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Cocaine-induced behavioral sensitization and conditioning in male Japanese quail

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Abstract

Repeated intermittent cocaine treatment often results in behavioral sensitization or an augmented response to cocaine. Cocaine-induced behavioral sensitization may be an important contributor to cocaine addiction and abuse. Some studies have also shown that conditioned drug effects may play a role in behavioral sensitization. The current experiment utilized a simplified discrimination paradigm to investigate behavioral sensitization and the role of conditioning in an avian species. Male Japanese quail received alternating injections of cocaine (10 mg/kg ip) paired with a context and saline injections paired with a different context. They were later given a cocaine challenge followed by and a saline challenge in the drug-paired context. Results showed that birds that received cocaine paired with one context also demonstrated behavioral sensitization to a cocaine challenge given after a withdrawal period and they developed conditioning to the drug-paired context. A saline control and a control group that received cocaine that was not paired with the test context failed to demonstrate sensitization or conditioning. The findings demonstrate visual discrimination learning and implicate the role of Pavlovian conditioning in behavioral sensitization.
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Chronic pre-exposure to cocaine may lead to a progressive and enduring enhancement of a motor stimulant effect, a phenomenon referred to as behavioral sensitization (e.g., Kalivas et al., 1998; Robinson and Berridge, 2001). Sensitization refers to the augmentation of a behavioral response to drugs of abuse that occurs with repeated administration and persists long after drug use is discontinued (e.g., Robinson and Becker, 1986). It is considered to be an important contributor to the addictive potency of cocaine (Robinson and Berridge, 1993). Cocaine-induced behavioral sensitization has been well documented in rats (e.g., Kalivas and Duffy, 1993; Post and Rose, 1976). Although less well documented, studies with avian species have demonstrated similar results. Hughes and McCormick (1993) found a dose-dependent increase in cocaine-induced locomotor activity and vocalizations in cockerels. More recent studies with Japanese quail have also demonstrated cocaine behavioral sensitization (Levens and Akins, 2001, 2004), including similar dose-dependent and temporal effects as those found in rodents (Geary and Akins, 2007).

Pavlovian conditioned drug effects appear to play an important role in behavioral sensitization. A number of studies have demonstrated the importance of conditioning in the development and expression of sensitization (Hinson and Poulos, 1981; Keller et al., 2002; Pert et al., 1990; Siegel et al., 1987; Wynne and Delius, 1995). The findings of these experiments indicate that when drugs are administered in association with a unique environment, contextual cues acquire the properties of a conditioned stimulus (CS), with the drug acting as the unconditioned stimulus (US). After pairing of the CS with the US, the CS (context) alone comes to elicit drug-like effects. When drugs serve as the US, the conditioned response resembles the unconditioned response, the drug-like response (Anagnostaras and Robinson, 1996; Hiroi and White, 1989; see

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also Martin-Iverson and Fawcett, 1996 for review). Several studies have demonstrated context specific conditioned locomotor activity to drugs (Beninger and Hahn, 1983; Hinson and Poulos, 1981; Levens and Akins, 2004; Post et al., 1981; Weiss et al., 1989).

The present experiment utilized a simplified drug discrimination procedure to assess behavioral sensitization and contextual conditioning. A typical drug discrimination procedure involves the use of an interoceptive drug cue to signal or "set the occasion" for when a CS will be reinforced (positive feature) or the use of the absence of a drug cue to signal or "set the occasion" for when that CS will not be reinforced (negative feature) (e.g., Holland, 1983; Rescorla, 1993). Previous research has shown that drugs can serve as positive features (Palmatier et al., 2004, 2005; Palmatier and Bevins, 2007) or negative features (Troisi and Akins, 2004; Bevins et al., 2006). The present experiment does not involve using the drug cue as an occasion setter but rather context appears to function as the CS and the cocaine drug state as the US.

The present experiment utilized an avian model to study discriminative learning and the role of Pavlovian conditioning in behavioral sensitization. The use of avian models to investigate drug effects may have additional relevance to human drug abuse because these studies involve using visual cues that may become conditioned to later elicit craving. Several studies have demonstrated that drug cues may become associated with a drug state through Pavlovian conditioning and that, in the absence of the drug, these cues may come to elicit conditioned physiological and subjective responses. These responses may initiate craving and trigger relapse (Childress et al., 1986, 1999; O'Brien et al., 1988).

In the present experiment, male Japanese quail were given alternating injections of cocaine in a chamber with distinct visual cues and saline in another. One control group received alternating injections of saline in each distinct chamber and another control group received alternating injections of cocaine and saline in their home cage. Later a cocaine challenge and a saline challenge were administered to assess cocaine sensitization and conditioning, respectively.

1. Methods

1.1. Subjects

Twenty male Japanese quail (*Coturnix japonica*) approximately 6–12 months old served as subjects. Quail was hatched (from eggs purchased from GQF Manufacturing; Savannah, GA) and raised at the University of Kentucky. After hatching, chicks were housed together in a heated brooder until sexual differentiation, 28–30 days posthatch. After sexual differentiation, males were individually housed in metal cages (50.8 × 25.4 × 21.4 cm). The birds were maintained on a 16:8 light/dark schedule with food and water available ad libitum. The experimental protocol for this experiment was approved by the University of Kentucky IACUC for the use of animal subjects and the procedures are in compliance with NIH "Guide for Care and Use of Laboratory Animals".

1.2. Apparatus

Sixteen standard locomotor activity chambers (28.6 long cm × 21.2 wide cm × 21.2 cm deep; Med Associates; Georgia, VT) were used to quantify locomotor activity. All chambers had wire mesh floors covered with brown paper and clear plastic ceilings. Half of the chambers had green and yellow alternating stripes on the walls (the colored context) and the other half had white walls (the white context). Thus, the two chambers were distinct. Each chamber had six photobeams that were approximately 6.4 cm apart and 3.2 cm above the floor. A Med Associates program (Georgia, VT) was used to collect photobeam breaks in 5 min increments.

1.3. Drugs

Cocaine hydrochloride (National Institute for Drug Abuse; Bethesda, MA), was mixed with saline (0.9% NaCl) at a volume of 3 ml/kg. (This volume was chosen to better control the precision of mixing and injecting since Japanese quail weigh less than rodents.) Cocaine was injected intraperitoneally (ip) at a dose of 20 mg/kg.

2. Procedure

Birds were randomly assigned to one of three groups: Paired Cocaine (PC), Saline (S), and Unpaired Cocaine (UC), with *n*s of 7, 6, and 7, respectively, and the experiment was conducted in two replications. Birds were given 1 day of habituation during which they were exposed to a white and a green and yellow striped context for 30 min each. Presentation of white and striped contexts was counterbalanced within each group. During discrimination training, group PC received alternating injections of cocaine and saline in either the striped or white context. Cocaine was paired with one of the contexts throughout training. Treatment was counterbalanced with context such that half of the birds received cocaine paired with the striped context and the other half had cocaine paired with the white context. Group S received the same treatment as group PC but was injected with saline and placed in the white context on alternating days with the striped context. The UC group received no injection prior to each locomotor session, but received alternating days of cocaine and saline administration in their home cage 2 h after the locomotor session. The purpose of this group was to determine whether cocaine injections in the home cage would result in sensitization to a cocaine challenge given in a context that was never paired with cocaine. Each session was 60 min. A total of 20 injections were given, one per day for 20 days. Photobeam breaks were collected for all groups during the locomotor sessions.

Following discrimination training, birds remained in their home cages for a 14-day withdrawal period. A cocaine challenge (10 mg/kg ip) was given to all groups. Group PC received the cocaine challenge in their previously trained cocaine-paired context. Half of groups UC and S received cocaine in one context and the other half in the other context. The challenge dose was half of the original training dose

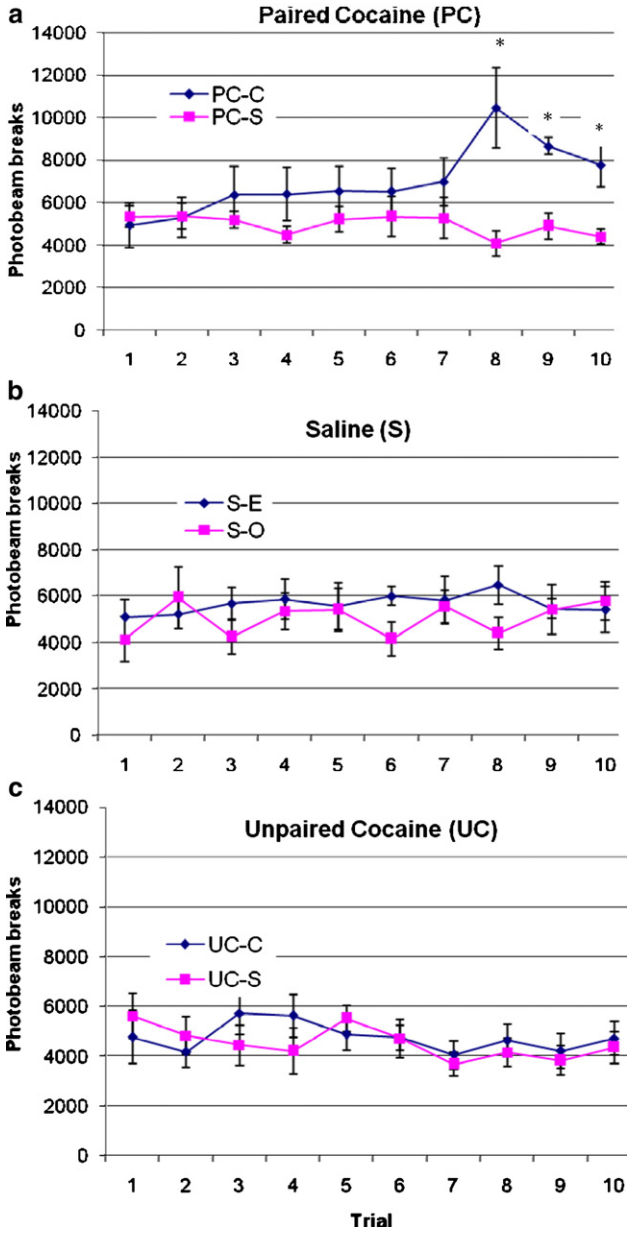

Fig. 1. a–c. Mean photobeam breaks during the 60 min locomotor activity session for trials 1–10. Fig. 1a. illustrates group PC on cocaine and saline alternating trials. The (*) indicates a significant difference between the saline and cocaine locomotor activity. Fig. 1b. represents group S on alternating even ("E") and odd ("O") days of saline trials. Fig. 1c. illustrates group UC on alternating days of receiving unpaired cocaine in the home cage or saline trials.

because this is a more conservative test of sensitization compared to giving the training dose. Following the challenge trial, two re-training days were given to re-establish activity levels to training levels. Re-training trials were conducted similarly to discrimination training and were, a day later followed by the administration of a saline challenge. Group PC received the saline challenge in the context that was paired with cocaine during discrimination training. For groups S and UC, administration of the saline challenge was counterbalanced between the contexts. The purpose of the saline challenge was to test for a Pavlovian conditioned effect indicated as an increase in locomotor activity in the absence of cocaine relative to control levels of locomotor activity. The significance level was set at $p<0.05$.

3. Results

Fig. 1a–c illustrates locomotor activity across training trials for groups PC (1a), S (1b), and UC (1c). For group PC, the figure shows locomotor activity during alternating cocaine and saline trials. For groups S and UC, the figure illustrates locomotor activity in alternating contexts, either on even (E) or odd (O) training trial days. Independent repeated-measures ANOVAs were conducted across trials for each group. Group PC showed an increase in locomotor activity during the last 3 cocaine administrations but not during saline administration. Post hoc analyses indicated that locomotor activity was greater for group PC compared to group S during the last 6 trials (trials 8–10 in Fig. 1a). A repeated-measures ANOVA resulted in a significant group X trial interaction for group PC, $F(2,24)=8.01$. Independent repeated-measures ANOVAs did not reveal significant group X trial interactions for either group S, $F(2,20)=0.97$, or group UC, $F(2,24)=0.49$.

During the cocaine challenge, all groups were administered 10 mg/kg cocaine. For group PC, the cocaine challenge was given in the cocaine-paired context. For groups S and UC, presentation of the challenge was counterbalanced between the two contexts. Fig. 2 illustrates the locomotor activity during the first trial versus the cocaine challenge for groups PC, S, and UC. We predicted that male quail that previously received cocaine in the trained context would show greater locomotor activity during the challenge than during the first trial, indicative of behavioral sensitization. We also predicted that neither of the control groups would show sensitization. Therefore, we conducted planned comparisons between trial 1 and the challenge trial for each group. Repeated-measures ANOVAs revealed a

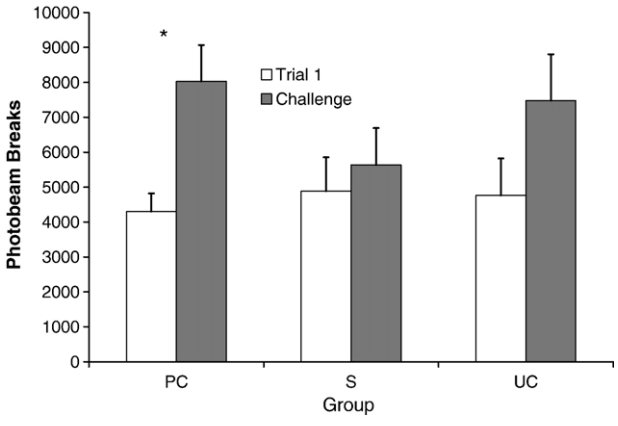

Fig. 2. Mean photobeam breaks during trial 1 of discrimination training and compared with the cocaine challenge. All groups were administered half the original training dose. Group PC was given the cocaine challenge in the cocaine-paired training context, while groups S and UC were counterbalanced between contexts. The (*) indicates a significant difference between trial 1 and the cocaine challenge.

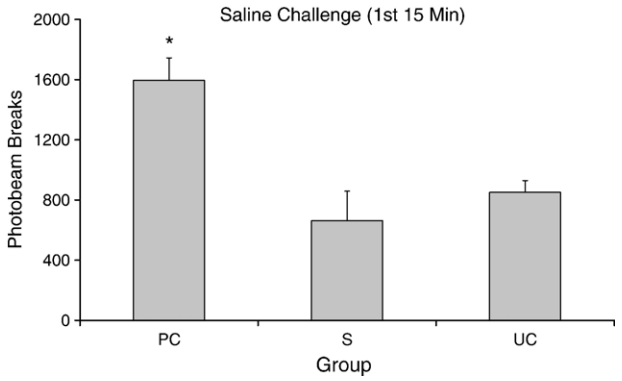

Fig. 3. Mean photobeam breaks for groups PC, S, and UC during the first 15 min of the 60 min saline challenge. Group PC received the saline challenge in the cocaine-paired context, while the context that groups S and UC received saline was counterbalanced between the two contexts. The (*) indicates that group PC is significantly difference from group S.

significant main effect of trial for group PC, $F(1,6)=30.93$, but there was no significant main effect of trial for groups S and UC, $F(1,5)=0.32$ and $F(1,6)=1.94$, respectively. Interestingly, group UC appears to show an increase in locomotor activity after the challenge trial compared to trial 1. This would have suggested that they had also been sensitized to cocaine but that sensitization was not context specific in the present experiment. However, the difference in responding on trial 1 versus the challenge trial was not statistically significant for group UC.

To test for a conditioned effect, a saline challenge was given after birds were given two additional re-training trials to re-establish training levels of responding. The saline challenge was given in the context previously trained with cocaine for the cocaine group. Half of groups UC and S received cocaine in one context and the other half in the other context. An ANOVA failed to reveal differences in locomotor activity during the 60 min saline challenge between any of the groups, $F(2,17)=0.92$. Means were 3915.86 (SEM=666.88), 5581.83 (SEM=1 395.3), and 4242.86 (SEM=585.91) for groups PC, S, and, UC, respectively. However, Carey and Damianopoulos (2006) observed a cocaine-conditioned drug effect with one saline injection during a 20 min test, suggesting that a conditioned effect might be evident early during a saline challenge. Therefore, in the current experiment, an analysis of the first 15 min of locomotor activity during the saline challenge was performed. Fig. 3 shows the locomotor activity response for each group during the first 15 min of the 60 min saline challenge. Results showed that the group that received cocaine paired with the context during training (group PC) demonstrated greater locomotor activity than the other groups, $F(2,17)=11.76$. Fischer's Protected LSD post-hoc analysis indicated that group PC had greater locomotor activity than groups S and UC but that neither of these groups differed from each other.

4. Discussion

In the current experiment, cocaine-induced behavioral sensitization was evident. Group PC showed increased locomotor activity to a challenge of half the training dose of cocaine compared to the first cocaine injection. Control groups did not demonstrate increased locomotor activity from the first injection to the challenge. These findings replicate previous findings in rodents (e.g., Davidson et al., 2002; Kalivas et al., 1998) and in aves (Geary and Akins, 2007; Levens and Akins, 2001, 2004; Hughes and McCormick, 1993).

A drug discrimination was also evident in the current experiment. Group PC demonstrated increased locomotor activity during cocaine sessions but not during saline sessions. The two control groups, one that received saline and the other that received cocaine in the home cage, did not show a pattern of discrimination. It has been well-established in the rodent literature that animals exhibit discriminations between drug states. (e.g., Stolerman and D'Mello, 1981; Childs et al., 2006). Palmatier et al. (2005) used a Pavlovian discrimination task in which rats were given nicotine followed by the presentation of a conditioned stimulus (CS) and subsequent sucrose presentation. In the absence of the drug, the same CS was presented but sucrose was withheld. As a result, anticipatory food seeking behavior developed to the CS that was presented on drug sessions but not on saline sessions. Similar findings have been reported by Troisi and Akins (2004) in male Japanese quail using drug discrimination and a sexual conditioning procedure. Male quail were given saline followed by presentation of a wooden block (the CS) that signaled copulatory opportunity with a female quail. On cocaine sessions, presentation of the wooden block signaled no copulatory opportunity with a female quail. Male quail demonstrated more approach to the CS during saline sessions than cocaine sessions. When the conditions were reversed such that cocaine sessions were followed by a CS and copulatory opportunity and saline sessions by a CS and no copulatory opportunity, male quail demonstrated more approach to the CS on cocaine sessions. Collectively, these studies indicate that drug states may serve as discriminative stimuli and/or occasion setters that come to predict certain outcomes or events.

The findings of the current experiment indicate that Pavlovian conditioning may play a role in cocaine-induced behavioral sensitization. To distinguish between the pharmacological and conditioning effects, a saline injection was administered in the drug-paired environment. Subjects that were given a saline challenge in a chamber where they had previously been given cocaine had greater locomotor activity compared to a control group that received saline there and a control group that received cocaine in the home cage. These findings support previous findings that have reported conditioned increases in locomotor activity in response to psychomotor stimulants (see Pert et al., 1990; Post et al., 1992 for reviews). In the current experiment, cocaine elicited a variety of physiological and behavioral responses, including increased locomotor activity that may have served as the unconditioned response. The Pavlovian conditioned cocaine effect was likely due to the contextual cues of the place where cocaine had been administered forming an association with the drug state. Therefore, in the absence of the drug (drug state), these contextual cues may come to elicit increased locomotor activity, the conditioned response.

In the present experiment, the cocaine group demonstrated greater locomotor activity during the saline challenge compared to the saline control group. The cocaine group also demonstrated

greater locomotor activity during the cocaine challenge than the saline group. It is likely that responding to the cocaine challenge by the cocaine group was a combination of contextual conditioning and cocaine-induced locomotor activity. The current findings do not provide conclusive evidence for a purely associative mechanism between contextual cues and a drug state. Nor do they provide evidence for a stimulus gating effect of cocaine sensitization in which the drug cue solely controls sensitization (Carey et al., 2005). However, the results of the saline test indicate that associative processes were involved in the induction of cocaine-induced sensitization in the current experiment.

In sum, the findings provide further evidence for cocaine-induced behavioral sensitization in an avian species. The findings are novel in that a simplified drug discrimination procedure was used to assess cocaine's effects on locomotor activity compared to when saline was administered. Unlike a previous study using male Japanese quail and a different procedure (a between-subjects design) in which the results provided tentative evidence for the role of conditioning (Geary and Akins, 2007), the current results provide stronger evidence for the role of conditioning in cocaine sensitization in this visually-oriented species. In general, these findings suggest that the use of an avian species in investigating drug effects and their conditioned associations with environmental cues may contribute to our understanding of human drug craving and relapse.

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