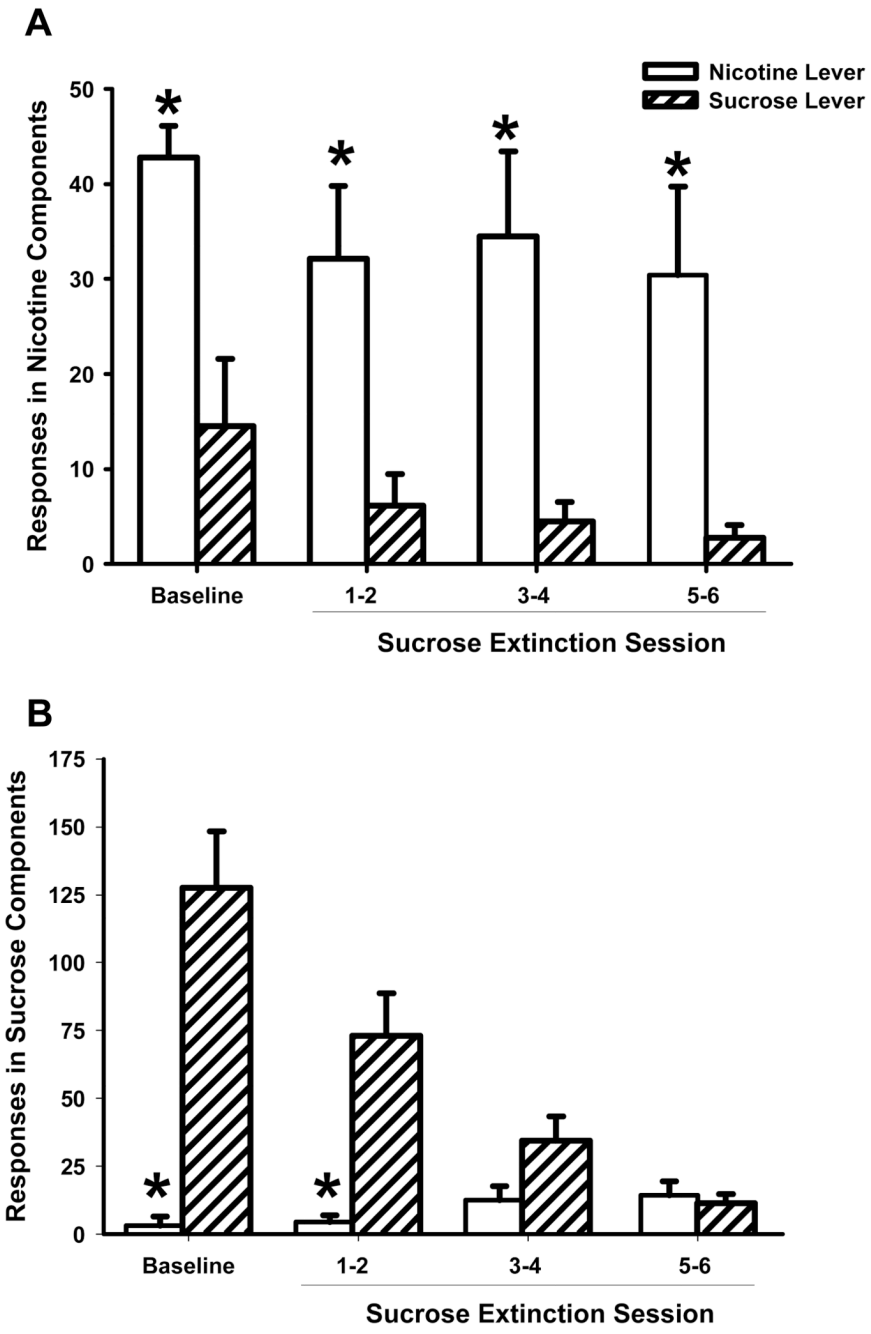

Figure 6. Effect of omitting the sucrose reinforcer on responding during the nicotine and sucrose components

Data are expressed as mean (± SEM; N=4) total number of responses during the nicotine components (Panel A) and sucrose components (Panel B) during the last session of sucrose-maintained responding (baseline), as well as the average of responding during sucrose extinction sessions 1–2, 3–4, and 5–6. * denotes a significant difference from responding on the sucrose lever.

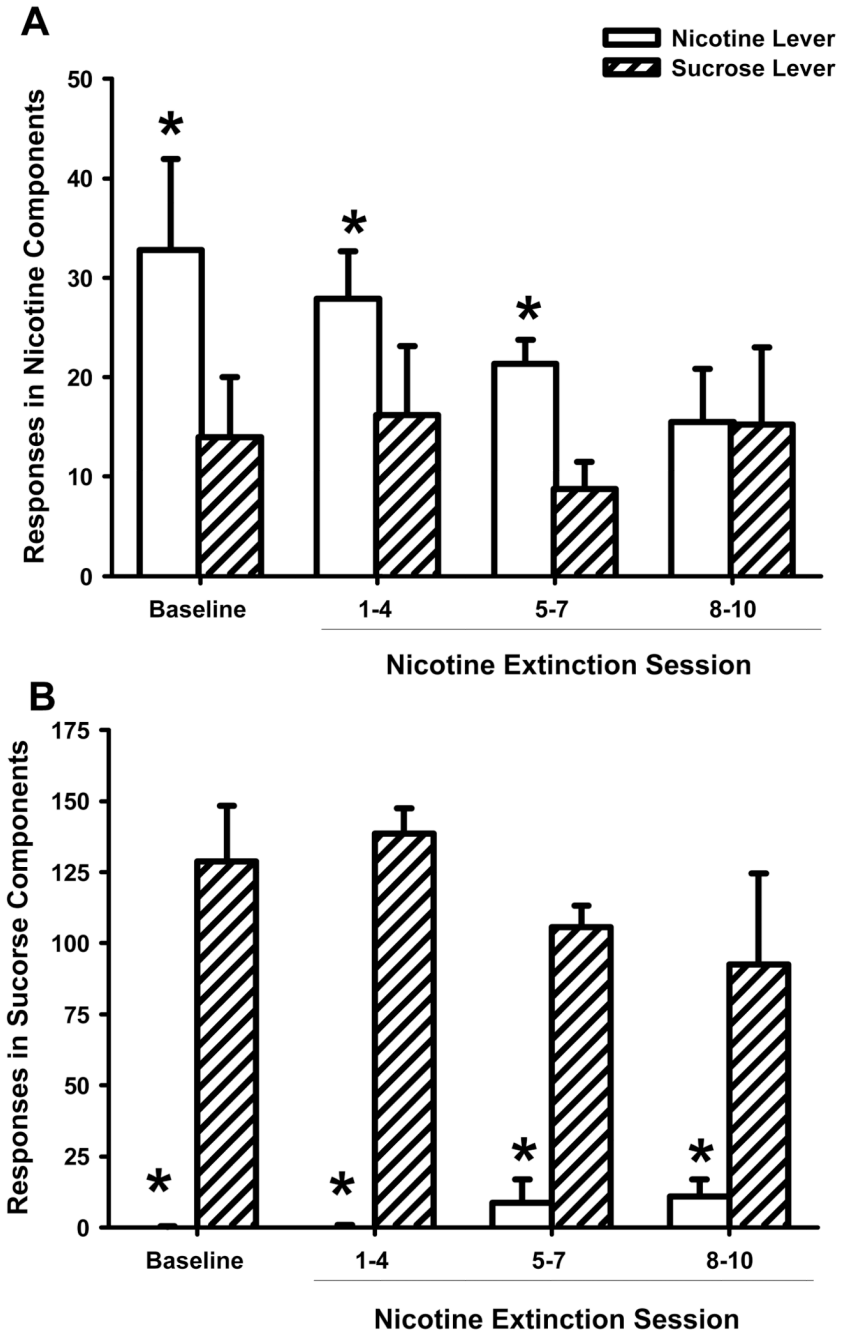

Figure 7. Effect of omitting the nicotine reinforcer on responding during the nicotine and sucrose components

Data are expressed as mean (± SEM; N=4) total number of responses during the nicotine components (Panel A) and sucrose components (Panel B) during the last session of nicotine self-administration (baseline), as well as the average of responding during nicotine extinction sessions 1–4, 5–7, and 8–10. * denotes a significant difference from responding on the sucrose lever, $p<.05$.