Pergamon

Pharmacology Biochemistry and Behavior, Vol. 51, No. 4, pp. 827-834, 1995

0091-3057/95 $9.50 + .00

0091-3057(95)00047-X

Intravenous Cocaine Self-Administration in the C57BL/6J Mouse

NICHOLAS J. GRAHAME,*[1] TAMARA J. PHILLIPS,*†
SUE BURKHART-KASCH*† AND CHRISTOPHER L. CUNNINGHAM*

*Department of Medical Psychology, Oregon Health Sciences University;
and †Research Service, VA Medical Center, Portland, OR 97201

Received 2 August 1994

GRAHAME, N. J., T. J. PHILLIPS, S. BURKHARDT-KASCH AND C. L. CUNNINGHAM. *Intravenous cocaine self-administration in the C57BL/6J Mouse.* PHARMACOL BIOCHEM BEHAV 51(4) 827-834, 1995.—Freely behaving C57BL/6J mice with intrajugular catheters were trained to nose-poke for cocaine (0.75 mg/kg per 5-μl infusion) under a fixed-ratio-10 schedule of reinforcement. Mice were given a choice between two nose-poke holes on opposite sides of the apparatus. Nose-pokes by experimental (O) subjects (operant group) were reinforced on only one side and reinforcer delivery coincided with the onset of a 10-s time-out light stimulus. Drug delivery to control subjects (yoked group) was determined by the behavior of O mice. Nose-poke rate increased in O subjects, whereas yoked subjects did not acquire the nose-poking response. Moreover, nose-poking was selective for the cocaine-paired side in O subjects. When saline infusions were substituted for cocaine (i.e., extinction), nose-poking in O subjects decreased, whereas yoked controls were unaffected. O subjects developed a preference for the drug-associated side of the apparatus during extinction. Overall, these data offer strong evidence of cocaine-directed behavior in the C57BL/6 inbred mouse strain. More generally, these findings support the feasibility of using intravenous self-administration to assess reinforcement in genetically well-defined populations.

Intravenous self-administration Cocaine Locomotor activity Place preference C57BL/6J mice Reinforcement Yoked control

MICE, relative to other experimental subjects, are an excellent model for the study of the genetic basis of alcohol and drug use. The existence of a variety of genetically well-defined, inbred and recombinant inbred strains allows for the identification of genes contributing to various drug-related behaviors (4). In addition, a variety of mouse lines exist that have been selected for differential responsiveness to drugs of abuse, including ethanol (5,12,15), opiates (2), and stimulants (19).

Intravenous (IV) self-administration of drugs of abuse has long been used as a model for studying drug reinforcement (22,23). Extensive work in a variety of species has allowed identification of many of the environmental and physiologic variables affecting IV drug self-administration (23). Unfortunately, identification of specific genes that might influence this behavior has been hampered, at least in part, by the lack of information about IV drug self-administration in well-characterized genetic animal models such as the inbred mouse.

Previous studies have indicated that mice will perform operant responses to obtain drugs of abuse IV. Two laboratories (6,11) used a procedure in which the tail vein served as the IV portal. Subjects nose-poking for drugs emitted more nose-pokes than yoked (Y) controls. However, these experiments required mice to be restrained by their tails during the entire self-administration session to prevent disruption of the tail vein cannula. Although these experiments provide evidence of drug reinforcement in mice, the behavior of these animals may have been affected by restraint stress, and restraint clearly precluded characterization of behaviors other than the operant (e.g., general activity). Moreover, although Criswell and Ridings (6) attempted to extinguish nose-poking by switching from morphine to saline infusions, experimental mice failed to decrease the frequency of nose-poke responding. No extinction was attempted by Kuzmin et al. (11).

In a recent report by Carney et al. (3), mice with chronically implanted jugular cannulae were allowed to locomote freely during drug administration sessions by use of a tethering system. The reinforcing potential of morphine, cocaine,

[1] Requests for reprints should be addressed to Dr. Nicholas J. Grahame, Department of Medical Psychology, L470, Oregon Health Sciences University, 3181 SW Sam Jackson Park Rd., Portland, OR 97201-3098.

methamphetamine, and pentobarbital in C57BL/6J mice was demonstrated by showing that animals increased lever-pressing rates when these drugs, but not when saline, were made contingent on lever-pressing. However, Y controls, that is, mice exposed to the same pattern of drug infusions in a non-response-contingent manner [e.g., (16)] were not included. Because the drugs used have well-known activity-enhancing effects in mice (18), it is unclear whether the response rate increases seen in these mice were due to these drugs' reinforcing effects or were instead manifestations of nonspecific increases in activity. Moreover, because activity data were not presented and cannula patency was not verified independently, conclusions about whether the animals were actually exposed to the planned contingencies are somewhat tenuous. Finally, the procedure of switching between vehicle and drug infusions in the same group of operant (O) subjects does not indicate whether the rate of the operant response depends on the response-reinforcer contingency or is instead due simply to the presence of the reinforcer. Y controls have long been used to assess whether a drug is serving as a reinforcer, and are best used in operant experiments in which the experimenter can be sure that the yoked animal receives the reinforcer (such as when the reinforcer is delivered by a cannula).

The purpose of the present experiment was to further develop the mouse IV self-administration model by addressing several of the issues raised in interpreting previous studies. Unrestrained C57BL/6J mice with jugular cannulae were placed in operant chambers with two nose-poke holes. A rapid infusion of cocaine was delivered when experimental mice (O) completed a fixed number of nose-pokes [fixed ratio (FR) schedule] into one nose-poke hole (designated "correct"). Nose-pokes in the opposite hole were not reinforced. Preferential nose-poking in the correct hole allowed differentiation between cocaine-contingent behavior and cocaine's locomotor-stimulating effects. As an additional means of determining whether the nose-poking response was contingent on cocaine reinforcement, mice in a control group (Y group) received cocaine infusions each time their yoked "master" from the O group earned a reinforcer. Although Y subjects might be expected to show increased nose-poking as a result of nonspecific activating effects of cocaine, they would not be expected to respond differentially to either nose-poke hole. Group differences in overall nose-poking rate and in preference for the O group–designated correct hole would presumably reflect the influence of the response-drug contingency. The experiment concluded with an attempt to extinguish the nose-poking response by replacing cocaine with saline. In addition to nose-pokes, general activity and side preference were automatically recorded during all phases of the experiment to better characterize this strain's behavior during cocaine self-administration.

METHOD

Subjects

Eighteen C57BL/6J male mice were obtained from the breeding colony at the Portland VA Medical Center, fewer than four generations removed from Jackson Laboratory stock. These mice were 7–9 weeks old and weighed from 20.4–27 g (mean, 24.1 g) at the beginning of the experiment. Prior to surgery, mice were housed in groups of four in polycarbonate cages (27.9 × 9.5 × 12.7 cm), with cob bedding, ad lib water and lab chow, and at an ambient temperature of 21 ± 1°C. After surgery, mice were housed individually in Thoren Shoebox cages (Hazleton, PA) except during operant sessions.

Surgical Procedure

Mice were anesthetized with 0.15 ml of a cocktail consisting of ketamine (17 mg/ml), acepromazine (3 mg/ml), and xylazine (6 mg/ml). Skin overlying the right clavicle and a strip on the midline of the dorsal surface were shaved and disinfected with Betadine solution (Norwalk, CT). A 0.7-cm incision was made in the skin overlying the jugular vein, the vein was exposed by blunt dissection, and two lengths of 5-0 surgical silk were threaded under the vein. A 1.5-cm incision was then made on the dorsal surface of the thorax, and a subcutaneous (SC) mouse saddle (IITC, Woodland Hills, CA) was implanted. The purpose of the saddle was to allow a stable dorsal exit for the cannula; without these saddles, mice frequently gnawed at the exit point for the cannula. This saddle was developed by James R. Weeks after trying numerous other methods of stabilizing the exit point for the cannula (James R. Weeks, personal communication, 1 November 1994). The saddle consisted of a 2-cm, 20-ga horizontal length of stainless-steel hypodermic tubing, with two inverted U-shaped, 4-cm wire legs mounted on either end of the tube. These legs were bent to conform to the shape of each mouse's thorax. A 1-cm piece of 27-ga hypodermic tubing, perpendicular to the SC horizontal tube, emerged from the animal's back to allow attachment to the drug-delivery tether. Silastic tubing (0.012-inch ID, 0.025-inch OD; 602-105; Dow Corning, Medland, MI) was run SC from the caudal aspect of the saddle to the jugular vein. The silastic cannula was then inserted through an incision in the jugular wall and advanced to a point just above the right atrium. The cannula was anchored to the vein with three lengths of suture, one of which, added after the catheter had been inserted, anchored the catheter and vein to muscle tissue immediately dorsal to the jugular. Cyanoacrylate glue was used to close both the dorsal and ventral skin incisions.

During and immediately after surgery, mice were kept at an internal body temperature of about 36°C (measured by rectal probe) using heating pads, until normal thermoregulation resumed. Shoebox cages were warmed with heating pads for 24 h after surgery. During recovery from surgery, cannulae were closed off with a cap placed over the external portion of the saddle, except during daily infusions of 0.02 ml heparin solution, given to reduce the probability of blood clotting in the cannulae. Furthermore, cannula patency was verified 48 h after surgery by infusion of Brevital (0.02 ml methohexital sodium, 10 mg/ml, Indianapolis, IN), an ultrashort, ultrafast-acting barbiturate. Mice that exhibited a delayed response to this infusion were eliminated from the study (see Results). Cannula patency was verified in this manner periodically (every 2–5 days), and at the end of each phase of training. This procedure was used because it was not always possible to withdraw blood from cannulae, even when patency was verifiable with Brevital. Animals generally recovered readily from this surgery and adapted well to the saddles, as demonstrated by rapid return to normal grooming and locomotor behavior. Training began 72 h after surgery.

Apparatus

Four identical acrylic and aluminum boxes (30 × 15 × 30 cm) were enclosed in separate, ventilated, light- and sound-attenuating enclosures. Infrared light sources and photodetectors (a total of six sets) were mounted opposite each other at 5-cm intervals on the long walls of each box, 2.2 cm above the floor. Three sets of photodetectors monitored activity on one half of the long (30 cm) side of the apparatus, whereas the

other three sets detected activity on the opposite half. A mouse was considered to have moved from one half to the other when all photobeams on one side were released and at least one beam on the opposite side was occluded. Total activity and time spent on each side were recorded automatically by computer.

One nose-poke hole with a diameter of 2.3 cm was mounted 1.5 cm above the floor in each of the 15-cm-wide aluminum ends of the box. A set of infrared light sources and photodetectors was mounted 1.5 cm inside each nose-poke hole. A response consisted of occlusion of the beam for at least 100 ms followed by cessation of beam interruption for at least 10 ms before the next response. A GE 47 bulb (powered by 5 VDC; Cleveland, OH) mounted behind the nose-poke hole provided indirect illumination of the hole during the 10-s time-out period following each drug infusion.

Subjects were tethered by Tygon tubing (Akron, OH) to a fluid swivel mounted in the center of the chamber lid, but were able to freely locomote around the boxes. Drug was advanced to the end of the tubing at the beginning of each session, and two drug deliveries were given at the beginning of each session to advance drug to the end of the IV cannula and give one priming dose of the drug.

Procedure

Subjects were randomly assigned to either O or Y groups. At the start of the experiment, squads consisted of three or four animals, with at least one subject from each group represented in each squad. One or two yoked animals were assigned to each operant subject. Following recovery from surgery, subjects were run in 1-h daily sessions between 1000 h and 0100 h in a test room separate from their colony rooms. Reinforcement consisted of 0.75 mg/kg cocaine dissolved in 5 μl saline delivered in <1 s using a pneumatic syringe pump (IITC). Drug solutions were replaced to account for changes in the animal's weight when the infusion dose exceeded 0.80 mg/kg or was <0.70 mg/kg.

All O subjects were started at FR-3; nose-poking was reinforced on only one side of the chamber, counterbalanced between animals in group O. Nose-pokes on the side in which reinforcement was available were considered to be "correct" nose-pokes. A partial reinforcement schedule was used from the start of the experiment because pilot studies indicated that a substantial number of nose-pokes were emitted as part of general exploratory activity on the first experimental day. Reinforcer delivery coincided with onset of a 10-s time-out period, during which responses were counted but had no scheduled effect. The time-out was signaled by illumination of the nose-poke hole light on the same side as that in which reinforcement was available. Otherwise, reinforcement was always available during acquisition sessions without any explicit signal of the contingency. Y subjects received drug administration and the light signal at the same time and on the same side as the paired O subject. For the purposes of graphing and statistical analysis, correct nose-pokes and time on reinforced side for group Y were considered to be nose-pokes on the same side as the light signaling reinforcement for each subject. There was no limit on the number of drug infusions possible during the session. The FR was incremented or decremented depending on each operant animal's behavior, until FR-10 was reached. Stable behavior was defined as $\geq$ 3 days of responding on the FR-10 schedule with $<$ a 20% change over the 3 days in the number of reinforcers obtained by each O subject. Following the acquisition of stable behavior, both groups were switched from cocaine to saline infusion to measure behavior during extinction of the nose-poke response.

RESULTS

Subject Attrition

Three subjects were eliminated from the experiment because their cannulae failed before experimental day 7. Fifteen mice (eight O and seven Y) completed at least 7 days of acquisition training and their data were used in the acquisition analyses reported subsequently. Six of those subjects were eliminated before completing the extinction phase, either because of cannula failure ($n = 2$), being yoked to an eliminated operant subject ($n = 2$), or poor health ($n = 2$). However, their cannulae were patent at least through day 7, as measured by a positive response to Brevital after day 7. Subjects eliminated as a result of poor health were eliminated more than 3 days after day 7, so that their illness likely had minimal impact on data collected up to day 7. The remaining nine subjects (five O and four Y) completed the entire experiment and showed a positive response to Brevital after the final session. Therefore, all mice were in good health and had cannula patency verified before being included in any data analyses.

Acquisition

The primary dependent variables during each of the first 7 days of acquisition were: total nose-pokes, percent correct nose-pokes, time spent on the correct side, total activity counts, and number of reinforcers. Some of these data are shown in Figs. 1-3; also see Table 1. Each dependent measure was analyzed by a separate Group $\times$ Session analysis of variance (ANOVA).

Total nose-pokes during each of the first seven acquisition sessions are depicted in Fig. 1. Group O increased total nose-pokes relative to group Y as a function of training sessions, providing between-groups evidence that nose-poking depended on the nose-poke-drug contingency. This finding was supported by an interaction of Group $\times$ Session [$F(6, 78) = 4.1$, $p < 0.001$], as well as a main effect of group [$F(1, 13) = 11.4$, $p < 0.01$]. Separate follow-up analyses for each group indicated that the increase in nose-poking over sessions was significant for group O [$F(6, 42) = 5.7$, $p < 0.001$] but

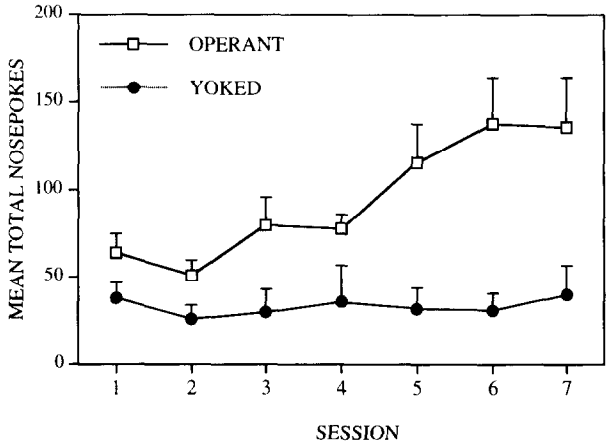

FIG. 1. Mean total nose-pokes emitted by subjects on both the reinforced and nonreinforced sides during each 60-min daily session. □, group O; ●, group Y. Bars denote standard error of the mean.

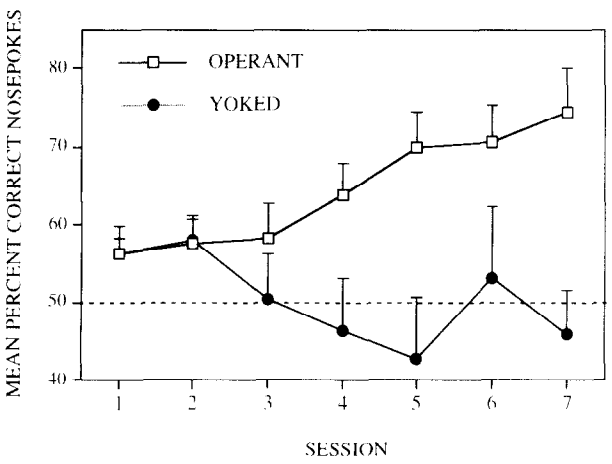

FIG. 2. Mean percent of nose-pokes emitted on the side reinforced with cocaine. □, group O; ●, group Y. Bars denote standard errors. The line at 50% represents chance responding.

not for group Y ($F < 1$). Increased nose-poking might be expected, in part, to result from the increasing FR demand. On day 1, the FR requirement was 3 for all group O subjects. By day 7, four subjects were at FR-10, one subject was at FR-6, and three subjects were at FR-4.

The percentage of correct nose-pokes was calculated by dividing nose-pokes on the reinforced side (including nose-pokes during the time-out periods) by total nose-pokes. As can be seen in Fig. 2, group O emitted a greater proportion of nose-pokes on the side reinforced with cocaine than did group Y, which tended to poke about equally often in both nose-holes. This observation was supported by a Group × Session interaction [$F(6, 77) = 4.1$, $p < 0.001$] and by a main effect of group [$F(1, 13) = 7.0$, $p < 0.05$]. For one mouse on day 5 in group Y, a zero nose-poke score made the percent score indeterminate, and this score was replaced by the median of the remaining subjects in that group on that day. Follow-up analyses showed that the increase in percent correct nose-pokes across sessions was significant in group O [$F(6, 42) = 5.3$, $p < 0.001$] but not in group Y. The finding that group O mice poked more frequently in the correct hole than in the incorrect hole provides additional (within-group) evidence of the control exerted by the nose-poke-drug contingency.

Figure 3 shows mean activity counts and mean reinforcers delivered over the first seven sessions. Locomotor activity of groups O and Y was similar, as indicated by a lack of a main effect of Group or a Group × Session interaction [each $F < 1$]. However, activity increased as a function of training sessions [$F(6, 78) = 3.6$, $p < 0.01$]. A one-way, repeated measures ANOVA was used to determine the effect of training on the number of reinforcers earned by group O subjects. This analysis showed no change in the number of reinforcers delivered across acquisition sessions ($F < 1$).

Time spent on the reinforced side of the operant chamber was not greatly affected by group assignment. In general, group O mice tended to spend more time on the reinforced side during acquisition than group Y mice (data not shown). However, the group effect did not reach significance [$F(1, 13) = 3.5$, $0.05 < p < 0.10$]. On day 7, mean time (± SEM) on the reinforced side was 2121 ± 136 s for group O and 1747 ± 94 s for group Y, out of a total of 3600 s.

The behavior of individual subjects for various response measures on day 7 is indicated in Table 1. These data indicate that most subjects in group O were nose-poking for cocaine, as demonstrated by a greater number of nose-pokes combined with more selective nose-poking than typical of subjects in group Y.

Extinction

The nine subjects who completed the entire experiment required 11–13 days to achieve the acquisition stability criterion on the FR-10 schedule. All subjects that maintained patent cannulae in group O for at least 2 weeks were able to meet the criterion for stable cocaine self-administration. To determine the effect of replacing cocaine with saline during the extinction phase, mean performance of each subject over its final 3 days of acquisition ("baseline") was compared to performance measured on each of the first 3 extinction days using Group × Session ANOVAs. Data for total nose-pokes, percent correct nose-pokes, and activity counts with number of reinforcers are shown in Figs. 4–6, respectively.

Figure 4 shows that extinction reduced nose-poking in group O, without affecting this behavior in group Y. ANOVA yielded significant main effects of Group [$F(1, 7) = 16.22$, $p < 0.005$] and Session [$F(3, 21) = 4.1$, $p < 0.05$] as well as a Group × Session interaction [$F(3, 21) = 3.1$, $p < 0.05$]. Follow-up comparisons indicated that group O differed from group Y during the final three acquisition sessions (i.e., baseline) [$F(1, 7) = 74.3$, $p < 0.001$] but not on the 3rd day of extinction ($p > 0.30$). Moreover, within-group contrasts between baseline and the 3rd day of extinction revealed a significant decrease in nose-poking in group O [$F(1, 21) = 15.29$, $p < 0.001$] but not in group Y ($F < 1$).

The percent correct nose-poking data (Fig. 5) indicate that group O emitted a greater percentage of their nose-pokes on the reinforced side than did group Y [$F(1, 7) = 22.6$, $p < 0.005$]. Somewhat unexpectedly, however, extinction did not diminish preference in group O—that is, there was no effect of Session, or a Group × Session interaction (each $p > 0.30$). Rather, both groups remained at about the same level of responding as during the baseline phase. Thus, despite showing a reduction in total nose-pokes during extinction, group O

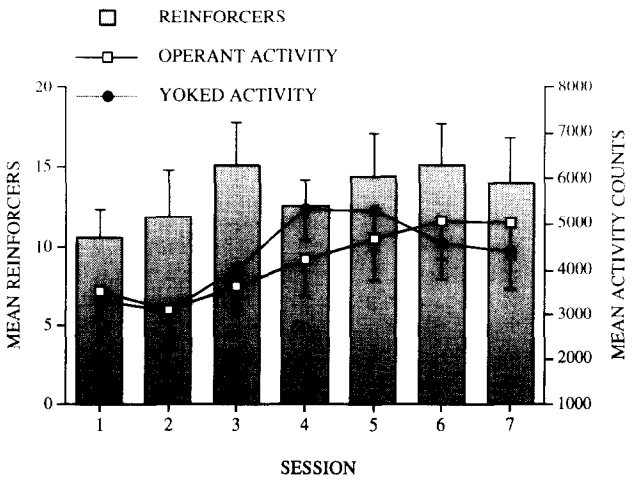

FIG. 3. Shaded bars indicate mean reinforcers administered to both groups, plotted on the left Y axis; lines depict mean activity scores for each group, plotted on the right Y axis. □, group O; ●, group Y. Bars denote standard errors.

TABLE 1

PERFORMANCE OF INDIVIDUAL SUBJECTS ON DAY 7

Group O Subject	Total NP*	% Corr. NP†	Time on Reinf. Side‡	Reinf.	FR
5/1	87	4.5	1413	8	4
5/3	197	79.2	2478	14	10
6/2	89	93.2	2433	18	4
6/4	114	61.4	2182	17	4
8/2	91	80.2	1878	7	10
7/1	39	71.8	1808	2	10
9/1	277	78.7	2425	20	10
9/3	191	87.9	2354	26	6
Group Y Subject				**Yoked to Subject**	
5/2	6	66.7	1982	5/3	N/A
5/4	9	44.4	2160	5/3	N/A
8/1	19	47.4	1689	8/2	N/A
8/3	38	63.2	1777	8/2	N/A
6/3	133	26.2	1464	6/4	N/A
7/2	48	37.5	1520	7/1	N/A
9/4	25	36.0	1636	9/3	N/A

*NP denotes nosepokes.
†Percent of total nosepokes on the side where cocaine was available.
‡Time on the side where cocaine was available; session duration was 3600 s.

mice continued to display a preference for the nosehole previously associated with cocaine delivery.

Activity data, presented in Fig. 6, were also affected by extinction. As might be expected, substitution of saline for cocaine decreased activity levels, as shown by a main effect of Session [$F(3, 21) = 18.8, p < 0.001$]. There was no main effect of Group or Group × Phase interaction (each $p > 0.10$). Number of saline infusions administered by group O during baseline and extinction was analyzed by repeated measures ANOVA. This analysis revealed a significant decrease over sessions in number of infusions [$F(3, 12) = 3.5, p < 0.05$].

On the other hand, time spent on the reinforced side was affected by switching from cocaine to saline infusion. Specifically, analysis of extinction data revealed a preference in group O for the side on which cocaine had previously been available. In contrast, group Y decreased time spent on the side associated with the cocaine signal. Group O's mean baseline score (± SEM) followed by scores on extinction days 1-3, respectively, were 2108 ± 182, 2480 ± 99, 2322 ± 253,

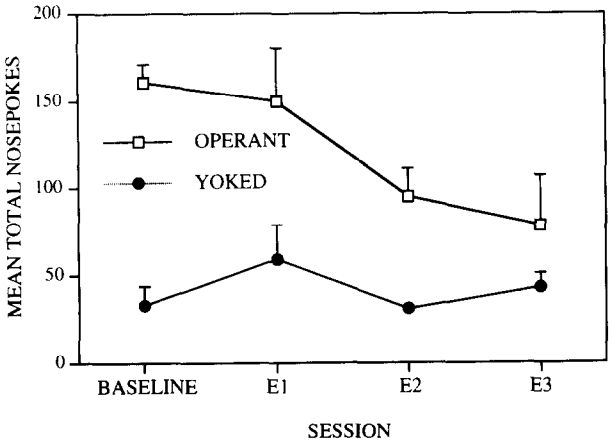

FIG. 4. Mean total nose-pokes emitted on both the reinforced and nonreinforced side during the final three acquisition sessions on the FR-10 schedule (Baseline) and each of three daily extinction sessions (E1, E2, and E3). □, group O; ●, group Y. Bars denote standard errors; where not visible, error bars are entirely contained within the plot symbol.

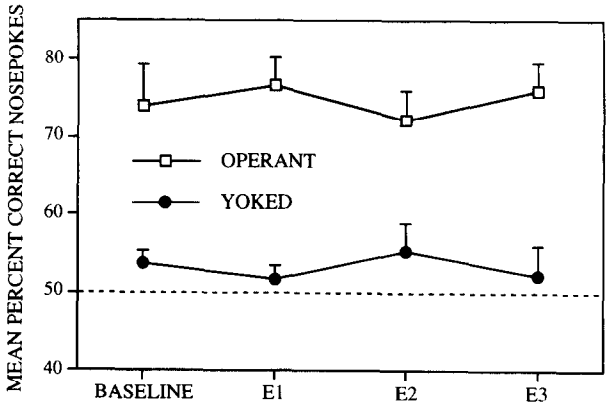

FIG. 5. Mean percent of total nose-pokes emitted on the reinforced side during the final three acquisition days (Baseline) and each of three extinction days (E1, E2, and E3). □, group O; ●, group Y. Bars denote standard errors. The line at 50% represents chance responding.

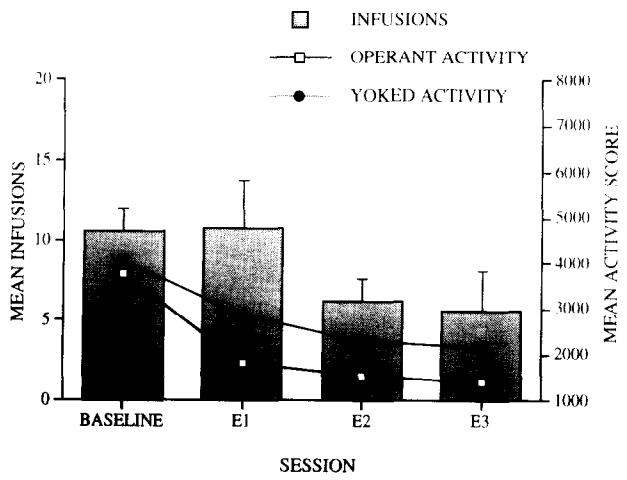

FIG. 6. Shaded bars indicate mean infusions (cocaine during baseline and saline during extinction) administered to both groups, plotted on the left Y axis; the lines depict mean activity scores for each group, plotted on the right Y axis. Data from the final three acquisition sessions (Baseline) and each of three extinction sessions are plotted. □, group O; ●, group Y. Bars denote standard errors.

and 2640 ± 216. Scores from the same days for group Y were 2060 ± 95, 1768 ± 44, 1937 ± 75, and 1582 ± 125. These findings yielded a main effect of Group [$F(1, 7) = 8.6$, $p < 0.05$] and a Group × Session interaction [$F(3, 21) = 6.9$, $p < 0.01$]. Follow-up comparisons indicated that group O differed from group Y during the third extinction session [$F(1, 7) = 15.5$, $p < 0.01$] but not during baseline ($F < 1$). Within-group contrasts showed that group O spent more time on the cocaine-paired side on the 3rd extinction day than during baseline [$F(1, 21) = 11.7$, $p < 0.005$], whereas group Y spent less time on the cocaine side on the 3rd extinction day than during baseline [$F(1, 21) = 7.6$, $p < 0.05$]. Enhanced expression of a latent side preference in group O may have been due to the lower activity levels seen during extinction than during the acquisition phase (see Discussion).

DISCUSSION

This experiment provided several kinds of evidence that nose-poking in C57BL/6 mice depended on a response-reinforcer contingency. First, group O mice showed a greater increase in total nose-poking over sessions than group Y mice, even though both groups were matched for amount and pattern of drug exposure. This finding indicates behavior of group O was dependent on a response-reinforcer contingency rather than the mere presence of drug. Second, group O mice showed a selective increase in nose-pokes directed toward the correct hole, whereas group Y mice did not. Finally, nose-poking by group O mice decreased when the response-drug contingency was eliminated during the extinction phase. Overall, these data offer strong support for the conclusion that nose-poking was maintained by reinforcing pharmacologic effects of cocaine and was not caused by nonspecific drug effects on general activity (17). To our knowledge, these data comprise the first demonstration of IV drug-self administration in freely locomoting mice with yoked controls and selective responding.

The lack of restraint in this experiment allowed characterization of cocaine-related behavior other than nose-poking, specifically locomotor activity and preference for the spatial location associated with cocaine delivery. Locomotor activity stimulated by cocaine increased over acquisition sessions (Fig. 3) and decreased over extinction sessions (Fig. 6). That the increase in activity occurred while the number of reinforcers remained constant may indicate sensitization (increased sensitivity) to the locomotor-activating effects of cocaine. Sensitization to the locomotor-activating effects of cocaine has been seen reliably in Pavlovian conditioning situations (20), and the parameters of the present operant conditioning preparation (i.e., reliable pairing of a particular context with drug administration) might be favorable to obtaining sensitization. Both groups O and Y (the latter of which received nonresponse-contingent cocaine administration) increased activity during the acquisition period. This finding contrasts with studies indicating that operant control of cocaine administration influences the development of changes in sensitivity to other effects of cocaine [e.g., toxic effects, see (7)].

Another outcome that emerged from the ability to assess unrestrained locomotor activity was the observation that during extinction, a preference was observed for the side on which cocaine had been available for group O. Cocaine-induced conditioned place preference has previously been reported in experiments involving nonresponse-contingent drug delivery in mice (18). In the present study, however, this preference emerged from operant training. That the preference was stronger during the extinction phase was somewhat unexpected. If side preference were simply a by-product of spending more time in the place where the subject nose-poked more often, one might have expected the preference to be greater during acquisition. However, group O mice displayed only a modest (nonsignificant) preference for the cocaine-paired side during acquisition, and side preference was enhanced rather than decreased by extinction. Moreover, this change in spatial preference occurred even though there was no change in the proportion of nose-poke responses emitted on the correct side during extinction. One way of explaining this effect might be that a Pavlovian conditioned place preference was formed in group O as a result of repeatedly receiving cocaine on a particular side of the chamber. Although mice presumably experienced cocaine intoxication on both sides of the apparatus, group O presumably was more likely to have been on the correct side immediately following reinforcement than was group Y. During acquisition, the locomotor activity elicited by cocaine may have interfered with the expression of this preference (21). According to this explanation, the conditioned place preference was revealed only in extinction because locomotor activity levels were lower in the absence of cocaine.

In contrast to O mice, Y mice decreased time spent on the cocaine-paired side during extinction. This effect is more difficult to interpret than the effect of extinction on O subjects. One possibility is that the decrease reflects extinction of a preference for the side on which the cocaine-associated time-out signal appeared. However, group Y's side preference did not differ from chance levels at any time during acquisition or extinction. In the absence of an acquired preference, it is difficult to argue that group Y demonstrated extinction of a preference. At present, therefore, the meaning of the behavior change shown by group Y is unclear.

One Pavlovian conditioning phenomenon that was not observed in the present experiment was sign-tracking (autoshap-

ing). In sign-tracking studies, subjects will learn to approach and often contact a localized visual cue paired with nonresponse-contingent administration of a reinforcer such as food (10). In the present experiment, group Y had the opportunity to acquire a sign-tracking response because the light presentations that signaled the time-out period for group O mice also signaled drug delivery for group Y mice. However, contrary to expectations based on a sign-tracking analysis, group Y mice showed no increase in nose-pokes, percentage of nose-pokes on the cocaine-paired side, or time spent on the cocaine-paired side during acquisition. Because autoshaping has previously been shown using food reinforcement in C57BL/6J mice (14), the present failure cannot be attributed to an inability to develop sign-tracking in this particular strain. Rather, in contrast to the predictions of some authors (13), it may be that drug reinforcers are simply unable to induce sign-tracking behavior.

Although group O showed several behaviors indicative of reinforcement by cocaine, they did not tend to increase the number of reinforcers administered across sessions. Although one would expect in many situations that acquisition would be reflected in a greater number of reinforcers administered over time, the present study began with a relatively simple operant response (i.e., FR-3 nose-poking), which may have been acquired quite rapidly by many subjects, perhaps during the first session. During subsequent sessions, response requirements were increased, but subjects were able to maintain the rate of reinforcement seen early in training. According to this view, little tolerance or sensitization developed to the reinforcing effects of cocaine during the period assessed in this experiment, so that mice showed a constant level of reinforcement across sessions.

Techniques used in the present experiment may allow future characterization of the genetic bases of drug craving and reinforcement. Although genetic differences in cocaine's reinforcing potential were not assessed in the present experiment, it is hoped that the mouse IV drug self-administration model developed here will prove instrumental in assessing genetic and other influences on sensitivity to drug reinforcement. There are obvious drawbacks to using the IV method of administration shown here, including the difficulty of maintaining cannula patency, which may or may not decrease with additional experience. However, the IV preparation demonstrated here as well as by (3) has certain advantages over oral self-administration for studying genetic differences in drug reward. First, the present procedure allowed the use of yoked controls (in addition to an inactive response manipulandum) to separate nonspecific activating effects of cocaine on nose-poking behavior from behavior resulting from the response-reinforcer contingency. Second, the IV procedure used here obviated the need to interpret effects caused by taste cues in oral self-administration, which can include unconditioned rejection of drug flavors before pharmacologic blood levels are reached (1). In addition, oral self-administration studies often use extensive forced preexposure to the drug solution (9) or exposure to combined solutions of ethanol and cocaine (8) before assessing cocaine's reinforcing effects. The present experiment assessed cocaine self-administration in drug-naive animals. Finally, given that humans in Western cultures tend to abuse cocaine via routes other than oral self-administration, modeling genetic differences in IV self-administration of cocaine in mice may prove a highly valid model for cocaine pharmacogenetics.

ACKNOWLEDGEMENTS

This research was supported by grants from the Department of Veterans Affairs, the Medical Research Foundation of Oregon, NIAAA P01 Grant AA08621 and NIDA Training Grant DA07262. Thanks are extended to Julie Broadbent for her assistance with surgical technique. All procedures were reviewed by and performed in accordance with guidelines from the OHSU Institutional Animal Care and Use Committee.

REFERENCES

1. Belknap, J. K.; Belknap, N. D.; Berg, J. H.; Coleman, R. Preabsorptive vs. postabsorptive control of ethanol intake in C57BL/6J and DBA/2J mice. Behav. Gen. 7:413–425; 1977.
2. Belknap, J. K.; O'Toole, L. A. Studies of genetic differences in response to opioid drugs. In: Crabbe, J. C.; Harris, R. A., eds. The genetic basis of alcohol and drug actions. New York: Plenum Press; 1991:225–252.
3. Carney, J. M.; Landrum, R. W.; Cheng, M. S.; Seale, T. W. Establishment of chronic IV drug self-administration in the C57BL/6J mouse. Neuroreport 2:477–480; 1991.
4. Crabbe, J. C. Genetic animal models in the study of alcoholism. Alcohol Clin. Exp. Res. 13:120–127; 1989.
5. Crabbe; J. C.; Phillips, T. J.; Kosobud, A.; Belknap, J. K. Estimation of genetic correlation: Interpretation of experiments using selectively bred and inbred animals. Alcohol Clin. Exp. Res. 14: 141–151; 1990.
6. Criswell, H. E.; Ridings, A. Intravenous self-administration of morphine by naive mice. Pharmacol. Biochem. Behav. 18:467–470; 1983.
7. Dworkin, S. M.; Volkmer, C.; Dworkin, S. I. Toxic consequences of cocaine are augmented by noncontingent drug administration. Soc. Neurosci. Abstr. 14:388.3; 1988.
8. George, F. R.; Elmer, G. I.; Meisch, R. A.; Goldberg, S. R. Orally delivered cocaine functions as a positive reinforcer in C57BL/6J mice. Pharmacol. Biochem. Behav. 38:897–903; 1991.
9. George, F. R.; Goldberg, S. R. Genetic differences in responses to cocaine. In: Clovet, D.; Asghar, K.; Brown, R., eds. NIDA Research Monographs, vol. 88. Rockville, MD: US Department of Health and Human Services; 1988:239–249.
10. Hearst, E.; Jenkins, H. M. Sign tracking: The stimulus-response relation and directed action. Austin, TX: Monograph of the Psychonomic Society; 1974.
11. Kuzmin, A.; Zvartau, E.; Gessa, G. L.; Martellota, M. C.; Fratta, W. Calcium antagonists isradipine and nimodipine suppress cocaine and morphine IV self-administration in drug-naive mice. Pharmacol. Biochem. Behav. 41:497–500; 1992.
12. McClearn, G. E.; Kakihana, R. Selective breeding for ethanol sensitivity: Short-sleep and long-sleep mice. In: McLearn, G. E.; Deitrich, R. A.; Erwin, V. G., eds. Development of animal models as pharmacogenetic tools. NIAAA Research Monograph no. 6. Washington, DC: U.S. Government Printing Office; 1981:147–159.
13. Newlin, D. B. A comparison of drug conditioning and craving for alcohol and cocaine. In: Galanter, M. ed. Recent developments in alcoholism, vol. 10: Alcohol and cocaine: Similarities and differences. New York: Plenum Press; 1992:147–164.
14. O'Connell, M. F. Autoshaping and food acquisition in mice: A genetic analysis. J. Comp. Physiol. Psych. 94:1149–1159; 1980.
15. Phillips, T. J.; Burkhart-Kasch, S.; Terdal, E. S.; Crabbe, J. C. Response to selection for ethanol-induced locomotor activation: Genetic analyses and selection response characterization. Psychopharmacology 103:557–566; 1991.

16. Pickens, R.; Meisch, R.; McGuire, L. E. Methamphetamine reinforcement in rats. Psychonom. Sci. 8:371–372; 1967.
17. Pickens, R.; Thompson, T. Cocaine-reinforced behavior in rats: Effects of reinforcement magnitude and fixed-ratio size. J. Pharmacol. Exp. Ther. 161:122–129; 1968.
18. Seale, T. W.; Carney, J. M. Genetic determinants of susceptibility to the rewarding and other behavioral actions of cocaine. J. Addict. Dis. 10:141–162; 1991.
19. Smolen, A.; Marks, M. J. Genetic selections for nicotine and cocaine sensitivity in mice. J. Addict. Dis. 10:7–28; 1991.
20. Stewart, J. Conditioned stimulus control of the expression of sensitization of the behavioral activating effects of opiate and stimulant drugs. In: Gormezano, I.; Wasserman, E. A., eds. Learning and memory: The behavioral and biological substrates. Hillsdale, NJ: Erlbaum; 1992:129–151.
21. Vezina, P.; Stewart, J. Morphine conditioned place preference and loco-motion: The effect of confinement during training. Psychopharmacology 93:257–260; 1987.
22. Weeks, J. R. Experimental morphine addiction: Method for automatic IV injections in unrestrained rats. Science 138:143–144; 1962.
23. Yokel, R. A. Intravenous self-administration: Response rates, the effects of pharmacological challenges, and drug dependence. In: Bozarth, M. A., ed. Methods of assessing the reinforcing properties of abused drugs. New York: Springer-Verlag; 1987:1–33.