Published in final edited form as:

Eur J Pharmacol. 2007 November 14; 573(1-3): 116–123.

Differential involvement of dopamine receptors in conditioned suppression induced by cocaine

Ivana Grakalic, Leigh V. Panlilio, Eric B. Thorndike, and Charles W. Schindler

DHHS/NIH/NIDA Intramural Research Program, Baltimore, MD

Abstract

Cocaine-paired stimuli can suppress food-reinforced operant behavior in rats, providing an animal model of conditioned drug effects. To study the neuropharmacological basis of this phenomenon, we examined the effects of various dopamine receptor antagonists on the acquisition and expression of cocaine-induced conditioned suppression in rats. Superimposed on an ongoing baseline of food-reinforced operant responding, a stimulus was paired with response-independent cocaine (3.0 mg/kg, i.v.) during each of 8 training sessions. To study acquisition, independent groups of rats were given saline, the dopamine D₁-like receptor antagonist R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH23390) (0.001–0.03 mg/kg, i.p.), or the dopamine D₂-like receptor antagonist eticlopride (0.001–0.03 mg/kg, i.p.) prior to each training session. To study expression, independent groups of rats were trained first, then given saline, SCH23390, eticlopride, or N-[4-(4-(2-methoxyphenyl)piperazinyl)butyl]-2-naphthamide (BP897) (a dopamine D₃ partial receptor agonist; 0.1–1.0 mg/kg, i.p.) before test sessions in which the stimulus was presented without cocaine. Pretreatment with either SCH23390 or eticlopride during acquisition reduced the direct suppressant effects of cocaine, but conditioning was blocked only in rats that were treated with SCH23390 during acquisition training. Expression of conditioning was attenuated only by eticlopride. Thus, dopamine at least partially mediates both the acquisition and expression of cocaine-induced conditioned suppression, with activation of dopamine D₁- and D₂-like receptors underlying these respective processes.

Keywords

Conditioned suppression; Cocaine; Rat; SCH 23390; Eticlopride; BP 897; food

1. INTRODUCTION

Classically conditioned effects of drug-related stimuli play an important role in drug abuse (Markou et al., 1993; Robinson and Berridge, 1993). Studies have demonstrated that stimuli associated with drugs can elicit drug craving, maintain the long chains of behavior involved in acquisition, preparation and ingestion of drugs, and lead to relapse after a period of abstinence (Panlilio and Schindler, 1997; Robbins et al., 1999; Schindler et al., 1988; Wallace, 1989). Innumerable studies of conditioned drug effects (e.g., conditioned locomotor activity and conditioned place preference) have successfully used a distinctive environmental context as a conditioned stimulus. However, such contexts are composed of many elements, and it is

Please address all correspondence to: Ivana Grakalic, Behavioral Neuroscience Research Branch, Preclinical Pharmacology Section, Intramural Research Program, National Institute on Drug Abuse, 5500 Nathan Shock Drive, Baltimore, MD 21224, Fax: (410) 550-1608, e-mail: igrakalic@mail.nih.gov

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

rarely clear which elements in these studies are actually involved in conditioning (cf. Panlilio and Schindler, 1997). This question is important because discrete stimuli and contextual or other compound stimuli can have divergent effects on the behavior involved in drug abuse (e.g., see Crombag et al., 2001; See et al., 1999; Zhou et al., 2005), and furthermore, discrete stimuli can be combined in specific ways to profoundly increase or decrease drug seeking (e.g., see Kearns et al., 2005; Panlilio et al., 1996, 2000a,b).

The conditioned suppression procedure provides an effective means of examining the classically conditioned effects of discrete stimuli associated with drugs. The conditioned effect is a disruption of ongoing food-reinforced operant responding. Traditionally, conditioned-suppression procedures have involved pairing an exteroceptive stimulus (e.g., a tone or light) with an aversive unconditioned stimulus, such as footshock, while an animal is engaged in operant behavior (e.g., bar-pressing or nose poking) maintained by a positive reinforcer (e.g., food). Following several such conditioned-unconditioned stimulus pairings, the rate of responding typically decreases in the presence of the conditioned stimulus (tone or light) alone. Estes and Skinner (1941) stated that suppression during the conditioned stimulus resulted because the conditioned stimulus was paired with a “disturbing” or aversive unconditioned stimulus such as footshock. Conversely, Azrin and Hake (1969) showed that conditioned suppression could also occur when pairing a conditioned stimulus with an appetitive unconditioned stimulus such as food, water, or reinforcing brain stimulation. This suggests that decreases in response rate result from a general emotional or motivational state during presentation of a stimulus that is paired with any reinforcer, whether the reinforcer is appetitive or aversive. Conditioned suppression has been demonstrated using a number of drugs as the unconditioned stimulus, such as chlorpromazine (Cameron and Appel, 1972), lysergic acid diethylamide (Cameron and Appel, 1972, 1976), psilocybin (Cameron and Appel, 1976), amphetamine (Duncan et al., 1989; Watanabe, 1990; Whitney and Trost, 1970), and pentobarbital (Duncan, 1997; Watanabe, 1990).

Schindler et al. (2000; see also Panlilio et al., 2000b) demonstrated that food-reinforced responding can be suppressed by a cocaine-paired stimulus, and that parameters such as dose and interstimulus interval (i.e., time between conditioned stimulus and unconditioned stimulus onset) can affect the development of this suppression. This cocaine-induced conditioned suppression may provide a useful model of the conditioned craving and other behavioral effects elicited by cocaine-paired stimuli in human drug abusers. However, to date there have been no neuropharmacological investigations of this phenomenon. Therefore, the purpose of the present study was to examine the involvement of dopaminergic receptors in cocaine-induced conditioned suppression. Specifically, this was assessed by investigating the effects of a dopamine D₁-like receptor antagonist R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH 23390), a dopamine D₂-like receptor antagonist (eticlopride), and a dopamine D₃ partial receptor agonist N-[4-(2-methoxyphenyl)piperazinyl]butyl]-2-naphthamide (BP 897) on the acquisition and expression of conditioned suppression induced by cocaine. Because BP 897 has been reported to disrupt expression of cue-induced drug-seeking behavior and reactivity to drug-associated stimuli, it was tested only on the expression of cocaine-induced conditioned suppression (Duarte et al., 2003; Frances et al., 2004; Le Foll et al., 2005; Pilla et al., 2003).

2. METHOD

2.1. Subjects

The subjects were experimentally naïve, male Sprague-Dawley rats ($n = 101$), approximately 250–300 g and 8 weeks of age at the beginning of the experiment. Subjects were single housed in plastic bins. They were maintained on a 12:12 hr reverse light-dark cycle (lights on at 2200 h) and at an ambient temperature of 23° C for the duration of the experiment. The animals had

free access to food and water. Once an animal reached a free-feeding weight of approximately 375 g, food access was limited to maintain weight at 85% of that free-feeding weight. Guidelines established by the Institutional Animal Care and Use Committee at the National Institute on Drug Abuse/Intramural Research Program and the Guide for the Care and Use of Laboratory Animals were followed at all times.

For intravenous (i.v.) drug administration, jugular-vein catheters were implanted according to the procedure described in detail by Panlilio et al. (1996). Briefly, approximately 4 cm of Silastic tubing (Dow Corning, 0.44 mm i.d., 0.9 mm o.d.) was inserted into the right jugular vein and connected to vinyl tubing (Dural Plastic conditioned stimulus, 0.5 mm i.d., 1.0 mm o.d.) that exited the back at the midscapular region, and was plugged with an obturator. Immediately following catheter implantation, a 20-mm nylon screw was cemented to the skull to serve as a head mount for connecting a metal spring protecting the catheter to the animal. Catheters were flushed before and after each training session with 0.1 ml of a saline solution containing 1.25 units/ml heparin and 0.08 mg/ml gentamicin.

2.2. Apparatus

Nine operant chambers (model E10-18, Coulbourn Instruments) were enclosed individually in sound-attenuation chambers. Each chamber had a grid floor and two nose-poke holes, one on each side of the food trough. The nose poke holes could be illuminated from inside the hole by a dim yellow light. Only the left nose-poke hole was used in the present study. A 4500-Hz auditory stimulus (model 628 Sonalert, NACC-Mallory, operated at 8.75 V) and a shielded house-light (model 1820, Sylvania) served as stimuli, and were situated above and between the nose-poke holes. Food pellets (Bio-Serv #F0021, 45 mg) were delivered into the food trough. Cocaine was delivered through Tygon tubing inside a metal spring, suspended through the ceiling from a single-channel fluid swivel (Alice King Chatham). This tubing was attached to a 10-ml syringe controlled by a motor-driven syringe pump (Harvard, model 22) outside the sound attenuation chamber. Experimental events were controlled by a MED-PC computer system (Med Associates).

2.3. Procedure

2.3.1. Basic procedure—Experimentally naïve Sprague-Dawley rats were trained to nose poke on a variable-interval (VI) 60-s schedule of food reinforcement. This schedule was used based on a previous conditioned suppression study using cocaine as the unconditioned stimulus (Schindler et al., 2000). Sessions were 60 min in duration. Once responding stabilized on this schedule, an i.v. catheter was implanted as described above. Following recovery from surgery, training was resumed on the VI schedule until responding was stable. After stabilization, conditioned-suppression training was begun. During training sessions, the food schedule continued to operate as before, but stimulus-cocaine pairings were superimposed on this baseline. Thirty min into each training session, a 70 s tone-light compound conditioned stimulus was presented, and a cocaine (3 mg/kg, i.v.) injection was begun 60 s into the stimulus. This dose of cocaine was chosen because it maintains a moderate level of conditioned suppression (Schindler et al., 2000), and therefore should be sensitive to either increases or decreases in suppression during treatment with the dopamine receptor antagonists.

2.3.2. Effects of SCH 23390 and eticlopride on acquisition of cocaine-induced conditioned suppression—In order to test the effects of dopamine receptor antagonists on the acquisition of conditioned suppression, receptor antagonist treatments were given during each of 8 days of training. Separate groups of rats were administered i.p. injections of the dopamine D₁-like receptor antagonist SCH 23390 (0.001, 0.003, 0.01 or 0.03 mg/kg), dopamine D₂-like receptor antagonist eticlopride (0.001, 0.003, 0.01 and 0.03 mg/kg) or saline immediately prior to each of 8 training sessions (n=34 for SCH 23390, n=35 for eticlopride,

n=13 for vehicle, with 6–12 rats trained at each dose of the receptor antagonists). Following training (Day 9), rats in all groups were tested for acquisition of conditioned suppression. During the test sessions, the tone-light compound was presented, but cocaine was not administered. No pretreatment drugs were administered.

2.3.3. Effects of SCH 23390, eticlopride, and BP 897 on expression of cocaine-induced conditioned suppression—Separate groups of animals were used to test the effects of SCH 23390 (n=12), eticlopride (n=11) and the dopamine D₃ partial receptor agonist BP 897 (n=8) on the expression of cocaine-induced conditioned suppression. Rats were initially trained with conditioned stimulus-cocaine pairings for 8 sessions with no receptor antagonist treatment. Then, a range of doses of SCH 23390 (0, 0.003, 0.01 or 0.03 mg/kg, i.p.), eticlopride (0, 0.001, 0.003, 0.01 or 0.03 mg/kg, i.p.), or BP 897 (0.1, 0.3 or 1 mg/kg, i.p.) was tested using a within-subjects design. Tests were performed twice/week, with the order of doses counter-balanced across animals and two additional training days (with conditioned stimulus-cocaine pairings but no receptor antagonist treatment) intervening between test sessions. Injections were given immediately before each test session for rats tested with SCH 23390 or eticlopride, and 30 min before each test session for rats tested with BP 897.

2.4. Drugs

Cocaine hydrochloride (NIDA, Baltimore, MD) was dissolved in sterile saline at a concentration of 4.0 mg/ml and injected intravenously (i.v.) at a rate of 3.0 ml/min. The duration of injection was adjusted for the weight of the animal (approximately 10 s). SCH 23390 and eticlopride were purchased from Sigma (St. Louis, MO). BP 897 was generously provided from the Bioprojet laboratory (Paris, France). SCH 23390, eticlopride and BP 897 were dissolved in sterile saline at a concentration of 0.1 mg/ml and administered intraperitoneally (i.p.). Doses are expressed as the salt. The saline vehicle was administered in a manner similar to that of the specific drug for which it served as a control.

2.5. Data Analysis

During each session, response rates were recorded during the 2 min period prior to the conditioned stimulus presentation (“pre-conditioned stimulus” period), the 1 min period during which the conditioned stimulus was presented but cocaine was not yet delivered (“conditioned stimulus” period), and the periods 0–2, 2–5, 5–10, 10–15, and 15–20 min following conditioned stimulus termination. Responding was not analyzed for the 10 s period during which cocaine was delivered at the end of the conditioned stimulus. In all cases where treatment effects were detected, suppression during the conditioned stimulus was not necessarily affected. However, responding resumed more rapidly after the conditioned stimulus ended following the pretreatment when compared to vehicle. The most effective treatments caused response rates to return to near baseline levels within about 5 min after the conditioned stimulus ended, while for vehicle pretreatment responding often returned to near baseline levels over 15 min after the conditioned stimulus ended. Thus, to compare the effects of pharmacological pre-treatments, suppression during each training or testing session was quantified as suppression time, the amount of time from the beginning of the conditioned stimulus until the rat’s response rate returned to at least 50% of the rate during the pre-conditioned stimulus period (as interpolated from the response rates in the periods described above). This measure provided a reasonable and valid measure of suppression because (1) pre-conditioned stimulus response rates did not differ significantly between groups; (2) preconditioned stimulus response rates did not change significantly over time within subjects, (3) response rates during the conditioned stimulus were always less than 50% of the pre-conditioned stimulus rate for all rats in the study; and (4) response rates following the conditioned stimulus usually returned to at least 50% of the pre-conditioned stimulus rate within 20 min of the end of the conditioned stimulus. If a rat’s response rate did not return to at least 50% of the pre-conditioned stimulus rate within 20 min

of the end of the conditioned stimulus (i.e., within 22 min of the start of the conditioned stimulus), a suppression time of 22 min was used.

For the 8 sessions of training in the acquisition experiments, data were combined into 2-session blocks for analysis and presentation. Response rates and suppression times were analyzed using Proc Mixed (SAS Institute, Cary, NC). For the response-rate data in Fig. 1, the Tukey-Kramer procedure was used to compare the rate during the pre-conditioned stimulus period to the rate in each of the other periods during the same block of training. For the data presented in Fig. 3, 4 and 5, the Dunnett procedure was used to compare each point to the vehicle-control condition, maintaining a 0.05 confidence level within each set of comparisons. Three rats were eliminated from the acquisition study because their pre-conditioned stimulus response rates were too low during training (i.e., less than 5 responses/min for 4 or more sessions); these included one rat that received saline pre-treatment, one that received 0.03 mg/kg SCH 23390, and one that received 0.01 mg/kg eticlopride. The SCH 23390 and eticlopride acquisition experiments were performed contemporaneously and used a single vehicle-treated control group.

3. RESULTS

3.1. Effects of SCH 23390 and eticlopride on acquisition of cocaine-induced conditioned suppression

Fig. 1 shows response rates in rats treated with saline before each training session. Throughout training, responding was suppressed not only in the presence of the conditioned stimulus, but after the conditioned stimulus had terminated and cocaine had been delivered. The duration of this post-conditioned stimulus suppression decreased over the course of training. Responding was significantly suppressed for 10 min after the cocaine injection in the first block of training, but for only 2 min by the last block of training. Because cocaine was delivered during all training sessions, it cannot be determined whether the suppression following presentation of the conditioned stimulus and cocaine was a conditioned or unconditioned effect in these sessions. Thus, the test session was conducted (with no cocaine delivered) to directly measure the conditioned effect. During the test, responding was significantly suppressed during the conditioned stimulus and during the 2-min period following the conditioned stimulus. Response rate data for the other groups in the study showed the same basic form seen in Fig. 1, with pre-conditioned stimulus rates remaining stable across blocks and the level of suppression during conditioned stimulus presentation remaining fairly stable across training. However, the groups differed in the duration of suppression after the stimulus ended. Thus, to facilitate analysis of these differences, suppression time was quantified as a single score for each session. Suppression times corresponding to the data in Fig. 1 can be seen in the vehicle group curves in Fig. 2.

During training, suppression-time curves for most groups showed a gradual decrease over the course of training (see Fig. 2). However, certain pre-treatments reduced the amount of time before responding resumed, shifting these curves downward. For SCH 23390 and eticlopride, respectively, there were significant main effects of pre-treatment [$F(4,39)=9.63$, $P<0.001$, and $F(4,36)=3.34$, $P<0.05$] and block of training [$F(3,117)=4.6$, $P<0.005$, and $F(3,110)=4.57$, $P<0.005$], but the interactions of pre-treatment and block of training were not significant ($P>0.38$ and $P>0.78$). When suppression times of each group were collapsed across blocks and compared to vehicle controls (see Fig. 3), it was found that two doses of SCH 23390 (0.01 and 0.03 mg/kg) and one dose of eticlopride (0.01 mg/kg) significantly shortened suppression times during training.

During testing (see Fig. 4), pre-treatments and cocaine delivery were both discontinued to measure conditioned suppression. Overall, suppression times during testing were well above zero, indicating that a conditioned effect was acquired in all groups. However, this conditioned

effect was attenuated (i.e., suppression times were significantly shortened) in rats that had received either 0.003 or 0.01 mg/kg of SCH 23390 during training [main effect of pre-treatment: $F(4,39)=4.51$, $P<0.01$]. Rats that received eticlopride ($P>0.35$) during training showed suppression times during the test that were not significantly different from rats that had received vehicle pre-treatment during training.

3.2. Effects of SCH 23390, eticlopride, and BP 897 on expression of cocaine-induced conditioned suppression

Expression tests (see Fig. 5) were conducted by giving pre-treatments only during testing, with cocaine delivery suspended. Only eticlopride had a significant effect on expression of conditioned suppression [$F(4,27)=4.98$, $P<0.01$], and this shortening of suppression times was only seen at one dose (0.01 mg/kg, the same dose that decreased suppression during training in the acquisition experiment). Neither SCH 23390 ($P>0.35$) nor BP 897 ($P>0.25$) altered expression.

4. DISCUSSION

Two facts should be mentioned about the conditioned suppression produced with this procedure. First, the novel conditioned stimulus initially suppresses responding by itself, regardless of whether it is paired with saline or cocaine. However, after eight training sessions, the stimulus no longer has an effect if it has been paired with saline, but it continues to suppress responding if it has been paired with cocaine (Panlilio et al., 2000b; Schindler et al., 2000). Thus, a traditional learning curve is not obtained during acquisition training. Rather, evidence of conditioning consists of the conditioned stimulus still producing substantial suppression at the end of training. Second, it should be noted that cocaine has a slower onset and offset of effect than footshock, the unconditioned stimulus traditionally used in conditioned-suppression studies. Thus, when cocaine is delivered at the end of the conditioned stimulus, both unconditioned and conditioned effects can occur after the conditioned stimulus has terminated. Therefore, it is important to evaluate suppression both during and after the conditioned stimulus.

Results of the present experiment suggest differential involvement of dopamine receptors in the acquisition and expression of conditioned suppression induced by cocaine. As in previous studies (Panlilio et al., 2000; Schindler et al., 2000), 3 mg/kg cocaine directly suppressed operant behavior in rats, and a stimulus paired with these injections produced a conditioned suppression of operant behavior. In the present study, pretreatment with either the dopamine D_1 -like receptor antagonist SCH 23390 or dopamine D_2 -like receptor antagonist eticlopride during acquisition training reduced the direct suppressant effects of this dose of cocaine. However, it is interesting that conditioning was blocked only in rats that had received the dopamine D_1 -like receptor antagonist SCH 23390 during acquisition training. In addition, only the dopamine D_2 -like receptor antagonist eticlopride prevented expression of conditioned suppression in pre-trained rats. Potential explanations of these effects are discussed below.

4.1. Direct suppressant effects of cocaine

Both SCH 23390 and eticlopride decreased the direct suppressant effects of cocaine. These treatment effects were comparable to those seen during extinction. That is, the short suppression times seen in these rats during the final training sessions of acquisition training were comparable to those seen in the control group when the conditioned stimulus was presented without cocaine during testing. As noted in the introduction, food-maintained responding can be suppressed by a conditioned stimulus paired with food, water, intracranial stimulation (Azrin and Hake, 1969; Hake and Powell, 1970) or a variety of drugs such as amphetamine and cocaine (Whitney and Trost, 1970; Duncan et al., 1989; Watanabe,

1990;Panlilio et al., 2000b;Schindler et al., 2000). According to the incentive-contrast effects theory, motivational effects of a strong positive reinforcer, like a high dose of cocaine, may be stronger than those of a weaker reinforcer, like a small food pellet (Grigson, 1997).

Alternatively, it is possible that cocaine suppresses responding for food because it acts as an aversive stimulus, like shock (Blackman, 1977). Thus, it is unclear whether the direct suppression of food-reinforced responding by cocaine results from appetitive or aversive effects of cocaine. However, it is clear that this direct suppression induced by cocaine is mediated in large part by the dopaminergic effects of cocaine, because this effect was attenuated by both SCH 23390 and eticlopride in the present study. This finding is consistent with earlier studies showing that both dopamine D₁ and D₂ receptor antagonists can block the reinforcing and/or aversive properties of cocaine within a variety of preparations (Adams et al., 2001;Caine et al., 1995;Cervo and Samanin, 1995;Epping-Jordan et al., 1998;Hubner and Moreton, 1991;Hunt et al., 1985;Morency and Beninger, 1986;Pruitt et al., 1995;Shippenberg and Heidbreder, 1995). However, the fact that neither dopamine receptor antagonist completely blocked the direct suppressant effects of cocaine suggests that other transmitter systems affected by cocaine (e.g., glutamate, serotonin) also contribute to these effects.

Another possibility is that the dopaminergic effects studied might be the consequence of stimulation of multiple dopamine receptors. For example, dopamine D₁-like receptor antagonists such as SCH 23390 bind to both dopamine D₁ and D₅ receptors, while dopamine D₂-like receptor antagonists such as eticlopride bind to dopamine D₂, D₃ and D₄ receptors. It is possible that the conditioned suppression effects studied here are due to an interaction involving both D₁-like and D₂-like receptor antagonists, in which case a receptor antagonist selective for only one of these dopamine receptor families might not be sufficient to completely block the direct or conditioned suppressant effects of cocaine.

4.2. Acquisition of conditioned suppression

Even though both SCH 23390 and eticlopride attenuated the direct suppressant effects of cocaine, only SCH 23390 attenuated acquisition of the conditioned effects of a cocaine-paired stimulus. First, it is possible that the dopamine D₁- and D₂-like effects of cocaine produce dissociable unconditioned responses, and only the dopamine D₁-like effects participate in conditioned suppression. Thus, blocking this dopamine D₁-like effect of cocaine during training may have been equivalent to using a less intense unconditioned stimulus, which is less effective in supporting learning (Kamin and Brimer, 1963).

A second, perhaps more parsimonious, possibility is that SCH 23390 directly interfered with the associative conditioned stimulus-unconditioned stimulus learning process. A number of studies have demonstrated that nonspecific or dopamine D₁-specific receptor antagonists can interfere with both appetitive and aversive conditioning induced by both drug and non-drug reinforcers (Azzara et al., 2001;Beninger and Miller, 1998;Dalley et al., 2005;Di Chiara, 2002;Eyny and Horvitz, 2003;Fenu and Di Chiara, 2003;Greba et al., 2001;Greba and Kokkinidis, 2000;Hiroi and White, 1991;Hunt et al., 1985;Inoue et al., 2000;Nader and LeDoux, 1999;Pruitt et al., 1995;Smith-Roe and Kelley, 2000), and several of these also found that dopamine D₂ receptor blockade did not prevent acquisition of conditioning (Caulliez et al., 1996;Cervo and Samanin, 1995;Fenu et al., 2001;Shippenberg and Heidbreder, 1995;Spina et al., 2006).

4.3. Expression of conditioned suppression

When rats were given conditioned suppression training prior to testing with dopamine receptor antagonists, the dopamine D₂-like receptor antagonist eticlopride attenuated the expression of conditioned suppression, but SCH 23390 and the dopamine D₃ partial receptor agonist BP 897 had no effect. It should be noted that expression of conditioned suppression was attenuated

only at one dose (0.01 mg/kg) of eticlopride, with lower and higher doses being ineffective. Although it is not clear why only this dose was effective, it should be noted that this was also the only dose of eticlopride that effectively attenuated the direct effects of cocaine in the acquisition study. Interestingly, when the highest dose (0.03 mg/kg) eticlopride was administered in the present study, there seemed to be a slight but non-significant increase in conditioned suppression, suggesting that this dose of eticlopride may affect motor activity. Indeed, both dopamine D₁ and D₂ receptor antagonists have well-documented effects on operant responding, and produce a variety of motor deficits when administered alone (e.g., Fowler and Liou, 1998;Schindler and Carmona, 2002;Wise, 2004;Rick et al., 2006). However, these effects were evident at higher doses (e.g., 0.1 mg/kg) than those used in the present study (0.001–0.03 mg/kg). Therefore, the present findings cannot be attributed to the direct effects of dopamine receptor antagonists on operant responding and/or locomotion.

This narrow range of effective doses may be related in some way to the biphasic effects of dopamine D₂ treatments in some previous studies (e.g., Berglind et al., 2006;de Oliveira et al., 2006). For example, Berglind et al. (2006) reported that infusion of a low dose of raclopride into the basolateral amygdala potentiated, while a higher dose attenuated cue-induced reinstatement of cocaine- seeking. This inverted U-shaped dose-effect function for raclopride may be due to the pre- and post-synaptic location of dopamine D₂ receptors and/or temporal changes of dopamine D₂ receptor occupancy. How or whether this interpretation relates to the present findings remains to be determined.

Using a wide variety of procedures, previous studies have shown that dopamine D₁ and/or D₂ receptor antagonists can interfere with the expression of conditioned drug effects (Adams et al., 2001;Baker et al., 2003;Cervo and Samanin, 1996;de Oliveira et al., 2006;Fontana et al., 1993;Guarraci et al., 1999;Hiroi and White, 1991;Inoue et al., 2000;Schenk and Gittings, 2003;Spina et al., 2006;Weissenborn et al., 1996). However, there has been little consistency across these studies in whether dopamine D₁ or D₂ receptor antagonists are more effective in blocking this expression. It may be that the specificity is determined by the details of the specific procedure used in each study.

In contrast, published studies have consistently demonstrated the involvement of dopamine D₃ receptors in drug-seeking behavior following presentation of drug-associated stimuli (Pilla et al., 1999;Di Ciano et al., 2003). Further, the dopamine D₃ receptor has also been implicated in reactivity to drug-associated stimuli in classical conditioning paradigms such as conditioned place preference (Vorel et al., 2002;Duarte et al., 2003;Le Foll and Goldberg, 2005;Le Foll et al., 2005) and conditioned hyperactivity (Le Foll et al., 2002,2003). Therefore, it was surprising that BP 897 did not modify the expression of cocaine-induced conditioned suppression in the present study. This discrepancy might be related to the nature of the baseline conditioned response. That is, the baseline was a conditioned decrease in behavior in the present study, but a conditioned increase in behavior in the cocaine self-administration and locomotor studies. Thus, BP 897 might be less effective in blocking a decrease in behavior than an increase.

4.4. Conclusion

Given the importance that conditioning plays in the acquisition, maintenance, extinction and relapse to drug abuse (Schindler et al., 1988), a clear understanding of a behavioral model such as conditioned suppression (with its well-defined conditioned stimulus parameters) can give an insight into the behavioral and pharmacological nature of that conditioning. This cocaine-induced conditioned suppression is probably based on the same process by which incentive value is conditioned to drug-related stimuli in humans. The results of the present study indicate that dopamine receptors play a critical role in the conditioning properties of cocaine as measured by conditioned suppression. Further, these results suggests that dopamine D₂-like

receptor ligands should be further evaluated as potential therapeutic agents for reducing conditioned drug effects, such as craving, that may contribute to drug abuse.

Acknowledgements

This research was supported by the Intramural Research Program of the NIH, NIDA.

References

- Adams JU, Careri JM, Efferen TR, Rotrosen J. Differential effects of dopamine antagonists on locomotor activity, conditioned activity and conditioned place preference induced by cocaine in rats. *Behav Pharmacol* 2001;12:603–611. [PubMed: 11856897]
- Azrin NH, Hake DF. Positive conditioned suppression: conditioned suppression using positive reinforcers as the unconditioned stimuli. *J Exp Anal Behav* 1969;12:167–173. [PubMed: 16811337]
- Azzara AV, Bodnar RJ, Delamater AR, Sclafani A. D₁ but not D₂ dopamine receptor antagonism blocks the acquisition of a flavor preference conditioned by intragastric carbohydrate infusions. *Pharmacol Biochem Behav* 2001;68:709–720. [PubMed: 11526968]
- Baker RM, Shah MJ, Sclafani A, Bodnar RJ. Dopamine D₁ and D₂ antagonists reduce the acquisition and expression of flavor-preferences conditioned by fructose in rats. *Pharmacol Biochem Behav* 2003;75:55–65. [PubMed: 12759113]
- Beninger RJ, Miller R. Dopamine D₁-like receptors and reward-related incentive learning. *Neurosci Biobehav Rev* 1998;22:335–245. [PubMed: 9579323]
- Berglind WJ, Case JM, Parker MP, Fuchs RA, See RE. Dopamine D₁ or D₂ receptor antagonism within the basolateral amygdala differentially alters the acquisition of cocaine-cue associations necessary for cue-induced reinstatement of cocaine-seeking. *Neuroscience* 2006;137:699–706. [PubMed: 16289883]
- Blackman, D. Conditioned suppression and the effects of classical conditioning on operant behavior. In: Honig, WK.; Staddon, JER., editors. *Handbook of operant behavior*. Prentice Hall; Englewood Cliffs, NJ: 1977. p. 340-63.
- Caine SB, Heinrichs SC, Coffin VL, Koob GF. Effects of the dopamine D-1 antagonist SCH 23390 microinjected into the accumbens, amygdala or striatum on cocaine self-administration in the rat. *Brain Res* 1995;692:47–56. [PubMed: 8548319]
- Cameron OG, Appel JB. onditioned suppression of bar-pressing behavior by stimuli associated with drugs. *J Exp Anal Behav* 1972;17:127–137. [PubMed: 5063082]
- Cameron OG, Appel JB. Drug-induced conditioned suppression: specificity due to drug employed as unconditioned stimulus. *Pharmacol Biochem Behav* 1976;4:221–224. [PubMed: 1265108]
- Caulliez R, Meile MJ, Nicolaidis S. A lateral hypothalamic D₁ dopaminergic mechanism in conditioned taste aversion. *Brain Res* 1996;729:234–245. [PubMed: 8876993]
- Cervo L, Samanin R. Effects of dopaminergic and glutamatergic receptor antagonists on the acquisition and expression of cocaine conditioning place preference. *Brain Res* 1995;673:242–250. [PubMed: 7606438]
- Cervo L, Samanin R. Effects of dopaminergic and glutamatergic receptor antagonists on the establishment and expression of conditioned locomotion to cocaine in rats. *Brain Res* 1996;731:31–38. [PubMed: 8883851]
- Crombag HS, Badiani A, Chan J, Dell'Orco J, Dineen SP, Robinson TE. The ability of environmental context to facilitate psychomotor sensitization to amphetamine can be dissociated from its effect on acute drug responsiveness and on conditioned responding. *Neuropsychopharmacology* 2001;24:680–690. [PubMed: 11331148]
- Dalley JW, Laane K, Theobald DE, Armstrong HC, Corlett PR, Chudasama Y, Robbins TW. Time-limited modulation of appetitive Pavlovian memory by D₁ and NMDA receptors in the nucleus accumbens. *Proc Natl Acad Sci U S A* 2005;102:6189–6194. [PubMed: 15833811]
- Di Ciano P, Underwood RJ, Hagan JJ, Everitt BJ. Attenuation of cue-controlled cocaine-seeking by a selective D₃ dopamine receptor antagonist SB-277011-A. *Neuropsychopharmacology* 2003;28:329–338. [PubMed: 12589386]

- de Oliveira AR, Reimer AE, Brandao ML. Dopamine D₂ receptor mechanism in the expression of conditioned fear. *Pharmacol Biochem Behav* 2006;84:102–111. [PubMed: 16780936]
- Di Chiara G. Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. *Behav Brain Res* 2002;137:75–114. [PubMed: 12445717]
- Duarte C, Lefebvre C, Chaperon F, Hamon M, Thiebot MH. Effects of a dopamine D₃ receptor ligand, BP 897, on acquisition and expression of food-, morphine-, and cocaine-induced conditioned place preference, and food-seeking behavior in rats. *Neuropsychopharmacology* 2003;28:1903–1915. [PubMed: 12915863]
- Duncan PM. Conditioned suppression of operant responding in response to a stimulus paired with pentobarbital injections. *Psychobiology* 1997;25:146–151.
- Epping-Jordan MP, Markou A, Koob GF. The dopamine D-1 receptor antagonist SCH 23390 injected into the dorsolateral bed nucleus of the stria terminalis decreased cocaine reinforcement in the rat. *Brain Res* 1998;784:105–115. [PubMed: 9518570]
- Estes WK, Skinner BF. Some quantitative properties of anxiety. *J Exp Psychol* 1941;29:390–400.
- Eyny YS, Horvitz JC. Opposing roles of D₁ and D₂ receptors in appetitive conditioning. *J Neurosci* 2003;23:1584–1587. [PubMed: 12629161]
- Fenu S, Bassareo V, Di Chiara G. A role for dopamine D₁ receptors of the nucleus accumbens shell in conditioned taste aversion learning. *J Neurosci* 2001;21:6897–6904. [PubMed: 11517277]
- Fenu S, Di Chiara G. Facilitation of conditioned taste aversion learning by systemic amphetamine: role of nucleus accumbens shell dopamine D₁ receptors. *Eur J Neurosci* 2003;18:2025–2030. [PubMed: 14622235]
- Fontana D, Post RM, Weiss SR, Pert A. The role of D₁ and D₂ dopamine receptors in the acquisition and expression of cocaine-induced conditioned increases in locomotor behavior. *Behav Pharmacol* 1993;4:375–387. [PubMed: 11224206]
- Fowler SC, Liou JR. Haloperidol, raclopride, and eticlopride induce microcatalepsy during operant performance in rats, but clozapine and SCH 23390 do not. *Psychopharmacology* 1998;140:81–90.
- Frances H, Le Foll B, Diaz J, Smirnova M, Sokoloff P. Role of DRD3 in morphine-induced conditioned place preference using drd3-knockout mice. *Neuroreport* 2004;15:2245–2249. [PubMed: 15371743]
- Greba Q, Gifkins A, Kokkinidis L. Inhibition of amygdaloid dopamine D₂ receptors impairs emotional learning measured with fear-potentiated startle. *Brain Res* 2001;899:218–226. [PubMed: 11311883]
- Greba Q, Kokkinidis L. Peripheral and intraamygdalar administration of the dopamine D₁ receptor antagonist SCH 23390 blocks fear-potentiated startle but not shock reactivity or the shock sensitization of acoustic startle. *Behav Neurosci* 2000;114:262–272. [PubMed: 10832788]
- Grigson PS. Conditioned taste aversions and drugs of abuse: a reinterpretation. *Behav Neurosci* 1997;111:129–136. [PubMed: 9109631]
- Guarraci FA, Frohardt RJ, Kapp BS. Amygdaloid D₁ dopamine receptor involvement in Pavlovian fear conditioning. *Brain Res* 1999;827:28–40. [PubMed: 10320690]
- Hake DF, Powell J. Positive reinforcement and suppression from the same occurrence of the unconditioned stimulus in a positive conditioned suppression procedure. *J Exp Anal Behav* 1970;14:247–257. [PubMed: 16811472]
- Hiroi N, White NM. The lateral nucleus of the amygdala mediates expression of the amphetamine-produced conditioned place preference. *J Neurosci* 1991;11:2107–2116. [PubMed: 2066777]
- Hubner CB, Moreton JE. Effects of selective D₁ and D₂ dopamine antagonists on cocaine self-administration in the rat. *Psychopharmacology (Berl)* 1991;105:151–156. [PubMed: 1839063]
- Hunt T, Switzman L, Amit Z. Involvement of dopamine in the aversive stimulus properties of cocaine in rats. *Pharmacol Biochem Behav* 1985;22:945–948. [PubMed: 2991950]
- Inoue T, Izumi T, Maki Y, Muraki I, Koyama T. Effect of the dopamine D(1/5) antagonist SCH 23390 on the acquisition of conditioned fear. *Pharmacol Biochem Behav* 2000;66:573–578. [PubMed: 10899372]
- Kamin LJ, Brimer CJ. The effects of intensity of conditioned and unconditioned stimuli on a conditioned emotional response. *Can J Psychol* 1963;17:194–198.
- Kearns DN, Weiss SJ, Schindler CW, Panlilio LV. Conditioned inhibition of cocaine seeking in rats. *J Exp Psychol Anim Behav Process* 2005;31:247–253. [PubMed: 15839780]

- Le Foll B, Frances H, Diaz J, Schwartz JC, Sokoloff P. Role of the dopamine D₃ receptor in reactivity to cocaine-associated cues in mice. *Eur J Neurosci* 2002;15:2016–2026. [PubMed: 12099907]
- Le Foll B, Goldberg SR, Sokoloff P. The dopamine D₃ receptor and drug dependence: effects on reward or beyond? *Neuropharmacology* 2005;49:525–541. [PubMed: 15963538]
- Le Foll B, Schwartz JC, Sokoloff P. Disruption of nicotine conditioning by dopamine D(3) receptor ligands. *Mol Psychiatry* 2003;8:225–230. [PubMed: 12610655]
- Markou A, Weiss F, Gold LH, Caine SB, Schulteis G, Koob G. Animal models of drug craving. *Psychopharmacology (Berl)* 1993;112:163–182. [PubMed: 7871016]
- Morency MA, Beninger RJ. Dopaminergic substrates of cocaine-induced place conditioning. *Brain Res* 1986;399:33–41. [PubMed: 3026573]
- Nader K, LeDoux JE. Inhibition of the mesoamygdala dopaminergic pathway impairs the retrieval of conditioned fear associations. *Behav Neurosci* 1999;113:891–901. [PubMed: 10571473]
- Panlilio LV, Schindler CW. Conditioned locomotor-activating and reinforcing effects of discrete stimuli paired with intraperitoneal cocaine. *Behav Pharmacol* 1997;8:691–698. [PubMed: 9832954]
- Panlilio LV, Weiss SJ, Schindler CW. Cocaine self-administration increased by compounding discriminative stimuli. *Psychopharmacology (Berl)* 1996;125:202–208. [PubMed: 8815954]
- Panlilio LV, Weiss SJ, Schindler CW. Effects of compounding drug-related stimuli: escalation of heroin self-administration. *J Exp Anal Behav* 2000a;73:211–224. [PubMed: 10784010]
- Panlilio LV, Weiss SJ, Schindler CW. Stimulus compounding enhances conditioned suppression produced by cocaine-paired stimuli. *Exp Clin Psychopharmacol* 2000b;8:6–13. [PubMed: 10743900]
- Pilla M, Paracheon S, Sautel F, Garrido F, Mann A, Wermuth CG, Schwartz JC, Everitt BJ, Sokoloff P. Selective inhibition of cocaine-seeking behavior by a partial dopamine D₃ receptor agonist. *Nature* 1999;400:371–375. [PubMed: 10432116]
- Pruitt DL, Bolanos CA, McDougall SA. Effects of dopamine D₁ and D₂ receptor antagonists on cocaine-induced place preference conditioning in preweanling rats. *Eur J Pharmacol* 1995;283:125–131. [PubMed: 7498301]
- Rick JH, Horvitz JC, Balsam PD. Dopamine receptor blockade and extinction differentially affect behavioral variability. *Behav Neurosci* 2006;120:488–492. [PubMed: 16719712]
- Robbins SJ, Ehrman RN, Childress AR, O'Brien CP. Comparing levels of cocaine cue reactivity in male and female outpatients. *Drug Alcohol Depend* 1999;53:223–230. [PubMed: 10080048]
- Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Rev* 1993;18:247–291. [PubMed: 8401595]
- Schenk S, Gittings D. Effects of SCH 23390 and eticlopride on cocaine-seeking produced by cocaine and WIN 35,428 in rats. *Psychopharmacology (Berl)* 2003;168:118–123. [PubMed: 12420155]
- Schindler CW, Carmona GN. Effects of dopamine agonists and antagonists on locomotor activity in male and female rats. *Pharmacol Biochem Behav* 2002;72:857–863. [PubMed: 12062575]
- Schindler CW, Katz JL, Goldberg SR. The use of second-order schedules to study the influence of environmental stimuli on drug-seeking behavior. *NIDA Res Monogr* 1988;84:180–195. [PubMed: 3147382]
- Schindler CW, Thorndike EB, Ma JD, Goldberg SR. Conditioned suppression with cocaine as the unconditioned stimulus. *Pharmacol Biochem Behav* 2000;65:83–89. [PubMed: 10638640]
- See RE, Grimm JW, Kruzich PJ, Rustay N. The importance of a compound stimulus in conditioned drug-seeking behavior following one week of extinction from self-administered cocaine in rats. *Drug Alcohol Depend* 1999;57:41–49. [PubMed: 10617312]
- Shippenberg TS, Heidbreder C. Sensitization to the conditioned rewarding effects of cocaine: pharmacological and temporal characteristic conditioned stimulus. *J Pharmacol Exp Ther* 1995;273:808–815. [PubMed: 7752084]
- Smith-Roe SL, Kelley AE. Coincident activation of NMDA and dopamine D₁ receptors within the nucleus accumbens core is required for appetitive instrumental learning. *J Neurosci* 2000;20:7737–7742. [PubMed: 11027236]
- Spina L, Fenu S, Longoni R, Rivas E, Di Chiara G. Nicotine-conditioned single-trial place preference: selective role of nucleus accumbens shell dopamine D₁ receptors in acquisition. *Psychopharmacology (Berl)* 2006;184:447–455. [PubMed: 16341849]

- Vorel SR, Ashby CR Jr, Paul M, Liu X, Hayes R, Hagan JJ, Middlemiss DN, Stemp G, Gardner EL. Dopamine D₃ receptor antagonism inhibits cocaine-seeking and cocaine-enhanced brain reward in rats. *J Neurosci* 2002;22:9595–9603. [PubMed: 12417684]
- Weissenborn R, Deroche V, Koob GF, Weiss F. Effects of dopamine agonists and antagonists on cocaine-induced operant responding for a cocaine-associated stimulus. *Psychopharmacology (Berl)* 1996;126:311–322. [PubMed: 8878347]
- Wallace BC. Psychological and environmental determinants of relapse in crack cocaine smokers. *J Subst Abuse Treat* 1989;6:95–106. [PubMed: 2746717]
- Watanabe S. Isodirectional conditioning effects of d-amphetamine and pentobarbital on schedule-controlled operant behavior in pigeons. *Pharmacol Biochem Behav* 1990;36:157–161. [PubMed: 2349257]
- Whitney, GD.; Trost, JG. Response disruption following amphetamine self- and programmed administration. In: Harris, RT.; McIsaac, MW.; Schuster, CR., editors. *Drug Dependence*. University of Texas Press; Austin, TX: 1970. p. 198-213.
- Wise RA. Dopamine, learning and motivation. *Nat Rev Neurosci* 2004;5:1–12.
- Zhou W, Zhang F, Tang S, Liu H, Gu J, Yang G. The dissociation of heroin-seeking patterns induced by contextual, discriminative, or discrete conditioned cues in a model of relapse to heroin in rats. *Psychopharmacology* 2005;181:197–206. [PubMed: 15830224]

Fig 1.

Acquisition of conditioned suppression in rats pre-treated with saline before each training session. Mean ($\pm$ SEM) response rates (responses per min) are presented for the following periods of time: 2 min prior to conditioned stimulus presentation ("Pre"), 1 min during conditioned stimulus presentation ("CS"), and 0–2, 2–5, 5–10, 10–15, and 15–20 min after the cocaine injection given at the end of the conditioned stimulus. Each block of training represents two sessions, and "Test" represents the ninth session, in which cocaine was not delivered. Asterisks indicate rates significantly ($P < 0.05$) lower than the preconditioned stimulus rate in the same block of training (or during the test session for the test data, on the right).

Fig 2.

Mean ($\pm$ SEM) suppression times over the course of acquisition training in groups treated with vehicle, SCH 23390, or eticlopride before each session. Legend indicates the pre-treatment drug and dose (mg/kg). Cocaine was delivered at the end of conditioned stimulus presentation during each session. Suppression times were measured from the beginning of the conditioned stimulus until the response rate returned to at least 50% of the pre-conditioned stimulus rate. SCH 23390 and eticlopride shared a vehicle-treated control group (*solid circles*); the data for this control group in Fig. 2 are derived from the same data in Fig. 1.

Fig 3.

Mean ($\pm$ SEM) suppression times during acquisition training, collapsed across blocks. Each curve in Fig. 2 is seen as a point in Fig. 3. *Asterisks* indicate that suppression times were significantly reduced ($P < 0.05$) in groups pre-treated with 0.01 or 0.03 mg/kg of SCH 23390 or 0.01 mg/kg of eticlopride.

Fig 4. Mean ($\pm$ SEM) suppression times during testing (Day 9) for conditioned effects in groups treated with vehicle, SCH 23390, or eticlopride before each session during acquisition training. During testing, no pre-treatment was given, and the conditioned stimulus was presented without cocaine delivery. *Asterisks* indicate that suppression times were significantly reduced ($P < 0.05$) in groups pre-treated with 0.003 or 0.01 mg/kg of SCH 23390.

Fig 5.

Mean ($\pm$ SEM) suppression times during testing for alterations in the expression of conditioned suppression when rats were pre-treated with vehicle, SCH 23390, eticlopride, or BP 897 before the test. Rats were given cocaine paired with the conditioned stimulus during each training session, but no cocaine was given during test sessions. Pre-treatments were given only before test sessions. Each rat was tested with only one pre-treatment drug, but doses within each drug were compared within subjects. Baseline training sessions (with cocaine delivered but no pre-treatment) were interspersed between test sessions. *Asterisk* indicates that suppression time was significantly reduced ($P < 0.05$) when 0.01 mg/kg of eticlopride was administered before the test session.