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# Differential involvement of dopamine receptors in conditioned suppression induced by cocaine

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**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 $D_1$-like receptor antagonist *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (SCH 23390) (0.001–0.03 mg/kg, i.p.), or the dopamine $D_2$-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, SCH 23390, eticlopride, or *N*-[4-(4-(2-methoxyphenyl)piperazinyl)butyl]-2-naphthamide (BP 897) (a dopamine $D_3$ partial receptor agonist; 0.1–1.0 mg/kg, i.p.) before test sessions in which the stimulus was presented without cocaine. Pre-treatment with either SCH 23390 or eticlopride during acquisition reduced the direct suppressant effects of cocaine, but conditioning was blocked only in rats that were treated with SCH 23390 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 $D_1$- and $D_2$-like receptors underlying these respective processes.
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## 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 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)

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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 (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 $D_1$-like receptor antagonist *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (SCH 23390), a dopamine $D_2$-like receptor antagonist (eticlopride), and a dopamine $D_3$ partial receptor agonist *N*-[4-(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).

## 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 h 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 Plasticonditioned 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