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Cocaine induces conditioned place preference and increases locomotor activity in male Japanese quail

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Abstract

The conditioned place preference (CPP) procedure is a popular method used for testing the rewarding properties of human drugs of abuse. Most CPP studies utilize mammalian models. However, avian species have better visual systems than rodent species, and because the cues that become associated with human drug-taking behavior are often visual, Aves might serve as an alternative animal model for investigating drugs of abuse. In three experiments, we examined the locomotor stimulant and rewarding effects of cocaine in adult male Japanese quail. In Experiment 1, cocaine increased locomotor activity relative to saline. In addition, behavioral sensitization was evident across repeated injections. In Experiment 2, CPP was established after six pairings of cocaine. Finally, the dopamine D_2 receptor subtype antagonist eticlopride did not attenuate acquisition of cocaine CPP in Experiment 3. Rather, subjects receiving pretreatment of eticlopride demonstrated a place preference for the cocaine-paired context. In contrast, pretreatment of eticlopride reduced cocaine-induced locomotor activity. The findings suggest that drug-reward processes may be highly conserved across species and that birds may serve as a viable model for investigating drug-reward processes especially with regard to the ability of cocaine to become associated with visual cues.

Keywords: Cocaine; Conditioned place preference; locomotor activity; Behavioral sensitization; Birds; Aves; Eticlopride; Dopamine antagonist

1. Introduction

There is an extensive literature using laboratory mammalian animal models to understand the neuropharmacological mechanisms of drug reward (see Bardo, 1998 for review). However, considerable controversy has surrounded the methods that are best for assessing the rewarding properties of drugs in animals. The self-administration (SA) paradigm is one of the most popular measures of drug reward (White et al., 1987). In the SA paradigm, delivery of the drug is made contingent on a response, and if the drug is rewarding, the preceding response should be repeated. Data on drug reinforcement gained through the SA technique are of great value. However, one disadvantage of the SA paradigm is that the subjects are required to perform a complex motor response while under the influence of a pharmacological treatment that may impair motor performance or perception. Alternatively, the conditioned place preference (CPP) procedure is widely used to assess the rewarding properties of drugs in the absence of potential pharmacological impairment during testing (e.g., (Bardo et al., 1995; Beach, 1957; Carr et al., 1989; Hoffman, 1989)). The typical CPP procedure consists of two (or more) different environments differentiated in terms of the number of stimulus modalities. One environment is consistently paired with drug treatment, while the other is paired with saline treatment. Following training, animals are tested in a drug-free state for their preference for the environment paired with the drug.

Although the SA paradigm better represents the addict's behavior of taking the abused drug, the CPP procedure involves classical conditioning (Siegel, 1979) and environmental cues (Johnson et al., 1998; Marlatt and Gordon, 1985; Modesto-Lowe et al., 1997), both of which are strongly related to drug abuse and relapse. From a clinical perspective, the SA paradigm is thought to model drug-taking behavior per se, whereas the CPP procedure serves as a model for cue-elicited conditioning. It has been suggested that the ability of cues associated with drugs of abuse to elicit place preference has important implications related to drug "craving" and relapse in humans (Hand et al., 1989; Neisewander et al., 1990).

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A drug of abuse commonly used in CPP studies is the psychostimulant cocaine. Cocaine, an indirect dopamine agonist, increases general locomotor activity at low to moderate doses (Antoniou and Kafetzopoulos, 1996; Elliott et al., 1987) in mammals. The acute motor effects of cocaine are related to increased dopaminergic transmission in the nigrostriatal and mesoaccumbens systems (Beninger, 1983; Kalivas et al., 1988). The mechanism of action of cocaine involves inhibition of the neuronal re-uptake of dopamine, norepinephrine, and serotonin (Hadfield and Nuggent, 1983; Reith et al., 1986). However, the rewarding effects of cocaine are attenuated by dopaminergic and not noradrenergic (de Wit and Wise, 1977; Yokel and Wise, 1975) or serotonergic antagonists (Lacosta and Roberts, 1993; Lyness and Moore, 1983), indicating that the neurotransmitter dopamine is involved in cocaine reward.

The rewarding effects of cocaine as mediated by dopamine have been demonstrated in both SA and CPP studies. Cocaine is self-administered by several mammalian species including rats (Stewart, 1984), mice (Rocha et al., 1998), monkeys (Slikker et al., 1984), and humans (McKim, 1997). In mammals, the dopamine receptor antagonists chlorpromazine, haloperidol, perphenazine, and pimozide block intravenous (iv) SA (de la Garza and Johanson, 1982; Johanson et al., 1976; Wilson and Schuster, 1972; Woolverton, 1986). In addition, cocaine-induced CPP has been established in mammals (Cervo and Samanin, 1995; Nomikos and Spyraki, 1988; Pruitt et al., 1995), and acquisition of CPP has been shown to be blocked with the D_1 antagonist SCH 23390 (Cervo and Samanin, 1995; Pruitt et al., 1995).

Although drug reward has been studied frequently in mammals, little research has been conducted on avian species. Hughes and McCormick (Hughes and McCormick, 1993) obtained psychomotor stimulatory effects of cocaine (0.0, 2.5, 5.0, 10.0, and 15.0 mg/kg ip) as measured by locomotor activity and vocalizations in young precocial domestic fowl. Furthermore, pigeons will maintain intravenous SA of cocaine (0.03 or 0.1 mg/kg/injection) on a fixed ratio (FR 50) schedule that can be decreased by the dopamine antagonist haloperidol (Winsauer and Thompson, 1991). In addition, CPP has been established in chicks with cocaine, morphine, and three amphetamine-like designer drugs (Bronson et al., 1996; Hughes et al., 1995). Therefore, there is reason to believe that drug-reward processes are conserved across species.

An avian model might be useful in studying cue-elicited drug conditioning especially with regard to visual cues. Although rodents have been the typical animal model used in drug-reward studies (see Bardo et al., 1995 for review) because of their limited visual capacity, they may not serve as an appropriate model for examining cue-elicited drug reward in which the most salient cues are visual. Quail have a well-developed visual system with color vision, and the perception of color is dominant over the perception of form and shape (Fidura and Gray, 1966). In this regard, domesticated Japanese quail (*Coturnix japonica*) may be ideal subjects to study drug reward that is primarily elicited by color visual cues.

In the present set of experiments, cocaine reward was investigated with male Japanese quail. Experiment 1 was conducted to examine the locomotor stimulant effects of cocaine. In Experiment 2, we used the CPP procedure to test the rewarding properties of cocaine. In a third experiment, we tested the effects of the D_2 dopamine receptor subtype antagonist eticlopride on the acquisition of cocaine-induced CPP and locomotor activity.

2. Experiment 1

Results from avian studies suggest that systemic cocaine administration within comparable dose range causes behavioral effects similar to those of mammalian species (Hughes and McCormick, 1993). However, the few studies available on cocaine-induced locomotor behavior in avian species have been conducted on chicks. Metabolic differences between chicks and adult fowl may result in different stimulant effects of cocaine. In order to make accurate cross-species comparisons, analysis of the stimulant effects of cocaine on adult birds is necessary. Adult chickens are not good captive animals for experimental purposes due to their large size. Therefore, adult quail were used in the present experiments because they are smaller and easier to handle. The purpose of Experiment 1 was to test the locomotor stimulant effects of cocaine on adult male Japanese quail. Subjects were injected with cocaine or saline, and their locomotor behavior was assessed. Psychomotor stimulant effects of cocaine should be evident if drug-treated birds show more locomotor activity than saline-treated birds.

2.1. Method

2.1.1. Subjects

A total of 12 male, drug-naive Japanese quail (*C. japonica*) ranging in age from 4 to 6 months served as subjects in the study. Birds were obtained from a colony at the University of Kentucky where they were hatched, raised, and kept in mixed-sex groups in brooders until 4–5 weeks of age before they were housed in individual wire-mesh cages (GQF Manufacturing; Savannah, GA). Lights in the laboratory were on from 6:00 a.m. to 10:00 p.m. daily. Food and water were available ad libitum. Animals were cared for, and the experimental procedures were conducted under the guidelines of the Institutional Animal Use and Care Committee at the University of Kentucky.

2.1.2. Apparatus

Six locomotor boxes, each 26.7 (long) × 22.9 (wide) × 43.2 cm (deep), were used in the present experiment. The boxes had plywood walls with wire-mesh floors and ceilings, and were bisected into four equal quadrants by two photobeams placed approximately 3.81 cm above the

floor. Each time a subject broke a beam, a computer program (prepared by Larry Hull from Lexington, KY) recorded it. The frequency of photobeam breaks was used as an index of locomotor activity. The computer program had a 0.2-s filter on the photobeams, so that if a subject stood in a beam, only one break was counted.

2.1.3. Drugs

Cocaine hydrochloride (National Institute on Drug Abuse; Bethesda, MD) was dissolved in physiological saline (0.9%) at a concentration of 5 mg/ml and injected intraperitoneally (ip) at a volume of 2-ml/kg body weight for a dose of 10 mg/kg.

2.1.4. Procedure

The 12 subjects were randomly assigned to either a cocaine ($n=6$) or saline group ($n=6$). Birds were weighed each day, injected intraperitoneally with either 10-mg/kg cocaine [Group Cocaine (C)] or saline [Group Saline (S)] and immediately placed in the boxes for 1 h. Six trials were conducted, one per day, with each group receiving the same treatment throughout the experiment. Subjects were assigned to the same box for each trial. Five extinction trials were conducted in the same manner as the conditioning trials except cocaine group subjects that received saline injections. Data were analyzed using a repeated-measures analysis of variance (ANOVA).

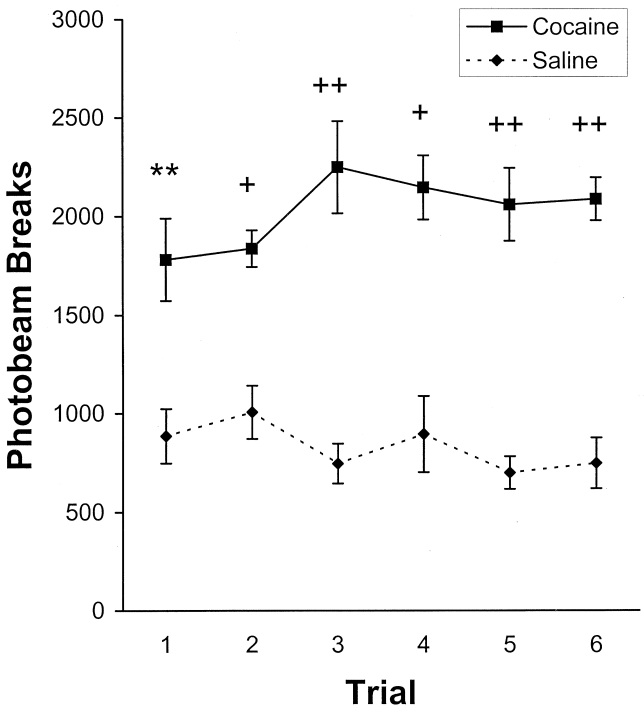

Fig. 1. Frequency of photobeam breaks (±S.E.M.) across six 1-h acquisition trials for subjects injected with either cocaine (10 mg/kg) or saline. $^{**}P<.01$; $^{+}P<.001$; $^{++}P<.0001$ vs. saline.

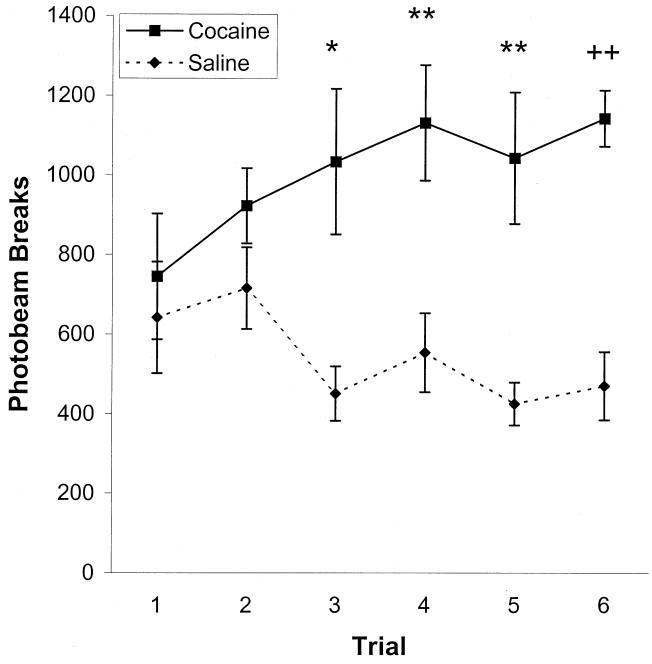

Fig. 2. Frequency of photobeam breaks (±S.E.M.) for the first 30 min of acquisition trials for subjects injected with either cocaine (10 mg/kg) or saline. $^{*}P<.05$; $^{**}P<.01$; $^{++}P<.0001$ vs. saline.

2.2. Results

Fig. 1 shows the frequency of photobeam breaks across trials for the cocaine and saline groups. Subjects injected with cocaine had significantly more photobeam breaks ($M=2026.92$, S.E.M. $=70.96$) than the saline control group

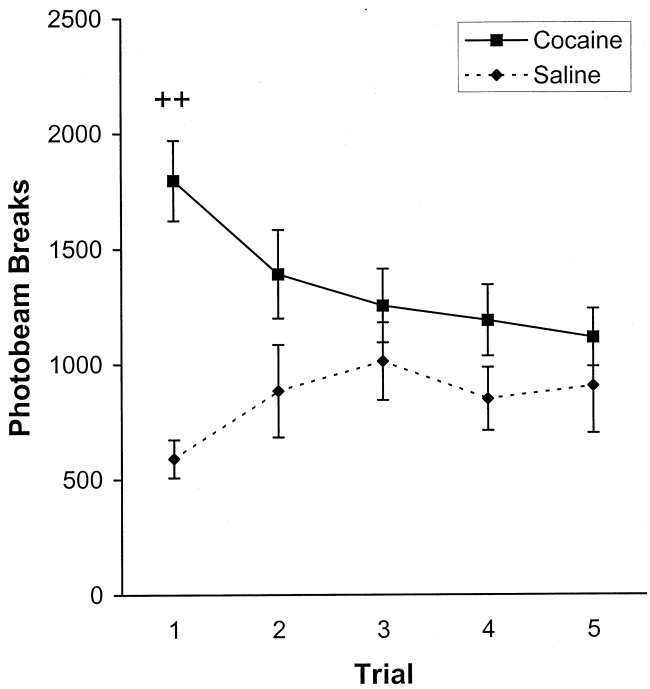

Fig. 3. Frequency of photobeam breaks (±S.E.M.) across extinction trials for the saline and cocaine groups. $^{++}P<.0001$ vs. saline.

[$M = 830.53$, S.E.M. = 53.84; $F(1,10) = 55.44$, $P < .0001$]. Independent ANOVAs performed on each trial indicated that Group C had significantly more locomotor activity than Group S during each trial [all F's(1,10) > 12.87, P's < .05]. No behavioral sensitization effects were evident across the 1-h trials for the cocaine group [$F(5,25) = 2.40$, $P = .07$]. However, behavioral sensitization was evident across trials during the first 30 min of each trial. Fig. 2 shows the frequency of photobeam breaks during the first 30 min of each 60-min trial. There was a significant trials × group interaction [$F(5,50) = 4.283$, $P < .005$]. Furthermore, independent repeated-measures ANOVAs indicated a significant increase in activity across trials for the cocaine group [$F(5,25) = 2.78$, $P < .05$] but no increase in activity across trials for the saline group [$F(5,25) = 2.43$, $P > .05$].

The number of photobeam breaks that the subjects made during extinction trials is represented in Fig. 3. Group C decreased photobeam breaks across trials, whereas Group S did not. A two-way (group × trials) repeated-measures ANOVA revealed a significant interaction [$F(4,40) = 6.91$, $P < .001$]. Independent ANOVAs indicated that cocaine and saline groups differed in locomotor activity only in the first extinction trial [$F(1,10) = 39.40$, $P < .0001$].

3. Experiment 2

CPP has been previously established in domestic chicks with both psychostimulants and morphine (Bronson et al., 1996; Hughes et al., 1995). (Hughes et al., 1995) demonstrated cocaine-induced CPP with chicks. In their study, cocaine (0.5, 1.0, and 2.0 mg/kg ip) was always paired with red stimulus cards, and saline was always paired with white stimulus cards. Six conditioning trials were conducted with chicks being placed in the test apparatus in pairs. Chicks that received cocaine paired with the red stimulus cards showed a significant preference for the red over the white stimulus cards. Although these results provide evidence for cocaine-induced place preference in domestic chicks, it may be of value to replicate this experiment using adult Aves. The purpose of Experiment 2 was to establish cocaine CPP in adult male Japanese quail. It was predicted that quail would prefer the context in which they received cocaine over a saline-paired context.

3.1. Method

3.1.1. Subjects

Subject characteristics and housing procedures were the same as in Experiment 1, except that 16 subjects were used. Subjects were naive to the drugs and to the CPP apparatus and procedure.

3.1.2. Apparatus

Two CPP boxes were constructed out of plywood and were 76.2 (long) × 21.6 (wide) × 45.7 cm (deep). Each box could be divided into three chambers by insertion of two vertical plywood dividers. Each of the two end chambers was 29.2 (long) × 21.6 cm (wide), while the middle chamber was 19.1 (long) × 21.6 cm (wide). The three chambers had distinct wall colors and floor textures. One end chamber had a wire-mesh floor and yellow-colored walls, while the other had green-colored walls and a brown paper floor. The middle chamber had white-colored walls and a white plywood floor. Although we used different floor textures and wall colors in each chamber to optimize the likelihood of the place preference, it is most probable that the colors of the chamber walls were the critical features of the chambers.

Japanese quail have excellent color vision (Mills et al., 1997), and it has been demonstrated that quail chicks show distinct preferences for different colors and color intensities (Kovach, 1974; Kovach and Wilson, 1975). Green and yellow were chosen because Japanese quail show a preference for colors in the middle range and short end of the color spectrum (Kovach, 1974; Kovach and Wilson, 1975). That is, quail chicks prefer yellow and green over blue and red. Therefore, we chose yellow and green to increase the saliency of the visual cues of the chambers and to optimize the likelihood of a response.

3.1.3. Procedure

3.1.3.1. Habituation. Because of the neophobic nature of Japanese quail, we conducted several habituation trials. During habituation, the CPP boxes were partitioned with the opaque dividers, and each bird was confined to one end chamber for 30 min. On the following day, subjects were placed in the other end chamber for 30 min. Subjects received two 30-min habituation sessions in each end chamber, one per day for 4 days.

Often, quail will not actively explore when first introduced into a new environment. Habituation to the CPP boxes therefore increased the likelihood that the subjects would sample both chambers. It is not likely that this amount of habituation will result in latent inhibition, the interference of conditioning when familiarization with a stimulus presented by itself impairs the subsequent conditioning of that stimulus. Cusato and Domjan (1998) found no latent inhibition in Japanese quail after 59 pre-exposures to a conditioned stimulus.

3.1.3.2. Preference tests

Preference tests were given before and after conditioning trials. Prior to conditioning, subjects were given free access to all of the chambers in two 15-min preference tests conducted on consecutive days to determine an initial context preference. Quail were placed in either the green or yellow chamber and allowed to move about the entire box. The purpose of starting each subject in the end rather than the middle chamber was to ensure that subjects sampled both end chambers. A place preference was evident as the chamber in which the subject spent the majority of its

time. Subjects not demonstrating a clear preference (defined as spending 40% or more of the time in one chamber) were randomly assigned to one of the two groups.

After conditioning trials, subjects were given another 15-min preference test. They were placed in the white chamber with removed dividers so that they had free access to the entire box. All preference test trials were videotaped.

3.1.3.3. Conditioning

During each conditioning trial, subjects were weighed, injected intraperitoneally with either 10-mg/kg cocaine (Group C; $n=9$) or saline (Group S; $n=7$), and immediately placed in the context that they least preferred (the trained context) for 30 min. The next day, subjects were placed in the opposite context (the untrained context) where they received a saline injection. This was repeated for six trials across 12 days.

3.1.3.4. Statistical analysis

To determine whether or not a place preference occurred, a preference score was calculated as the (time in trained context)/(time in trained context + time in untrained context). The greater the preference score, the more time that the subject spent in the trained context after conditioning. For saline subjects, the trained context was their initially non-preferred context. Data were analyzed using a two-factor repeated-measures ANOVA with group (cocaine vs. saline) as the between-subjects factor and test (pre- vs. post-test) as the within-subjects factor. Independent one-way repeated-measures ANOVAs were conducted for the main effect of test.

3.2. Results

Fig. 4 represents the mean preference scores for both groups. The preference scores for subjects in Group C significantly increased from pre- ($M=0.33$, S.E.M. $=0.07$) to post-test ($M=0.75$, S.E.M. $=0.10$), whereas preference scores of Group S did not differ from pre- ($M=0.31$, S.E.M. $=0.07$) to post-test [$M=0.18$, S.E.M. $=0.06$; $F(1,14)=21.03$]. Independent one-way repeated-measures ANOVAs revealed that the increase in preference scores was significant for the cocaine [$F(1,8)=37.62$, $P<.0005$] but not for the saline group [$F(1,6)=1.53$], thus demonstrating that cocaine CPP was obtained.

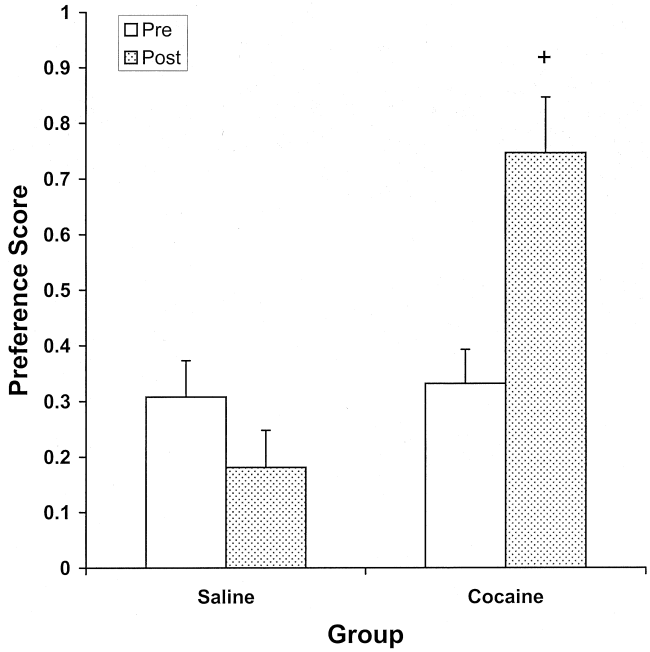

Fig. 4. Preference scores (±S.E.M.) of pre- and post-tests for subjects injected with either cocaine (10 mg/kg) or saline. $^{+}P<.001$ vs. pre-test.

4. Experiment 3

Despite the paucity of drug research with avian species, there has been an abundance of work investigating avian dopamine systems. Dopamine receptors have been identified via autoradiography in several avian species (Ball et al., 1995; Dietl and Palacios, 1988; Richfield et al., 1987). Ball et al. (1995) found that D_1 receptors were enriched in the lobus parolfactorius and the paleostriatum augmentatum of Japanese quail. These areas are believed to be homologous to the mammalian caudate–putamen (Parent, 1986; Reiner et al., 1984), which is also enriched with D_1 receptors (Camps et al., 1990; Richfield et al., 1987). Similarly, the regional distribution of D_2-like receptors in quail brain as measured by homogenate binding is similar to that of mammals (Levens et al., in press). Dopamine has also been measured via fluorometric assay or high-performance liquid chromatography in several avian species (Balthazart et al., 1992; Barclay and Harding, 1990). Furthermore, tyrosine hydroxylase (TH), the biosynthetic enzyme of dopamine, has been localized in the brain of several species via immunohistochemistry (Bailhache and Balthazart, 1993; Bottjer, 1993). In Japanese quail, TH fibers have been co-localized with areas containing high D_1 receptor densities (Ball et al., 1995). Identification of dopamine and the localization of D_1 and D_2 dopamine receptor subtypes in avian species suggest that dopaminergic mechanisms are conserved from mammalian to avian species. Hence, drugs of abuse that have dopaminergic mechanisms of action may be rewarding in Aves as well as in mammals.

Evidence for the role of dopamine in mediating drug reward comes in part from studies using dopamine antagonists. Some of these studies have demonstrated that acquisition of drug-induced CPP may be blocked by pretreatment with a dopamine antagonist. For example, acquisition of amphetamine-induced CPP has been blocked with the D_1 antagonist SCH 23390 (Bardo et al., 1999; Hiroi and White, 1991; Hoffman and Beninger, 1989; Leone and Di Chiara, 1987) as well as with the D_2 antagonists metoclopramide (Hiroi and White, 1991; Hoffman and Beninger, 1989),

haloperidol (Leone and Di Chiara, 1987), α-flupenthixol (Hiroi and White, 1991), sulpiride (Hiroi and White, 1991), and eticlopride (Bardo et al., 1999). Similarly, cocaine-induced CPP has been attenuated with the D_1 antagonist SCH 23390 (Cervo and Samanin, 1995; Pruitt et al., 1995). Thus, some mammalian studies suggests that dopamine antagonists block cocaine-induced CPP.

Eticlopride is a selective D_2 dopamine receptor antagonist that has been shown to reduce locomotor activity (Bardo et al., 1999; Fowler and Liou, 1998) and block psychostimulant reward (Bardo et al., 1999; Pruitt et al., 1995). The purpose of Experiment 3 was to investigate the effects of eticlopride on cocaine-induced CPP and locomotor activity in adult male Japanese quail. We hypothesized that eticlopride would block the acquisition of cocaine-induced CPP and increased locomotor activity induced by cocaine.

4.1. Method

4.1.1. Subjects

Subject characteristics and housing procedures were the same as in Experiments 1 and 2, except that 28 male quail were used as subjects. Subjects had not been previously exposed to drugs or to the CPP paradigm. Data for one subject were omitted from the analyses because it did not sample both contexts during pre- and post-tests.

4.1.2. Apparatus

The apparatus was similar to that of Experiment 2, except that the CPP boxes were also equipped to measure locomotor behavior. As in Experiment 1, boxes were divided into four quadrants by two photobeams located approximately 3.81 cm above the floor.

4.1.3. Procedure

Habituation and conditioning procedures were the same as in Experiment 2, except that subjects were randomly assigned to one of four groups: Group Eticlopride/Cocaine (E/C; $n=8$), Group Cocaine (C; $n=8$), Group Eticlopride (E; $n=6$), and Group Saline (S; $n=6$). Prior to conditioning, subjects were given a preference test using the same procedure as in Experiment 2. Every other day on conditioning trials, subjects were weighed, and Groups E/C, E, and C were injected with eticlopride (1 mg/kg ip), eticlopride, and saline, respectively. About 15 min later, Groups E/C and C received cocaine injections (10 mg/kg ip), while Group E received a saline injection. Group S received two saline injections at 15 min apart, and at the same time, the other groups were injected. All subjects were immediately placed into their least preferred context (the trained context) after the second injection.

On alternate days, all birds received two saline injections at 15 min apart and were placed in the opposite context (the untrained context). Six drug-conditioning and six saline trials were conducted. After 12 days, a preference test similar to that of Experiment 2 was given, and the same calculation was used to derive preference scores: (time in trained context)/(time in trained context + time in untrained context). The same analyses were performed as in Experiment 2. Locomotor activity was recorded when subjects were in the green and yellow contexts during conditioning trials but not during preference tests.

4.2. Results

4.2.1. Place preference

Preference scores for all of the groups during pre- and post-tests are shown in Fig. 5. All groups showed an increase in preference scores from pre- to post-test. This resulted in a significant main effect of trial [$F(1,23)=10.11$, $P<.005$] but not of group [$F(3,23)=0.27$]. The group × trial interaction was also not significant [$F(3,23)=0.70$]. Independent ANOVAs conducted on the pre- and post-test preference scores of each group indicated that both Groups E/C and C increased their preference for the trained context from pre- to post-tests [$F(1,7)=10.65$ and $F(1,6)=6.56$, $P<.05$, respectively], whereas Groups E and S did not [$F(1,5)=1.15$ and 0.34, respectively].

4.2.2. Locomotor activity

Fig. 6 shows the mean number of photobeam interruptions across drug trials for the four groups. Cocaine significantly increased locomotor activity compared to the other three groups, while eticlopride attenuated this increase. There was a significant main effect of group [$F(3,23)=10.99$, $P<.0001$] but not of trial [$F(5,115)=2.27$] or a group × trial trial interaction [$F(15,115)=1.58$]. A separate two-factor

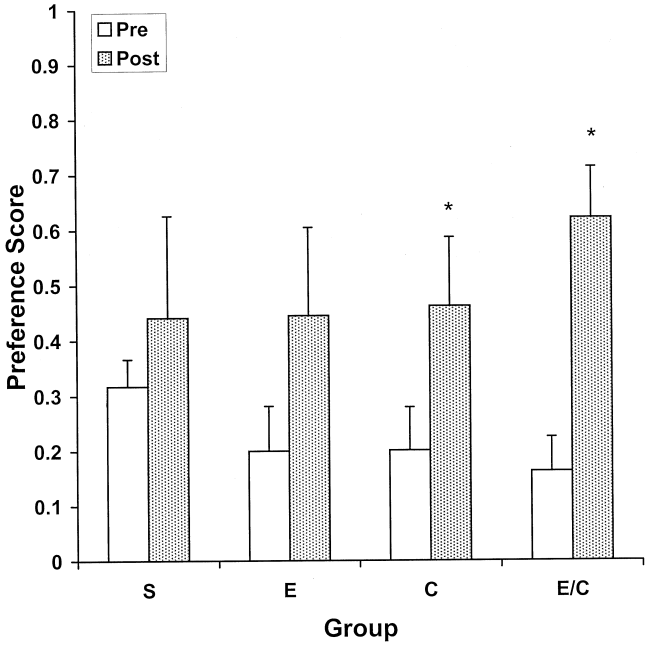

Fig. 5. Preference scores (±S.E.M.) of pre- and post-tests for Groups S, E, C, and E/C. * $P<.05$ vs. pre-test.

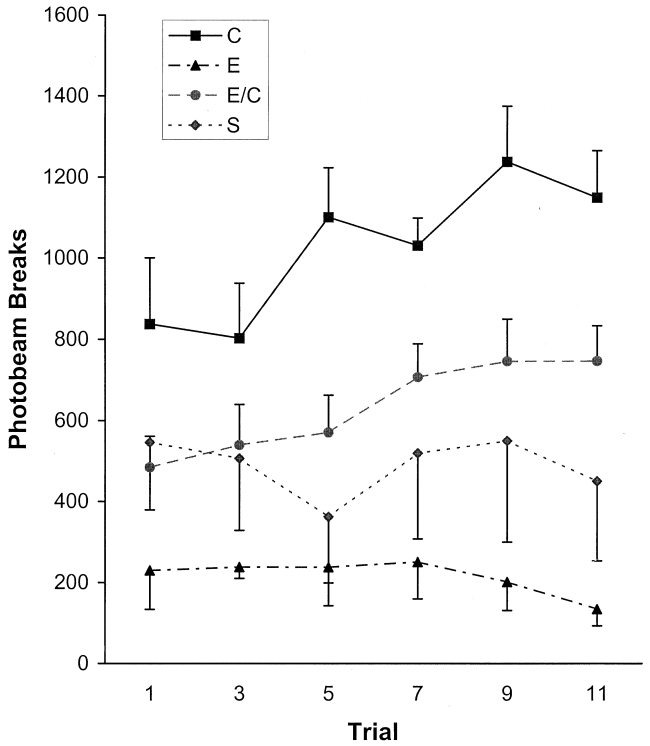

Fig. 6. Frequency of photobeam breaks (±S.E.M.) for the six 30-min drug trials for Groups S, E, C, and E/C.

repeated-measures ANOVA demonstrated that Group C had greater locomotor activity ($M = 1026.60$, S.E.M. = 54.60) than Group E/C [$M = 632.10$, S.E.M. = 38.30; $F(1,13) = 16.19$, $P < .005$], indicating that eticlopride impaired cocaine-induced locomotor activity. In addition, activity significantly increased across trials for Group C [$F(5,30) = 3.162$, $P < .05$] but not for Group E/C [$F(5,35) = 1.795$, $P = .14$], further suggesting that eticlopride hindered cocaine-induced locomotor effects. Moreover, Group E ($M = 211.97$, S.E.M. = 29.16) did not have significantly lower activity than Group S [$M = 488.81$, S.E.M. = 75.34; $F(1,10) = 2.08$, $P = .18$], although this may have been the result of a floor effect in Group E (Fig. 6).

5. General discussion

The present studies investigated the locomotor stimulant and rewarding effects of cocaine in male Japanese quail. Systemic cocaine administration increased general locomotor activity compared to saline. In addition, cocaine induced a CPP after six pairings of cocaine with that context. Although acquisition of that preference was not blocked by pretreatment of the dopamine D_2 antagonist eticlopride, cocaine-induced locomotor activity was blocked by eticlopride. The findings suggest the following: (1) cocaine has a stimulant effect on avian species, (2) cocaine is rewarding to avian species, and (3) dopaminergic mechanisms of the locomotor stimulant effect of cocaine may be conserved between mammalian and avian species.

In mammals, repeated administration of cocaine results in augmented stimulant effects of the drug that are manifest as cocaine-induced hyperactivity or generalized locomotor activity (Post and Rose, 1976; Reith et al., 1987). Similar to previous studies with mammalian species and one avian study (Hughes and McCormick, 1993), systemic cocaine injections increased general locomotor activity and resulted in behavioral sensitization in the present study. This increased locomotor activity may be related to increased dopaminergic transmission in dopamine-related brain areas, just as increased locomotor activity in mammals is thought to be related to increased dopaminergic transmission in the nigrostriatal and mesoaccumbens systems (Beninger, 1983; Kalivas et al., 1988).

Conditioned locomotor activity was evident in the first extinction trial of Experiment 1 (see Fig. 3). In the absence of cocaine, locomotor activity was just as high as during the conditioning trials when cocaine was administered. Although this conditioned effect disappeared by the second extinction trial, similar results have been found with rats (Carey and Gui, 1998), and our effect is stronger than that found in rats in some cases (e.g., Pickens and Crowder, 1967). This conditioned drug effect is believed to stem from Pavlovian processes where an association is formed between contextual stimuli (CS) and the unconditioned locomotor response (US). Although the exact neurochemical mechanisms of this process are not completely understood, it seems likely that it is conserved between mammals and Aves.

As indicated by a significant place preference, cocaine was found to be rewarding to male quail. Again, these results are similar to those found in mammalian species (see Hoffman, 1989 for review) and domestic chicks using morphine and psychostimulants (Bronson et al., 1996; Hughes et al., 1995). In mammals, dopaminergic mechanisms particularly in the mesolimbic system mediate reward processes in general as well as those of drugs of abuse. Avian species may have similar dopaminergic mechanisms to mediate reward processes. The dopaminergic system of birds has been found to consist of the same or comparable receptor subtypes and similar regional distribution of the subtypes as mammals (Ball et al., 1995; Balthazart et al., 1992; Levens et al., in press). One uncertainty concerning reward circuitry in the avian brain is the homology of dopamine-related brain structures to those of the mammalian brain. One can only speculate that the cocaine-induced CPP that resulted in the present study reflects cocaine effects on dopaminergic mechanisms in homologous avian brain regions. Regardless, our results clearly indicate similar behavioral effects of cocaine in birds as those found in mammals.

In the present study, pretreatment of eticlopride followed by cocaine did not attenuate the CPP, but it blocked cocaine-induced locomotor activity and sensitization. Subjects that were given pretreatment of eticlopride followed by cocaine

showed a significant place preference for the cocaine-paired context. This place preference was similar to that of subjects that received cocaine alone. In contrast, subjects that received eticlopride pretreatment followed by cocaine had depressed locomotor activity across acquisition trials compared to subjects that received cocaine alone. Subjects that received cocaine alone also showed sensitization to the cocaine locomotor effects, whereas Group E/C did not. Furthermore, eticlopride alone did not result in significantly lower activity than saline, although the lack of variability in activity across trials for Group E suggests a floor effect.

Although we predicted that the D_2 antagonist eticlopride would block cocaine CPP, our findings are consistent with some of the previous literature in rats. Several studies have reported an inability to block cocaine-induced CPP with systemic administration of D_2 antagonists (Cervo and Samanin, 1995; Morency and Beninger, 1986; Spyraki et al., 1982). Cocaine CPP was blocked with pimozide but only when cocaine was microinjected directly into the lateral ventricle (Morency and Beninger, 1986). Furthermore, while cocaine CPP was attenuated in 10-day-old rats with systemic injections of sulpiride and eticlopride, they failed to attenuate cocaine CPP in 17-day-old rats (Pruitt et al., 1995). Thus, most of the mammalian literature indicates that in general the D_2 dopamine receptor subtype antagonists administered intraperitoneally or subcutaneously do not attenuate cocaine CPP. Our findings with birds are in agreement with these previous findings.

Another possible explanation for the failure of eticlopride to block cocaine CPP might be related to the aversive properties of cocaine. Cocaine has anxiogenic effects as measured by several animal paradigms (DeVries and Pert, 1998; Ettenberg and Geist, 1991). Evidence suggests that these effects may be mediated in part by the mesolimbic dopamine system. Both cocaine (Kalivas et al., 1988) and stress (Miczek et al., 1999) individually increase extracellular dopamine levels in the mesolimbic system. For example, Miczek et al. (1999) found that rats generalized between cocaine and social defeat stress by responding on the drug-appropriate lever after social defeat stress. Presumably, the rats were generalizing between the anxiogenic effects of cocaine and those produced by social defeat stress. Therefore, if there is dopaminergic involvement in the anxiogenic effects of cocaine, it is possible that eticlopride attenuated some of these effects in the current study, thereby increasing the reward incentive of the cocaine-paired context for Group E/C.

The inability to attenuate cocaine-induced CPP with eticlopride in the present study could also have been due to novelty effects. Eticlopride reduced cocaine-induced locomotor activity; thus, it is possible that it prevented habituation to the cocaine-paired context on conditioning days (Bardo et al., 1999). Therefore, on test day, this context may have been more novel to the quail, making them more likely to prefer it. It has been shown that rats prefer a novel over a more familiar environment (Bardo et al., 1989; Parker, 1992). However, there are two reasons that suggest that this was not the case in the present study. First, each subject received two habituation trials to the cocaine-paired context in the absence of any drug in addition to the time spent in that context during the six conditioning trials. Thus, a preference for novelty appears unlikely due to the amount of exposure to the cocaine-paired context. Second, the eticlopride alone group did not show a place preference. If eticlopride was blocking novelty, one would expect this group to also demonstrate a place preference.

That eticlopride failed to block cocaine-induced CPP but blocked cocaine-induced locomotor activity might be indicative of a dissociation between the mechanisms mediating locomotor stimulant and reward effects in an avian species. Dissociation of these mechanisms has been found in mammals. For example, low doses of eticlopride (0.02 mg/kg iv) block amphetamine-induced locomotor activity but not CPP in rats (Bardo et al., 1999). Admittedly, however, the likelihood that our findings are a result of the dissociation between the mechanisms is speculative. It is just as likely that our dose of eticlopride was high enough that it was ineffective at blocking the cocaine-induced place preference despite a decrease in cocaine-induced locomotor activity. Similar results have been found in rats, indicating that a high (but not a low) dose of the dopamine antagonist haloperidol did not attenuate methylphenidate-induced CPP despite a marked decrease in locomotor activity (Mithani et al., 1986).

Finally, the inability to attenuate cocaine CPP with eticlopride may also implicate the involvement of other transmitters in cocaine reward. Recent evidence suggests that serotonergic mechanisms may play a modulatory role in cocaine reward. Dopamine transporter (DAT) knockout mice self-administer cocaine at levels similar to wild-type mice (Rocha et al., 1998), and they demonstrate a CPP to cocaine-paired stimuli (Sora et al., 1998). Since cocaine inhibits neuronal re-uptake of dopamine, serotonin, and norepinephrine, it is likely that serotonin or norepinephrine is also involved in cocaine reward.

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