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Reinstatement of Extinguished Cocaine-Taking Behavior by Cocaine and Caffeine

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WORLEY, C. M., A. VALADEZ AND S. SCHENK. *Reinstatement of extinguished cocaine-taking behavior by cocaine and caffeine.* PHARMACOL BIOCHEM BEHAV **48**(1) 217–221, 1994.—Interactions between caffeine and cocaine have been demonstrated in a number of behavioral paradigms. The present study was undertaken in an attempt to determine whether caffeine could reinstate extinguished cocaine-taking behavior in rats. Experienced self-administering rats were first put through extinction training. The rats were then given an injection of either saline, cocaine (5.0, 10.0, or 20.0 mg/kg), or caffeine (5.0, 10.0, 20.0, 40.0 mg/kg). Both cocaine and caffeine induced a dose-dependent increase in the number of responses made on the previously cocaine-associated lever. These results confirm findings that the originally self-administered drug can serve as a prime to reinstate drug-taking behavior, and that nondopaminergic agonists can also provide an effective prime to reinstate responding. Potential mechanisms for these effects are discussed.

Cocaine Caffeine Self-administration Reinstatement Extinction
Reinstatement of cocaine-taking behavior

AFTER the initial detoxification procedure in cocaine abusers relapse rates are typically high (14). However, relatively little is known about factors that may contribute to relapse. Animal models have contributed to the identification of factors that may control drug taking. For example, animals returned to environments that had been associated with cocaine (18,22), suggesting that stimuli that have been associated with the reinforcing effects of a drug became effective conditioned reinforcers. Cues that had been paired with morphine (4) or cocaine (6) in a self-administration context reinstated extinguished responding in rats. Thus, these conditioned reinforcers were able to prime behavior that had previously resulted in drug delivery.

It has been shown that many noncontingently administered drug primes, including the originally self-administered drug and also the dopamine agonist, bromocriptine, have been potent stimuli for reestablishing extinguished drug-taking behavior in laboratory animals (5,6,24,26). In an early study (8), self-administration of amphetamine by monkeys, once extinguished by saline substitution, was readily reinstated by an experimenter-delivered infusion of amphetamine. In experienced cocaine self-administering monkeys that had been extinguished by substituting saline for the cocaine solution, cocaine, methylamphetamine, morphine, and codeine reinstated responding for saline infusions (19).

One antecedent to reinstatement of extinguished drug-taking behavior may be the control of operant responding by the discriminative stimulus properties acquired by the drugs during self-administration training. If so, one would expect drugs that fully or partially substitute for cocaine in a drug discrimination procedure to be effective at reinstating extinguished responding in cocaine-dependent rats. However, it is also expected that drugs that share only specific relevant reinforcing stimulus properties with cocaine would serve to reinstate drug-taking behavior.

One such drug may be caffeine. Interactions between the behavioral effects of cocaine and caffeine have been demonstrated in tests of motor activity (12,17) and drug discrimination (7,9). Further, rats that were preexposed with caffeine had shorter latencies to acquire cocaine self-administration, suggesting that the prior exposure sensitized rats to cocaine's reinforcing effects (11). As a result of these interactions at the behavioral level, we suggested that caffeine may be capable of priming reward-relevant circuitry so that cocaine's reinforcing effects may become potentiated. If so, this neurochemical prime may be an effective stimulus leading to reinstatement of extinguished cocaine-taking behavior.

To test this possibility, a modified version of an animal model of reinstatement was utilized (5,6). An initial phase established intravenous self-administration of cocaine. The

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next phase involved withdrawal of drug reinforcement to extinguish the operant to obtain drug infusions. Finally, the abilities for saline, caffeine, or cocaine to reinstate the extinguished behavior were compared.

METHOD

Subjects

Male Sprague-Dawley rats (Harlan, TX) weighing 350–400 g were used. The rats were individually housed in standard plastic hanging rodent cages, where rat chow and water were available ad lib. The temperature controlled (22°C ± 1°C) colony room was maintained on a 12 L : 12 D cycle with lights on at 0730.

Surgery

Following a 1 week acclimation period, the rats were deeply anesthetized with separate injections of ketamine (60 mg/kg, IP; Fort Dodge Laboratories, Inc., Fort Dodge, IA) and sodium pentobarbitol (20 mg/kg, IP; Sigma Chemical Co., St. Louis, MO). A Silastic cannula was inserted into the external jugular vein (25), passed subcutaneously, and the distal end was mounted on top of an exposed portion of the rat's skull. The 22 gauge metal tubing on the distal end of the cannula was affixed to the skull with dental acrylic, secured by four screws mounted into the skull. The rats were allowed to recover for 3 days prior to the training phase. Cannulae were flushed daily with 0.10 ml physiological saline solution containing heparin (1.25 U/ml; Elkins-Sinn, Inc., Cherry Hill, NJ), penicillin G Na (250,000 U/ml; Bristol-Myers Squibb Co., Princeton, NJ), and streptokinase (4000 U/ml; Kabivitrum, Inc., Franklin, OH) to maintain catheter patency and to prevent infection.

Apparatus

Sixteen operant chambers (Med Associates, ENV-001) were equipped with two levers. Depression of the active lever resulted in the delivery of a 0.10 ml intravenous infusion of cocaine HCl dissolved in 0.9% saline (NIDA, Research Triangle Park, NC) delivered over 12 s. Coincident with drug delivery was the illumination of a house light located directly above the lever. Drug delivery was via motorized pumps (Razel, model A, 1 rpm motor) and was controlled by IBM compatable computers interfaced with the operant chambers through the OPN software package (21). Depressions of the inactive lever were recorded but did not result in the delivery of a drug infusion.

Procedure

Phase 1: acquisition of self-administration. This initial phase consisted of 8–10 days access to cocaine. At the start of each daily 2 h session, the rats were placed in the operant chambers where they received an experimenter delivered infusion of cocaine (0.25 mg/kg/infusion). Thereafter, infusions were delivered on a FR1 schedule following depression of the active lever. Approximately 80% of the rats responded on the active lever 30 or more times per session with less than 10 inactive lever responses, and were included for subsequent testing.

Phase 2: test day. During the first 60 min of the test day, cocaine (0.25 mg/kg/infusion) was available for self-administration. Following this period, the rats were disconnected from the infusion line, the cannulae were flushed with 0.10 ml of the heparinized saline, penicillin, and streptokinase solution, and the pumps were turned off, allowing lever depressions to be recorded without a drug consequence. During this time, lever depressions still produced the light stimulus that had been paired with the cocaine infusion. Thus, extinction of behavior leading to drug delivery and extinction to the conditioned reinforcing properties of the light was accomplished. This extinction session continued until there were no active lever responses for a 60 min period. Extinction was accomplished within 5 h following inactivation of the syringe pumps. The rats then received an intraperitoneal injection of either the saline vehicle, caffeine (5.0, 10.0, 20.0, or 40.0 mg/kg, salt weight; Sigma Chemical Co., St. Louis, MO), or cocaine HCl (5.0, 10.0, or 20.0 mg/kg, salt weight). Following the injection, extinction conditions were maintained and the number of lever depressions was recorded until the rats failed to respond for 60 min.

At the completion of testing, the patency of catheter lines was confirmed by infusing sodium pentobarbitol (20 mg/kg, IV). An immediate loss of the righting reflex verified catheter patency. Only data from rats that passed this test were included for analysis. Some rats were tested with more than one drug treatment. Under these conditions, a 2 day period of

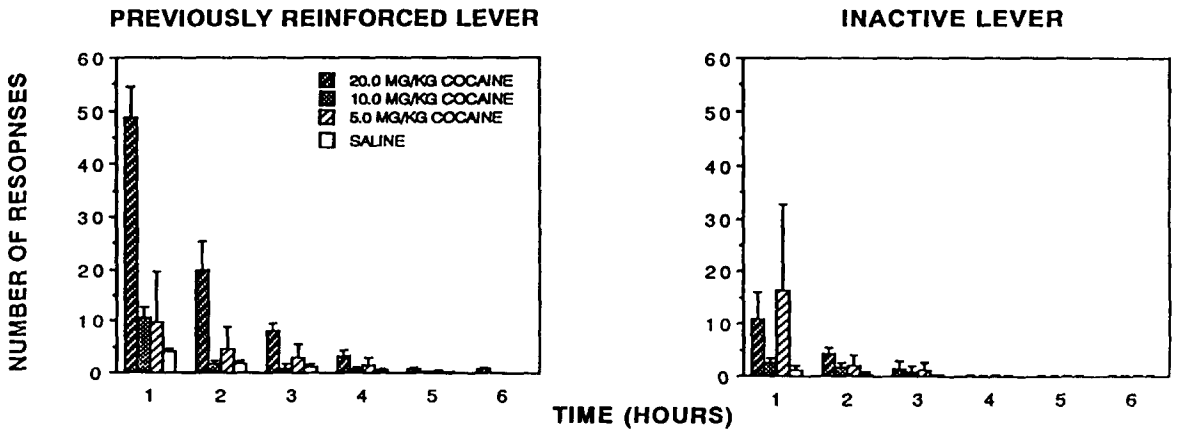

FIG. 1. Mean number (±SEM) of previously cocaine-associated (left panel) or inactive (right panel) lever responses as a function of time following the injection of saline or the various doses of cocaine.

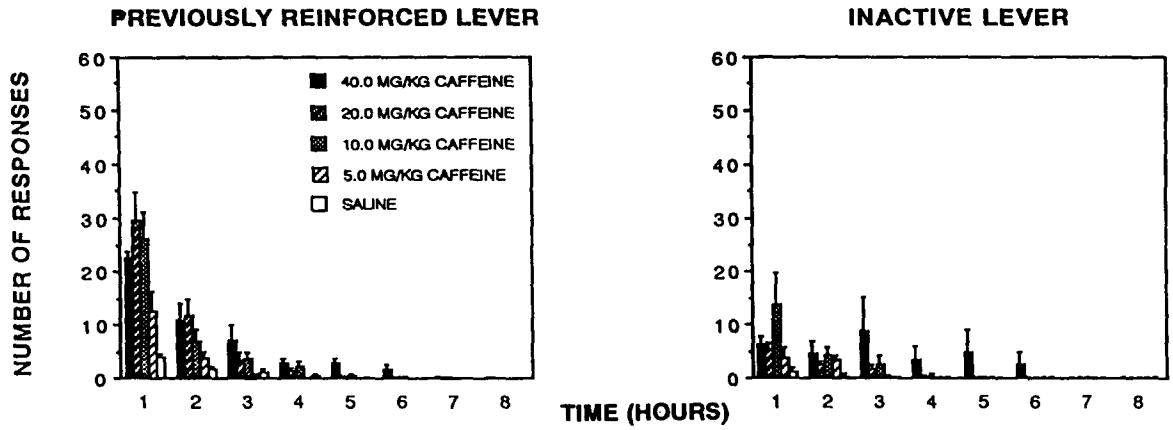

FIG. 2. Mean number (±SEM) of previously cocaine-associated (left panel) or inactive (right panel) lever responses as a function of time following the injection of saline or the various doses of caffeine.

limited access cocaine as in Phase 1 separated the tests. For these rats, the order of treatment presentation was random. The final number of subjects per group was 12 for the saline controls, 3-6 for all other groups.

RESULTS

Figure 1 shows the average number of responses made as a function of time following the injection of saline or the various doses of cocaine. The left panel shows responses on the previously cocaine-associated lever and the right panel shows responses on the previously inactive lever. An injection of saline produced an average of four active lever responses during the first hour. For the remainder of the session an average of less than two active lever responses per hour were made. Following the saline injection, inactive lever responses were also low.

Cocaine induced a dose-dependent increase in responding on the active lever. A three-way ANOVA (dose × hour × lever) revealed a significant interaction between all three variables, $F(15, 110) = 11.727$, $p < 0.001$. Tukey post hoc tests demonstrated that active lever responding following 20.0 mg/kg cocaine was significantly higher than following saline for 2 h following the injection ($p < 0.05$). The increase in responding produced by the lower cocaine doses was not statistically significant. During the first hour, inactive lever responses for the cocaine-treated rats (20.0 mg/kg) were also higher than for saline-treated rats ($p < 0.05$), although this difference disappeared by hour 2. Thus, during the second hour the effect of cocaine was specific to the previously cocaine reinforced lever.

Figure 2 shows the average number of responses as a function of time following the injection of saline or the various doses of caffeine. As in Fig. 1, the left panel shows responses made on the previously cocaine-associated lever and the right panel shows responses made on the previously inactive lever. The number of responses made when caffeine (20.0 mg/kg and 40.0 mg/kg) was injected was less than when cocaine served as the prime but was, nonetheless, substantially higher than when saline was administered, and the effect persisted for several hours. A three-way ANOVA (dose × hour × lever) revealed a significant interaction between all three variables, $F(28, 182) = 3.821$, $p < 0.001$. Tukey post hoc tests demonstrated that active lever responding following 20.0 and 40.0 mg/kg caffeine was significantly higher than following saline for 2 h and for 3 h, respectively ($p < 0.05$). The increase in active lever responding produced by the lower caffeine doses (5.0 mg/kg and 10.0 mg/kg) was statistically higher than responding produced by saline for 1 h following the injection. There were no significant differences in inactive

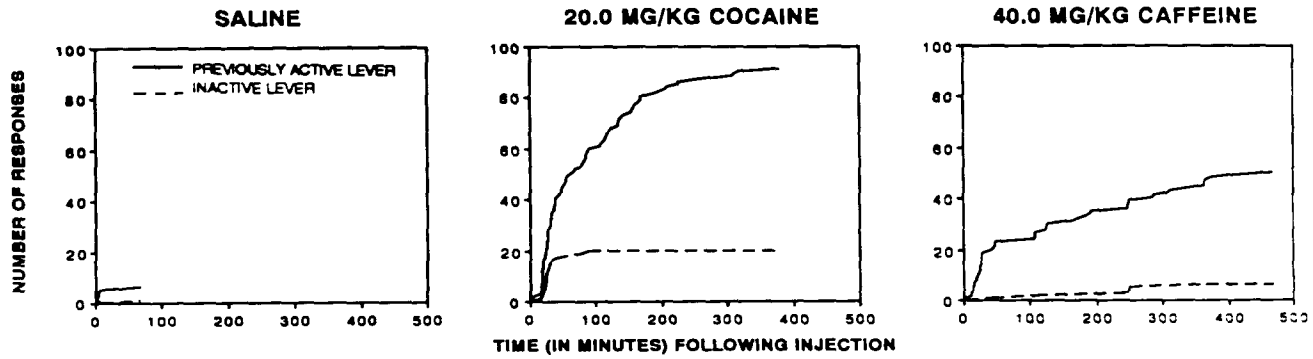

FIG. 3. Cumulative number of previously cocaine-associated and inactive lever responses for a typical rat from the saline control group and from each of the groups that received the highest doses of cocaine and caffeine tested.

lever responding for the caffeine-treated (5.0 mg/kg, 20.0 mg/kg, and 40.0 mg/kg) rats and the saline-treated rats; however, the dose of 10.0 mg/kg produced significantly higher inactive lever responding than saline.

Figure 3 shows the cumulative number of active and inactive lever responses for a typical rat from the saline control group and from each of the groups that received the highest doses of cocaine and caffeine tested. Saline-treated rats rapidly reached the 60 min criterion of nonresponding, indicating the end of the test phase. However, cocaine-injected rats required nearly 6 h and caffeine-injected rats required nearly 8 h to achieve the criterion of 60 min nonresponding following injection. Responding following the cocaine and caffeine injections was primarily restricted to the previously cocaine-associated lever. Both cocaine- and caffeine-induced responding was characterized by a strong burst of responses the first hour, followed by a steady increase in responding for 2-3 hours postinjection.

DISCUSSION

The present data confirm and extend previous findings that certain classes of drugs can serve to reinstate extinguished drug-taking behavior. The ability for cocaine to effectively reinstate extinguished cocaine-taking behavior was not surprising because others had shown that noncontingent administration of the originally self-administered drug produced potent reinstatement of extinguished drug-taking behavior (5, 6,8,19). These effects may be due to the ability for the drug to prime reward-relevant circuitry and to initiate drug-like effects that led to reinstatement. When cocaine (20.0 mg/kg) served as the prime, the first hour following the injection was characterized by a general increase in motor activity, with responding on both the active and inactive levers significantly higher than responding produced by saline. Responding became restricted to the previously cocaine reinforced lever after the first hour. Caffeine also potently and dose-dependently reinstated extinguished cocaine-taking behavior, suggesting that it may also be capable of priming cocaine-related reward circuitry.

It should be pointed out that the reinstatement paradigm is not merely a drug discrimination procedure. Rather, there are important differences between the two approaches. The drug discrimination paradigm is enormously time consuming and takes many trials to establish (1). In contrast, the reinstatement procedure is relatively time efficient, due to the specific aspect of the stimulus properties of the drug that provides the discriminative cue (the reinforcing property). A most critical difference is that drug discrimination is based on food reinforced behaviors, whereas the reinstatement procedure examines cocaine's reinforcing properties by using the cocaine self-administration procedure. Therefore, the reinstatement is tied specifically to the stimulus properties of the drug that are related to cocaine's reinforcing properties.

An understanding of the mechanisms for these priming effects is of great interest to understanding the basis for reinstatement of drug-taking behavior. The positive-reinforcing incentive explanation of drug-taking behavior (23) asserts that a self-administered drug causes a positive affective state and, thus, becomes reinforcing through its action on the CNS. If so, then perhaps the noncontingent injection of cocaine or caffeine induced positive affect, and the drug-taking behavior that had been associated with positive affect was reinstated. However, even though caffeine can produce a discriminative cue (10), caffeine is not a readily self-administered drug, and only weakly generalizes to a cocaine discriminative training cue (9). It seems that caffeine's effects are not easily explained by the positive-reinforcing incentive explanation of drug-taking behavior.

Cocaine's reinforcing effects are widely accepted as being due to dopamine uptake blockade, particularly in the mesocorticolimbic dopamine system (16). This effect may also underlie the reinstatement of cocaine-taking behavior observed in the present study and in previous work (5,6,8,19).

It has been suggested that reinstatement of cocaine-taking behavior is accomplished by drugs that share cocaine's dopamine agonist properties (26). Because caffeine, a weak dopaminergic agonist at best (3,13), potently reinstated cocaine-taking behavior, other mechanisms must also account for these effects.

Caffeine, particularly when administered in low doses, produces many of its behavioral effects via adenosine receptor blockade (2,20), although other mechanisms may also be activated (10). It is of interest that there is a high density of A2 receptors in the nucleus accumbens (15), which raises the possibility that A2 receptor antagonism in this terminal region of the mesolimbic dopamine system underlies the reinstatement effects produced by caffeine. This hypothesis awaits further investigation using specific adenosine receptor probes.

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