Caffeine, Acting on Adenosine A<sub>1</sub> Receptors, Prevents the Extinction of Cocaine-Seeking Behavior in Mice<sup>1</sup>

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ABSTRACT

Drug-naive DBA/2 mice were trained to self-administer cocaine (40 μg/kg/infusion) i.v. by nose poking. The number of nose-poke responses was higher in mice receiving response-contingent injections of cocaine (active group) than in yoked controls or in animals receiving response-contingent saline injections. Twenty-four hours after the training session (cocaine or saline self-administration), mice were injected i.p. with saline, cocaine, caffeine, 1,3-dipropyl-8-cyclopentyl xanthine (DPCPX), 8-cyclopentyl theophylline (8-CPT), 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine (SCH 58261), or 9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-c]quinoxalin-5-amine (CGS 15943) and placed again in exactly the same operant boxes as during the training session but without response-contingent i.v. infusions. Saline injection elicited similar responding in animals from the active group and from the yoked control group. A low dose of cocaine (5 mg/kg) or caffeine (3 mg/kg), but not higher doses, produced greater responding in the active group than in the yoked control group during a single extinction trial. The adenosine A<sub>1</sub>-receptor antagonists DPCPX and 8-CPT and the nonselective antagonist CGS 15943 partially reproduced the effect of a low dose of caffeine on the cocaine-associated behavior in a dose-dependent manner and did not alter the nose-poke activity of yoked control mice in the extinction experiment. In contrast, the adenosine A<sub>2a</sub>, antagonist SCH 58261, in doses above 1 mg/kg, reduced nose-poke activity equally in active and yoked control animals. This confirms that a drug from a different pharmacological class (adenosine-receptor antagonist) can induce behavior changes similar to the effects of the original self-administered drug (indirect dopamine-receptor agonist). The data also suggest that the effects of caffeine on cocaine-seeking behavior might be related to interaction with adenosine A<sub>1</sub> receptors, but not A<sub>2a</sub> receptors.

Cocaine-dependent individuals tend to relapse into cocaine use even after long periods of abstinence. It is important to find factors that contribute to such a relapse. Animal studies have shown that drug-seeking behavior can be elicited not only by a low dose of the original self-administered drug (Gerber and Stretch, 1975; de Wit and Stewart, 1981; Stewart and Wise, 1992; Self et al., 1996), but also by drugs from pharmacological groups other than that of the original self-administered drug (Slikker et al., 1984).

Previously, it was shown that caffeine reinstated responding during an extinction phase in rats trained to self-administer cocaine (Worley et al., 1994; Schenk et al., 1996) or potentiated cocaine effects on the reinstatement of self-administration behavior (Self et al., 1996). The nature of this cue is not understood presently. Caffeine does not generalize well to cocaine in a drug-discrimination paradigm, and it is poorly self-administered (Griffiths and Woodson, 1988; Gauvin et al., 1990). However, cocaine substituted for the caffeine-discriminative stimulus in rats trained to discriminate caffeine from saline (Holtzman, 1986). Thus, how caffeine and cocaine can substitute for each other, and to what extent, remains to be determined.

Of all of the known primary biochemical mechanisms of action of caffeine, only antagonism of adenosine effects on adenosine A<sub>1</sub> and A<sub>2a</sub> receptors is known to be important at concentrations of caffeine that produce behavioral stimulation (see Fredholm, 1995). Adenosine A<sub>1</sub> receptors are widely distributed in the brain (Johansson et al., 1993) and regulate the release of several neurotransmitters (Fredholm and Dunwiddie, 1988). In contrast, adenosine A<sub>2a</sub> receptors are largely restricted to neurons that release γ-aminobutyric

ABBREVIATIONS: DPCPX, 1,3-dipropyl-8-cyclopentyl xanthine; 8-CPT, 8-cyclopentyl theophylline; SCH 58261, 5-amino-7-(2-phenylethyl)-2-(2-furyl)pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine; CGS 15943, 9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-c]quinoxalin-5-amine; DMSO, dimethyl sulfoxide.
acid that also express dopamine D2 receptors (Fink et al., 1992; Svenningsson et al., 1997a), and there is a functionally important negative interaction between adenosine A2A and dopamine D2 receptors (see Ferré et al. 1997). Perhaps secondarily to its primary effects, caffeine also influences and interacts with a host of transmitter systems in the brain (see Daly, 1993).

Recently, it was shown that an agonist at dopamine D2 receptors, but not one at D1 receptors, could reinstate cocaine-seeking behavior (Self et al., 1996). Given that D2 and A2A receptors are colocalized, that they are mutually antagonistic (Ongini and Fredholm, 1996; Ferré et al., 1997), and that an A2A-receptor antagonist can mimic the stimulation of motor behavior produced by a D2-receptor agonist (Svenningsson et al., 1995), it seemed possible that the ability of caffeine to reinstate the extinguished cocaine-seeking behavior is mainly associated with its ability to block A2A receptors. To test this possibility, we used a technique of the extinction of cocaine-associated behavior based on a model of one-session initiation of i.v. cocaine self-administration in naive mice (Kuzmin et al., 1997). With this model, we tested the ability of cocaine and caffeine to prevent the extinction of cocaine reward-associated responding in mice withdrawn from contingent reinforcing infusions of cocaine. Also, we examined the ability of selective adenosine A1-receptor antagonists, 1,3-dipropyl-8-cyclopentyl xanthine (DPCPX) and 8-cyclopentyl theophylline (8-CPT), a selective A2A-receptor antagonist, 5-amino-(2-phenylethyl)-2-(2-furyl)pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine (SCH 58261; see Ongini and Fredholm, 1996), and a nonselective, nonxanthine, adenosine-receptor antagonist, 9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-c]quinazolin-5-amine (CGS 15943) to mimic the effect of caffeine.

Materials and Methods

Animals. All experiments were carried out on male DBA/2 mice (18–22 g). Animals were obtained from the breeding farms in Rappolo, Russia and Bomholtgård, Denmark and kept under standard laboratory conditions with unlimited access to food and water. They were housed 12 mice per cage in a light-controlled room (12 h light/dark cycle; light on at 10:00 AM) at 21°C and 60% humidity. The experiments were approved by the respective regional animal ethics boards.

Drugs. Caffeine (as free base; Sigma Chemical Co., St. Louis, MO) and cocaine (as hydrochloride; Sigma Chemical Co.) were dissolved in warm saline and injected i.p. just before the experiment. SCH 58261 (a gift from Dr. Ennio Ongini, Schering-Plough, Milan, Italy), CGS 15943 (Research Biochemicals International, Natick, MA) and DPCPX (Research Biochemicals International) were dissolved in dimethyl sulfoxide (DMSO), and stock solutions were diluted in saline so that the final concentration of DMSO was 20% (v/v). 8-CPT (Research Biochemicals International) was dissolved in 0.1 M NaOH and the pH of the solution was adjusted to 7.2 with 0.01 N HCl. Cocaine HCl for i.v. self-administration was dissolved in saline and the pH of the solution was adjusted to 7.2 with 0.01 N NaOH. Doses of cocaine refer to the salt.

Intravenous Self-Administration Method. The model of one-session initiation of i.v. self-administration of cocaine was used (40 μg/kg/infusion; 1.6 μl per infusion; fixed ratio = 1; naive mice; Kuzmin et al., 1997). This unit dose was chosen on the basis of preliminary testing in the range from 10 to 80 μg/kg/infusion. Mice were tested in pairs in cages (8 x 8 x 8 cm) that had a frontal hole (1.5 cm) for nose poking fitted with infrared sensors (3 mm into the hole from the inner surface of the cage) interfaced to an operating computer that controlled the automatic syringe pump. Mice were partially immobilized by fixing their tails with adhesive tape to the horizontal surface. The tails protruded through a vertical slot in the back wall of the box.

First, the number of nose-poke reactions during a 10-min period was recorded for all experimental animals. On the basis of this 10-min test, pairs of mice were chosen so that animals in pairs exhibited an approximately equal level of nose-poke activity.

Thereafter, the matched pairs were placed into the experimental boxes, and cannulas (external diameter, 0.4 mm) were introduced into the lateral tail veins of both of the animals in the pair. To verify the proper placement of the cannulas, test infusions (3 x 1.6 μl) were made. Improper positioning of the needle resulted in a local paling of the tail. In this case, the needle was removed and reintroduced. When all of the cannulas were properly placed, the response-contingent injections were activated. Each nose poke of one mouse (called the active mouse) resulted in a response-contingent infusion of 1.6 μl of cocaine solution (40 μg/kg/infusion) or saline to both the active mouse and the yoked control. The injection had a constant duration of 1 s. Nose pokes of the yoked control animal were counted but had no programmed consequences. The self-administration session lasted 30 min. After this period, the animals were labeled and returned to their home cages.

The number of nose pokes by both active and yoked control animals was counted and analyzed. Because of the differences in the initial (i.e., before drug treatment) nose-poke activity between pairs, a ratio (r) criterion was used as a quantitative measure of the reinforcing effect of the drug solution (Kuzmin et al., 1997). This was calculated as the log10 of the ratio between the cumulative number of nose-pokes by the active and the yoked control mouse in the matched pair during a 30-min self-administration session. Logarithms were used to normalize the distribution of the data. The rationale of this criterion is that if the infused solution has a reinforcing effect, the number of operant reactions (nose pokes) in active mice will exceed that in the yoked control animal, independent of the stimulatory effects of the drug, and the log10 of the ratio will become a positive value.

Not all of the pairs of mice acquired cocaine self-administration according to the r criterion (about 15–25% of pairs in groups failed to reach self-administration criterion). In the present study, only those pairs of mice with the r criterion greater than the upper 95% confidence limit of the mean r criterion obtained in the saline self-administration experiments were selected for the extinction experiments.

Extinction of Cocaine Reward-Associated Responding. On day 2, extinction sessions were carried out. Just before placement into boxes, both active and yoked control mice were injected i.p. (1 ml/kg) with saline or one of the compounds investigated: cocaine HCl (2.5–20 mg/kg); caffeine (3–30 mg/kg); CGS 15943 (1.25–5 mg/kg); SCH 58261 (0.312–2.5 mg/kg); DPCPX (1.25–5.0 mg/kg); or 8-CPT (5–20 mg/kg). There were six pairs in the treatment groups except in the groups given cocaine and caffeine treatment (n = 8). After the injection, pairs of mice were placed into exactly the same experimental boxes and partially immobilized as in the initiation experiments (day 1), but the needles were not introduced and cocaine infusions were not activated. As in the initiation session, the number of nose pokes of each mouse in the pair was counted and analyzed.

Data Analysis and Statistics. The number of nose pokes for active and yoked control mice in each treatment group was analyzed with two-way ANOVA; mode (active mice versus yoked control mice) and dose (saline/vehicle and drug treatments) were the independent factors. Next, paired comparison (two-tailed Student’s t test) was performed to compare active and yoked control activity within each group of drug-injected animals and nose-poke activity in active and yoked control animals between the drug-treated group and a saline/vehicle-treated group. The whole study was designed as a between-subjects (independent groups) experiment (i.e., each treatment we describe was done on a single set of animals).

Next, the r criterion was analyzed in a similar way: one-way ANOVA for the r criteria (with drug treatment as an independent
factor), followed by paired comparison of the $r$ criteria (two-tailed Student's $t$ test) in an extinction experiment with corresponding ratios in the acquisition experiment and the extinction ratio in the control group (treated with saline/vehicle).

**Results**

**Initiation of Cocaine/Saline Self-Administration.** The initial nose-poke activities (in a 10-min trial without infusions) did not differ significantly between the different groups of animals. The mean number of nose-poke responses in the various groups ranged from 23.88 ± 14.13 to 31 ± 12.87 during 10 min of testing (lowest and highest levels for different groups; mean ± S.E.M.). However, it is important to note that the difference in nose-poke activity between individual pairs was significant (from 16 to 88 in 10 min; lowest and highest activity in pairs). Two-way ANOVA showed no difference between treatment groups ($p = 0.49$) or between active and yoked control mouse subgroups ($p = 0.86$), and there was no interaction between group and subgroup factors. From this, it can be concluded that the matching between and within experimental groups was successful. Thus, any reinforcing action of the contingent infusions of the drug solution was expected to result in a larger number of nose pokes by the active mouse than by the yoked control.

Cocaine (40 µg/kg/infusion), when made contingent to the nose pokes of one animal in the pair, selectively and significantly increased the operant responses of active animals ($t = 13.55; p < .001; 276$ degrees of freedom), whereas the responses of the yoked control animals remained unchanged and did not differ from the activity of the control animals in groups with saline self-administration. This translates into a highly significant ($p < .01$) increase of the $r$ criterion (i.e., the logarithm of the ratio of nose-poke responses in treatment versus control group; see Materials and Methods) in mice given cocaine on day 1 ($0.27 ± 0.01, 148$ selected pairs) as compared with that in mice offered saline self-administration ($−0.92 ± 0.01; 32$ selected pairs). This was interpreted as a strong reinforcing effect of this particular unit dose of cocaine. In addition, the results show that a 30-min experimental session with the nose-poke operant procedure is sufficient for mice to acquire cocaine self-administration behavior. For the extinction experiments, only pairs of mice that reached the criterion for the acquisition of cocaine self-administration were used (see Materials and Methods).

**Extinction.** On day 2, mice were given i.p. injections of different drugs or saline/vehicle and placed into exactly the same experimental boxes as in the initiation experiment. In the groups injected with saline/vehicle, the number of nose pokes was not significantly different between active and yoked control mice (see Figs. 1-3). The ratio between the response rate in the two paired mice was close to 1 and, hence, the $r$ criterion did not differ from 0. This was interpreted as the extinction of the behavior acquired on day 1 in the absence of response-contingent cocaine infusions. Injection of the vehicle for adenosine antagonists (20% DMSO) produced a parallel decrease in nose pokes in both active and yoked control mice as compared with saline-treated animals. However, the $r$ criterion remained on the same level after vehicle treatment as after saline treatment.

**Effects of Cocaine in the Extinction Phase.** Injection of cocaine had a dose-dependent influence on the operant pattern in mice trained to self-administer cocaine (Fig. 1). Administration of cocaine at the dose of 5.0 mg/kg increased specifically the nose-poking response of the active animals that had learned to self-administer cocaine on day 1 ($p < .05$ as compared with the saline-treated group). ANOVA revealed a significant effect of cocaine on the number of nose pokes in the active mice ($F_{4,29} = 4.31; p = .007; n = 8$ animals per group). However, this effect of cocaine (5.0 mg/kg) was not seen in the active animals trained for saliné self-administration on day 1 (data not shown) or in yoked control animals that were trained with either cocaine or saline infusion on day 1. With the higher doses of cocaine (10 and 20 mg/kg), there were no significant differences in nose-poking activity between the animals in the pair.

ANOVA also showed a significant influence of cocaine dose on $r$ criteria ($F_{4,29} = 8.79; p = .00008$). Paired comparison revealed a significant difference between $r$ criteria in the initiation experiment versus the extinction experiment in the groups injected with saline ($p = .002$) or the two highest doses of cocaine (10 mg/kg, $p = .008; 20$ mg/kg, $p = .00001$). In groups injected with 2.5 or 5.0 mg/kg cocaine, the $r$ criterion in the extinction experiment did not differ significantly from that in the initiation experiment. However, it was only in the group injected with 5.0 mg/kg cocaine that the $r$ criterion during extinction differed significantly from that in the group injected with saline ($p < .05$). Of special importance is...
the fact that cocaine (5.0 mg/kg) did not increase the value of the \( r \) criterion in mice that had been trained to self-administer saline during the initiation experiment.

**Effects of Caffeine in the Extinction Phase.** ANOVA failed to reveal a significant effect of caffeine treatment on the number of nose pokes in groups. However, there was a significant influence of caffeine dose on the level of the \( r \) criterion in the extinction experiment (\( F_{(3,26)} = 7.04; p = .001; n = 8 \) animals per group) with a significant increase of the \( r \) criterion (\( p < .01 \), compared with the saline-injected group) in the group injected with caffeine (3 mg/kg). Paired comparison revealed a significant difference between \( r \) criteria in the initiation versus the extinction experiment in the groups injected with saline (\( p = .002 \)) or with caffeine at the doses of 10 mg/kg (\( p = .0002 \)) and 30 mg/kg (\( p = .002 \); Fig. 1, bottom). There was no difference between the \( r \) criteria in initiation and extinction sessions in the group treated with caffeine at the dose of 3 mg/kg. Thus, it was concluded that i.p. administration of caffeine at a dose of 3 mg/kg maintained the cocaine-oriented pattern of behavior in mice, in the absence of contingent, reinforcing cocaine infusions, and prevented the extinction of cocaine-oriented behavior, whereas higher doses of caffeine lacked this effect. To find the reason for these effects, paired comparison of the nose pokes in particular groups was performed. Among the active mice trained to self-administer cocaine, those injected with caffeine (3 mg/kg) exhibited more nose-poke responses than did those injected with saline (\( p = .05 \); Fig. 1). No such difference was seen in active mice trained for saline self-administration or in either of the yoked control groups. The highest dose of caffeine (30 mg/kg) increased responding in both active mice and yoked controls, but the effect did not reach statistical significance (\( p = .08 \)). Thus, caffeine at the low dose (3 mg/kg) tended to increase specifically nose-poke responses of the active animals that had learned to self-administer cocaine on day 1.

**Effects of the Nonselective Adenosine-Receptor Antagonist CGS 15943 in the Extinction Phase.** First, we examined whether the effect of caffeine could be reproduced by CGS 15943, a structurally unrelated antagonist, at adenosine \( A_1 \) and \( A_{2A} \) receptors. CGS 15943 is approximately 1000-fold more potent than caffeine at both adenosine \( A_1 \) and \( A_{2A} \) receptors (see Ongini and Fredholm, 1996). However, it is much less water-soluble and, hence, its apparent volume of distribution is much higher. For this reason, it must be given in vivo in doses close to those of caffeine. As seen in Fig. 2, in none of the CGS 15943-treated groups (6 animals per group) was the \( r \) criterion different from that obtained in the initiation experiments, whereas such a difference was observed in the vehicle-treated group. Although ANOVA failed to show a significant influence of the drug on the nose-poke activity in the extinction experiment, we tested whether the increase in the \( r \) criterion with the highest dose of CGS 15943 was because of an increase in nose-poke responding in active mice or because of a decrease in activity in yoked control mice. The former was the case (\( p < .05 \); active and yoked control groups were compared with Student’s \( t \) test). Thus, we concluded that CGS 15943 tends to prevent the extinction of the cocaine-oriented behavior.

**Effects of the Adenosine \( A_{2A} \)-Receptor Selective Antagonist SCH 58261 in the Extinction Phase.** Next, we examined the effect of a highly selective adenosine \( A_{2A} \)-receptor antagonist, SCH 58261, which is structurally related to CGS 15943 (see Ongini and Fredholm, 1996). This compound is at least as potent as CGS 15943 as an antagonist at \( A_{2A} \) receptors, but at least 100 times less potent at \( A_1 \) receptors. Therefore, it was given in doses similar to those of CGS 15943. As seen in Fig. 2, lower doses of this compound had little effect on nose-poke responding, whereas higher doses produced a marked decrease. ANOVA revealed a significant effect of SCH 58261 injection on nose-poke responses in both active and yoked control mice (\( F_{(4,25)} = 22.5; p < .0005 \) and \( F_{(2,25)} = 20.5; p < .0005 \) respectively; 6 animals per group). There was a significant reduction (compared with vehicle-injected group) of the operant activity in the groups injected with SCH 58261 at doses of 1.25 or 2.5 mg/kg (\( p < .01 \) for both doses). Treatment with 0.312 and 0.625 mg/kg had no effect on operant responses in either active or yoked control mice (comparison with vehicle-treated group). ANOVA also failed to reveal any significant effect of SCH 58261 on the \( r \) criteria in the extinction experiment. Paired comparison of the levels of \( r \) criteria in the initiation versus the extinction experiments revealed that in all of the groups injected with SCH 58261, the \( r \) criteria in the extinction experiments differed significantly from those in the initiation...
of the initiation experiment versus the extinction experiment in the groups injected with vehicle (\(p = .03\)) or with DPCPX at the doses of 1.25 mg/kg (\(p = .005\)) and 2.5 mg/kg (\(p = .02\)). There was no difference between \(r\) criteria in initiation and extinction sessions in the group treated with DPCPX at a dose of 5.0 mg/kg. Thus, it could be concluded that i.p. administration of DPCPX at the dose of 5.0 mg/kg prevented the extinction of the cocaine-oriented behavior.

Next, we examined the effects of 8-CPT (Fig. 3). 8-CPT is some 10-fold less potent than DPCPX on both \(A_1\) and \(A_{2A}\) receptors (see Daly, 1993). It also is more water soluble, and therefore was given in 4-times higher doses than DPCPX. There was a significant dose-dependent influence of 8-CPT on nose-poke responding in mice (\(F_{(3.44)} = 3.37; p < .05\)). Among active mice trained to self-administer cocaine, those injected with 8-CPT at 5 and 10 mg/kg showed a tendency (\(p < .1\)) to increased nose-poke responding compared with those injected with vehicle. Paired comparison of the levels of \(r\) criteria in initiation versus the extinction experiments revealed that in groups treated with 8-CPT at 5 and 20 mg/kg, \(r\) criteria in the extinction experiments differed significantly from those in the initiation experiments (\(p = .008\) and \(p = .03\), respectively), whereas in the group injected with 10 mg/kg 8-CPT, there was no difference between \(r\) criteria in initiation and in the extinction experiments. Thus, 8-CPT tends to prevent the extinction of the cocaine-oriented behavior.

**Discussion**

This study shows that the initiation and the extinction of cocaine-related behavior can be studied not only in rats, but also in mice. A single, 30-min session in mice established self-administration of cocaine by means of directed nose pokes. This behavior was rapidly extinguished in mice that received only saline/vehicle injections during testing on a subsequent day. However, an i.p. injection of cocaine at 5.0 mg/kg, a dose known to exert a reinforcing effect in a conditioned place-preference paradigm in mice (Kuzmin et al., 1997), was able to stabilize the higher level of nose-poke activity in active animals compared with the yoked control animals even when withdrawn from the contingent cocaine infusions. The ability of cocaine to effectively maintain cocaine-oriented behavior in the absence of response-contingent injections of cocaine was not surprising because others have shown that noncontingent administration of the self-administered drug produces potent reinstatement of extinguished drug-taking behavior (Gerber and Stretch, 1975; de Wit and Stewart, 1981; Slikker et al., 1984; Self et al., 1996). Conversely, the highest dose of cocaine used in the present study (20 mg/kg), a dose that does not induce conditioned place preference in mice (Kuzmin et al., 1997), lacked a priming effect during the extinction session. Thus, it can be speculated that only reinforcing doses of the drugs are able to prevent an extinction of drug-related behavior.

In our experiments, caffeine at the dose of 3 mg/kg i.p. also effectively prevented the extinction of a cocaine-induced operant pattern. This finding confirms and extends previous data showing the ability of caffeine to reinstate cocaine-
seeking behavior in rats (Worley et al., 1994; Schenk et al., 1996). However, Self and coworkers (Self et al., 1996) found that caffeine at the dose we used for this study (3 mg/kg) lacked appreciable priming ability, whereas caffeine at 10 mg/kg tended to enhance cocaine priming. A probable explanation is that in our mouse strain, the caffeine dose-response curve is slightly shifted to the left compared with the rat strain used by Self and coworkers (Self et al., 1996), but there also are differences in the experimental procedure. Whereas in our study there was a 24-h delay after the cocaine self-administration session, in the cited study, the extinction and the effects of priming caffeine injections were studied 2 to 4 h after the cocaine self-administration session.

Whereas the lower dose (3 mg/kg) of caffeine had a clear-cut priming effect, a high dose (30 mg/kg) of caffeine induced an increase in nose poking in both active and yoked control mice, but this activation was not selective and lacks priming effect, according to the r criteria. Because caffeine has a biphasic reinforcing/aversive activity depending on the dose used (Brockwell et al., 1991), the dose of 30 mg/kg in mice might have some aversive properties. In fact, our preliminary experiments with the aid of the conditioned place-preference technique also showed that whereas caffeine at the dose of 3 mg/kg produced conditioned place-preference in mice, the dose of 30 mg/kg produced a strong place-aversion reaction (A.K., unpublished observation).

The ability of a low dose of caffeine to prevent the extinction of a cocaine-related behavior cannot be explained simply by its psychomotor stimulant properties (Schenk et al., 1989) because an increased responding was observed only in active animals and not in the yoked control mice. Thus, the effect of caffeine is related to an association of the operant responses with cocaine infusions on day 1 of the experiment. Moreover, it has been shown previously that the motor-activating effect of caffeine is not correlated with its ability to reinstate cocaine-associated responding (Schenk et al., 1989), and that with repeated administration, the effectiveness of a dose of caffeine in increasing motor activity remains unchanged, whereas its ability to reinstate cocaine-associated responding decreases (Schenk et al., 1996).

It is important to note that the nose-poke activity does not mirror the general motor activity in mice. In fact, administration of high doses of cocaine (Kuzmin et al., 1997) that produce definite stimulatory effect usually resulted in a decrease in the number of nose pokes in the technique used. This gives a unique opportunity to study selectively the reinforcing effects of drugs because the increase in nose-poke activity was found only in mice that actively self-administer cocaine and not in animals from the yoked control group.

It is possible that some component of the effects of caffeine can modify cocaine-sensitive systems, possibly by the activation of the central dopaminergic systems (Ferré et al., 1991; Garrett and Holtzman, 1994). As mentioned, it is generally believed that a major primary neurochemical effect of caffeine is the blockade of adenosine receptors (Daly, 1993; Fredholm, 1995), and this effect has been demonstrated as critical for the acute stimulant effects of caffeine (Kaplan et al., 1989; Holtzman, 1991). It also has been shown that activation of adenosine receptors can antagonize the effects of dopamine at both D1 and D2 receptors (see Ferré et al., 1997). There is a high density of A2A receptors in the accum-
fects of A1- and A2A-receptor antagonists on cocaine-associ-
ated responding.

Adenosine A1 receptors have a widespread distribution in the brain of most species (Fastbom et al., 1987) and are present in the nucleus accumbens, where they selectively counteract responses to dopamine D1-receptor agonists (Ferré et al., 1996). In addition, adenosine A1 receptors are located on the nerve terminals of several types of neurons and inhibit the release of many neurotransmitters (Fredholm and Dunwiddie, 1988). Thus, an adenosine A1 antagonist might increase the release of endogenous dopamine (Stoner et al., 1988), which is known to activate dopamine receptors. Furthermore, adenosine A1 antagonists enhance signaling via such receptors (see Ferré et al., 1996). Such activation of dopaminergic transmission is known to be part of the re-

tponse to cocaine (Self and Nestler, 1995). However, recent findings have shown that reinstatement of cocaine-associated behavior by cocaine priming does not correlate with the dopaminergic neurotransmission in the accumbens nucleus (Neisewander et al., 1996). Furthermore, as noted above, a D1 agonist did not reinstate cocaine-seeking behavior (Self et al., 1996), although D1 agonists were shown to maintain self-administration behavior in rats (Weed et al., 1997). Thus, the schemes for explaining the actions of caffeine as well as selective adenosine-receptor antagonists in terms of interactions with known mechanisms involving dopamine receptors, schemes that work very well in explaining the actions of these compounds on motor behavior (see Ferré et al., 1997), break down when trying to explain the effects of these drugs in the extinction model of cocaine-seeking behav-

ior. Consequently, the mechanism of the maintenance of drug-seeking behavior also must incorporate other neuro-

transmitter systems and probably other neuronal circuits (Koob and Le Moal, 1997). It also might be speculated that the receptors involved in reinforcement (D1 for cocaine and, possibly, A2A for caffeine) are different from the receptors involved in the processes of extinction and reinstatement (D2 for cocaine and A1 for caffeine).

In summary, the present results show that a mouse model can be successfully used to study the mechanisms of the extinction of drug-seeking behavior during withdrawal from the response-contingent reinforcement. Given the increasing availability of genetically modified mouse strains, this type of experimental model might prove valuable in future studies of the genetic mechanisms involved in establishment, mainte-
nance, extinction, and reinstatement of drug-seeking behav-

ior. With this model, we demonstrated a clear-cut biphasic effect on the extinction of cocaine-seeking behavior when using both the self-administered drug (cocaine) and a drug from a completely different drug class (caffeine). Finally, low, behaviorally stimulant doses of caffeine can prevent the ex-


tinction of cocaine-seeking behavior, possibly via the block-
ade of A1 adenosine receptors.

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