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Factors That Determine a Propensity for Cocaine-Seeking Behavior during Abstinence in Rats

Michael A. Sutton, B.Sc., David A. Karanian, M.A., and David W. Self, Ph.D.

Individual differences in locomotor responses to novelty and psychostimulants, and sensitization following repeated drug exposure, predict increased sensitivity to the reinforcing effects of psychostimulants and are thought to underlie vulnerability to drug addiction. This study tested whether these factors determine another core feature of drug addiction, the propensity for drug-seeking behavior during abstinence in rats with prior cocaine-self-administration experience. Low and high response groups for each of these factors were determined in outbred rats by the median locomotor response to novelty and amphetamine prior to cocaine self-administration (pre-test), and to amphetamine during abstinence (post-test). Cocaine-seeking behavior during abstinence was measured by the level of drug-paired lever responding during extinction, and also during reinstatement induced by cocaine-associated cues, an amphetamine priming injection, and footshock stress. Animals with low and high locomotor responses to novelty and the amphetamine pre-test showed similar levels of cocaine-seeking behavior during extinction and reinstatement testing. Locomotor responses to amphetamine following cocaine self-administration (post-test) also failed to determine amphetamine's ability to reinstate cocaine-seeking behavior. Conversely, high levels of amphetamine-induced reinstatement were associated specifically with escalating cocaine intake during prior self-administration. These animals also developed locomotor sensitization to amphetamine following cocaine self-administration (post-test vs. pre-test), but the capacity to develop locomotor sensitization was not sufficient to determine a propensity for cocaine-seeking behavior. The findings suggest that the relationship between locomotor responses to novelty, amphetamine and behavioral sensitization a,nd the propensity for cocaine-seeking behavior during abstinence is complex, while the level of drug intake during prior self-administration is a primary determinant of this behavior.

[Neuropsychopharmacology 22:626–641, 2000]

KEY WORDS: *Drug addiction; Reinstatement; Self-Administration; Cocaine; Sensitization; Novelty; Craving; Stress*

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Received March 30, 1999; revised September 22, 1999; accepted December 23, 1999.

Studies on the etiology of drug addiction have utilized several animal models that assess the propensity to develop addictive behavior. These studies have found that heightened and prolonged locomotor responses to novelty (Piazza et al. 1989; Bardo et al. 1996; Pierre and Vezina 1997), and to an initial amphetamine challenge (Piazza et al. 1989), predict a propensity to acquire self-administration of low doses of psychostimulants. Another factor thought to contribute to drug addiction is behavioral sensitization, a phenomenon where repeated drug exposure increases the sensitivity and magnitude of behavioral responses to the drug. Although sensitization is most often studied using loco-

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655 Avenue of the Americas, New York, NY 10010

0893-133X/00/$–see front matter
PII S0893-133X(99)00160-8

motor responses as a dependent variable, it is thought to encompass other qualities of psychostimulants such as reinforcing efficacy and incentive value (Robinson and Berridge 1993). Indeed, repeated exposure to psychostimulants also increases the propensity to acquire psychostimulant self-administration at low doses (Horger et al. 1990 1992; Piazza et al. 1990), and enhances the reinforcing efficacy of psychostimulants during maintenance of self-administration (Mendrek et al. 1998).

While these effects could contribute to the initiation and maintenance of drug self-administration habits, there is very little data bearing on their relationship to another core feature of addiction: the propensity or vulnerability to relapse during abstinence from chronic drug self-administration. The propensity for relapse during abstinence has been modeled in both extinction and reinstatement paradigms (e.g., see Self 1998; Self and Nestler 1998, Tran-Nguyen et al. 1998). These paradigms may represent valid models of drug craving because similar environmental and pharmacological stimuli trigger both drug-seeking behavior in animals and drug craving in humans (e.g., Jaffe et al. 1989; Robbins et al. 1997, Sinha et al. 1999). Moreover, these paradigms may have predictive validity in evaluating anti-craving compounds for use in the treatment of addiction (Fuchs et al. 1998).

In the extinction paradigm, drug-seeking behavior is measured by the magnitude and persistence of non-reinforced responding at a drug-paired lever following acquisition of drug self-administration. Following extinction testing, the ability of specific experimenter-delivered stimuli to elicit or "reinstate" drug-paired lever responding is measured. Reinstatement of cocaine-seeking behavior can be induced by priming injections of drugs (*e.g.*, Gerber and Stretch 1975; De Wit and Stewart 1981), presentation of drug-associated stimuli or "cues" (De Wit and Stewart 1981; Meil and See 1997; Fuchs et al. 1998), and following brief periods of intermittent footshock stress (Erb et al. 1996; Ahmed and Koob 1997).

In this study, we tested whether individual differences in locomotor responses to novelty, amphetamine, and the capacity to develop locomotor sensitization would determine a propensity for cocaine-seeking behavior during abstinence. Low and high response groups were determined in outbred rats by the median locomotor response to novelty and amphetamine before cocaine self-administration (pre-test), and by the median locomotor response to amphetamine during abstinence (post-test). Low and high response groups for each factor were compared for the level of cocaine-seeking behavior during extinction, and during reinstatement testing with cocaine-associated cues, amphetamine, and footshock stress. The capacity to develop locomotor sensitization also was compared in these response groups (post-test vs. pre-test). Conversely, the propensity for cocaine-seeking behavior was compared to other behavioral measures in low and high groups determined by the median response to amphetamine-induced reinstatement. Finally, the strength of association between several behavioral measures, including locomotor responses, drug intake, and responding during extinction and reinstatement, was assessed by correlation.

METHODS

Subjects

Fifty-six outbred male Sprague-Dawley rats (Charles River, Kingston, NY), weighing 300–325 grams on arrival, were used in this study. Animals were individually housed in a climate-controlled environment (21°C) with a 12-hr light-dark cycle (lights on at 7:00 A.M.). Subjects were allowed free access to food and water, except during food training and initial cocaine self-administration (see below). All animals were maintained according to the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985).

Surgery

Animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) supplemented with atropine sulfate (0.10 mg/animal, s.c.), prior to surgical implantation of a chronic indwelling intravenous catheter. The catheters consisted of Silastic® tubing (0.02″ i.d., 0.037″ o.d.; Green Rubber, Woburn, MA) treated with tridodecylmethyl ammonium chloride (TDMAC) heparin (Polysciences Inc., Warrington, PA). Each catheter was secured at the jugular vein with Mersiline surgical mesh (General Medical, New Haven, CT) and passed subcutaneously to exit the back through 22 gauge tubing embedded in dental cement on a Marlex surgical mesh base (Bard Inc., Cranston, RI). Following surgery, animals received a prophylactic injection of penicillin (60,000 IU/0.2 ml, i.m.), and antibiotic ointment was applied daily to the exit wound. Catheters were flushed daily with 0.2 ml of heparinized (20 IU/ml) bacteriostatic saline containing gentamycin sulfate (0.33 mg/ml) to prevent clogging and curb infection.

Apparatus

Locomotor activity was recorded in circular test chambers with a runway 10 cm wide, equipped with four pairs of photocells located at 90° intervals around the 56 cm perimeter. The operant test chambers (Coulbourn Instruments, Lehigh, PA) used for self-administration, extinction, and reinstatement testing were contextually distinct from the locomotor test chambers, and located in a different room. Each of these chambers was en-

closed in a Styrofoam encased unit, along with an infusion pump assembly. The infusion pump assembly consisted of a Razel Model A pump equipped with a 20-ml glass syringe that was connected to a fluid swivel (Stoelting #1) by Teflon tubing. Tygon® tubing connected the swivel to the animal's catheter assembly, and was enclosed by a metal spring. Each operant chamber contained two levers (4 cm × 2 cm, located 2 cm off the floor); during self-administration testing, a 20 g lever-press response at the active lever delivered an intravenous infusion of cocaine, and produced no programmed consequence at the inactive lever. Each cocaine infusion (1.0 mg/kg/infusion) was delivered over 10-s in a 0.1 ml volume. During the infusion period, a cue light (above the lever) was illuminated and the house lights were extinguished. The infusion period was followed by an additional 5-s time-out interval where responding at the active lever produced no programmed consequences. The illumination of the house lights signaled the end of the 15 s infusion/time-out interval.

Procedure

Seven groups of eight rats/group were received and tested at separate times over the course of seven months. To hasten acquisition of cocaine self-administration, animals were food deprived and trained to press the active lever for 45-mg sucrose pellets until they reached a criterion performance (100 correct responses) on three successive days. Animals were then fed *ad lib* before surgical catheterization and allowed one week recovery. Following recovery, all rats underwent an identical sequence of behavioral testing (see Figure 1). First, animals were tested on a Friday in an initial 5-hr (7:00A.M.–12:00P.M.) locomotor test session in the dark. Locomotor responses measured during the first two hours represented the novelty response. Then, rats were removed from the apparatus and given a subcutaneous (s.c.) injection of saline (1 ml/kg), and locomotor responses to the saline injection were measured over the third hour. At the end of the third hour, rats were again removed and injected with amphetamine (0.5 mg/kg, s.c.), and locomotor responses to amphetamine were measured for the final two hours of the session (amphetamine pre-test). Subcutaneous amphetamine injections (rather than i.p. cocaine) were used to assess locomotor responses because amphetamine produces longer and more reliable locomotor responses in a given animal across multiple treatments.

Beginning the following week, rats were allowed to acquire cocaine self-administration (1.0 mg/kg, i.v.) under a fixed-ratio 1: time-out 15 sec schedule of reinforcement in 10 daily 4-hr test sessions conducted 5 days/week (7:30–11:30A.M.). During the first week, animals were maintained at a constant body weight by food restriction to facilitate acquisition. On days 6–10, they were fed *ad lib* and given a single priming injection of cocaine at the beginning of the session. Catheter patency was verified at the end of the self-administration phase by intravenous infusion of the short-acting barbiturate sodium methohexital (1.0 mg/ml); a positive test was indicated by rapid onset of brief anesthesia. Following the self-administration phase, animals were placed in the same operant test chambers for five days of extinction testing in 4-hr sessions at the same time of day, where responses at the drug-paired lever were recorded but produced no programmed consequence.

During the following week, the ability of specific

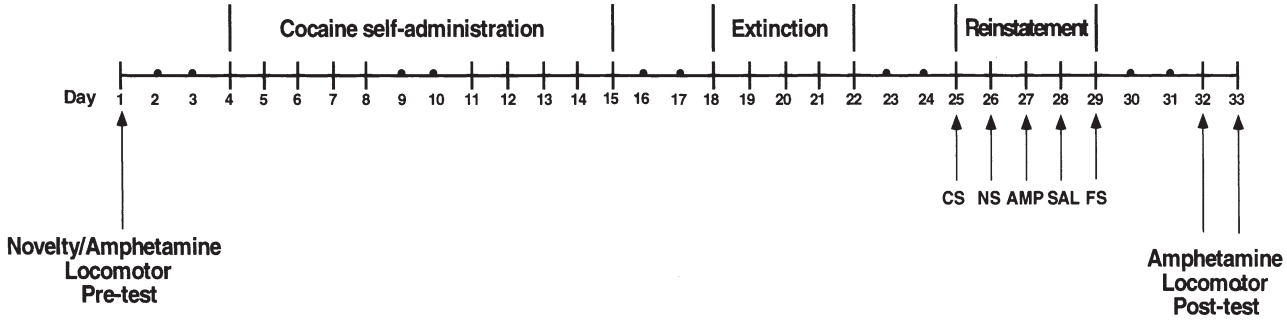

Figure 1. Time course of the behavioral testing procedure. Each stroke along the abscissa denotes an independent experimental test session conducted on separate days, as indicated below the line. The black dots denote days without behavioral testing. Pre- and post-tests for locomotor activity were conducted over five hours. Responses were measured during two hours of novelty/habituation, one hour following a saline injection, and two hours following an amphetamine injection (0.5 mg/kg). A dose of 1.0 mg/kg amphetamine was used in the second post-test at the end of the experiment. Cocaine self-administration, extinction, and reinstatement testing were conducted in 4-hr test sessions. During reinstatement testing, five different experimenter-delivered stimuli were given on consecutive days, each immediately following three hours of additional extinction conditions. Reinstatement baselines were determined by the level of drug-paired lever responding for the 1-hr period immediately prior to reinstatement testing (see Table 1). CS, conditioned stimuli (cocaine-associated cues), NS, novel stimulus (not drug-associated), AMP amphetamine (0.5 mg/kg, s.c.), SAL saline (1 ml/kg, s.c.), FS footshock stress.

stimuli to induce cocaine-seeking behavior was assessed in five consecutive reinstatement test sessions. Each reinstatement test session consisted of four hours of extinction conditions (7:30–11:30A.M.) where responses at the drug-paired lever produced no consequence. The first three hours served to extinguish residual responding to low levels prior to each reinstatement test. Response baselines were determined by the number of drug-paired lever responses during the third hour, immediately prior to each reinstatement test (Table 1). Each of five reinstatement test stimuli were given either prior to, or during, the fourth hour of the test session, and the level of drug-paired and inactive lever responding was measured throughout the fourth hour. The five reinstatement test stimuli were given in the following sequence: 1) CS (conditioned stimuli), cues specifically associated with cocaine infusions during self-administration (*i.e.*, cue-light on, house-light off, pump sound along with a saline infusion) presented over 10 seconds every 2 minutes during the fourth hour of the reinstatement test session; 2) NS, a novel stimulus (4 kHz, 76 dB tone) presented, like the CS, over 10 seconds every 2 minutes during the fourth hour; 3) Amph, an amphetamine priming injection (0.5 mg/kg, s.c.) given at the beginning of the fourth hour of the session; 4) Sal, a saline priming injection (1 ml/kg, s.c.) given at the beginning of the fourth hour; 5) FS (footshock stress), a 0.25 mAmp/0.5 s footshock delivered every 30 seconds for a 15-min period immediately prior to the fourth hour of the session. This duration and amperage of footshock stress produced marked startle responses in all animals tested. Control stimuli were spaced between effective reinstating stimuli to reduce the possibility of residual response carry-over from one effective stimulus to another. Footshock stress was tested last to avoid the effects of aversive conditioning of the test environment on subsequent test stimuli. Reversing the order of CS and NS presentation does not alter the differential ability of these stimuli to induce drug-paired lever responding under these conditions (D.W. Self, unpublished data).

On the following Monday, animals were tested for locomotor responses to amphetamine during the post-test (0.5 mg/kg, s.c.), under conditions identical to the novelty/amphetamine pre-test. To test for a possible ceiling to locomotor responses, a second locomotor post-test was conducted the next day at a higher dose of amphetamine (1.0 mg/kg, s.c.).

Post-Hoc Determination of Low and High Response Groups

The test population was screened for adequate acquisition of cocaine self-administration. The criterion for acquisition was defined as self-administration of 30 or more cocaine infusions over the last three days of self-administration testing. Of the 56 animals tested, four animals were excluded from the analysis due to one death and three catheter failures. Sixteen of the remaining 52 animals failed to meet acquisition criterion and were classified as *non-responders.* Response profiles for the non-responders were compared with the remaining group of 36 *self-administering* animals across all behavioral tests, and are shown in Figure 2.

The 36 self-administering animals represent the test population used to determine "Low" and "High" response groups ($n = 18$/group) based on the median locomotor response to novelty (reported but not shown), and the median locomotor response to 0.5 mg/kg amphetamine during the pre-test (Figure 3), where both tests were conducted before cocaine self-administration. In other analyses, low and high response groups were determined by the median locomotor response to the 0.5 mg/kg amphetamine post-test (Figure 4), and the median number of drug-paired lever responses during amphetamine-induced reinstatement (Figure 5), both conducted during abstinence from cocaine self-administration (see Figure 1 for time points).

Data Analysis

Locomotor responses to novelty, saline, and the amphetamine pre- and post-tests in low and high response groups were compared using a 2-factor (group × test condition) analysis of variance (ANOVA) followed by a

Table 1. Drug-Paired Lever Responding Immediately Prior to Reinstatement Testing[a]

	Novelty		Pre-test		Post-test		Amph Rstmt	
Primer	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**
CS	7.1 (2.1)	8.9 (2.3)	4.9 (1.6)	11.1 (2.5)*	5.7 (2.3)	10.3 (2.0)	5.2 (1.4)	10.8 (2.6)
NS	4.3 (1.5)	1.8 (0.8)	4.3 (1.5)	1.7 (0.8)	4.1 (1.6)	1.9 (0.7)	2.3 (1.1)	3.8 (1.4)
Amph	2.3 (0.6)	3.9 (1.5)	2.1 (0.8)	4.1 (1.4)	2.5 (1.1)	3.7 (1.2)	3.7 (1.0)	2.5 (1.4)
Saline	5.2 (2.5)	2.1 (0.9)	3.4 (1.2)	3.9 (2.4)	3.1 (1.7)	5.5 (2.6)	4.7 (2.6)	2.7 (0.7)
Footshock	3.1 (2.5)	1.8 (1.0)	2.7 (1.6)	2.3 (1.1)	1.8 (0.6)	1.8 (1.0)	1.7 (1.0)	3.3 (1.7)

[a]Mean (± SEM) responses at the drug-paired lever during the 1-hr period preceding each reinstatement test (hr 3 of the 4-hr reinstatement test session). CS, cocaine-associated cues; NS, novel stimulus; Amph, amphetamine. Asterisk signifies different from low response group by simple effect test (*$p \leq .05$).

test of simple effects for group on each test condition. The level of sensitization was analyzed by comparing locomotor responses to 0.5 mg/kg amphetamine during the pre- and post-tests with paired t-tests. Group comparisons for cocaine self-administration and extinction responding were analyzed separately with 2-factor (group × test day) ANOVA followed by tests of simple effects for group on each test day. Dependent measures were the number of self-infusions during cocaine self-administration and the number of responses made at the drug-paired lever during extinction. The average daily cocaine intake (total number of infusions/10 days × 1 mg/kg/infusion) for each group is illustrated in Figures 2B–5B; a main effect of group represents a statistically significant difference for this measure. Separate analyses were conducted for drug–paired and inactive lever responses during reinstatement with a 2-factor ANOVA (group × stimulus), followed by tests for simple effects for group on each stimulus. Comparisons between the CS and the NS (control), and between the amphetamine priming injection or footshock stress and the saline injection (control), were conducted separately in the self-administering and non-responding groups with Wilcoxon signed ranks tests of related measures. Finally, several behavioral measures were compared in the 36 self-administering animals using Pearson product-moment correlation coefficients (see Table 2).

RESULTS

Comparison of Self-administering Animals with Non-responders

In this analysis, animals that met acquisition criterion (n = 36) were compared with non-responders ($n = 16$). Figure 2A shows that both non-responders and the self-administering animals exhibited similar locomotor responses to novelty and the 0.5 mg/kg amphetamine pre-test prior to self-administration. However, only the self-administering group of animals exhibited a mean 47 ± 13 % sensitization of locomotor responses to amphetamine between the pre- and post-tests. The self-administering group also exhibited greater locomotor responses at a higher dose of amphetamine during the second locomotor post-test (main effect of group, $F_{4,200} = 61.5$, $p < .001$; group × locomotor test interaction, $F_{4,200} = 2.53$, $p < .05$). Given that both groups exhibited further increases in locomotor responses at the 1.0 mg/kg amphetamine, sensitization in non-responders was not precluded by a locomotor response ceiling.

Since self-administration data was used as an independent variable (see Methods), these data are shown but were not analyzed statistically (Figure 2B). Acquisition of cocaine self-administration in the self-administering group is reflected by higher and increasing levels of self-administration, whereas non-responders showed lower and decreasing levels of self-administration. During extinction testing, the self-administering group showed higher levels of non-reinforced drug-paired lever responding than non-responders (main effect of group, $F_{1,50} = 7.28$, $p = .01$). Higher responding in the self-administering group was found over the first three days of extinction testing (Figure 2B), but converged with non-responders over the last two test days (group × test day interaction, $F_{4,200} = 3.36$, $p = .01$).

Figure 2C shows the level of drug-paired lever responding induced by the five reinstatement test stimuli. Overall, the self-administering group showed greater drug-paired lever responding than non-responders during reinstatement testing (main effect of group; $F_{1,50} = 4.94$, $p < .05$). The differential ability of these stimuli to induce responding in the two groups resulted in a group × test stimulus interaction ($F_{4,200} = 6.06$, $p < .001$). Presentation of cocaine-associated cues induced modest, but greater drug-paired lever responding in the self-administering group when compared both to non-responders (Figure 2C) and to a similar schedule of novel stimulus presentation ($p < .001$). Similarly, a priming injection of amphetamine induced markedly greater responding in the self-administering group when compared both to non-responders (Figure 2C) and to a saline injection ($p < .001$). A brief period of intermittent footshock stress immediately prior to testing induced a modest, but greater level of drug-paired lever responding than a saline control injection in the self-administering group ($p < .05$). Footshock stress also induced extremely high responding in 2 of the 16 non-responders (Figure 2C), thereby preventing the detection of statistical differences between the two groups. However, footshock stress, the amphetamine priming injection, and cocaine-associated cues all failed to induce statistically significant drug-paired lever responding in non-responders when compared to the saline or novel stimulus controls (all p values $> .05$).

Overall *inactive* lever responding was significantly different among reinstatement test stimuli (main effect of stimulus, $F_{4,200} = 4.47$, $p < .05$), primarily due to enhanced inactive lever responding following exposure to footshock stress (not shown). Footshock-induced inactive lever responding was lower than drug-paired lever responding (12.8 ± 8.24 and 5.7 ± 1.22 lever-presses in non-responders and self-administering animals, respectively), but was significantly different from the saline injection control in self-administering animals ($p < .05$). However, the amphetamine priming injection and cocaine-associated cues induced very low levels of inactive lever responding that were not different from control stimuli (p values $> .05$), and were similar between self-administering and non-responding groups (means ranged from 2.4–4.6 and 0.25–2.0 lever-presses, respectively). Thus, the amphetamine priming injection and cocaine-associated cues induced responding selectively at

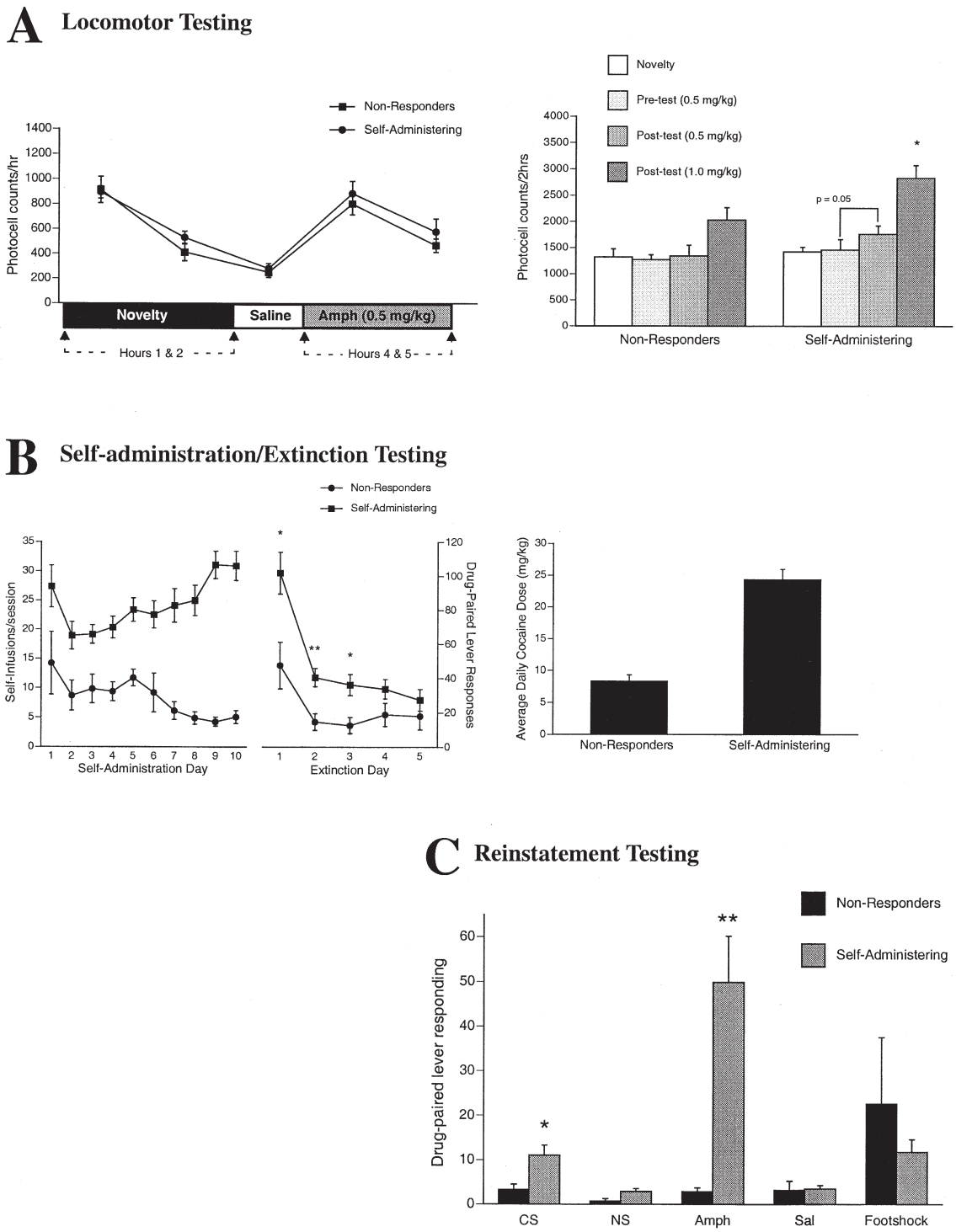

Figure 2. Comparison of behavioral responses in groups determined by criterion for acquisition of cocaine self-administration (⩾ 30 responses on days 8–10 of self-administration testing). This criterion effectively distinguished the level of cocaine self-administration in the self-administering (n = 36) and non-responding (n = 16) groups, as shown in the left panel of **(B)**. Results from locomotor testing are shown in **(A)**. The left panel shows the mean (± SEM) photocell crossings/hour for each hour of the initial 5-hr locomotor test for novelty and the amphetamine pre-test. The right panel shows two hour totals (mean + SEM) of photocell crossings during exposure to novelty, or following an amphetamine challenge during the pre- and post-tests. **(B)** The left panel shows the mean (± SEM) number of cocaine infusions for each of the ten 4-hr self-administration test sessions, and number of non-reinforced, drug-paired lever responses during each of the five 4-hr extinction test sessions. The right panel shows the average daily intake of cocaine during self-administration testing. **(C)** The mean (+ SEM) number of non-reinforced drug-paired lever responses are shown for five consecutive 1-hr reinstatement tests. Reinstatement tests consisted of

the drug-paired lever and only in self-administering animals, while footshock stress also induced greater, albeit less selective, drug-paired lever responding in this group.

The criterion for acquisition effectively screened self-administering animals from the test population, as indicated by drug-paired lever responding during reinstatement testing. Therefore, the 36 self-administering animals were used in all subsequent analyses to determine low and high response groups. Low and high response groups were determined by the median locomotor response to novelty (see below), to the amphetamine pre-test (Figure 3), to the amphetamine post-test (Figure 4), and to the median number of drug-paired lever responses during amphetamine-induced reinstatement (Figure 5). Results from these comparisons are described below.

Amphetamine Pre-test: "Low" vs. "High" Groups

Behavioral measures from low and high response groups (n = 18/group) determined by the median locomotor response to the 0.5 mg/kg amphetamine pre-test are shown in Figure 3. Figure 3A shows that animals with high pre-test responses also exhibited higher locomotor responses to novelty when compared to the low pre-test group, while responses to a saline injection did not differ (main effect of group $F_{1,34} = 8.98$, $p < .01$; group × test condition interaction, $F_{3,102} = 3.76$, $p = .01$). Post-test locomotor responses to amphetamine also were greater in high relative to low pre-test groups, indicating that this phenotypic distinction persisted throughout the experiment. However, only the low pre-test group developed locomotor sensitization to 0.5 mg/kg amphetamine following cocaine self-administration, as indicated by a mean 87 ± 19% increase from pre- to post-test responses (Figure 3A). The lack of sensitization in the high amphetamine pre-test group was not precluded by a locomotor response ceiling, because these animals showed further increases in locomotor responses to the 1.0 mg/kg amphetamine post-test.

The differential capacity to develop locomotor sensitization also cannot be accounted for by differences in cocaine intake during self-administration (Figure 3B), since both low and high pre-test groups self-administered similar amounts of cocaine over the 10 days of testing (main effect of group, $F_{1,34} = 2.89$, $p > .05$; group × test day interaction, $F_{9,306} = 0.80$, $p > .05$). In fact, the low pre-test group (that developed sensitization) averaged somewhat fewer self-infusions during the first half of training. Both low and high groups exhibited an initial decrease followed by a gradual increase in cocaine self-administration, resulting in a main effect of test day ($F_{9,306} = 5.25$, $p < .001$). There also was no difference in drug-paired lever responding during extinction testing between low and high amphetamine pre-test groups (main effect of group, $F_{1,34} = 0.07$, $p > .05$). Although a significant group × test day interaction was found ($F_{4,136} = 2.90$, $p < .05$), a test for simple effects revealed no difference in responding on any given day during extinction testing.

Figure 3C shows that low and high amphetamine pre-test groups exhibited similar drug-paired lever responding during reinstatement testing (main effect of group, $F_{1,34} = 0.04$, $p > .05$; group × stimuli interaction $F_{4,136} = 0.47$, $p > .05$). Thus, heightened locomotor responses to the amphetamine pre-test failed to predict a propensity for cocaine-seeking behavior during both extinction and reinstatement tests.

Response to Novelty: "Low" vs. "High" Groups

Low and high novelty response groups (n = 18/group) exhibited response profiles remarkably similar to the amphetamine pre-test groups described above. Thus, these data are reported but are not shown. Animals with high locomotor responses to novelty also showed significantly greater locomotor responses to the amphetamine pre-test ($p < .05$) and the post-test at 1.0 mg/kg ($p < .001$), but not to a saline injection ($p = .151$) or the post-test at 0.5 mg/kg ($p = .08$), resulting in a main effect of group ($F_{1,34} = 12.46$, $p < .001$) and a group × locomotor test interaction ($F_{4,136} = 4.25$, $p < .01$). The phenotypic distinction between low and high novelty response groups persisted throughout testing, since locomotor responses to a second "novelty" test differed ($p < .01$) during the post-test (1348 ± 89 and 1884 ± 140 photocell counts for low and high groups, respectively). However, only the low novelty response group developed locomotor sensitization following cocaine self-administration, as indicated by a mean 57 ± 15% increase in locomotor responses between the pre- and post-tests with 0.5 mg/kg amphetamine ($t_{17} = 3.89$, $p < .001$; $t_{17} = 0.47$, $p > .05$, for low and high groups, respectively). Low and high novelty groups self-administered equivalent amounts of cocaine throughout self-administration testing (main effect of group, $F_{1,34} = 0.64$, $p > .05$; group × test day interaction, $F_{1,34} = 0.52$, $p > .05$), and responded equally during extinction testing (main effect of group, $F_{1,34} = 0.01$, $p > .05$; group × test day interaction, $F_{1,34} = 1.67$, $p > .05$). Cocaine-associated cues, an amphetamine priming injection, and footshock

intermittent presentation of cocaine-associated cues (CS), similar presentation of a novel (non-associated) stimulus (NS), or immediately following a 0.5 mg/kg s.c. priming injection of amphetamine (Amph), an equivalent volume of saline (Sal), and 15-min of intermittent footshock (FS). *$p \leq .05$, **$p \leq .01$, ***$p \leq .001$, difference between groups by simple effects or main group effect. Locomotor pre-and post-tests with amphetamine (0.5 mg/kg) were compared by paired t-tests.

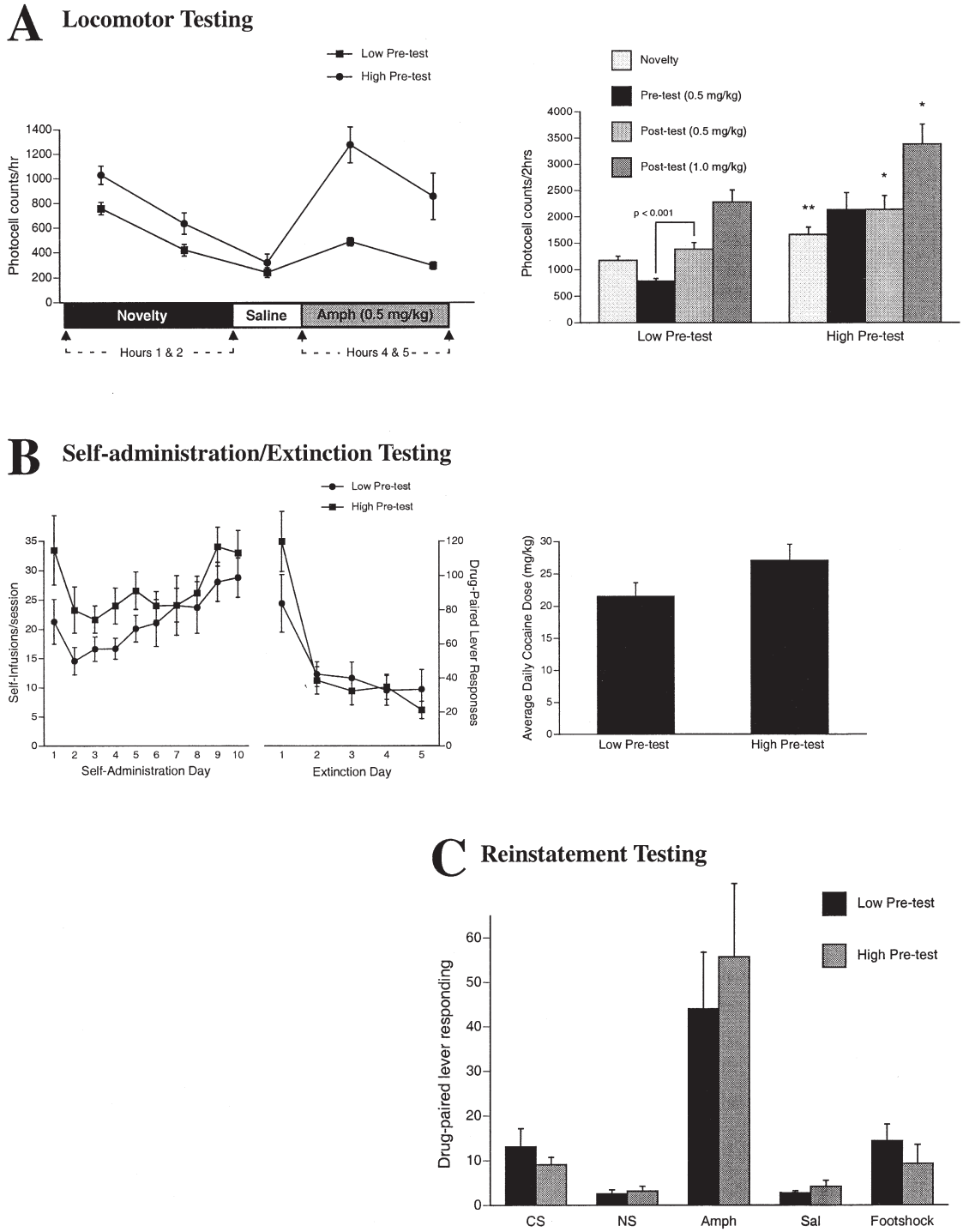

Figure 3. Comparison of behavioral responses in the 36 self-administering animals (see Figure 2) when "low" and "high" response groups (n = 18/group) were determined by the amphetamine locomotor pre-test (0.5 mg/kg) as an independent variable. This determination resulted in an approximate 3-fold difference in mean amphetamine pre-test responses between groups (shown as black bars in panel A). Data are presented as described in Figure 2.

stress also induced similar responding at the drug-paired lever during reinstatement testing in low and high novelty groups (main effect of group, $F_{1,34} = 0.02$, $p > .05$; group × stimulus interaction, $F_{4,136} = 0.17$, $p > .05$). Thus, heightened locomotor responses to novelty failed to predict the level of cocaine-seeking behavior during extinction and reinstatement testing in self-administering animals.

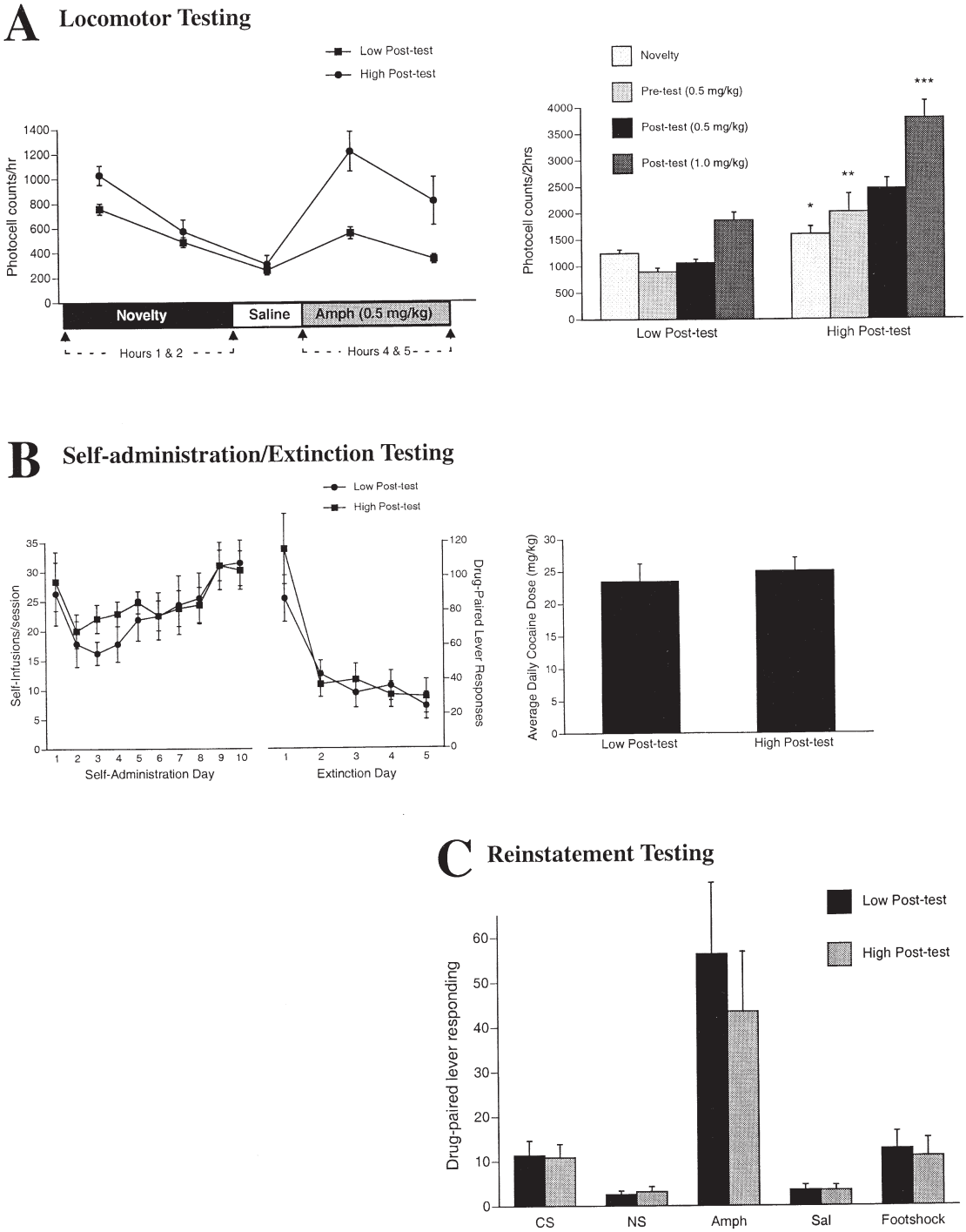

Figure 4. Comparison of behavioral responses in the 36 self-administering animals (see Figure 2) when "low" and "high" response groups (n = 18/group) were determined by the amphetamine locomotor post-test (0.5 mg/kg) as an independent variable. This determination resulted in an approximate 2.5-fold difference in mean amphetamine post-test responses (shown as black bars in panel A). Data are presented as described in Figure 2.

Amphetamine Post-test: "Low" vs. "High" groups

In order to test the potential impact of both initial sensitivity and behavioral sensitization following cocaine self-administration, locomotor responses to the 0.5 mg/kg amphetamine post-test were used to determine low and high response groups (n = 18/group). However, despite the fact that the high post-test group exhibited twice the locomotor response to amphetamine of the low post-test group (Figure 4A), the same dose of amphetamine induced similar levels of drug-paired lever

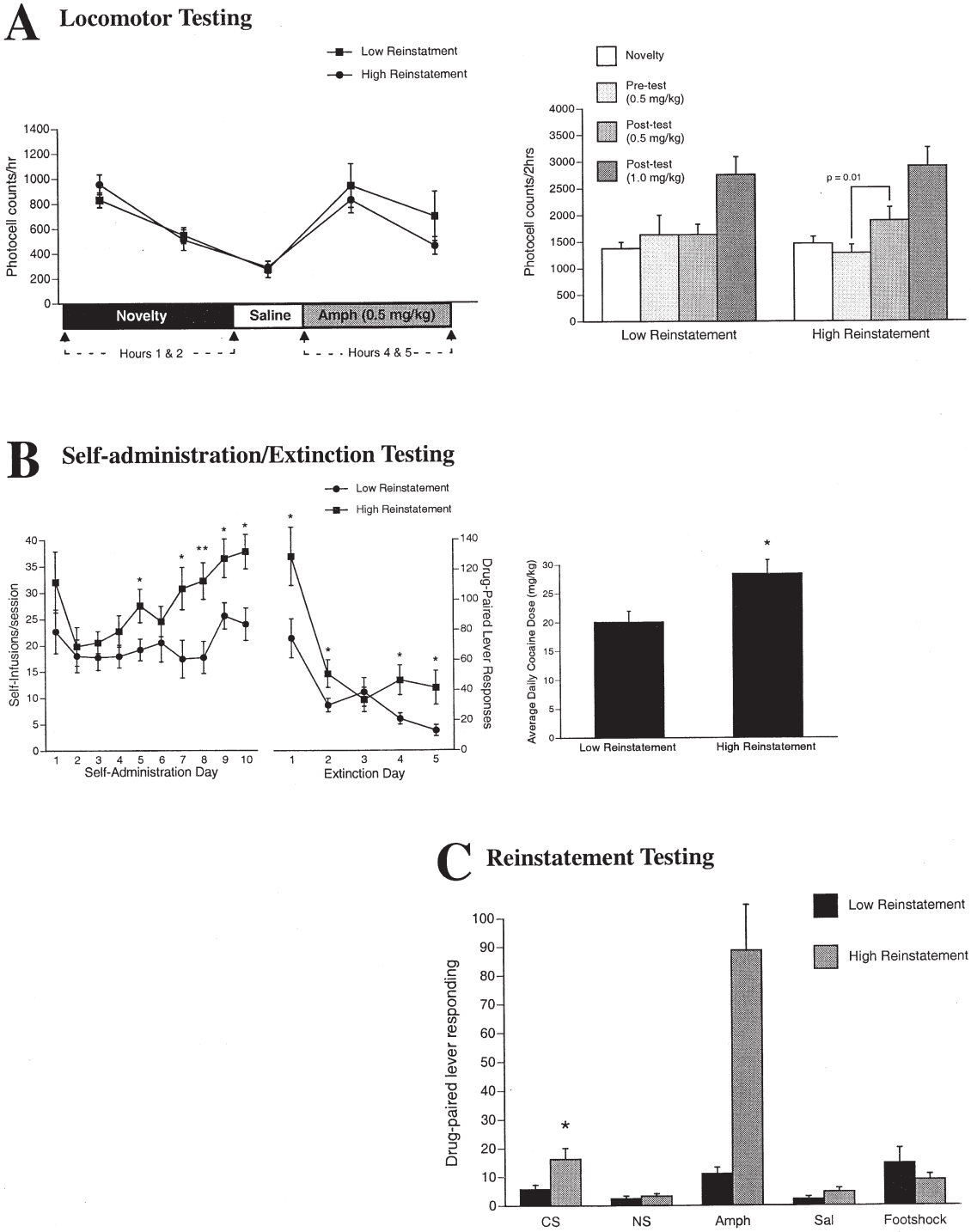

Figure 5. Comparison of behavioral responses in the 36 self-administering animals (see Figure 2) when "low" and "high" response groups (n = 18/group) were determined by the level of amphetamine-induced reinstatement (0.5 mg/kg) as an independent variable. This determination resulted in an approximate 8-fold difference in mean amphetamine-induced reinstatement of drug-paired lever responding as shown in panel C. Data are presented as described in Figure 2.

responding during reinstatement testing (Figure 4C). Reinstatement induced by cocaine-associated cues and footshock stress also was similar between low and high post-test groups (main effect of group, $F_{1,34} = 0.38$, $p > .05$; group × stimulus interaction, $F_{4,136} = 0.33$, $p > .05$). Even when low and high groups were determined by the bottom and top third of amphetamine post-test responses (not shown), there was no difference in amphetamine-induced reinstatement between these low and high post-test groups (62.0 ± 22.1 and 59.7 ± 18.4

drug-paired lever responses, respectively), despite a 3-fold difference in locomotor responses to amphetamine (922 ± 61.5 and 2873 ± 209 photocell counts, respectively).

Figure 4B shows that low and high amphetamine post-test groups (determined by the median) self-administered similar amounts of cocaine throughout the 10-day training period (main effect of group, $F_{1,34} = 0.20$, $p > .05$; group × test day interaction, $F_{9,306} = 0.92$, $p > .05$), and exhibited similar levels of drug-paired lever responding during extinction testing ($F_{1,34} = 0.30$, $p > .05$; group × test day interaction, $F_{4,136} = 1.49$, $p > .05$). In contrast, Figure 4A shows that locomotor responses to novelty and the amphetamine pre-test prior to cocaine self-administration were greater in the high post-test group (main effect of group, $F_{1,34} = 21.48$, $p < .001$; group × locomotor test interaction, $F_{3,102} = 14.79$, $p < .001$). Although the overall profile of the amphetamine post-test groups is similar to the novelty and amphetamine pretest groups, neither low nor high amphetamine post-test groups developed locomotor sensitization following cocaine self-administration (Figure 4A), indicating that these groups represent distinct sub-populations from groups determined by novelty and amphetamine pre-test responses.

Amphetamine-induced Reinstatement: "Low" vs. "High" Groups

In this analysis, we tested whether high levels of cocaine-seeking behavior were associated with any other behavior measured in the experiment. Animals were divided into low and high response groups (n = 18/group) based on the median number of drug-paired lever responses induced by amphetamine during reinstatement testing (Figure 5C). Figure 5A shows that both low and high amphetamine reinstatement groups exhibited similar locomotor responses across all locomotor tests (main effect of group, $F_{1,34} = 0.02$, $p > .05$; group × locomotor test interaction, $F_{4,36} = 0.95$, $p > .05$). However, only the high amphetamine reinstatement group developed sensitized locomotor responses to 0.5 mg/kg amphetamine, as indicated by a mean 66 ± 21% increase in post-test compared to pre-test responses. The low amphetamine reinstatement group showed no evidence of sensitization.

The high amphetamine reinstatement group also exhibited greater levels of cocaine intake during prior self-administration than the low reinstatement group ($F_{1,34} = 7.44$, $p = .01$). Initially, both groups self-administered similar amounts of cocaine, but cocaine intake in the high reinstatement group escalated throughout testing, whereas cocaine intake in the low reinstatement group remained lower and more steady (Figure 5B). The high reinstatement group self-administered significantly more cocaine during five of the last six days of self-administration training. The ~50% greater increase in cocaine intake in the high reinstatement group cannot be explained by major differences in body weight, since both low and high groups averaged 418 ± 10.3 g and 448 ± 12.1 g on the last day of self-administration testing, respectively ($p > .05$). Following self-administration, the high reinstatement group also exhibited greater drug-paired lever responding during extinction than the low group (main effect of group, $F_{1,34} = 5.58$, $p < .05$; group × test day interaction; $F_{4,136} = 3.57$, $p < .01$); higher responding persisted throughout four of the five extinction test days (Figure 5B).

In addition to amphetamine-induced reinstatement (the independent variable), Figure 5C shows that cocaine-associated cues, but not footshock stress, induced greater drug-paired lever responding in the high reinstatement group when compared to the low group, resulting in a significant group × stimulus interaction ($F_{3,102} = 3.41$, $p < .05$). However, baseline response rates in the 1-hr period prior to reinstatement testing also were somewhat greater in the high reinstatement group compared to the low group (Table 1). Nonetheless, only the high, and not the low, amphetamine reinstatement group further increased responding from baseline levels during presentation of cocaine-associated cues.

Reinstatement Baselines

Since low and high response groups did not always extinguish to similar levels during extinction testing, it was important to determine reinstatement baselines during the 1-hr period immediately prior to each reinstatement test (Table 1). Baseline response rates were low (<5 responses/hour) and similar between groups for most reinstatement tests. However, baseline response rates prior to testing with cocaine-associated cues were somewhat greater in "High" groups from all low/high determinations except novelty. Only the high reinstatement group showed further increases in response to cocaine-associated cues, whereas only the low pre- and post-test groups showed further increases. Thus, the level of baseline responding did not entirely predict the ability of cocaine-associated cues to further increase drug-paired lever responding.

Correlation among Locomotor Activity, Cocaine Self-administration, and Reinstatement

Although many of the predicted relationships were not found, certain behavioral measures were significantly correlated with locomotor responses to novelty, the amphetamine pre-test, locomotor sensitization, and the level of cocaine-seeking behavior during abstinence. The strongest positive correlations were between locomotor responses to novelty and the amphetamine pre-

Table 2. Pearson Correlation Matrix[a]

	Locomotor Responses							Priming Stimulus		
Variable	**Novelty**	**Pre-test**	**Post-test**	**Post/Pre**	**$S.A._{avg}$**	**$Ext._{day1}$**	**$Ext._{day5}$**	**CS**	**Amph**	**FS**
Novelty	—	**0.608****	**0.551****	−0.090	0.117	0.187	−0.112	0.086	0.040	0.053
Pre-test	—	—	**0.670*****	**−0.433***	0.085	0.117	−0.120	−0.079	−0.080	−0.036
Post-test	—	—	—	0.175	0.185	0.260	0.104	−0.004	0.175	0.131
Post/Pre	—	—	—	—	0.045	0.018	0.221	0.220	0.183	0.002
$S.A._{avg}$	—	—	—	—	—	**0.464****	**0.455****	0.284	0.300	−0.123
$Ext._{day1}$	—	—	—	—	—	—	**0.645*****	0.381	**0.419***	0.272
$Ext._{day5}$	—	—	—	—	—	—	—	**0.638*****	0.138	0.036
CS	—	—	—	—	—	—	—	—	0.259	−0.055
Amph	—	—	—	—	—	—	—	—	—	−0.095

[a]Correlation coefficients (Pearson's Product Moment) for multiple comparisons of test variables. Correlation coefficients with p values $\leq .1$ are indicated in bold. Asterisks indicate p values with error corrected for multiple (10) comparisons: *$p \leq .10$, **$p \leq .05$, ***$p \leq .01$. Abbreviations: SA_{avg}, average daily cocaine self-administration over 10 days; Ext., non-reinforced drug-paired lever responding on the first and fifth day of extinction; CS, cocaine-associated cues; Amph, amphetamine; FS, footshock stress.

and post-tests (Table 2). Amphetamine pre-test responses were negatively correlated with the development of locomotor sensitization following cocaine self-administration (post-test/pre-test, an index of sensitization). Although high levels of amphetamine-induced reinstatement were associated with locomotor sensitization (Figure 5A), the degree of locomotor sensitization (post-test/pre-test) failed to correlate with the level of drug-paired lever responding during extinction and reinstatement testing (Table 2).

In contrast, drug-paired lever responding during extinction testing (first and last test day) was positively correlated with the level of cocaine intake during prior self-administration, and with subsequent reinstatement induced by cocaine-associated cues. The level of drug-paired lever responding during the first, but not last, day of extinction was positively correlated with the ability of amphetamine to reinstate drug-paired lever responding. However, the association of higher drug intake during self-administration with higher drug-paired lever responding during amphetamine and cue-induced reinstatement (as shown in Figure 5) failed to correlate significantly. The ability of footshock stress to reinstate drug-paired lever responding was not significantly correlated with any other parameter measured.

DISCUSSION

The propensity for drug craving and relapse to drug use during abstinence is a primary pathological disturbance in drug addiction. In the present study, we identified a specific sub-population of self-administering rats that reflect this propensity by exhibiting greater and more persistent cocaine-seeking behavior during extinction testing, and during reinstatement induced by an amphetamine priming injection (Figure 5C). Similarly, these animals showed greater increases in responding during presentation of cocaine-associated cues relative to the low reinstatement group. However, baseline response rates prior to cue testing also were higher in this group (Table 1), which may have facilitated their responsiveness to the cues. In any event, these animals apparently are more sensitive to the ability of both conditioned environmental and pharmacological stimuli to maintain and reinstate cocaine-seeking behavior during abstinence, which may reflect their ability to activate a common neural substrate. Although the mechanism for cue-induced reinstatement is not entirely understood, both conditioned cues and pharmacological stimuli may utilize the mesolimbic dopamine system during reinstatement of drug-seeking behavior (see Self and Nestler 1998 for review), and increased dopamine levels have been detected in the amygdala during extinction testing (Tran-Nguyen et al. 1998). Somewhat surprisingly, this sub-population of animals did not differ in footshock stress-induced reinstatement. It is unlikely that greater responding was precluded by a ceiling effect, because higher shock amperages or longer periods of footshock stress both produce greater responding at the drug-paired lever (Erb et al. 1996; D.W. Self, unpublished data). It also is unlikely that these animals were less sensitive to the sensation of footshock, since all animals exhibited marked startle responses to the footshocks. The lack of correlation between footshock-induced reinstatement and any other measure of cocaine-seeking behavior is consistent with the idea that footshock stress may utilize dopamine-independent pathways to reinstate drug-seeking behavior (Shaham and Stewart 1996; Erb et al. 1998; Self and Nestler 1998).

The only behavioral measure specifically associated with the level of cocaine-seeking behavior during abstinence was the level of cocaine intake during prior self-administration. As shown in Figure 5B, both low and high amphetamine reinstatement groups initially self-administered similar amounts of cocaine, but the level of cocaine intake in the high reinstatement group esca-

lated throughout self-administration testing. In addition, the magnitude and persistence of cocaine-seeking behavior during extinction testing was positively correlated with the level of drug intake during self-administration. Escalating drug intake is a hallmark of drug dependence (DSM-IV 1994), and is suggested to reflect an upward shift in the "hedonic set point" that signifies a change from controlled to uncontrolled drug-taking during self-administration binges (Ahmed and Koob 1998). Association of escalating cocaine intake with a propensity for drug-seeking behavior during abstinence encompasses two major features of the addicted state. The critical association of these features suggests that this animal model could be useful to identify genetic and neurobiological determinants of addictive behavior in future studies.

Although both low and high amphetamine reinstatement groups actively engage in cocaine self-administration, it is possible that the low group would achieve similar levels of drug intake (and drug-seeking behavior) over a longer training period. However, we have observed a similar positive association between higher levels of cocaine intake and higher cocaine-seeking behavior during extinction testing when animals are allowed to achieve stabilized rates of self-administration (D.W. Self, unpublished data). Taken together, these results suggest that animals escalating to higher stabilized levels of cocaine intake rapidly become less sensitive (tolerant) to factors that limit cocaine intake during self-administration, and compensate by taking infusions at shorter time intervals. This interpretation is supported by the fact that similar rate increases are produced by lowering the infusion dose over this dose range (e.g., Self et al. 1998), and cocaine intake is increased by pre-treating animals with dopamine antagonists under similar fixed-ratio schedules of reinforcement (*e.g.*, De Wit and Wise 1977). Our findings suggest that decreased sensitivity to the rate-limiting effects of cocaine also may impart a propensity for cocaine-seeking behavior during abstinence. Alternatively, these factors could alter the propensity for cocaine-seeking behavior indirectly, through neuroadaptations produced by higher levels of cocaine exposure. In contrast, sensitivity to amphetamine-induced locomotion apparently is unrelated to sensitivity to cocaine's rate-limiting effects, as both low and high locomotor response groups exhibited similar levels of cocaine intake (Figure 3B and 4B).

Heightened locomotor responses to novelty and amphetamine predict the subsequent ability of threshold doses of amphetamine and cocaine to support self-administration (Piazza et al. 1989; Piazza and Le Moal 1996; Pierre and Vezina 1997), although they do not predict greater sensitivity to cocaine reward as measured by place conditioning (Gong et al. 1996). In our study, the dose of cocaine used (1.0 mg/kg/infusion) was well above threshold for acquisition and maintenance of self-administration (e.g., Horger et al. 1990). Thus, our findings agree with others in that both low and high response groups readily acquire cocaine self-administration at higher doses (Piazza and Le Moal 1996). In addition, these low and high response groups exhibited many characteristics first reported by Piazza et al. (1989), including (1) a strong positive correlation between locomotor responses to novelty and the amphetamine pre-test, and (2) low, but not high, responders develop sensitization as a result of cocaine self-administration, despite equivalent levels of cocaine intake over the acquisition period. This latter effect may reflect a differential capacity to develop sensitization in low responders, rather than a pre-existing sensitized state in high responders, because sensitization was not precluded by a locomotor response ceiling in high responders. In contrast, "non-responders" exhibited similar locomotor responses to novelty and the amphetamine pre-test as the overall group of self-administering animals (Figure 2A). Factors that prevented acquisition of cocaine self-administration in this group are unknown, but could involve increased sensitivity to the aversive effects of cocaine at a relatively high training dose.

Heightened locomotor responses to novelty and the amphetamine pre-test failed to predict higher levels of cocaine-seeking behavior during extinction and reinstatement testing (Figures 3B and 3C and see Results). Conversely, animals with high levels of amphetamine-induced reinstatement showed similar locomotor responses to novelty and the amphetamine pre-test as the low reinstatement group (Figure 5A). Moreover, there was no correlation between these locomotor responses and the level of cocaine-seeking behavior during extinction and reinstatement testing (Table 2). Thus, under conditions where both low and high responders acquire self-administration, and voluntarily take similar amounts of cocaine, these factors are not associated with a propensity for cocaine-seeking behavior during abstinence. Furthermore, the phenotypic distinction between low and high responders was still detectable at the end of the testing procedure (Figure 3A), although sensitization in low responders partially diminished the magnitude of this difference, and could account for similar propensities for cocaine-seeking behavior between these groups.

However, individual locomotor responses to amphetamine during abstinence from cocaine self-administration also failed to predict amphetamine's ability to reinstate cocaine-seeking behavior (Figure 4C). Conversely, animals exhibiting low and high levels of amphetamine-induced reinstatement exhibited similar levels of amphetamine-induced locomotion in the post-test (Figure 5A). It is unlikely that a response ceiling for either locomotion or reinstatement obscured a positive association between these variables, because higher

doses of amphetamine are reported to induce even greater responding during reinstatement testing (De Wit and Stewart 1981), and both low and high amphetamine post-test groups showed greater locomotor responses at a higher dose of amphetamine (Figure 4A). Although both locomotion and reinstatement are thought to be mediated by amphetamine-induced dopamine release, it is possible that these behaviors are differentially modulated by amphetamine effects on norepinephrine and serotonin release. Another possibility could involve the differential involvement of D_1 and D_2 dopamine receptors in amphetamine-induced locomotion and reinstatement. Thus, while activation of either D_1 or D_2 receptors induce locomotor responses, only D_2 receptor activation reinstates cocaine-seeking behavior, and D_1 receptor activation blocks cocaine-induced reinstatement (Wise et al. 1990; Self et al. 1996, De Vries et al. 1999). Therefore, it is possible that sensitivity to amphetamine's locomotor activating effects are determined by the combined sensitivity of both D_1 and D_2 receptors, whereas differences in amphetamine-induced reinstatement are determined primarily by the sensitivity of D_2 receptors.

Previous studies conducted between groups of sensitized and non-sensitized animals found that drug-induced reinstatement of cocaine- or heroin-seeking behavior is detectable only when sensitized locomotor responses to the same drug are evident (De Vries et al. 1998; De Vries et al. 1999; Vanderschuren et al. 1999). In contrast, the present within-group study found clear and prominent amphetamine-induced reinstatement in the absence of detectable locomotor sensitization to amphetamine (post-test vs. pre-test). Thus, amphetamine effectively reinstated cocaine-seeking behavior in the high amphetamine pre-test group (Figures 3A and 3C), and also in both low and high amphetamine post-test groups (Figure 4A and C), with little or no evidence of locomotor sensitization. These findings suggest that sensitization to locomotor activating effects of amphetamine is not necessary for amphetamine to reinstate cocaine-seeking behavior.

However, the relationship between locomotor sensitization and the *propensity* for cocaine-seeking behavior apparently is complex, since we found that the development of sensitization either is associated, or not associated, with this propensity depending on the subpopulation examined. Thus, animals exhibiting high, and not low, amphetamine-induced reinstatement also developed sensitized locomotor responses to amphetamine (Figure 5A). In contrast, animals with low novelty and amphetamine pre-test responses also developed sensitization, but without a greater propensity for amphetamine-induced reinstatement (Figures 3A and 3C). Moreover, there was no correlation between the degree of locomotor sensitization (post-test/pre-test) and any index of cocaine-seeking behavior during abstinence (Table 2). These findings suggest that while locomotor sensitization is associated with a propensity for cocaine-seeking behavior during abstinence, sensitization is not sufficient to determine this propensity. Interestingly, these different subpopulations may develop sensitization for different reasons, such as a greater level of cocaine intake (high amphetamine reinstatement group), or a biological predisposition (low amphetamine pre-test group).

Although cocaine and amphetamine may induce sensitization through different neural mechanisms (Vezina 1996; Steketee 1998), cross-sensitization between these drugs was found in the present study. However, since our study compared individual responses to amphetamine in both locomotor and reinstatement tests, differential sensitivity to amphetamine and cocaine cannot account for our findings. Moreover, since only self-administering animals, and not non-responders, exhibited both sensitization and reinstatement, the emergence of these features is related specifically to acquisition of cocaine self-administration, and is not an artifact of the surgical or testing procedures. It also is unlikely that a positive association was obscured by the 5-day interval between the amphetamine reinstatement and locomotor post-tests, since cocaine-induced sensitization is reported to be present at one week of abstinence and persist for at least one month (Henry and White 1995; Heidbreder et al. 1996).

One potential mechanism thought to contribute to the long-term expression of behavioral sensitization is supersensitivity of D_1 receptor responses in nucleus accumbens neurons (Henry and White 1991). If so, then locomotor sensitization could reflect the level of D_1-receptor supersensitivity, whereas sensitivity to amphetamine-induced reinstatement may be more directly related to D_2 receptor sensitivity, as suggested earlier. Another consideration is that this study was designed to measure sensitization to the "unconditioned" locomotor-activating effects of amphetamine, and so the potential contribution of conditioned factors to sensitization was not addressed (Browman et al. 1998). Conditioned drug effects are known to play a major role in cocaine craving in humans (Grant et al. 1996; Childress et al. 1999), and may play a greater role in determining sensitivity to the incentive/motivational properties of drugs and drug-associated stimuli than sensitization to unconditioned drug effects.

An addictive phenotype encompasses many behavioral features, including a propensity to initiate and maintain drug-taking, to escalate drug intake during self-administration binges, and to relapse during abstinence. Previous studies suggest that heightened locomotor responses to novelty and amphetamine, as well as behavioral sensitization, are important factors in initiating drug self-administration (at least at low doses), but our findings suggest that these factors do not directly determine a propensity for either escalation of

drug intake or relapse to cocaine-seeking behavior during abstinence. Instead, our results suggest that biological factors that contribute to, or result from, escalating drug intake during self-administration also contribute to an increased propensity for relapse during abstinence. The elucidation of biological factors that determine these important features of addiction remains a challenge for future investigation.

ACKNOWLEDGMENTS

We wish to acknowledge Michela Marinelli and Cindy Brandon for helpful comments and discussion pertaining to this study. This work was supported by United States Public Health Service Grants DA 10460 and DA 08227, and by the Abraham Ribicoff Research Facilities of the Connecticut Mental Health Center.

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