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Acquisition of lever pressing for cocaine in C57BL/6J mice: effects of prior Pavlovian conditioning

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Abstract

The purpose of this study was to determine (1) if C57BL/6J (C57) mice would lever-press for intravenous cocaine infusions in a limited-access paradigm without previously establishing the instrumental response with natural reinforcers and (2) if prior Pavlovian conditioning of cocaine to the response contingent stimulus complex used in the cocaine self-administration sessions would facilitate acquisition of lever responding for cocaine. After implanting jugular catheters, some mice received Pavlovian conditioning during which 12 passive cocaine infusions (0.1 or 1 mg/kg unit doses) were paired with the tone/light/pump sound stimulus complex used in the self-administration sessions. The remaining mice simply began the cocaine self-administration sessions for 0.1 or 1 mg/kg unit doses of cocaine. Twenty-seven of the 33 mice with patent catheters acquired stable lever responding within an average of 5 to 6 days without previously establishing the instrumental response with natural rewards. Prior Pavlovian pairing of cocaine with the response contingent stimulus complex used in the self-administration sessions did not influence the acquisition of cocaine self-administration at the highest cocaine dose (1 mg/kg). This conditioning procedure using the low cocaine dose (0.1 mg/kg/infusion) reduced the number of mice acquiring cocaine self-administration to 50%, and the number of mice developing stable response patterns was only 25%. The results establish that C57 mice can acquire cocaine self-administration over several unit doses in a limited-access paradigm without previously establishing the instrumental response with natural reinforcers. Furthermore, prior pairing of response contingent cues with cocaine via Pavlovian conditioning did not facilitate the acquisition of cocaine self-administration.

Keywords: Drug abuse; Cocaine addiction; Pavlovian transfer; Cocaine self-administration; Animal models

1. Introduction

Several recent studies indicate that techniques are available for intravenous self-administration of abused substances by mice (Grahame et al., 1995; Roberts et al., 1997; Caine et al., 2002; Highfield et al., 2002; Fuchs et al., 2003). The availability of many genetically modified strains of mice provides impetus for developing intravenous self-administration techniques in this species (e.g., Rocha et al., 1998a; Caine et al., 2002). In spite of this need, intravenous self-administration in the mouse remains relatively undeveloped compared to the rat or monkey species. One limitation for intravenous self-administration of drugs by mice is the relatively short duration of jugular catheter patency. This limitation necessitates establishing conditions that facilitate the acquisition of intrumental responses for intravenous drug administration.

One common method to promote rapid acquisition of operant behaviors for drug reward in rodents is to establish the instrumental response with natural reinforcers before implanting jugular catheters, and several studies have used the procedure to facilitate the acquisition of cocaine self-administration in mice (Rocha et al., 1998a,b; Caine et al., 1999, 2002; Fuchs et al., 2003). This training procedure creates an association of the instrumental response with a positive reinforcer other than cocaine (e.g., sucrose or food). The problem created by this procedure is that it is unclear whether the lever-response–food association extinguishes within the time frame of catheter patency for a mouse and the mouse is truly responding for cocaine reinforcement.

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The C57BL/6J (C57) strain of mice is commonly used in laboratory studies and much is known about how they respond to cocaine in a variety of paradigms. Most published studies on lever responding for intravenous cocaine by C57 mice have established the lever response with food or sucrose reward before cocaine self-administration. Although two studies indicate that mice can initiate lever pressing for cocaine without prior conditioning, one was a continuous housing paradigm (Carney et al., 1991) and the other used Swiss Webster mice (Tsibulsky and Norman, 2001). Thus, whether C57 mice will acquire stable self-administration of cocaine using an infrequently emitted unnatural response such as the lever press during a limited access paradigm remains to be established.

The present study was conducted to determine (1) if C57 mice would acquire lever responding for cocaine without prior training of the instrumental response using natural reinforcers and (2) whether Pavlovian conditioning pairing cocaine with the response contingent stimuli used in cocaine self-administration sessions before initiating the sessions would facilitate the acquisition of lever pressing for cocaine. It was hypothesized that the cocaine-conditioned cues established by the prior Pavlovian conditioning would serve as a conditioned reward in the operant paradigm, thus facilitating acquisition of the instrumental response, particularly in the early self-administration sessions.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice obtained from Jackson Laboratories (Bar Harbor, ME) were housed individually in an AALAC-accredited animal facility and were maintained on a 12-h reverse-light cycle (lights on at 1800 h). The mice were approximately 60 days of age at the beginning of the experiment and were tested during the dark phase of their circadian cycle. Food and water were available ad libitum except as noted below. All experimental protocols were approved by the Institutional Animal Care and Use Committee and were consistent with the guidelines of the NIH *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 80-23, revised 1996).

2.2. Equipment

The self-administration chambers used in this study (model ENV-307A) were enclosed in sound-attenuating cabinets, both manufactured by MedAssociates (Georgia, VT). Two levers were inside the chamber on the same wall and elevated 6.5 cm above the grid floor (lever model ENV-310). Two grams of force on a lever defined a response. Following a response on the active lever, cocaine infusions were delivered on a FR1 schedule of reinforcement. A response on the active lever (1) terminated the house light, (2) initiated a 2-s infusion of cocaine (19 μL), and (3) initiated a 2-s compound conditioned stimulus consisting of a tone (2900 Hz, ENV-323A), a yellow LED stimulus light (ENV-321M) located directly above the active lever, and the infusion pump noise (model PHM-100). The house light remained off for an additional 20-s timeout period, indicating that cocaine was not available. Responses on the alternative lever were recorded but had no programmed consequences. Finally, the jugular catheter port located on the mouse skull was connected to the infusion pump via a single channel fluid swivel (Instech Laboratories, 375 series).

2.3. Catheter construction

The catheters were designed to be inserted into the right jugular vein and have a skull-mounted access port using an adaptation of a previously published procedure (Kelley et al., 1997). The bodies of the catheters were constructed of Silastic tubing (0.30 mm i.d., 0.64 mm o.d.; Dow Corning). A small bead of silicon adhesive placed near the end of the tubing served as an anchor point for the sutures. The access port of the catheter was constructed of 22-gauge stainless steel tubing cut and polished to a final length of 1.5 cm (Small Parts, Inc.). The stainless steel tubing was smoothly bent at 90° degrees near the middle of its length. Additionally, the portion of the steel tubing that would lie parallel to skull was smoothly bent into a U shape so that the open end of the tubing faced 180° from its original position, but would still be parallel to the skull. This shaping procedure provided a flat, stable base for mounting the access port to the skull. The exposed end of the access port extended vertically from the skull and, when not in use, was occluded by a short piece of tubing sealed at one end.

2.4. Catheterization surgery

Catheterization was completed using an adaptation of a previously described procedure (Kelley et al., 1997). In brief, mice were anesthetized with a mixture of xylazine (6 mg/kg) and ketamine (120 mg/kg). The right jugular vein was exposed by blunt dissection and then phlebotomized. The catheter tip was inserted approximately 1.2 mm and placed near the atrium of the heart. The catheter was secured to the vein using 5-0 silk sutures. Internal adipose and connective tissue on either side of the catheter were sutured together over the catheter so that it was secured in the subcutaneous space. The incision was closed using cyanoacrylate adhesive. The catheter passed subcutaneously in a direct path to an incision on top of the skull. The catheter's access port was secured to the skull using a light-cured resin as previously described (Groseclose et al., 1998). On the day of surgery, the mice were given a single intravenous dose of cefazolin (50 μl, 100 mg/ml) and this was followed by heparinized saline (20 μl, 90 U/ml).

2.5. Catheter maintenance and patency

Following surgery, the catheters were flushed twice daily with 20 μl of heparinized saline (30 U/ml first flush and 90 U/ml second flush). Catheter patency was assessed every 6 days, or as changes in behavior indicated, using a bolus infusion of methohexital HCl (20 μl, 6.25 mg/ml). Rapid sedation was regarded as a positive test. Any mice with delayed or negative tests were removed from the study.

2.6. Experimental procedure

Upon arrival, mice were habituated to the handling necessary for catheter maintenance and connection to the infusion pump by daily handling for 5 to 7 days before catheterization surgery. After 48 h of postsurgical recovery, the mice were introduced to the operant chambers. The experiment involved four groups of mice differing according to the cocaine unit dose per infusion (0.1 mg/kg "low" dose and 1 mg/kg "high" dose), and according to whether or not the mice were exposed to Pavlovian conditioning ("paired"), or not ("nonpaired") before cocaine self-administration sessions. Mice in the paired groups received a Pavlovian conditioning procedure before the operant self-administration session. This conditioning procedure consisted of a single 75-min session in the operant chamber with the levers covered. During this session, the mice received 12 randomly timed passive infusions of cocaine at the 0.1 or 1 mg/kg unit dose, depending on group assignment, each paired with the light/tone/pump cue complex described for the active lever response. For the remaining daily, 2-h cocaine self-administration sessions, mice in the paired groups were treated just as the nonpaired mice, and cocaine was delivered only following a lever press on the active lever. Mice in the two nonpaired groups received no Pavlovian conditioning, but were allowed to acquire lever responding for cocaine at 0.1 or 1 mg/kg unit dose, depending on group assignment, beginning with the first session in the chamber.

A distinguishing characteristic for all of the groups is that no cocaine priming or food enticements were used to promote lever-pressing behavior. To increase the probability of lever pressing, after the first self-administration session mice were given only 2 g of food for 1 day, and 5 g/day for the next 4 days. Thereafter, food access was unlimited.

2.7. Data analysis

The primary data for the experiment were responses on the active lever that resulted in a cocaine infusion, total active lever presses and total inactive lever presses. These data were used to determine whether or not the mice acquired cocaine self-administration, the intake of cocaine (mg/kg) per session, whether responding was stable across sessions, and correct lever choice. The criteria for acquisition of cocaine self-administration was set at ≥ 10 active lever presses that delivered an infusion for $\geq$ 5 sessions. Since deviation from baseline is important for some experimental manipulations, the development of stable responding was also evaluated. Stability was defined as 3 consecutive days in which the mean number of active lever presses resulting in cocaine infusions did not vary more than 20% and at least 80% of the total responses were on the active lever.

Data were analyzed with 2×2 between-groups analysis of variance (ANOVA) for the Dose and Training Procedure factors. Newman–Keuls tests were applied for post hoc comparisons. The assumption of homogeneity of error variance across the groups was assessed with Bartlett's chi-square test. In the event of heterogeneity of error variance across groups, data were transformed ($\log_e$) before analysis. Probabilities less than .05 ($P < .05$) were considered statistically significant.

3. Results

Of the 42 mice that began the study, three mice from the low-dose groups (0.1 mg/kg) were eliminated because of nonpatent catheters and their data were excluded from analysis. For the remaining mice, data comparing the two different procedures (paired and nonpaired) across two doses (0.1 mg/kg, "low" and 1 mg/kg, "high") on the acquisition and stability of cocaine self-administration are summarized in Table 1. This table summarizes data from

Table 1

Dose (mg/kg unit dose of cocaine)	Group[a]	n[b]	No. of mice meeting criteria		No. of sessions to meet criteria ($X \pm$ S.E.M.)		Active lever presses ($X \pm$ S.E.M.)
			Acquisition	Stable resp.	Acquisition	Stable resp.	Acquisition
0.1	P	8	4	2	3 ± 1.3	6	102 ± 19
0.1	NP	11	9	8	4.3 ± 0.7	5.9 ± 0.7	107 ± 15
1	P	7	7	5	2.1 ± 0.6	5.2 ± 0.6	30 ± 2.9
1	NP	7	7	6	2.3 ± 0.5	5.7 ± 0.8	29 ± 2.1

Acquisition refers to mice meeting the acquisition criteria.
Stable resp. refers to mice meeting the stability criteria.
[a] P, paired group, refers to mice in the Pavlovian conditioning group; NP, nonpaired group, refers to mice that did not receive Pavlovian conditioning.
[b] Number of mice included in the group.

the four groups on (1) the numbers of mice meeting the criteria, (2) the number of sessions to meet criteria performance, and (3) the number of active lever presses resulting in cocaine infusions per session based on the average of the last three sessions.

For both the acquisition and stability measures, the prior Pavlovian conditioning procedure reduced the proportion of mice self-administering the low dose of cocaine. As noted in Table 1, only 50% of the mice in the paired low-dose group met the acquisition criteria in comparison to 88% for the nonpaired low-dose group, and 100% for each of the high-dose groups. Chi-square analysis of these data indicated that the number of mice acquiring cocaine self-administration varied according to training procedure and dose [$\chi^2 = 8.6$, $P = .04$], with dose being the most influential factor [$\chi^2 = 5.4$, $P = .02$]. Although the paired low-dose group differed from each of the high-dose groups [$\chi^2 = 5.4$, $P = .02$], it did not differ significantly from nonpaired low-dose group [$\chi^2 = 2.2$ $P = .14$]. Mice meeting the stability criterion in the different groups followed a similar pattern with stable cocaine self-administration across days depending on training procedure and dose. However, in this case, chi-square analysis across the four groups reached only a .06 level of significance [$\chi^2 = 3.2$, $P = .06$]. Fewer mice in the paired low-dose group met the stability criteria in comparison to nonpaired mice in either the low-dose [$\chi^2 = 4.2$, $P = .04$] or the high-dose [$\chi^2 = 5.5$, $P = .02$] groups.

The data for the number of sessions to reach the beginning of the five consecutive days required to meet the acquisition criteria were analyzed with a 2(dose) × 2(procedure) ANOVA. Dose influenced the number of sessions required for meeting the acquisition criteria [$F(1,23) = 43.5$, $P < .0001$], with mice in the high-dose groups requiring fewer sessions. However, neither the pairing procedure [$F(1,23) < 1$] nor its interaction with dose [$F(1,23) < 1$] influenced the sessions required to acquire the response. In addition, the number of sessions required to meet the stability criteria was not influenced by either dose or training procedure, with all groups requiring approximately five to six sessions to meet the stable self-administration criteria. These data were not evaluated statistically because only two mice in the paired, low-dose group reached stability.

Active lever presses resulting in cocaine infusions by mice meeting acquisition criteria are shown in the last column of Table 1. These data were transformed to comply with the homogeneity of error variance assumption ($\chi^2 = 26.5$, $P < .0001$). A 2(dose) × 2(procedure) ANOVA on the transformed data indicated that the mice made fewer reinforced responses for the high than for the low cocaine dose during the last three sessions of responding [$F(1,23) = 82.8$, $P < .0001$]. However, neither training procedure [$F(1,23) < 1$] nor its interaction with dose influenced the number of reinforced responses. The groups were also compared on percent of total responses made on the cocaine contingent lever. The low-dose groups averaged 86% and 81% for the paired and nonpaired mice, respectively, compared to 92% and 93% for the comparable high-dose groups. However, a 2(dose) × 2(training procedure) ANOVA on the transformed data indicated that training procedure [$F(1,23) < 1$], dose [$F(1,23) = 2.9$], and their interaction [$F(1,23) < 1$] did not significantly influence this measure.

Fig. 1 illustrates the acquisition of lever pressing behavior in the four groups of mice. The median lever presses (active or inactive) are shown across sessions. The median lever presses are shown due to the large variance in lever presses, particularly within the first two to three sessions in the low-dose, paired group, which was composed of only four mice. Fig. 1 underscores the findings shown in Table 1 that the pairing procedure used in this experiment did not

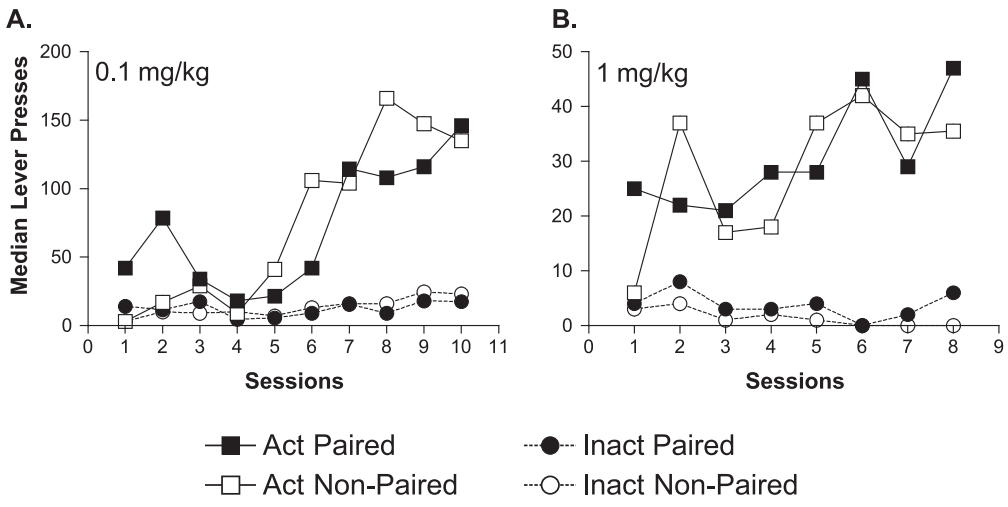

Fig. 1. Time course of acquisition for intravenous cocaine reinforcement for the paired (closed symbols) and nonpaired (open symbols) groups of mice. Median active lever presses (squares) and median inactive lever presses (circles) are shown for all four groups of mice.

improve acquisition of lever-pressing behavior. In addition, it also illustrates that the mice readily acquired lever-pressing behavior directed at the appropriate lever within a few sessions without operant training before surgery, regardless of training procedure.

Data from a fifth group of mice ($n=6$) that had undergone the pairing procedure and acquisition with a 0.3 mg/kg unit dose of cocaine was compared with the mice in the paired low-dose and paired high-dose groups. Four of the mice from the 0.3 mg/kg group met the acquisition criteria in an average of 2.5 days and two of these mice developed stable responding in an average of 4.5 days. For the mice that acquired responding, the active lever presses that yielded infusions were intermediate to those of the other paired groups (64.8 ± 13.1), indicating an orderly dose–response function in these three groups of mice. Bartlett's test of the response data in these three paired groups was significant ($\chi^2=7.6$, $P=.0224$) and these data were transformed ($\log_e$) before further analysis. A one-way ANOVA of these data indicated an effect of dose [$F(2,12)=17.6$, $P=.0003$] and post hoc analysis with Newman–Keuls test confirmed that all three groups differed from one another ($P<.05$), confirming an orderly dose–response function for the three paired groups of mice. Finally, the amount of cocaine intake for these three groups was evaluated (9.75 ± 1.6, 18.3 ± 3.6, and 34 ± 2.4 mg/kg/session $\pm$ S.E.M. for the low, middle and high doses, respectively). The cocaine intake data met the homogeneity of variance requirement and was not transformed ($\chi^2=1.6$). A one-way ANOVA on the cocaine intake data supported the systematic increase in the amount of cocaine obtained with increasing unit dose noted in Fig. 1 [$F(2,14)=23.2$, $P<.0001$]. Newman–Keuls post hoc tests again indicated that the groups differed from one another ($P<.05$).

4. Discussion

C57 mice in this study acquired lever pressing for intravenous cocaine in a limited-access paradigm without previously establishing the instrumental response with natural reinforcers, such as food, water, sucrose, etc., as was reported for mice continuously maintained in operant chambers (Carney et al., 1991). Cocaine self-administration was acquired over a 10-fold range of cocaine unit doses (0.1–1 mg/kg) with only mild food restriction during the initial phase of training, and without cocaine priming injections or food enticements. Moreover, most of the mice in this study exhibited good lever discrimination and stable response patterns within five to six sessions. The decreasing number of infusions obtained by the mice as the unit dose of cocaine increased is commonly reported in cocaine self-administration studies (LeSage et al., 1999) and indicates that the unit doses used in our study were on the descending limb of an inverted U function. Our findings that fewer mice receiving the low dose met the acquisition criteria and that mice in this group took slightly longer to meet criteria at the lower unit dose suggest that it was a borderline reinforcing unit dose for C57 mice. The reduced time to acquire the response at the higher unit dose compared with the lower unit dose is consistent with previous reports for cocaine (Gerrits and van Ree, 1995; Carroll and Lac, 1997). Prior Pavlovian conditioning of cocaine to stimuli contingent on cocaine-producing responses in cocaine self-administration sessions had no effect on acquisition of lever pressing for cocaine at the high cocaine unit dose and reduced the number of mice acquiring the response for the low dose of cocaine.

In spite of the reduced proportion of mice in the low-dose groups meeting cocaine self-administration acquisition criteria and the slight increase in the number of sessions required to meet these, the percent responses on the correct lever did not differ significantly from the high-dose groups. Thus, it appears that the 0.10 mg/kg unit dose provided interoceptive stimuli sufficient for discrimination by those mice that met the criteria.

Slightly less than 10 mg/kg per 2-h session was sufficient to maintain responding by most of the C57 mice in the two low-dose groups, which is similar to intake by this strain over a 23-h period in an earlier study (Carney et al., 1991). On the other hand, the number of responses made for cocaine delivered on the continuous reinforcement (CRF) schedule of in our experiment has similarities and differences to that reported for C57 mice when cocaine was delivered on an FR2 schedule (Rocha et al., 1998b). In the former study, mice obtained approximately 17 infusions of cocaine per hour over a 90-min period when a 1.0 mg/kg dose was delivered on the FR2 schedule, an amount that closely approximates the 14–15 infusions per hour over the 2-h session for the same unit dose of cocaine delivered on the CRF schedule in our study. In contrast, when a 0.25 mg/kg dose was delivered on the FR2 schedule in the former study, mice obtained approximately eight infusions per hour compared to approximately 55 infusions per hour on the CRF schedule in our experiment. In addition to reinforcement schedule, other methodological differences in these two studies might account for different amounts of cocaine self-administered. In the former study, mice had experience lever pressing for 2 mg/kg unit doses of cocaine before introducing the lower doses; whereas, in our study the mice only had experience self-administering one unit dose of cocaine. Thus, it is possible that negative contrast might have contributed to the low response output in the previous study.

The amount of cocaine obtained over the 2-h session in our study also differs from that reported for C57 mice performing a more natural nose-poke response for cocaine (Kuzmin and Johansson, 2000). Nose-poke responses for cocaine unit doses ranging from 0.12 to 0.24 mg/kg produced approximately 20 mg/kg over a 30-min session. In comparison, lever responding over the longer 2-h session in our study for the 0.3 mg/kg unit dose produced approx-

imately 18 mg/kg, and about 10 mg/kg for the 0.1 mg/kg unit dose. These differences suggest that the use of a more difficult and unnatural instrumental response such as the lever response may reduce the amount of cocaine self-administered. A recent report, however, indicates that the self-administration of a cocaine + heroin mixture (speedball) was similar for both the nose-poke and lever responses (David et al., 2001). Thus, the apparent discrepancies at low doses may be due to methodological differences such as self-administration history or the use of a more difficult instrumental response such as the lever press. Nevertheless, the fact remains that C57 mice will self-administer cocaine delivered at several unit doses and on different reinforcement schedules using different operant procedures.

Although Pavlovian conditioning enables conditioned cues to maintain established instrumental responding for natural and drug reinforcers during periods of extinction (Hyde, 1976; Dickinson and Dawson, 1987; Dickinson et al., 2000; Hall et al., 2001), the procedure used in the current experiment did not facilitate the acquisition of lever responding for cocaine. In contrast to our hypothesis, prior Pavlovian conditioning did not facilitate the acquisition of cocaine self-administration at different unit doses. In fact, at the lower unit dose, the procedure reduced the percentage of mice meeting the acquisition criteria to 50% in comparison to 73% for the nonpaired controls, and only half of those developed stable responding. Clearly, the Pavlovian conditioning procedure used in this experiment did not facilitate acquisition of cocaine self-administration and hindered acquisition and the development of stable responding in most mice at the low cocaine dose.

The reasons that Pavlovian conditioning interfered with the acquisition of cocaine self-administration of the low dose are not provided by our experiment. Insufficient interoceptive discriminative, or hedonic cues associated with low dose is not likely accountable for the absence of Pavlovian transfer since the most mice acquired cocaine self-administration of the low dose in the nonpaired group. A negative contrast effect might account for the poor acquisition of self-administration of the low cocaine dose. In contrast to the 12 infusions over a 75-min period for the Pavlovian conditioning session, the rate of self-administered cocaine was insufficient to promote responding. The reduced amount of cocaine may have created a negative contrast effect similar to that observed when rodents are switched from high to low sucrose solutions and manifests as a reduction in the instrumental behavior to obtain the solution (e.g., (Weinstein, 1978; Flaherty and Rowan, 1989; Timberlake and Engle, 1995)). Thus, in the present study, it is possible that the amount of self-administered cocaine provided less "reward" for some of the mice in the low-dose group than was obtained during the conditioning procedure. Further experimentation is needed to address this interpretation.

In conclusion, C57 mice in this study acquired lever pressing for intravenous cocaine at unit doses of 0.1 mg to 1 mg/kg in a limited-access paradigm without previously establishing the instrumental response with natural reinforcers, such as food, water, sucrose, etc. The Pavlovian conditioning paradigm used to facilitate the acquisition of cocaine self-administration was not effective at either cocaine dose and, in fact, appeared to be detrimental to the acquisition and development of stable cocaine self-administration.

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